

Poster Session, July 18, 12:15 pm –2:15 pm

Abstracts and Biosketches

Ozone Inhalation Promotes CX3CR1-dependent Maturation of Resident Lung Macrophages

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Inhalation of ambient ozone alters populations of lung macrophages. However, the impact of altered lung macrophage populations on the pathobiology of ozone remains poorly understood. We hypothesized that sub-populations of macrophages modulate the response to ozone. We exposed C57BL/6J mice to ozone (2 ppm x 3hr) or filtered air. 24 h after the exposure, the lungs were harvested and digested and the cells underwent flow cytometry. Analysis revealed a novel macrophage subset present in ozone exposed mice, which were distinct from resident alveolar macrophages (AM) and identified by enhanced Gr-1+ expression (Gr-1 Macs). Further analysis identified that Gr-1+ Macs exhibited high expression of MARCO, CX3CR1, and NQO1. Gr-1+ Macs were present in the absence of CCR2, suggesting that they were not derived from a CCR2-dependent circulating intermediate. Using PKH26-PCL to label resident phagocytic cells, we demonstrated that Gr-1 Macs were derived from resident lung cells. This new subset was diminished in the absence of CX3CR1. Interestingly, CX3CR1-null mice exhibited enhanced responses to ozone, including increased airway hyperresponsiveness (AHR), accumulation of 8-isoprostanes and protein carbonyls, and increased expression of cytokines (CXCL2, IL-1 β , IL-6, CCL2, and TNF- α). Our results identify a novel subset of lung macrophages, which are derived from a resident intermediate, dependent upon CX3CR1, and appear to protect the host from the biological response to ozone.

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Role of Pattern Recognition Receptors in Organic Dust-Induced Airway Inflammation

Jill A. Poole

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Rationale: Organic dust exposure in the agricultural industry results in significant airway disease. Since we have recently demonstrated high concentration of bacterial peptidoglycan in large animal farming environments, we examined the role of innate immune receptors/sensors of peptidoglycan cell wall products, Toll-like receptor 2 (TLR2) and nucleotide oligomerization domain 2 (NOD2), in regulating organic dust-induced inflammation.

Methods: Monocytes were exposed to swine facility organic dust extract (ODE) and NOD2 expression by real-time PCR and Western blot was evaluated. The role of NF- κ B was evaluated by EMSA and NF- κ B pathway inhibitors. Isolated primary lung macrophages from NOD2 knock-out (NOD2^{-/-}), TLR2^{-/-}, and wild-type (WT) mice were *ex vivo* stimulated with ODE and cytokine/chemokines levels were quantitated by ELISA. Utilizing an established *in vivo* model, bronchoalveolar lavage (BAL) and lung tissues were collected from WT and KO mice challenged once or repetitively for 3wks to ODE or saline.

Results: Relative mRNA and protein expression of NOD2 increased following ODE stimulation, which was dependent on the NF- κ B pathway. NOD2^{-/-} lung macrophages demonstrated significantly enhanced

cytokine production following ODE stimulation. Airway neutrophils and BAL CXCL1 levels were significantly enhanced in NOD2^{-/-} mice following a one-time ODE exposure. After repetitive exposure, there was a significant increase in lung parenchymal inflammation indices in NOD2^{-/-} mice. In contrast, inflammatory outcomes were reduced in the absence of TLR2. Airway neutrophil influx, cytokine release, and bronchiolar inflammation were diminished in TLR2^{-/-} mice following ODE challenges.

Conclusions: NOD2 expression is enhanced following organic dust exposure by an NF- κ B-dependent pathway. NOD2 appears to selectively negatively regulate organic dust-induced pro-inflammatory consequences whereas TLR2 is a selective positive regulator.

Macrophage MMP-9 Gene Expression is Modulated by Rac1 Via Inhibition of SP-1 at Ser-586

A Brent Carter

University of Iowa

Asbestosis, which is the most debilitating asbestos-related lung disease, is a prototypical form of pulmonary fibrosis. Aberrant matrix deposition is a hallmark of asbestosis and is characterized by an imbalance between matrix deposition and degradation. The release of H₂O₂ by alveolar macrophages plays an integral role in the pathogenesis of aberrant matrix deposition in pulmonary fibrosis. An emerging target for ROS, such as H₂O₂, is the family of matrix metalloproteinases (MMPs) which regulate the fibrotic phenotype. Rac1, a small GTPase, is a key second messenger that is linked to the generation of H₂O₂. We have found that alveolar macrophages from patients with asbestosis generate greater than 10-fold more H₂O₂ and express greater than 30-fold less MMP-9 compared to normal subjects. This decrease in expression was associated with decreased macrophage-derived matrix metalloproteinase-9 (MMP-9) activity in BAL fluid and increased collagen deposition by fibroblasts exposed to BAL fluid. Compared to wild type (WT) macrophages, Rac1 null macrophages have significantly greater *MMP-9* gene expression which was due, in part, to increased SP-1 transcriptional activity. We have identified a PEST domain (575-595) in SP-1 with a score of 6.1 containing one threonine (T578) and two serine residues (S586 and S587). As ERK, a serine/threonine kinase, is constitutively active in Rac1 null cells and increases MMP-9 promoter activity, we hypothesized that SP-1-driven *MMP-9* gene expression is modulated by Rac1 and ERK at the threonine and/or serine residues. Transfection of macrophages with SP-1 containing mutations at these sites revealed that, compared to the empty vector, constitutively active Rac1 suppressed MMP-9-luciferase in cells over expressing WT SP-1 and SP-1 mutants T578A and S587A but not in cells expressing the S586A mutant. To examine whether these results were due to ERK, macrophages were transfected with MEK1, an upstream kinase of ERK, and SP-1_{WT} or SP-1_{S586A}. Over expression of MEK1 significantly increased SP-1_{WT}-driven MMP-9 promoter activity but did not alter MMP-9 promoter activity in cells expressing the S586A mutant suggesting that this is the site of ERK-mediated phosphorylation. Phosphorylation at this residue increased the stability of SP-1 as demonstrated by decreased half-life of SP-1_{S586A}. Furthermore, inhibition of ERK activity decreased abundance of SP-1_{WT} but not that of SP-1_{S586A}. In aggregate, these novel observations suggest that SP-1 is a critical target of Rac1 and ERK in the regulation of *MMP-9* gene expression, which regulates the development of the fibrotic phenotype.

Gender influences the response to experimental silica-induced lung fibrosis in mice.

Cheryl L. Fattman

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Accumulating evidence suggests that gender can have a profound effect on incidence and severity of a variety of pulmonary diseases. To address the influence of gender on the development of silica-induced pulmonary fibrosis, we instilled 0.2 g/Kg silica into male and female C57BL/6 mice and examined the fibrotic and inflammatory response at 14 days post-exposure. Both silica-exposed male and female mice had significant increases in total lung hydroxyproline compared to saline controls. However, silica-exposed female mice had significantly less total lung hydroxyproline than silica-exposed male mice. Interestingly, silica-exposed female mice had significantly more inflammatory cells, the majority of which were macrophages, as well as higher levels of the macrophage-specific chemokines MCP-1 and CCL9 in whole lung lavage compared with silica-exposed male mice. We also show that at baseline, estrogen receptor α (ER α) mRNA expression is lower in female mice than in male mice and that ER α mRNA expression decreased by silica exposure. Finally we show that the response to ovariectomized female mice to silica instillation is similar to that of male mice. These observations together show that gender influences the lung response to silica.

Shared Mechanisms of Pulmonary Lymphocyte Activation by Bacteria and Toxicants

Michael Borchers

University of Cincinnati

The underlying hypothesis of this project is that chronic toxicant exposure activates pathways that have evolved to recognize pathogens and pathogen induced injury. Specifically, we have identified a receptor on cytotoxic lymphocytes, NKG2D, which recognizes ligands induced on pulmonary epithelial cells and responds by killing ligand expressing cells and initiating inflammatory cascades mediated primarily through natural killer cells. We hypothesized that this toxicant induced mechanism contributed to the pathogenesis of chronic obstructive pulmonary disease and proposed to further examine the role of NKG2D in the response to infections in the context of toxicant exposures. the development of COPD. The specific aims of this project are to 1) Determine the role of NKG2D receptor activation in the clearance of pulmonary infection, 2) Define the contribution of toxicant-induced NKG2D ligands to cytotoxic lymphocyte activation *in vitro*, and 3) Define the consequences of conditional NKG2D ligand expression on pulmonary epithelial cells *in vivo*. These shared mechanisms represent potential disease points that may shift the immune response from protective to pathological. This suggests that, in the context of toxicant exposure, immune balance is shifted and the imbalance results in increased pulmonary damage. This evidence includes the findings that toxicant exposure renders natural killer (NK) cells hyperresponsive to viral ligands and stimulating cytokines. These findings are important because NK cells are critical in the defense against infection through direct cytolysis of infected cells and the secretion of mediators that shape subsequent inflammatory and immune responses. The contribution of the proposed research is expected to be a detailed understanding of how toxicant exposure renders NK cells hyperresponsive to infection. The studies have advanced our current concepts by identifying the underlying mechanisms of how toxicants compromise some immune system functions, yet paradoxically hypersensitize other specific immune responses.

Protease Activated Receptor-1 and Hematopoietic Cell Tissue Factor are Required for Hepatic Steatosis in Mice Fed a High Fat Diet

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Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of obesity and Metabolic Syndrome and contributes to an increased risk of cardiovascular disease and liver-related morbidity and mortality. Several studies suggest that patients with obesity and Metabolic Syndrome generate higher levels of thrombin, an indication of coagulation cascade activation. However, the role of the coagulation cascade in Western diet-induced NAFLD has not been investigated. Utilizing an established mouse model of Western diet-induced NAFLD, we tested the hypothesis that the thrombin receptor protease activated receptor-1 (PAR-1) and hematopoietic cell-derived tissue factor (TF) contribute to hepatic steatosis. In association with hepatic steatosis, plasma thrombin-antithrombin levels and hepatic fibrin deposition increased significantly in C57Bl/6J mice fed a Western diet for 3 months. PAR-1 deficiency reduced hepatic inflammation, particularly monocyte chemoattractant protein-1 (MCP-1) expression and macrophage accumulation. In addition, PAR-1 deficiency was associated with reduced steatosis in mice fed a Western diet, including reduced liver triglyceride accumulation and CD36 expression. Similar to PAR-1 deficiency, hematopoietic cell TF deficiency was associated with reduced inflammation and reduced steatosis in livers of low density lipoprotein receptor-deficient (LDLr^{-/-}) mice fed a Western diet. Moreover, hematopoietic cell TF deficiency reduced hepatic fibrin deposition. These studies indicate that PAR-1 and hematopoietic cell TF are required for liver inflammation and steatosis in mice fed a Western diet.

Mechanisms of Mast Cell Directed Carbon Nanotube Toxicity

Jared M. Brown

East Carolina University

Due to their unique physical and chemical characteristics, the use of nanomaterials has increased dramatically in recent years leading to a growing need for research examining their potential impact on the environment and human health. Multi-walled carbon nanotubes (MWCNT) represent an important nanomaterial with wide ranging applications. Mast cells are well recognized for their role in allergy, asthma and cardiovascular disease. The overall aim of this grant is to examine the activation of mast cells in the pulmonary and cardiovascular systems following exposure to MWCNTs.

We have examined the ability of MWCNTs to activate bone marrow-derived mast cells (BMMCs) as measured by degranulation and cytokine production. Additionally, we compared pulmonary inflammation, lung fibrosis, lung function, aortic vascular reactivity, and myocardial ischemia/reperfusion injury between C57BL/6 and B6.Cg-Kit^{W-sh} mast cell deficient mice following MWCNT instillation. MWCNT exposure did not alter BMMC degranulation *in vitro*, however, MWCNTs stimulated production of pro-inflammatory cytokines including osteopontin. C57BL/6 mice instilled with MWCNTs developed pulmonary inflammation and lung fibrosis with recruitment of macrophages, neutrophils and eosinophils. In contrast, B6.Cg-Kit^{W-sh} mast cell deficient mice displayed minimal lung inflammation and fibrosis. Further, MWCNT exposure in C57BL/6 mice resulted in impaired lung function as demonstrated by increased airway resistance and tissue damping; and decreased lung compliance. The increase in airway resistance was mast cell dependent.

Consistent with the pulmonary effects of MWCNTs, cardiovascular effects following MWCNTs exposure were mast cell dependent. C57BL/6 mice exposed to MWCNTs exhibited impaired aortic vascular relaxation to forskolin and altered constrictor responses to norepinephrine. These changes in vascular reactivity were not present in B6.Cg-Kit^{W-sh} mice. Lastly, we have shown that instillation of MWCNTs into lungs of C57BL/6 mice can exacerbate an episode of myocardial ischemia/reperfusion injury

whereas expansion of the infarct was not observed in B6.Cg-*Kit*^{W-sh} mast cell deficient mice following MWCNT exposure. These findings demonstrate that MWCNTs can direct mast cell production of pro-inflammatory mediators that lead to adverse pulmonary effects, including impaired lung function, as well as adverse cardiovascular effects such as alteration of vascular reactivity and exacerbation of myocardial ischemia/reperfusion injury.

This work is novel in that it identifies an unrecognized, yet prominent mechanism by which CNTs lead to toxicity. Understanding the role of mast cells in response to nanomaterials will allow us to design better models and *in vitro* screening tools to predict nanomaterial toxicity. Lastly, this work provides an important translational application in that by elucidating the proposed mechanism, we will provide support for the use of mast cell directed strategies, such as cromolyn sodium, for early intervention after exposure to prevent subsequent inflammation and fibrosis. This work supported by NIEHS RO1 ES019311.

The Enzymatic Degradation of Carbon Nanomaterials

Alexander Star

University of Pittsburgh

Carbon nanotubes (CNTs) and graphene are endowed with mechanical strength and unique electronic properties both of which continue to be explored for the development of novel electronic, composite, sensor, and medical applications. As products incorporating these carbon nanomaterials become commercialized and the demand for CNTs and graphene increases, so too does the risks of human exposure. Given *in vitro* and *in vivo* data demonstrating the potential toxicity of CNTs and graphene, contact with these carbon-based nanomaterials remains particularly problematic. In an effort to mitigate the possible toxicological effects of these carbon nanomaterials before they enter the human body, this research project aims to develop a mechanistic understanding of the enzymatic degradation of graphene and both single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs).

The Protective Role of Nrf2 in Arsenic-Induced Toxicity and Carcinogenicity

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Nrf2 has emerged as a key adaptive response system for protection against ROS and electrophilic intermediates. Nrf2 controls the expression of cytoprotective genes through the antioxidant response element in their promoter regions to elicit the cellular defense response. The activation of the Nrf2 pathway is important in protecting cells against the deleterious effects caused by environmental toxins. Unfortunately, the “dark-side” of Nrf2 has been revealed recently, indicating that constitutive activation of Nrf2 due to somatic mutations in Nrf2 or Keap1, creates an environment conducive to cancer cell growth and thus, contributes to chemoresistance.

Canonical activation of Nrf2 by chemopreventive compounds is through the modification of cysteine residues in Keap1, a negative regulator of Nrf2. However, new evidence suggests that cysteine residues are not essential in Nrf2 activation in response to certain Nrf2 inducers, including arsenic. The non-canonical mechanism of Nrf2 activation by arsenic is mediated through autophagy deregulation. Arsenic upregulates p62 and increases autophagosome formation, which is required for arsenic mediated Nrf2

activation. This presentation will discuss the distinct mechanisms of Nrf2 activation by arsenic and chemopreventive compounds and the strategies of choosing appropriate Nrf2 inducers for protection against arsenic-induced toxicity and carcinogenicity.

Association between arsenic exposure from drinking water and serum levels of cardiovascular biomarkers

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BACKGROUND: Epidemiologic studies of mechanisms underlying cardiovascular disease risk in populations exposed to arsenic at low-to-moderate levels are lacking.

OBJECTIVE: We investigated the relationship between arsenic exposure from drinking water and serum levels of matrix metalloproteinase-9 (MMP-9), myeloperoxidase (MPO), plasminogen activator inhibitor-1 (PAI-1), soluble E-selectin (sE-selectin), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular adhesion molecule-1 (sVCAM-1), markers of systemic inflammation and endothelial dysfunction, in an arsenic-exposed population in Bangladesh.

METHODS: We conducted a population-based cross-sectional study using baseline data from 668 participants randomly selected from participants who were >30 years in the Health Effects of Arsenic Longitudinal Study (HEALS), a prospective cohort study involving 20,033 participants in Araidhazar, Bangladesh. Arsenic exposure was measured in drinking water from wells and spot urine samples. Serum levels of biomarkers were measured using Luminex xMap technology.

RESULTS: Participants were exposed to well arsenic ranging from 0.1-500.6 µg/L, with an average of 50.5 µg/L, for an average of 9.1 years. Well arsenic, a long-term measure of arsenic exposure in the study, was positively associated with serum levels of sVCAM-1 (P=0.01 for trend). For every one unit increase in log-transformed well arsenic (ln µg/L) there was an increase of 0.02 (95% CI, 0.01–0.03) in log-transformed sVCAM-1 (ln ng/mL). Urinary arsenic was also positively related to serum levels of sVCAM-1 (P<0.01 for trend). Every one unit increase in log-transformed urinary arsenic (ln µg/g creatinine) was related to a rise of 0.04 (95% CI, 0.01–0.07) in log-transformed sVCAM-1 (ln ng/mL).

CONCLUSIONS: The findings indicate an effect of chronic arsenic exposure from drinking water on vascular inflammation and endothelial dysfunction and also suggest a potential mechanism underlying the association between low-to-moderate levels of arsenic exposure and cardiovascular disease.

Paradoxical roles of Nrf2 activation in arsenic-induced pancreatic β-cell dysfunction and insulin resistance

Jingbo Pi

The Hamner Institutes for Health Sciences

There is growing evidence that chronic exposure of humans to inorganic arsenic (iAs), a potent environmental oxidative stressor, is associated with the incidence of Type 2 diabetes (T2D). Nuclear

factor E2-related factor 2 (Nrf2) is a CNC-bZIP transcription factor that is well established as a master regulator in the cellular adaptive response to oxidative stress. Although cytotoxic, reactive oxygen species (ROS) also function as important intracellular signaling molecules to activate cellular responses to a variety of physiological stimuli, including glucose-stimulated insulin secretion (GSIS) in pancreatic β -cells and insulin action in insulin responsive cells. Therefore, we propose that Nrf2-mediated antioxidant response plays paradoxical roles in β -cell function and insulin signaling trasduction: (1) It protects the cells from oxidative damage and possible cell death, thus minimizing oxidative damage-related impairment in insulin secretion and action; (2) Since ROS signaling triggered by glucose could be an important component involved in insulin secretion and action, the induction of endogenous antioxidants in the presence of oxidative stress may blunt the signals, resulting in reduced GSIS and insulin resistance. iAs and its methylated trivalent metabolites are potent oxidative stressors and robustly activate Nrf2-mediated antioxidant response, but at the levels typically observed in human exposures, they are not likely to reach cytotoxic concentrations sufficient to cause overt oxidative damage, especially when endogenous antioxidant enzymes can be actively induced. Therefore, blockade of ROS signaling in premise 2 is potentially more relevant to the etiology of T2D in the context of low-level environmental iAs exposure, whereas premise 1 might be associated with protecting cells from acute toxicity induced by high doses of arsenic.

Metabolism of the Brominated Components of the PentaBDE Replacement Mixture, Firemaster 550, in human liver microsomes

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Introduction. Firemaster [®]550 (FM 550) is a commercial flame retardant mixture used as a replacement for PentaBDE in polyurethane foam. It contains two brominated flame retardants, 2,3,4,5 tetrabromoethylhexyl benzoate (TBB) and 2,3,4,5 tetrabromo-bis-ethylhexyl phthalate (TBPH), along with organophosphate flame retardants. The brominated components of FM 550 have been detected at high concentrations in house dust, thus suggesting that human exposure is occurring, similar to PentaBDE. After exposure, FM 550 may be metabolized to compounds structurally similar to known toxicants, increasing the potential toxicity of the mixture.

Materials and Methods. Human liver microsomes (HLM) were purchased from Invitrogen (Carlsbad, California). Tetrabromobenzoic acid was synthesized by reflux of tetrabromophthalic anhydride in DMSO, and tetrabromo-monoethylhexyl phthalate was generously provided by Dr. Kim Boekelheide (Brown Univ.). TBB and TBPH were purchased from Accustandard (New Haven, CT). Triiodobenzoic acid and tetrachloro-monoethylhexyl phthalate were purchased from Sigma (St. Louis, MO). HLM samples were incubated for 30 min with TBB or TBPH, and extracted with Oasis HLB solid phase extraction cartridges, methyl-derivitized with diazomethane, and analyzed using GC/ECNI-MS and GC/EI-MS in scan mode. Potential metabolites detected using GC/MS were synthesized and an LC/MS/MS method was developed using structurally similar compounds as internal standards.

Results. Tetrabromobenzoic acid (TBBA) was detected as a metabolite of TBB using GC/MS with derivitization. Using a more robust LC/MS/MS method with triiodobenzoic acid as an internal standard, the kinetics of TBBA formation in HLM were calculated, giving an approximate K_M of 5 μ M and V_{max} of 100 pmol min⁻¹ mg protein⁻¹. In previous studies, bis-2-diethylhexyl phthalate (DEHP), the non-brominated analog of TBPH, was rapidly hydrolyzed to form mono-ethylhexyl phthalate (MEHP). Even though metabolites for TBPH were not detected using GC/MS in this study, the brominated analog of

MEHP (tetrabromo-monoethylhexyl phthalate) was included in the LC/MS/MS method using tetrachloro-monoethylhexyl phthalate as an internal standard. Even in incubations with high concentrations of microsomal protein and TBPH, this metabolite was below the detection limit of 1 pmol/mL.

Discussion. The metabolic conversion of TBB to TBBA may result in increased potential toxicity of TBB, while reducing the *in vivo* half-life of TBB. Although no toxicological information is available for TBBA, it is structurally similar to brominated phenols, which have been shown to inhibit deiodinase enzymes in HLM and competitively bind to thyroid transport proteins and receptors. The enzymatic hydrolysis of the ether bond of TBB to form TBBA is mechanistically similar to the hydrolysis of DEHP to form MEHP and is likely mediated by carboxylesterases.

Surprisingly, the brominated analog of DEHP, TBPH, did not appear to undergo similar hydrolysis of an ethylhexyl chain and instead appeared to be resistant to metabolism in HLM. Assuming similar uptake after ingestion, it is likely that TBPH will have a longer half-life in the body than TBB based on hepatic microsomal clearance. The toxicity and potential bioaccumulation of TBB, its metabolite TBBA, and TBPH will be evaluated in ongoing studies.

***In Utero* Exposure to Bisphenol A: Effects on the Fetal Epigenome**

Dana Dolinoy

It is increasingly recognized that exposure to chemical, nutritional, and behavioral factors alters gene expression and affects health and disease by not only mutating promoter and coding regions of genes, but also by modifying the *epigenome* — modifications to DNA that confer an additional layer of heritable gene regulation that lead to disease when deregulated. Moreover, such exposures have been directly linked with subsequent disease formation through epigenetic mechanisms. Until recently, most attempts to elucidate the effects on the epigenome following nutritional and environmental exposures, including *in utero* exposures, were either 1) candidate gene driven or based on epigenetic techniques with limited genome coverage/sensitivity, 2) restricted in dose-response assessment, and/or 3) confined to animal models. Drawing upon data from multiple epigenomics platforms and focusing on bisphenol A (BPA) as a representative environmental exposure, we explore dose- and species-dependent methylation effects and associated perturbations in physiological and cellular endpoints. Epigenomic data from animal models, human clinical samples, and epidemiological studies now indicate that BPA-induced alterations vary between species and across dose, a revelation that should be considered in human health risk assessment. In addition, epigenomic profiling will facilitate the identification of biomarkers of exposure, enabling clinicians to identify at-risk individuals prior to disease onset.

Enhanced Nrf2 Activation Attenuates Fasting-Induced Fatty Liver

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Non-Alcoholic fatty liver disease (NAFLD) affects 20% of the U.S. population. Approximately 15 to 20% of patients with fatty liver develop a severe form of hepatic disease known as nonalcoholic steatohepatitis (NASH). The high prevalence of fatty liver and NASH warrants research into the signaling mechanisms responsible for hepatic lipid accumulation. Nuclear factor-erythroid 2-related factor 2

(Nrf2, Nfe2l2), a bZIP family transcription factor, is protective against xenobiotic-related toxicity and chemical-induced carcinogenesis. However little information about Nrf2 function on lipid metabolism is available. Male C57BL/6 and Keap1-KD mice were fed *ad libitum* or withheld food (fasted) for 24 hours to determine whether enhanced Nrf2 activation in liver could prevent fasting-induced steatosis. In C57BL/6 mice, fasting increased hepatic triglyceride content 240%, and serum triglyceride concentrations 21% compared to fed mice. In contrast, keap1-kd mice were resistant to fasting, with hepatic triglyceride increased 1.3 fold, and serum triglyceride levels decreased about 6% compared to fed Keap-KD mice. Fasting increased serum free fatty acid levels 110% in C57B/6, but only 61% Keap1-KD mice. Keap1-KD mice exhibit decreased basal expression of FAS, ACC-1 and SCD-1, genes important for regulating lipid synthesis, compared to C57BL/6 mice. Fasting decreased FAS, ACC-1 and SCD-1 mRNA expression in liver to a much greater degree in Keap1-KD compared to C57BL/6 mice, 54%, 31% and 81% respectively. These data demonstrated that keap1-kd mice are resistant to fasting-induced steatosis. Future work will focus on the mechanism by which Nrf2 activity modulates fatty acid synthesis gene expression in liver and promotes resistance to fasting. In conclusion, enhances Nrf2 activation Keap1 knockdown prevents fasting induced steatosis, pointing to an important (NIH 3R01ES016042-02S2).

Trm9 Catalyzed tRNA Modifications Link Translation to the DNA Damage Response

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Transcriptional and post-translational signals are known mechanisms which promote efficient responses to DNA damage. We have identified *S. cerevisiae* tRNA methyltransferase 9 (Trm9) as an enzyme that prevents death and damage-induced cell cycle abnormalities via translational enhancement of DNA damage response proteins. Trm9 methylates the uridine wobble base of tRNA^{ARG(U_{CU})} and tRNA^{GLU(U_{UC})}. We used computational and molecular approaches to predict that Trm9 enhances the translation of transcripts over-represented with specific arginine and glutamic acid codons. We found that translation elongation factor 3 (*YEF3*) and the ribonucleotide reductase (*RNR1* and *RNR3*) large subunits are over-represented with specific arginine and glutamic acid codons, and demonstrated that Trm9 enhances Yef3, Rnr1, and Rnr3 protein levels. We have demonstrated by *RNR1* over expression and codon optimization approaches that the translation of this transcript is increased during the G1 to S transition and it is most likely error prone and slow in Trm9 deficient cells. We propose that Trm9-specific tRNA modifications are a highly conserved mechanism to enhance codon-specific translation elongation and promote increased levels of key DNA damage and stress response proteins. Ultimately our studies of Trm9 like activities in yeast, mice and humans have identified a translation-associated mechanism that can optimize stress signaling and plays an important role in human health.

The Role of Human DNA Polymerase Eta and Mutagenic Response to Oxidative Stress

Scott D. McCulloch

North Carolina State University

Oxidative stress is caused by a number of environmental agents such as polycyclic aromatic hydrocarbons like dioxin, heavy metals, pesticides, and ultraviolet and ionizing radiation. Reactive oxygen species are created by normal cell processes and in elevated levels by oxidative stress. They cause multiple types of DNA lesions, the most common being 8-oxo-guanine (8-oxo-dG). This lesion is highly mutagenic due to its ability to base pair with the incorrect base adenine in a manner that escapes detection by the proofreading activity of the replicative polymerases. Translesion synthesis (TLS) by specialized polymerases of the Y-family is therefore a necessary component of DNA replication. Direct bypass of damage allows completion of DNA replication in the face of lesions that would otherwise stall the replication fork, collapse of which can lead to strand breaks and other gross chromosomal changes. TLS past several DNA lesions by Y-family members occurs with higher efficiency compared to replicative polymerases, making them the preferred choice for ensuring replication is completed, even though the fidelity of many TLS polymerases is relatively low. We have previously demonstrated that human pol eta performs efficient bypass of 8-oxo-dG, but also observed that the bypass occurred with remarkably low fidelity. Errors were generated during ~50% of all bypass events. The most frequent error was adenine misinsertion opposite 8-oxo-dG, which as noted above frequently goes undetected by the proofreading activities of replicative polymerases due to its structural similarity to a correct base pair. This implies that if pol eta suppresses mutagenesis during 8-oxo-dG bypass the fidelity must be substantially altered somehow. We hypothesize that the replication accessory proteins alter the outcome of pol eta bypass either by association with pol eta or the DNA. We aim to determine which factors may influence the fidelity of the bypass reaction and also to determine what role, if any, pol η plays in the generation of mutations during times of oxidative stress when the number of 8-oxo-dG bypass events would be greatly elevated. The goals of this proposal are: 1) to determine the effects of replication proteins on the efficiency and fidelity of 8-oxo-dG bypass by human pol η; 2) to reconstitute and characterize the 'complete' 8-oxo-dG lesion bypass reaction *in vitro*; 3) to identify and characterize human pol η mutants, including known SNPs, that display altered fidelity for 8-oxo-dG bypass; and 4) to determine the mutation rate of wild type and pol η deficient cells, and cells expressing mutant forms of pol η, under conditions of oxidative stress. The long term objectives of this proposal are to determine the molecular mechanisms that modulate the efficiency and fidelity of 8-oxo-dG bypass in human cells, and ultimately the mutagenesis caused by oxidative DNA damage.

The deacetylase SIRT1 positively regulates global genome nucleotide excision repair

Yu-ying He

University of Chicago

Disruption of the nucleotide excision repair (NER) pathway by mutations can cause xeroderma pigmentosum, a syndrome predisposing affected individuals to development of skin cancer following UV radiation in sunlight. The xeroderma pigmentosum C (XPC) protein is essential for initiating global genome NER by recognizing the DNA lesion and recruiting downstream factors. Here we show that inhibition of the deacetylase and longevity factor SIRT1 impairs global genome NER through suppressing the transcription of XPC in a SIRT1 deacetylase-dependent manner. SIRT1 enhances XPC expression by reducing AKT-dependent nuclear localization of the transcription repressor of XPC. Finally, we demonstrate that SIRT1 levels are significantly reduced in UV damage-associated human skin tumors from Caucasian patients, a population at highest risk. These results suggest that SIRT1 acts as a tumor suppressor through its role in DNA repair.

Causes and phenotypic consequences of gene copy number variation

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Indiana University at Bloomington

Recent studies indicate copy number variation (CNV) represents a large source of the genetic variation observed in human populations and have uncovered strong associations between CNV and disease, including complex phenotypes. However, the environmental contributions to CNV remain unknown, in part because there are few animal models available for environmental genomics studies, which seek to understand how genome structure and function evolve in response to environmental change. Our group makes use of the NIH model, *Daphnia*, to test the central hypothesis that exposure to environmental contaminants increase the rate of mutations giving rise to CNV, and that this variation has functional consequences on gene expression, phenotype, fitness, and population structure. To accomplish this goal we are developing mutation accumulation (MA) lines derived in the absence and presence of cadmium in order to define the spectra of CNV and measure the per generation rate at which they spontaneously arise in individuals. We are studying past populations that have been captured and preserved in lake sediments. Here we scan genomes from populations that have adapted to over a century of mining pollution in order to characterize the magnitude, distribution, functional consequences, and evolutionary path of CNV in relation to the metal adapted phenotype. Finally, we are conducting quantitative trait loci experiments to determine the functional significance of CNV by establishing cause and effect relationships between copy number variants and metal tolerance. Collectively, these studies quantitatively assess whether environmental exposure affects the risk for spontaneous CNV, and do so in context of their contributions to individual health parameters that influence tolerance (i.e., adaptation and susceptibility) and disease. Answers to these questions have profound implications for the long-term health of human populations that are living longer and doing so in the presence of a greater diversity of chemicals that can modify DNA.

Remote Microvascular Dysfunction after Particulate Matter Exposure

Timothy R. Nurkiewicz

West Virginia University

Epidemiological evidence indicates that acute pulmonary exposure to airborne pollutants such as particulate matter (PM) increases the risk of pulmonary and cardiovascular morbidity and mortality. This implies that PM affects extra-pulmonary tissues, as evidenced by the occurrence of cardiovascular dysfunction on high pollution days. Furthermore, Federal Criteria Documents for PM have provided a wealth of evidence demonstrating PM dependent effects on the cardiovascular system. However, despite its obvious importance in regulating the delivery of cells and molecules to all tissues, and in the etiology of most cardiovascular diseases, my laboratory conducts the lone investigations that explore how systemic microvascular function is affected by pulmonary PM exposure. The overall aim of this project is to determine if there is a true causal link between the inflammatory events that follow PM exposure and the disruption of endothelium-dependent dilation. Central hypothesis: Inflammatory mechanisms govern the systemic microvascular dysfunction that follows ultrafine PM exposure, and the severity of this dysfunction is augmented in clinically relevant populations. Intravital microscopy and isolated vessels will be used to test this hypothesis in the spinotrapezius muscle, bone marrow, and subendocardial circulations of rats and mice exposed to diesel exhaust particles (DEPs) or ultrafine titanium dioxide. The role of gender and age in determining the severity of these effects will also be studied. Various in vivo and in vitro techniques will be used to measure microvascular reactivity after

PM exposure, and to characterize pathological changes at this crucial level of the circulation. DEPs are mobile source emission air pollutants representative of particles that humans are exposed to on a regular basis. Ultrafine titanium dioxide is a commonly used nanoparticle found in cosmetics, paints and various protective coatings. A better understanding of how these particles affect remote microvascular function will provide mechanistic insight into pathologic changes that contribute to cardiovascular morbidity and mortality. Moreover, these studies may provide a biological basis for the epidemiological associations between air pollution and cardiovascular dysfunction. A fundamental understanding of these mechanisms is vital to the prevention and treatment of life-threatening cardiovascular events, and will contribute to control strategy development.

Particulate matter induced lung inflammation impairs fibrinolysis and activates coagulation through different mechanisms

Gokhan M. Mutlu

Northwestern University

Rationale: Exposure of humans to ambient particulate matter (PM) air pollution causes increased premature mortality attributable to ischemic cardiovascular events. In humans and rodents, our group and others have reported that PM exposure results in the systemic activation of coagulation and impaired fibrinolysis. We sought to determine whether the activation of coagulation and impairment of fibrinolysis are mediated through similar or distinct mechanisms.

Methods: Adult, male C57BL/6 and IL-6 knock out (IL-6^{-/-}) mice were exposed to either concentrated ambient PM less than 2.5 μm (CAPs) or filtered air (FA) 8 hours daily for 3 days or were exposed to either a well-characterized urban PM or PBS via intratracheal instillation. Twenty four hours later, blood and bronchoalveolar lavage fluid were collected and lungs and white adipose tissue were harvested for measurement of coagulation and the levels of plasminogen activator-1 (PAI-1), the major inhibitor of thrombolysis.

Results: Exposure to CAPs or urban PM resulted in the IL-6 dependent activation of coagulation systemically and locally in the lungs assessed by protein and mRNA expression of tissue factor. PAI-1 antigen and mRNA levels were higher in the lung and adipose tissue of mice treated with CAPs or PM compared with FA or PBS controls, respectively. The elevation in PAI-1 level was similar between wild-type and IL-6^{-/-} mice but was attenuated after treatment with etanercept, a TNF-α inhibitor. However, etanercept did not prevent the PM-induced prothrombotic state.

Conclusions: Exposure to PM results in a TNF-α-dependent increase in PAI-1 and an IL-6-dependent activation of coagulation. IL-6 does not play a role in PM-induced impairment of fibrinolysis. These results suggest that distinct mechanisms link PM-induced lung inflammation with the development of a prothrombotic state.

Diesel Exhaust Emissions Induce Systemic Lipid Peroxidation and Loss of HDL Anti-oxidant and Anti-inflammatory Properties

Jesus Araujo

David Geffen School of Medicine, University of California, Los Angeles

Exposure to ambient particulate matter is associated with increased cardiovascular ischemic events and greater degrees of atherosclerosis. We have shown that exposure of apoE^{-/-} mice to Concentrated Ambient Particles (CAPs) in the fine and ultrafine range led to the development of dysfunctional high-density lipoproteins (HDL) that it was more pronounced among mice exposed to concentrated ultrafine particles where it was accompanied by enhanced aortic atherosclerosis as compared with mice exposed to concentrated fine particles (PM2.5). *Our principal hypothesis is that exposure to ambient PM results in dysfunctional HDL and enhanced atherosclerosis via the induction of systemic prooxidant and proinflammatory effects and that a decreased antioxidant response will significantly promote the degree of HDL dysfunction and development of atherosclerosis.* Our ONES proposal was designed to test this hypothesis via three specific aims. In specific aim 1, we proposed to characterize the nature of HDL changes induced by the exposure to air particulate matter, using diesel exhaust (DE) emissions as the model pollutant. The current work aims to determine whether apoE^{-/-} mice exposed to DE undergo changes in their HDL functional status similar as those observed in mice subjected to CAPs inhalations. In addition, we tested whether alteration in HDL could be due to oxidative modifications of lipoprotein components. Thus, we exposed male apoE^{-/-} mice (n=12-13/group) to diesel exhausts (DE) at 300 µg /m³ for two weeks, filtered air (FA) for two weeks or diesel exhausts for two weeks, followed by FA for one week (DE+FA). Plasma HDL was separated by dextran-sulfate precipitation. HDL anti-oxidant capacity was assessed by a DCF-based cell fluorescent assay that evaluated HDL ability to inhibit LDL oxidation. DCF fluorescence was measured in relative fluorescence units (RFU). HDL from DE-exposed mice exhibited 23,053.2±2,844 RFU, significantly higher than FA-exposed mice (10,282 ±1,135 RFU, p<0.001) but similar to the HDL from DE+FA-exposed mice (22,448 ±3,115 RFU). Notably, HDL from the DE and DE+FA mice promoted rather than inhibited LDL oxidation, supporting the notion that DE induced the development of prooxidant dysfunctional HDL. Decreased HDL anti-oxidant capacity correlated with increased levels of plasma 8-isoprostanes, liver malondialdehyde levels (p<0.05) and subsequent upregulation of hepatic antioxidant genes. Likewise, HDL from DE and DE+FA mice exhibited loss of their anti-inflammatory capacity, as determined by a monocyte chemotactic assay. In conclusion, DE emissions induced systemic prooxidant effects that led to the development of prooxidant and proinflammatory HDL. Generation of dysfunctional HDL might be one of the mechanisms how air pollution leads to enhanced atherosclerosis

The NFκB p50 radical and neurotoxic microglial activation

Michelle L. Block

Virginia Commonwealth University

Reactive oxygen species (ROS) are key mediators of microglia-mediated dopaminergic (DA) neurotoxicity. However, the precise mechanisms through which ROS change microglia to a neurotoxic phenotype are poorly understood. Using immuno-spin trapping (IS), we began to explore the potential targets (protein radicals) in microglia modified by ROS to result in an enhanced pro-inflammatory response. Primary microglia cultures activated with diverse triggers (10 ng/ml LPS, 250 nM α synuclein, & 0.5µM paraquat) at concentrations previously shown to be neurotoxic to DA neurons through microglial activation resulted in increased levels of total protein radicals, as measured by IS ELISA. Analysis by confocal microscopy revealed that microglia exposed to neurotoxic amounts of LPS (10 ng/ml), INFγ(100U/ml), and soluble neuron injury factors expressed increased protein radical levels that were localized to both the nucleus and the cytosol. Immuno-precipitation with the anti-DMPO antibody and western blot analysis for NFκB p50 revealed that the NFκB p50 radical is present in the cytosol of

neurotoxically activated microglia exposed to 10 ng/ml LPS and soluble neuron-injury factors. TNF α levels in whole brain homogenate and serum were enhanced in NF κ B p50^{-/-} mice in response to IP administration of LPS (5mg/kg), indicating that a lack of functional NF κ B p50 amplifies both systemic and neuro-inflammation. NF κ B p50^{-/-} mixed- glia cultures also showed elevated levels of TNF α . Substantia nigra brain sections stained for IBA-1 revealed that NF κ B p50^{-/-} mice have an activated morphology in saline treated controls (at rest). LPS-induced changes in microglial morphology were also more pronounced in NF κ B p50^{-/-} mice, as depicted by greater increases in staining intensity and ameboid shape. Together, these data support that NF κ B p50 plays a key role in neuroinflammation and microglial activation, where the NF κ B p50 radical may be a common factor in neurotoxic microglial activation.

Contributors' Biographies

John W. Hollingsworth

Duke University

Dr. John W. Hollingsworth is an Associate Professor of Medicine and an Assistant Professor of Immunology at Duke University Medical Center. Dr. Hollingsworth received his medical degree, attended residency, and served as chief resident at the University of Texas Medical Branch. He completed a fellowship in Pulmonary and Critical Care Medicine at Duke University Medical Center in 2004. The goal of Dr. Hollingsworth's research program is to better understand the interaction between exposure to common environmental factors and host vulnerability to complex heritable lung disease. His laboratory has two major interests. *First*, work has focused on using exposure to the urban air pollutant ozone to better understand the fundamental mechanisms that regulate airway hyperresponsiveness and innate immunity. Using this approach, the lab has identified that hyaluronan, a cellular matrix component, contributes to the development of reactive airways disease through interaction with genes of innate immunity. These studies have provided novel insight into the pathogenesis of reactive airways disease. *Second*, we are interested in both the impact of common environmental exposures during vulnerable periods of development on prevalence of disease and the mechanisms that common exposures can alter heritable risk of complex immunological lung disease. Work has focused on the impact of common exposures on modification of epigenetic marks and provides fundamental insight into effects of environmental exposures on transgenerational inheritance of complex disease. Dr. Hollingsworth has contributed to 41 peer reviewed manuscripts. He has received continuous support from the NIH since 2001 and his lab is currently primarily supported by the Outstanding New Environmental Scientist (ONES) Award from the NIEHS. He is an active member of the American Thoracic Society's Environmental Health Policy Committee. He currently serves as an editorial board member for both the *American Journal of Respiratory Cell and Molecular Biology* and *Inhalation Toxicology*. His program is dedicated to understanding the role of common environmental exposures and host factors in the development of lung disease.

Stephania Cormier

Louisiana State University Health Sciences Center in New Orleans

Dr. Stephania Cormier is an Associate Professor in the Department of Pharmacology & Experimental Therapeutics at Louisiana State University Health Sciences Center in New Orleans (LSUHSC-NO). She is currently joint appointed in the Department of Pulmonary and Critical Care Medicine and maintains an adjunct appointment with the Department of Pathobiological Sciences at the Louisiana State University School of Veterinary Medicine. Prior to this position, she held the position of Assistant Professor in the Department of Biological Sciences at LSU in Baton Rouge. She also held an appointment as a Visiting Scientist at Mayo Clinic. Dr. Cormier is actively involved in teaching activities, committee participation and mentoring at LSU and LSUHSC-NO. She is interested in how adult airways disease such as asthma and COPD results, in part, from environmental insult that occurs during a critical window of pulmonary and immunological immaturity. These insults can be airborne pollutants or respiratory viruses. The long-term objective of her laboratory is to realize the initiators of the immune and pathophysiological changes that occur during early stages of pulmonary airways disease so that more effective interventions and therapy might be developed. Her research has been funded by the NCR, NIAAA, NIAID, NIEHS, NHLBI, and Energex Corporation. Along with her numerous publications and presentations, Dr. Cormier has served as an Editorial Board Member for *Analytical Biochemistry*, a journal referee for a number of journals, and a proposal referee for USDA, Lytmos Corporation, NCI, NHLBI, and NIAID. Dr. Cormier was also the founding President for the South Louisiana Chapter of the Association for Women in Science. Dr. Cormier has an Honors B.S. from the University of Southwestern Louisiana and a Ph.D. from Louisiana State University School of Medicine. She was a postdoctoral fellowship from the Mayo Clinic.

Jill A. Poole

University of Nebraska Medical Center

Dr. Jill A. Poole is an Associate Professor of Medicine in the Pulmonary, Critical Care, Sleep & Allergy Division at the University of Nebraska Medical Center (UNMC). During medical school at UNMC, she was awarded a one year clinical research training opportunity at the National Institute of Health whereby she worked in the Institute of Allergy and Infectious Disease. She completed an Internal Medicine residency program at Washington University/Barnes Jewish Hospital in St. Louis, Missouri and fellowship training in Allergy, Asthma, and Immunology at National Jewish Medical Center in Denver, Colorado prior to joining UNMC faculty in the summer of 2005. Dr. Poole sees patients with allergic disorders, eczema, urticaria, rhinitis, sinusitis and asthma. She has active research interest with funding

from the National Institute of Environmental Health Sciences since 2007 to study agricultural exposure-induced lung disease. She has developed models to understand the complex, chronic innate immune adaptation response to organic dust exposure. Due to findings of a potential critical role for components of Gram-positive bacteria within the organic dust samples, her research is now focused on targeting specific innate immune pattern recognition receptors of Gram-positive bacteria and/or peptidoglycan. She has also received funding through the National Institute of Occupational and Safety Health (NIOSH), American Academy of Allergy, Asthma & Immunology, and Intramural grants at UNMC.

A Brent Carter

University of Iowa

A. Brent Carter received his medical degree at the University of Missouri. This was followed by post-graduate training as a resident in internal medicine and as a fellow in pulmonary and critical care medicine at the University of Iowa Carver College of Medicine. Dr. Carter is currently an Associate Professor in Internal Medicine at the University of Iowa and is the ICU Director at the Iowa City Veterans Administration Medical Center. Dr. Carter's research interests are related to the regulation of inflammatory and fibrotic responses that occur in the lung in models of environmental lung injury and pulmonary fibrosis. In particular, Dr. Carter studies the role of asbestos exposure in the development of lung injury. Asbestosis, which is the most debilitating asbestos-related lung disease, is a prototypical form of pulmonary fibrosis leading to 4000 deaths annually, despite tight regulatory controls to limit exposure. Dr. Carter's studies include investigations dealing with the role of reactive oxygen species and antioxidant enzymes in the development of inflammatory/fibrotic response and the regulation of the signal transduction pathways in macrophages that induce the development of pulmonary fibrosis.

Cheryl L. Fattman

University of Pittsburgh

Dr. Cheryl L. Fattman is an Assistant Professor of Environmental and Occupational Health at the University of Pittsburgh Graduate School of Public Health. She obtained her PhD in Molecular Pharmacology from the University of Pittsburgh School of Medicine. She subsequently completed a post-doctoral fellowship in the Department of Pathology, also at the University of Pittsburgh School of Medicine, where she developed her interest in the pathophysiology of pulmonary fibrosis and the role of oxidative stress in this disease process. Her current work uses mouse models to determine the role of the antioxidant enzyme extracellular superoxide dismutase (ECSOD, Sod-3) in silica-induced pulmonary

fibrosis. She also has a strong interest in the influence that gender has on the development and regulation of the characteristic lung inflammation and fibrosis associated with this disease.

Michael Borchers

University of Cincinnati

Dr. Borchers laboratory examines the fundamental biology of the pulmonary innate immune response to environmental exposures. This work is conducted at many levels including molecular biology, cell biology, pulmonary physiology, and immunology; and will increase our understanding of the environmental agents that alter immunologic mechanisms that regulate the susceptibility and progression of chronic pulmonary diseases. My research program examines lung pathophysiology from two sides. One component examines how the airway epithelium, the site of host contact with the environment, responds to environmental stimuli and stresses to potentially activate the innate and acquired immune system. The second component examines the effector functions of the lymphocyte subsets in the lung in response to environmental exposures. These studies are particularly relevant to persons chronically exposed to cigarette smoke. Defining the changes in the immune system after long term cigarette smoke exposure will yield a better understanding of its impact on other aspects of pulmonary pathology such as chronic exacerbations in response to infection and increased susceptibility to cancer.

James P. Luyendyk

University of Kansas Medical Center

Dr. Luyendyk received his B.S. in Biochemistry from Colorado State University in 2000 and Ph.D. in Pharmacology/Toxicology from Michigan State University in 2004. Dr. Luyendyk was a post-doctoral fellow in the laboratory of Nigel Mackman at The Scripps Research Institute until 2007. Dr. Luyendyk is currently an Assistant Professor in the Department of Pharmacology, Toxicology and Therapeutics at The University of Kansas Medical Center. His work is focused on understanding the regulation and role of blood coagulation in chronic liver disease and acute hepatotoxicity.

Jared M. Brown

East Carolina University

Dr. Jared Brown is an Assistant Professor at East Carolina University School of Medicine. He received his doctorate in Toxicology from the University of Montana in 2004 under the guidance of Dr. Andrij Holian and was a postdoctoral fellow with Dr. Dean Metcalfe in the Laboratory of Allergic Diseases at the National Institutes of Health, NIAID, from 2004-2008. Dr. Brown's research interest is how nanomaterials may influence mast cell activation leading to allergic disease and inflammatory conditions in the lung and cardiovascular systems. Dr. Brown is author/co-author on more than 20 peer-reviewed publications and has served as an ad-hoc member on NIEHS special emphasis panels for nanoparticle testing. Dr. Brown was a recipient of the Outstanding New Environmental Scientist Award from NIEHS in 2010 and also serves as project director on a NIEHS U19 funded Center for Nanotechnology Health Implications Research. In addition, Dr. Brown has received funding from the North Carolina Biotechnology Center for nanotoxicology studies and for the development of an inhalation center at ECU. Dr. Brown is an active member of the Society of Toxicology, American Thoracic Society and the American Association for Immunologists.

Alexander Star

University of Pittsburgh

Alexander Star is an assistant professor at the University of Pittsburgh. His research interests are in nanomaterials and their biomedical applications. He received his B.Sc. and Ph.D. degrees in chemistry from Tel-Aviv University in 1994 and 2000, respectively. He then spent two years as a postdoctoral fellow in California NanoSystems Institute at University of California, Los Angeles. From 2002 to 2005, Alex was a Senior Scientist and Manager of Applications Development at Nanomix, Inc.- a nanotechnology startup company - where he worked on development and commercialization of carbon nanotube products.

Donna D. Zhang

University of Arizona

Donna D. Zhang, Ph.D., is an associate professor in the department of Pharmacology and Toxicology at the University of Arizona. She got her Ph.D. from the Department of Environmental Health Sciences, Nelson Institute of Environmental Medicine at New York University Medical Center (advisor: Max Costa). Her post-doctor training was with David Warheit, at DuPont-Haskell Laboratories, Newark, DE. She had worked as a research assistant professor in Biochemistry Department at the University of Missouri for 6 years before she joined University of Arizona in 2005. The research in her laboratory is focused on (i) mechanisms of arsenic-mediated toxicity and carcinogenicity, (ii) mechanistic regulation of the Nrf2-Keap1 signaling pathway, (iii) the dual role of Nrf2 in cancer prevention and promotion, and (iv) the development of therapeutic drugs targeting the Nrf2-Keap1 pathway for disease prevention/treatment.

She has published 34 peer-reviewed papers since 2005. Her expertise in the arsenic/Nrf2 research has resulted in her inclusion as a member of various NIH and NSF study sections and a reviewer for European grant applications (AICR, Wellcome Trust, FCT, CR-UK, CR-Italian, ISS). She is the president of MWSOT and an editorial board member of Toxicology and Applied Pharmacology.

Yu Chen

New York University School of Medicine

Dr. Chen is an Associate Professor of Epidemiology. Her research focuses on how environmental and dietary factors are related to the risk of chronic diseases, including cancer and cardiovascular disease. Dr. Yu Chen leads several epidemiologic studies of the influence of risk factors related to systemic inflammation on chronic diseases. She is carrying out a multidisciplinary case-control study of the association between periodontal disease and gastric precancerous lesions (R21). With funding from American Heart Association and National Heart, Lung and Blood Institute, Dr. Chen is conducting case-control studies nested in the New York University Women's Health Study (NYUWHS) to investigate the association between the serum levels of taurine, a nutrient and popular ingredient in energy drinks, and the risk of coronary heart disease and stroke. Dr. Chen is also a recipient of the Outstanding New Environmental Scientist Award (ONES) from the National Institute of Environmental Health Sciences (NIEHS) to study the interactions between arsenic exposure from drinking water and genetic susceptibility related to inflammation and oxidative stress in cardiovascular disease. Building upon previous collaboration, this project utilizes resources of the Health Effects of Arsenic Longitudinal Study (HEALS) in Bangladesh. Dr. Chen is teaching epidemiology methods at NYU. She received her PhD with distinction in Epidemiology from Columbia University in 2005. Dr. Chen is an author of 80 journal articles.

Jingbo Pi

The Hamner Institutes

Jingbo Pi received his MD in China Medical University in 1990 and Ph.D. on Environmental Medicine in the University of Tsukuba, Japan in 2002. Dr. Pi did Post-doctoral training in Dr. Michael P. Waalkes' lab at NCI at NIEHS (2002-2004) and Sheila Collins' lab at CIIT Centers for Health Research (2004-2007). Currently Dr. Pi is an Associate Investigator in the Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences at Research Triangle Park. Dr. Pi's current research involves: (1) Mechanisms of Type 2 diabetes with particular emphasis on the paradoxical roles of reactive oxygen species (ROS) and Nuclear factor E2-related factor 2 (Nrf2) in pancreatic β -cell function, insulin signaling

and adipogenesis; (2) Pathogenic role of environmental oxidative stress in the development of obesity and Type 2 diabetes; (3) Regulatory mechanism of Nrf2-mediated antioxidant response and its application in chemical toxicity testing; and (4) Regulatory role of Nrf1 in cellular adaptive response to environmental oxidative stress, glucose homeostasis and energy metabolism. These studies are expected to lead to exploring pharmacologic interventions directed at ROS production and Nrf2/Nrf1 transcriptional activity for prevention and treatment of metabolic disorders.

Heather M. Stapleton

Duke University

Dr. Stapleton received her PhD in environmental chemistry from the University of Maryland in 2003. Her research interests are focused on examining the sources, fate, transport and metabolism of halogenated organic contaminants in the environment. Her current research projects focus on the sources of exposure to flame retardant contaminants, particularly in children. She also has active research programs in species specific differences in the metabolism of brominated flame retardants. Analytical methods employed in Dr. Stapleton's laboratory include gas chromatography, liquid chromatography and mass spectrometry. (ESP)

Dana Dolinoy

University of Michigan at Ann Arbor

Dr. Dana Dolinoy is the Searle Assistant Professor of Environmental Health Sciences at the University of Michigan School of Public Health. Dr. Dolinoy's research investigates how nutritional and environmental factors interact with epigenetic gene regulation to shape health and disease. Using the viable yellow agouti mouse as an epigenetic biosensor, Dr. Dolinoy has shown that genistein, the major phytoestrogen in soy, increases DNA methylation of the *Agouti* gene, resulting in decreased incidence of adult-onset obesity, diabetes, and cancer. She has also shown that both methyl donors, such as folic acid, betaine and choline, and genistein counteract DNA hypomethylation caused by bisphenol a (BPA), an endocrine active agent used to make polycarbonate plastic, showing that simple dietary changes can protect against the deleterious effects of environmental toxicants on the fetal epigenome. Dr. Dolinoy holds a BA in environmental sciences from Duke University, a MS in environmental health from the Harvard School of Public Health, and a PhD in Genetics and Genomics and Integrated Toxicology from Duke University.

Angela L. Slitt

University of Rhode Island

Angela Slitt is an assistant professor in the Department of Biomedical Sciences in the College of Pharmacy at the University of Rhode Island. Dr. Slitt received a B.S. with Honors in Molecular and Cellular Biology and a Ph.D. Pharmaceutical Sciences from the University of Connecticut. Her graduate work in the laboratory of Dr. Steven Cohen focused on biochemical mechanisms of acetaminophen-induced liver and kidney injury. She was the recipient of an American Liver Foundation Predoctoral Fellowship and was a Boehringer Ingelheim Predoctoral Fellow during this time. In 2000, Dr. Slitt joined the laboratory of Dr. Curtis Klaassen at the University of Kansas Medical Center in the Department of Pharmacology, Toxicology, and Experimental Therapeutics as a NIH postdoctoral trainee. Her postdoctoral work focused on aspects of drug transporter expression in models of liver injury and microsomal enzyme induction. Her NIH NRSA postdoctoral fellowship examined induction of drug transporters and altered vectorial excretion of acetaminophen metabolites. After an extended maternity leave, Dr. Slitt joined the University of Rhode Island faculty in 2007. Her Transition into Independent Position award has addressed the role Nuclear Factor E2 Related-Factor 2 in cholesterol metabolism, biliary cholesterol excretion, and gallstone formation. Her NIH ONES award has been addressing how caloric restriction affects the Nrf2-Keap1 pathway, hepatic transport processes, and Bisphenol A excretion. ARRA funded supplements to Dr. Slitt's ONES award have allowed for successful summer research experiences, giving five high school, four undergraduate, and two high school teachers exposure to scientific research in academia. Other funded projects include studies of diabetes and obesity on drug transporter expression and evaluation of plant and food-derived polyphenolic compounds for anti-inflammatory activity. Along with research activities, Dr. Slitt is a URI Grand Challenges Teaching Fellow, teaching URI freshman about aspects of environmental health and is Pharmacology/Toxicology track coordinator.

Thomas J. Begley

State University of New York at Albany

Thomas Begley is an associate professor of Nanobiosciences at the College of Nanoscale Science and Engineering at the University at Albany, State University of New York. He is also a member of the Cancer Research Center and RNA Institute at UAlbany. Dr. Begley earned a B.S. in Molecular Biology and Biochemistry ('94) and PhD in Biological Sciences ('00) from the University at Albany, State University of New York. He was a post-doctoral associate in Cancer Biology at the Harvard School of Public Health. In addition, he was an NIH post-doctoral fellow and Merck Computational and Systems Biology fellow in the Biological Engineering Division at the Massachusetts Institute of Technology. His lab uses a multidisciplinary approach to study molecular signaling pathways associated with the DNA damage response, as deficiencies in this response are associated with cancer onset and progression. The long term goals of his research are to develop methodologies to identify individuals susceptible to environmentally induced cancers and to identify protein-based therapeutic targets for cancer treatment.

Dr. Begley is a member of the American Association for the Advancement of Science, the American Chemical Society, The Society for Toxicology, and the Environmental Mutagen Society; he has served on the editorial board for *Mutation Research* and *Reviews in Mutation Research*, Elsevier; and he has served as an ad hoc reviewer for *DNA Repair*. In 2006 he was awarded a James D. Watson Award from New York State and he was a recipient of the Outstanding New Investigator Award (ONES) from NIEHS. He is funded by the National Institutes of Health to study cellular responses to environmental carcinogens and to study potential tumor suppressor pathways in colon cancer.

Scott D. McCulloch

North Carolina State University

The research interests of the McCulloch laboratory focus on the role of DNA polymerases in mutagenesis, specifically those mutations resulting from exposure to genotoxic insults. One polymerase in particular, pol eta, has been strongly implicated as being crucial for preventing mutations by the UV-light induced *cis-syn* cyclobutane pyrimidine dimer adduct, as evidenced by the extreme sunlight sensitivity displayed by *Xeroderma pigmentosum variant* patients who naturally lack the enzyme. However, pol eta displays incredibly low fidelity when bypassing this lesion. This has led to the idea that multiple factors are involved in the complete bypass reaction, including but not limited to proofreading, replication accessory proteins, and the mismatch DNA repair pathway. Current work focuses on the role of pol eta in the bypass of oxidative lesions and also on the factors that dictate polymerase expression. Dr. McCulloch obtained his Doctorate in Toxicology at the University of Kentucky under the guidance of Dr. Guo-Min Li, investigating the mechanism of DNA mismatch and loop repair. He worked as a post-doctoral researcher under Dr. Thomas Kunkel at the National Institute of Environmental Health Sciences, studying the mechanisms of pol eta during TT dimer bypass.

Yu-ying He

University of Chicago

Yu-Ying He is an Assistant Professor of Medicine at the University of Chicago. She received her PhD in Chemistry in 2000 from the Chinese Academy of Sciences in China. Then she accepted the Humboldt Research Fellowship and spent one year at the University of Erlangen-Nuerenburg in Germany. In 2001, she came to the NIEHS for her postdoctoral training with Dr. Colin Chignell. In 2007, she joined the faculty in the Department of Medicine at the University of Chicago. During her research career, Yu-Ying He has received several awards, including the first NIEHS Science Day Early Career Award, the NIH Fellows Award for Research Excellence (FARE), and the American Skin Association Research Scholar Award. Her research interests are in the genetic and environmental determinants of genomic stability in skin cells.

Sarah Delaney

Brown University

Dr. Delaney received her B.A. in Chemistry from Middlebury College in 1999 and Ph.D. in Chemistry from the California Institute of Technology in 2004, working in the laboratory of Jacqueline Barton. Dr. Delaney was a Damon Runyon postdoctoral fellow in the laboratory of John Essigmann at the Massachusetts Institute of Technology until 2007. Dr. Delaney is currently an Assistant Professor in the Department of Chemistry at Brown University. Her research interests are focused on understanding the role of oxidation of DNA nucleobases in expansion of genetically unstable trinucleotide repeat sequences such as CAG/CTG.

Jason H. Bielas

Fred Hutchinson Cancer Research Center

Dr. Bielas is an Assistant Member in the Molecular Diagnostics Program at Fred Hutchinson Cancer Research Center (FHCRC) and holds an Affiliate Assistant Professorship in Department of Pathology at the University of Washington. Dr. Bielas has had a long-standing interest and commitment to discerning the relationship between mutagenesis, aging, and cancer. Dr. Bielas earned his Ph.D. with Distinction and the Governor General of Canada's Gold Medal in 2004 from the Department of Biology at York University in Toronto. Together with doctoral thesis advisor, Dr. John A. Heddle, he developed novel methods to measure DNA repair and mutation to delineate the relationship between proliferation and mutagenesis. Following his doctoral work, Jason pursued postdoctoral training in the laboratory of Dr. Lawrence A. Loeb. Here, at the University of Washington, Jason's primary research focused on the role of a mutator phenotype in carcinogenesis, where he continued to develop novel methods to monitor mutagenesis, including the Random Mutation Capture (RMC) assay, which demonstrates that tumors exhibit point mutation instability (PIN), and that mitochondrial point mutations do not limit natural lifespan. During his tenure as graduate student and postdoctoral fellow, Dr. Bielas received a number of awards, including a graduate scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC), an Ontario Graduate Scholarship in Science and Technology, the RH Haynes Scholarship for Academic Excellence, and Postdoctoral Fellowships from NSERC, the Canadian Institutes of Health Research, and the Terry Fox Foundation. Since opening his laboratory at the FHCRC, Jason has received a New Scholar Award from the Ellison Medical Foundation, a New Investigator Award from the Department of Defense, and an Outstanding New Environmental Scientist (ONES) (RO1) Award from the National Institute of Environmental Health Sciences.

Joseph Shaw

Indiana University at Bloomington

Joseph R. Shaw, Ph.D. earned his doctoral degree from the Center for Toxicology at the University of Kentucky in 2001. He received post-doctoral training at Dartmouth College working to bridge studies of genes and their relationship to toxicologically relevant phenotypes with those that describe the effects environmental pollution on populations. He joined the faculty of the School of Public and Environmental Affairs at Indiana University, Bloomington in 2007. There his research group seeks to discover critical, specific and causative molecular toxicological and disease pathways resulting from complex environmental exposures. They embrace new high-throughput molecular techniques and couple these with evolutionary theory, and sophisticated statistical analysis and informatics in order to integrate toxic-response across levels of biological organization. Current projects *(i)* dissect variation in disease and toxicant response within and between populations; *(ii)* identify the evolutionary mechanisms and partition of the costs of physiological acclimation and long-term population level adaptation to chemical stress, especially metals, *(iii)* elucidate the molecular underpinnings of evolved metal tolerance, and *(iv)* understand the causes and phenotypic consequences of gene copy number variation in metal stressed populations (<https://daphnia.cgb.indiana.edu/Projects#Projects-19>).

Timothy R. Nurkiewicz

West Virginia University

Timothy R. Nurkiewicz earned his B.S. in Exercise Science from the Pennsylvania State University in 1990. He received his M.S. in Exercise Physiology from West Virginia University in 1992. After serving in cardio-pulmonary rehabilitation at Allegheny General Hospital in Pittsburgh, Pennsylvania, he returned to West Virginia University and earned his Ph.D. in Physiology in 1999. Dr. Nurkiewicz performed his post-doctoral training at Texas A&M University in the Department of Medical Physiology. While trained as a microvascular physiologist, he has a broad background ranging from bench top in vitro experiments/techniques to clinical interactions. Currently, Dr. Nurkiewicz is an Associate Professor in the Department of Physiology and Pharmacology at the West Virginia University School of Medicine, where he is also a member of the Center for Cardiovascular and Respiratory Sciences. Dr. Nurkiewicz's research program focuses on the systemic microvascular effects of pulmonary exposure to particulate matter and nanoparticles (cardiovascular toxicology and nanotoxicology), and has been continuously supported by extramural funding from the Health Effects Institute and National Institute of Health. He serves on the Editorial Board of four toxicology journals; is an ad hoc reviewer for numerous journals as well as study sections; and participates in various committees and consortiums at the University, State and National levels. Dr. Nurkiewicz is the Past-President (and a founding member) of the Cardiovascular Toxicology Specialty Section, and the President-Elect of the Allegheny-Erie Regional Chapter of the Society of Toxicology.

Gokhan M. Mutlu

Northwestern University

Dr. Mutlu is an Associate Professor of Medicine in the division of Pulmonary and Critical Care Medicine at Northwestern University Feinberg School of Medicine. He serves as an Associate Editor for the *American Journal of Respiratory and Critical Care Medicine*. He has received the ONES Award in 2006 on his research project focusing on understanding the mechanisms by which ambient particulate matter air pollution causes thrombosis. He has over 80 publications including 15 papers directly related to the ONES Award. He has served as a reviewer for NIH and is an active member of the American Thoracic Society Scientific Advisory Committee.

Jesus A. Araujo,

University of California, Los Angeles

Dr. Jesús A. Araujo was born in Caracas, Venezuela where he got his M.D. degree Magna Cum Laude at the Central University of Venezuela and M.Sc. degree in Immunology at the Venezuelan Institute for Scientific Research. He subsequently completed internal medicine residency training at Beth Israel Medical Center, Albert Einstein College of Medicine in New York and a Cardiology fellowship at UCLA Medical Center in Los Angeles. He also obtained a Ph.D. degree in Molecular Biology from the University of California, Los Angeles and is currently an Assistant Professor of Medicine and director of Environmental Cardiology at the David Geffen School of Medicine at UCLA. Dr. Araujo's research interests focus on: i) dissecting the mechanisms how exposure to air particulate matter promotes HDL dysfunction, atherosclerosis and ischemic heart disease, ii) study of gene-environment interactions relevant to vascular oxidative stress and atherosclerosis.

Jason R. Richardson

University of Medicine and Dentistry of New Jersey

Jason Richardson, M.S., Ph.D. is an Assistant Professor at in the Department of Environmental and Occupational Medicine at Robert Wood Johnson Medical School and Resident Member of the Environmental and Occupational Health Sciences Institute. He received his M.S. and Ph.D. degrees from Mississippi State University where he conducted research on mixtures of organophosphate pesticides and the developmental neurotoxicity of organophosphates during critical periods of development. He then completed postdoctoral training in Molecular Neuroscience and Neurotoxicology at Emory University where he focused on the role of pesticide exposure in Parkinson's disease. His research at EOHSI focuses on the role of environmental exposures during development and how such exposures interact with genetic susceptibility to produce neurological disease. Dr. Richardson has authored or co-authored 33 publications in the areas of developmental neurotoxicology, neurodegenerative disease, and pesticides. He has received the Outstanding New Environmental Scientist Award from the National Institute of

Environmental Health Sciences and a Young Scientist Award from the American Society for Pharmacology and Experimental Therapeutics. Dr. Richardson is currently a member of the editorial boards of Toxicological Sciences, Neurotoxicology and Teratology, Current Molecular Pharmacology, and Neurotoxicology, and is an Associate Editor for BMC Neurology. He has served as a grant reviewer for several NIH panels, the Michael J. Fox Foundation for Parkinson's Disease Research, Autism Speaks, and the United Kingdom Parkinson's Disease Society.

Michelle L. Block

Virginia Commonwealth University

Dr. Block graduated from Iowa State University in 1994 and received her Ph.D. in Genetics from Penn State University in 2002. She began her post doc experience in a joint position between the EPA and NIEHS/NIH from 2002-2003 working on air pollution and CNS effects with Dr. Bellina Veronesi and Dr. Jau-Shyong Hong. From 2002-2007, she completed her postdoctoral work in the laboratory of Dr. Jau-Shyong Hong at NIEHS/NIH. At present, Dr. Block is an Assistant Professor in the Department of Anatomy and Neurobiology at Virginia Commonwealth University. Her research focuses the biology of microglia, the resident innate immune cell in the brain, in neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. Dr. Block's group focuses on identifying the triggers (environmental and endogenous) that initiate deleterious microglial activation, revealing the redox mechanisms through which microglia cause neurotoxicity, and applying these findings towards the identification of biomarkers of neurotoxic exposures and the development of therapeutic compounds capable of halting the progression of neurodegenerative disease