Department of Environmental Medicine
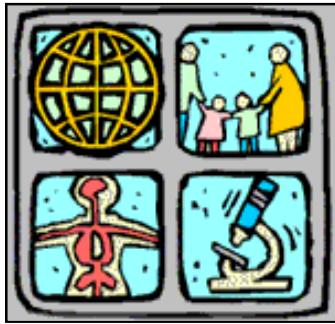
Toxicology Training Program Annual Retreat

Schedule of Events and Abstracts

University of Rochester
School of Medicine and Dentistry

June 22, 2017

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Toxicology Retreat Schedule

Time	Event	Room
8:15-9:30	Workshop 1: **"Connecting the Dots in Decision Making and Public Outreach"** by Steven Gilbert PhD, DABT	Northeastern 1-9525
9:30-10:45	Workshop 2: **"Abstracts for Everyone"** by Dina Markowitz and Laura Shum	Northeastern 1-9525
11:00-12:00	Keynote Address: **"Environmental Health in the Age of Precision Medicine"** Andrea Baccarelli MD, PhD	Class of '62
1:30-3:00	Platform Presentations	Ryan Case Method Room (1-9576)
3:00-4:30	Poster Session	Sarah Flaum Atrium

<u>Keynote Speaker</u>
11:00-12:00 in Class of '62 Auditorium (G-9425)

Andrea Baccarelli, M.D., Ph.D.
Chair and Leon Hess Professor, Department of Environmental Health Sciences
Columbia University
Mailman School of Public Health

Environmental Health in the Age of Precision Medicine

Schedule of Student and Postdoctoral Fellow Platform Presentations

1:30-3:30 in Ryan Case Method Room

Time	Speaker	Title
1:30-1:45	**William Domm** (Postdoctoral Fellow) Advisor: Michael A. O'Reilly, Ph.D.	Early life oxygen exposure leads to changes in influenza A virus-macrophage interactions in adult mice
1:45-2:00	**Jessica Meyers** (5th year student) Advisor: B. Paige Lawrence, Ph.D.	Aryl hydrocarbon receptor activation during development reduces dendritic cell responses later in life
2:00-2:15	**Lisa Prince** (5th year student) Advisor: Matthew D. Rand, Ph.D.	A 24-Hour Exposure to MeHg Causes a Persistent Inhibition of Myogenin Expression and C2C12 Myoblast Differentiation
2:15-2:30	**Tyler Beach** (4th year student) Advisor: Jacob N. Finkelstein, Ph.D.	Persistent and progressive lung injury is linked to recurring DNA damage, cellular senescence and a pro-fibrotic epithelial response

2:30-2:45	**Brian Palmer** (4th year student) Advisor: Lisa A. DeLouise, Ph.D., M.P.D.	Topical skin treatment with nanoparticles modulates the contact hypersensitivity response in a murine model
2:45-3:00	**Shannon Lacy** (3rd year student) Advisors: Richard P. Phipps, Ph.D. and Patricia J. Sime, M.D.	Activated human lung fibroblasts produce prostaglandins with paracrine anti-fibrotic effects delivered via exosomes

Abstracts of Student and Postdoctoral Fellow Platform Presentations
Ryan Case Method Room (1-9576)

***1:30-1:45* William Domm** (Postdoctoral Fellow)
Research Advisor: Michael A. O'Reilly, Ph.D.
Early life oxygen exposure leads to changes in influenza A virus-macrophage interactions in adult mice
William Domm, Michael A. O'Reilly

Children who were born preterm have greater pulmonary morbidity and are more likely to be re-hospitalized following a respiratory viral infection than children born full term. Mechanisms by which preterm birth increases the severity of infection are poorly understood. Since preterm infants are often exposed to excess oxygen at birth, we have been using mice to understand how high oxygen exposure at birth alters host-pathogen interactions between the lung and influenza A viruses (IAV) later in life. Previous work from our laboratory has shown how young adult (8 week old) mice exposed to 100% oxygen (hyperoxia) between birth and postnatal day 4 exhibit alveolar simplification. They also develop greater pulmonary inflammation and fibrotic lung disease when infected with a sublethal dose of IAV (HKx31, H3N2) than siblings exposed to room air. Using IAV (PR8, H1N1) expressing mCherry reporter protein to track virally infected cells, we discovered neonatal hyperoxia does not increase primary or early infection of airway or alveolar epithelial cells. Instead, mCherry expression in macrophages and macrophage cell death was increased in mice exposed to hyperoxia at birth compared to room air controls. Consistent with these in vivo findings, macrophages isolated from mice exposed to neonatal hyperoxia were more readily infected with IAV ex vivo than room air controls. Additional evidence suggests changes in interstitial macrophages that direct tissue repair in adult mice exposed to hyperoxia at birth. Since macrophages are long-lived immune cells that are important for maintaining pulmonary homeostasis and directing tissue repair, our findings suggest neonatal oxygen-dependent changes in macrophages may contribute to a poor outcome in the lungs response to IAV infection in adult mice. Supported by: NIH T32-ES0-07026, R01 HL091968

***1:45-2:00* Jessica Meyers** (5th year student)
Research Advisor: B. Paige Lawrence, Ph.D.

Aryl hydrocarbon receptor activation during development reduces dendritic cell responses later in life

Jessica Meyers, Bethany Winans, Lisbeth Boule and B. Paige Lawrence

The developing immune system is susceptible to environmental insults, which can contribute to altered immune function later in life. Epidemiological studies and work in animal models indicate that early life exposure to aryl hydrocarbon receptor (AHR) binding chemicals leads to persistent changes in immune function at maturity. For example, developmental exposure to a prototype AHR ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), durably deregulates T cell responses to influenza A virus (IAV) infection. During primary infection, lung resident dendritic cells (DCs) acquire antigen, mature, and emigrate to the lung-draining mediastinal lymph nodes (MLN), where they activate naïve T cells. However, the impact of developmental exposure on these DC responses to infection is poorly understood. We report here that developmental AHR activation changes DC distribution, trafficking, and function later in life. While developmental exposure did not change the number of conventional DCs (cDCs) or plasmacytoid DCs (pDCs) in the lung prior to or after infection, there were two-fold fewer cDCs and pDCs in the MLN. To determine whether developmental exposure affects DC trafficking, we fluorescently tagged DCs in the lung, and enumerated lung-derived DCs in the MLN. Significantly fewer lung-derived DCs were detected in the MLN of developmentally exposed offspring, indicating that early life activation of the AHR reduces DC's ability to migrate from the site of infection to the site of T cell activation. To determine whether reduced migration reflects reprogramming of intrinsic properties of DCs, we generated bone marrow derived DCs (BMDCs) from developmentally exposed mice, and measured their migration towards a gradient of the chemokine CCL21. Fewer BMDC from developmentally exposed mice migrated towards CCL21, compared to BMDCs from offspring of control dams. A key regulator of DC trafficking is the receptor for CCL21: chemokine receptor 7 (CCR7). CCR7 was not reduced on DCs in the lung prior to or after infection; however, subtle reductions in CCR7 expression were observed on cDCs in the MLN and on BMDCs from developmentally exposed mice. Thus, while developmental exposure reduces DC trafficking, factors in addition to CCR7 likely drive this change. In separate studies, we determined that developmental exposure hampers the ability of DCs to stimulate naïve virus-specific CD8+ T cells. In contrast, it did not affect DCs' capacity to activate naïve CD4+ T cells. This suggests that in addition to changing DC migration, early life AHR

activation preferentially affects DC functions that are essential for or unique to DC:CD8+ T cell interactions. Given that DCs regulate T cell responses in a broad spectrum of infectious and non-infectious diseases, the discovery that maternal exposure to an AHR ligand perturbs DC function has broad implications for understanding how the early life environment shapes the integrated function of the immune system.

2:00-2:15 **Lisa Prince** (5th year student)
Research Advisor: Matthew D. Rand, Ph.D.
A 24-Hour Exposure to MeHg Causes a Persistent Inhibition of Myogenin Expression and C2C12 Myoblast Differentiation
Lisa Prince and Matthew D. Rand

MeHg is a ubiquitous environmental toxicant, and fish consumption is the main route of exposure. MeHg exposure can cause both motor and cognitive defects, with children exposed in utero being most vulnerable. Decades of studies have focused on MeHg as a developmental neurotoxicant, but few have examined the neuromuscular system in its entirety. Data from our lab has shown that MeHg disrupts development of both neurons and muscles in Drosophila and preferentially upregulates a Notch target gene that is predominantly expressed in developing muscles. We further demonstrated that MeHg tolerance and susceptibility in developing flies associate with core genes in pathways of muscle and neuromuscular development, including components of Notch signaling. Notch signaling is a conserved pathway for directing myogenesis. Therefore, we hypothesized that MeHg can impact developing muscle by upregulating Notch target genes involved in myogenesis. We tested this hypothesis by exposing differentiating C2C12 mouse myoblasts to MeHg and analyzing muscle cell morphology and expression of Notch target genes and myogenic regulatory factors (MRFs). A 24-hour MeHg exposure was found to cause a persistent reduction in myotube formation, as well as a persistent and specific inhibition of one MRF, myogenin. Surprisingly, MeHg was shown to repress Notch target gene expression in the C2C12 model. However, MeHg also caused a reduction in genes involved in mitochondrial biogenesis and mtDNA transcription and translation, which are associated with the regulation of myogenesis. Future studies aim to further investigate the role of MeHg-induced mitochondrial toxicity on inhibiting myogenin and myoblast differentiation. We anticipate our findings will advance the understanding of the role that muscle tissue plays as a developmental

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target for MeHg toxicity. Supported by NIEHS R01 ES007026 and NIEHS P30 ES001247.

2:15-2:30 **Tyler Beach** (4th year student)
Research Advisor: Jacob N. Finkelstein, Ph.D.
Persistent and progressive lung injury is linked to recurring DNA damage, cellular senescence and a pro-fibrotic epithelial response
<u>Tyler A. Beach</u>, Carl J. Johnston, Angela M. Groves, Jaqueline P. Williams and Jacob N. Finkelstein

Radiation induced lung injury (RILI) is a common outcome in patients requiring radiation treatment for lung cancer. Approximately 43% of patients receiving radiation treatment at a dose of 15 Gy or greater will experience some type of RILI, either in the form of acute pneumonitis and/or late onset pulmonary fibrosis. Known effects of radiation injury include DNA damage, inflammation, and a cellular senescence among the exposed cell population, events associated with onset and progression of fibrosis in lung tissue. Resident Club cells and Type 2 Alveolar Epithelial cells (AEC2) play an active role in tissue repair through their ability to proliferate in response to injury, and as such are likely to be damaged by ionizing radiation. Therefore, we sought to determine the role of pulmonary epithelia in the development and progression of radiation induced pulmonary fibrosis (RIPF). We have hypothesized that following radiation induced immediate and recurrent DNA damage, there is continuous cellular senescence, and both a pro-fibrotic and inflammatory phenotype in the surviving epithelial cells. Whole lung tissue and isolated pulmonary (CD326+) epithelia were collected from fibrosis prone C57BL/6J female mice exposed to12.5Gy thorax only γ-radiation and examined at 24 hrs, 1,4,12, and 32 weeks post radiation treatment by RNA sequencing and histological analysis. In the weeks/months following radiation exposure we observed a loss of both Club cells and Type 2 epithelia. DNA damage as evidenced by γH2A.X foci was persistently increased in whole lung tissue compared to non-irradiated controls, and this data was further supported by an increased abundance of DNA Damage Response (DDR) associated transcripts such as p53bp1 and xrcc1. Transcripts indicating cell cycle arrest and cellular senescence such as cyclin dependent kinase inhibitors Cdkn1a, and Cdkn2b were similarly upregulated in both whole lung tissue and pulmonary epithelia following RT, suggesting cellular senescence in the surviving cells. Epithelial transcripts associated with mediating the immune/injury response appear to diminish over time as the population of those cells decrease. In contrast growth factors Ctgf, Vegf, Fgf, and Pgdf are persistently increased in transcript abundance

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following radiation, and are potentially important contributors of fibrosis. The initial results of this study have revealed ROS production and activity, as well as stimulation or suppression of immune responses as possible targets for mitigation of fibrotic outcomes following radiation exposure.

2:30-2:45 **Brian Palmer** (4th year student)
Research Advisor: Lisa A. DeLouise, Ph.D., M.P.D.
Topical skin treatment with nanoparticles modulates the contact hypersensitivity response in a murine model

Brian C. Palmer, Sarah Phelan, Samreen Jatana, Lisa A. DeLouise

Nanoparticle engineering is a growing field due to the vast array of applications. For example, titanium dioxide and zinc oxide nanoparticles are included in a number of sunscreens for their ultraviolet light filtering properties, and silica based nanoparticles and carbon nanomaterials are being researched as potential drug delivery vehicles through skin. The dermal toxicity of these nanoparticles needs to be tested, and since skin is exposed to a number of potential allergens we find it equally important to examine the effect of nanoparticle application on immune system activation after allergen exposure. Here we examine the Type IV immune response using the mouse contact hypersensitivity (CHS) model after sensitization and challenge with dinitrofluorobenzene (DNFB). We have found that a number of small, spherical, negatively charged nanoparticles ameliorate the skin swelling responses in the CHS challenge phase. The reduction in skin swelling is associated with reductions in mast cell degranulation and reduced inflammatory cytokines (IL-6, IL-1β, and KC) in the skin. Conversely, we have found that some high aspect ratio, carboxylated carbon nanotubes may exacerbate the skin swelling in a CHS response. The doses tested in these studies are similar to other nanoparticle based topical therapies already in use, and they suggest that prolonged use of some nanoparticles can alter the skin immune activation. On-going work seeks to elucidate the molecular and cellular mechanisms of action and exploration of nanoparticles as a potential preventative treatment for contact dermatitis, but will also focus on carbon nanotubes since they exacerbate CHS responses which may present an interesting toxicological concern due to their increased use in biomedical science.

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2:45-3:00 **Shannon H. Lacy.** (3rd year student)
Research Advisor: Richard P. Phipps, Ph.D. and Patricia J. Sime, M.D.

In vitro and In vivo Activated human lung fibroblasts produce prostaglandins with paracrine anti-fibrotic effects delivered via exosomes

Shannon H. Lacy, Collynn F. Woeller, Thomas H. Thatcher, Patricia J. Sime, and Richard P. Phipps

A critical step in the pathogenesis of lung scarring, or pulmonary fibrosis, is the differentiation of human lung fibroblasts (HLFs) into contractile myofibroblasts that proliferate and secrete excessive extracellular matrix (ECM) in the interstitium, impairing gas exchange. We recently demonstrated that HLFs produce prostaglandin (PG) mediators that activate the transcription factor peroxisome proliferator-activated receptor-gamma, raising the possibility that HLFs may also exert anti-fibrotic effects. Here, we used direct contact and non-contact co-culture systems to test the hypothesis that HLFs activated with interleukin-1 beta (IL-1β) produce antifibrotic PGs with both autocrine and paracrine effects. We found that IL-1β-activated HLFs inhibit transforming growth factor-beta (TGFβ)-induced myofibroblast differentiation and ECM production by both the IL-1β treated cells (i.e., autocrine signal) and by adjacent naïve HLFs (i.e., paracrine signal). Extracellular vesicles, and exosomes in particular, are increasingly recognized as a highly efficient and biologically important means of intercellular communication. Yet, the production of exosomes by HLFs has not been rigorously studied. We used nanoparticle tracking analysis to characterize the extracellular vesicles produced by HLFs, and found that concentrated exosomes, particularly from IL-1β treated HLFs, exert potent anti-fibrotic effects. Finally, we used targeted lipidomics to investigate the eicosanoid content of concentrated exosomes from untreated and IL-1β treated HLFs, and confirmed the presence of several anti-fibrotic PGs in the exosomes of IL-1β treated HLFs. This work opens the way for future research into exosome-mediated intercellular signaling in the lung, and may inform the development of urgently needed novel therapies for fibrotic lung diseases. Supported by NIH grants R01 HL127001, R01 HL120908, T32 HL066988, and P30 ES001247; the Greg Chandler and Guy F. Solimano Fibrosis Research Fund; and the Doran Family Endowment.

List of the Student and Postdoctoral Fellow Posters

3:00-4:30 in Sarah Flaum Atrium

[#1] Identification of novel immune pathways altered by aryl hydrocarbon receptor in mouse and human dendritic cells during influenza A virus infection
Anthony M. Franchini, Ph.D. Advisor: *B. Paige Lawrence*

[#2] Role of infiltrating monocytes in the development of radiation induced pulmonary fibrosis
Angela Groves, Ph.D.   Advisor: *Jacob N. Finkelstein*

[#3] Salinomycin reduces radiation-induced capsular fibrosis associated with silicone implants in a mouse model
Katie L. Lannan, Ph.D.   Advisor: *Richard P. Phipps*

[#4] Behavioral modulation of Neurotoxicity: Behavior-Dependent Effects of Developmental Lead (Pb) and Prenatal Stress (PS) on Epigenetic Modifications in Adult Brain
Marissa Sobolewski, Ph.D. Advisor: *Deborah A. Cory-Slechta*

[#5] Gestational Particulate Matter Exposure Disrupts the Trajectory of White Matter Development
Carolyn R. Klocke, Ph.D.   Advisor: *Deborah A. Cory-Slechta*

[#6] Aryl Hydrocarbon Receptor Inhibitors Rescue Impaired Anti-Viral Responses to Dung Biomass Smoke in Human Small Airway Epithelial Cells
Claire E. McCarthy   Advisor: *Patricia J. Sime*

[#7] Embryonic iron deficiency has a lasting effect on adult neural structure
Michael Rudy   Advisor: *Margot Mayer-Proschel*

[#8] Suppression of Anti-viral Responses in Lung Epithelial Cells Exposed to Tobacco Smoke is Mediated through the Aryl Hydrocarbon Receptor
Parker Duffney   Advisors: *Richard Phipps and Patricia J. Sime*

[#9] miR338-3p Negatively Regulates Lactate Dehydrogenase and Inhibits Myofibroblast Differentiation
Jennifer L. Judge   Advisor: *Patricia J. Sime*

[#10] Live imaging of microglia, dendritic spines, and their interactions in adolescent mouse cortex after brain growth spurt alcohol exposure
Elissa L. Wong   Advisor: *Ania K. Majewska*

[#11] The Effects of Ultrafine Particulates from Air Pollution on Microglia in a Mouse Model of Alzheimer's Disease
Denise Herr   Advisor: *Alison Elder*

[#12] The ancestral environment shapes antiviral CD8+ T cell responses across generations
Christina M. Post   Advisor: *B. Paige Lawrence*

[#13] Triggering the aryl hydrocarbon receptor during development durably programs CD4+ T cell responses
Catherine Burke   Advisor: *B. Paige Lawrence*

[#14] Effects of ultrafine particulates from air pollution on the progression of Alzheimer's disease
Kelly Hanson   Advisor: *Jacob N. Finkelstein*

[#15] Ultrafine particulate matter air pollution effects on learning and memory in an Alzheimer's disease mouse model
Katrina Jew   Advisor: *Alison Elder*

[#16] Developmental activation of the aryl hydrocarbon receptor (AHR) has multi- and transgenerational effects on antiviral immunity
Keith Morris-Schaffer   Advisor: *Deborah A. Cory-Slechta*

[#17] Effect of UVR and UVR Filters in Modulating AhR Dependent Skin Immune Responses
Sarah J. Phelan   Advisor: *Lisa A. DeLouise*

[#18] Aerosolized Gold Nanoparticle Suspensions and Spark-Generated Gold Nanoparticles Do Not Readily Enter the CNS
Candace Wong   Advisor: *Alison Elder*

[#19] Reproductive and Developmental Toxicity from Maternal Exposure to Estrogenic Endocrine Disrupting Chemicals
Jasmine Reed   Advisor: *Martha Susiarjo*

[#20] Neonatal ultrafine particulate matter exposure adversely impacts white matter development
Timothy R. Smyth   Advisor: *Steve Georas*

[#21] Transgenerational Epigenetic Inheritance of Behavioral Alterations in Mice Exposed to Lead and Stress
Kadijah Abston   Advisors: Martha *Susiarjo & Deborah A. Cory-Slechta*

[#22] Effects of vitamin B6 deficiency of epigenetic and genetic regulation in mice
Ashley Fields   Advisor: *Martha Susiarjo*

[#23] Metabolism and Pulmonary Fibrosis: A Potential Role for TG2
Ashley R. Rackow   Advisor: *Patricia J. Sime*

[#24] Exploring regulatory crosstalk between AHR and HIF-1α in hematopoiesis
Keegan L. Vaughan   Advisor: *B. Paige Lawrence*

Abstracts of the Student and Postdoctoral Fellow Poster Session

3:00-4:30 in Sarah Flaum Atrium

[#1] **Anthony M. Franchini, Ph.D.** (Postdoctoral Fellow)
Research Advisor: B. Paige Lawrence, Ph.D.

Identification of novel immune pathways altered by aryl hydrocarbon receptor in mouse and human dendritic cells during influenza A virus infection
Anthony M. Franchini, Jason R. Myers, B. Paige Lawrence

Dendritic cells (DC) are critical mediators of the immune response to viruses. They are equipped with the capacity to present viral antigens and prime naïve T cells. Activation of the aryl hydrocarbon receptor (AHR), an environment-sensing transcription regulator, perturbs DC function during influenza A virus (IAV) infection, severely reducing their ability to serve as antigen presenting cells. The mechanism that drives this is unclear, and AHR gene targets in DCs are poorly characterized. Thus, we used unbiased gene expression profiling to identify genes and signaling pathways in DCs that are modulated by in vivo AHR activation. Specifically, sorted DCs from infected mice treated with the prototypical AHR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or control were used for transcriptome analysis by RNA-seq. Differentially expressed genes include hallmark AHR target genes, such as cyp1a1, and ido1. Other significantly affected pathways primarily relate to immune function, including extracellular sensing and leukocyte trafficking, revealing potentially novel AHR regulatory targets. Notably, AHR activation reduced DC-SIGN (cd209a) and ccl17 expression. Flow cytometry and ELISAs confirm diminished DC-SIGN levels on DCs in the infected lung, and significantly reduced CCL17 production during infection. Human monocyte-derived DCs (hmoDCs) showed analogous changes in gene expression following TCDD and IAV exposure. Similar to transcriptional changes in mice, AHR activation elevated cyp1a1 and ido1, and decreased cd209a expression levels in hmoDCs. Collectively, this shows AHR activation exerts regulatory control over both immunoregulatory and stimulatory pathways in DCs and thus, anti-viral immune responses during primary IAV infection. As DCs are responsible for initiating the immune response in many disease states, these findings have broad implications regarding how AHR regulates DC function. Supported by: NIEHS grants R01 ES017250, R01 ES023260, P30 ES07026, and T32 ES007026

[#2] **Angela Groves, Ph.D.** (Postdoctoral Fellow)
Research Advisor: Jacob Finkelstein, Ph.D.

Role of infiltrating monocytes in the development of radiation induced pulmonary fibrosis

<u>Angela Groves,</u> Carl Johnston, Jackie Williams, Jacob Finkelstein

In response to radiation (RT) induced lung injury the lung releases inflammatory cytokines and chemotactic mediators to recruit immune cells to execute inflammatory and wound healing responses. However, microenvironmental alterations can occur in which this response becomes dysregulated impairing the return to homeostasis, leading the development of chronic inflammation, pneumonitis and pulmonary fibrosis (PF).   The extent to which recruitment of inflammatory monocytes, which are bone marrow derived and express CCR2, contribute to RT induced PF has not been fully investigated and we hypothesized that RT induced PF is reliant on this population. To test this, CCR2-/- chimeric mice were exposed to 9.75 Gy thoracic RT. PF development was evaluated and alterations in pulmonary macrophage subpopulations were assessed by flow in immune cells enriched from lung digests collected at 12 – 18 wk following RT. In WT > CCR2-/- and WT syngeneic mice fibrotic foci were observed in the periphery of the lungs by 12 and 16 wk following RT, respectively. In contrast, in CCR2-/- > WT and CCR2-/- syngeneic mice no PF was observed for at least 22 wk post exposure and correlated with decreased numbers of infiltrating and interstitial macrophages compared to controls at this time point. Macrophage phenotype was further analyzed in resident subpopulations. In all chimera groups RT decreased the percentage of F4/80+ cells, expressed on mature macrophages and monocytes, suggesting the presence of less mature macrophages following RT exposure. In WT syngeneic chimeras the percentage of Ly6C+ cells, an inflammatory cell marker, and CD206, indicative of an alternatively activated phenotype, were similar to 0 Gy controls. Mice that had received CCR2-/- bone marrow, however, showed a decreased proportion of Ly6C+ cells, suggesting CCR2+ cells contribute to an inflammatory macrophage phenotype in the RT lung, while reduced proportions of CD206+ cells were noted in CCR2-/- mice following RT, regardless of bone marrow type, suggesting resident lung populations influence this phenotype. These results suggest infiltrating monocytes influence resident macrophage phenotype and play a role in the development of RT induced PF. Supported by: R01 AI101732, U19 AI091036, P30 ES001247 and T32 ES007026

[#3] **Katie L. Lannan, Ph.D.** (Postdoctoral Fellow)
Research Advisor: Richard P. Phipps, Ph.D.

Salinomycin reduces radiation-induced capsular fibrosis associated with silicone implants in a mouse model

<u>Katie L. Lannan</u>, Trevor C. Hansen, Collynn F. Woeller, Richard P. Phipps

Breast cancer is the most common malignancy diagnosed in women, affecting approximately 1 in 8 women in the U.S. One therapeutic strategy combines mastectomy and adjuvant radiotherapy, with reconstruction of the affected breast using saline or silicone implants. Over 80,000 women in the US chose implant-based breast reconstruction in 2014, and over 300,000 implant-based breast augmentations were performed. Unfortunately, silicone implants can cause a foreign body response and lead to pathological scarring process, called capsular contracture, which involves abnormal firmness, severe pain, and breast disfigurement. Approximately 40-70% of women who received implant-based breast reconstruction with radiotherapy develop capsular contracture, and the incidence rate for implant-based breast augmentation is reported to be as high as 50%. To date, no truly effective therapeutic strategies exist to combat implant-induced scar formation. Our lab has identified that the coccidiostatic antibiotic, salinomycin, acts as a potent anti-scarring agent in vitro. In the present study, we utilized a mouse model of radiation-induced capsular fibrosis associated with silicone implants to evaluate salinomycin's ability to reduce scarring in vivo. To this end, silicone discs were impregnated with salinomycin or control solvent and placed submuscular on the dorsum of the mouse. Mice received 0 Gy or 15Gy gamma, then implants and surrounding tissue were harvested after 16 weeks. Histological evaluation of the peri-implant tissue from both the irradiated and non-irradiated mice revealed a fibrous capsule comprised of a dense network of collagen. Salinomycin significantly reduced scar formation, evidenced by decreased capsule thickness. Our exciting new data suggest that salinomycin may be an attractive new anti-scarring therapeutic for the prevention of periprostetic capsular fibrosis. This work was supported in part by T90 DE021985 and the Wilmont Cancer Center Pilot Project Program.

[#4] **Marissa Sobolewski, Ph.D.** (Postdoctoral Fellow)
Research Advisor: Deborah Cory-Slechta, Ph.D.

Behavioral modulation of Neurotoxicity: Behavior-Dependent Effects of Developmental Lead (Pb) and Prenatal Stress (PS) on Epigenetic Modifications in Adult Brain

Marissa Sobolewski, G. Varma, K. Conrad, J. Schneider, and Deborah Cory-Slechta

Lead (Pb) and prenatal stress (PS) are well known to alter the development of the central nervous system (CNS), resulting in altered behavioral function into adulthood. In fact, recently these adverse CNS outcomes have been shown to have an epigenetic basis. Less considered however, is how behavioral experience may modulate the effects of developmental exposure to Pb or PS on the epigenome. In fact, the type of behavioral experience (BE), either positive or negative, could differentially modulate the epigenetic effects of Pb±PS exposure. To explore this, animals were developmentally exposed to Pb±PS. Female C57/Bl6 mice were randomly assigned to receive drinking water containing 0 or 100 ppm Pb acetate for 2 months prior to breeding and through lactation and were assigned to non-stress (NS) or PS conditions (i.e., restraint stress 3x/day for 30 min/day from gestational day 15-18) groups, yielding 4 treatment groups: 0-NS, 0-PS, 100-NS and 100-PS. Offspring were separated into non-behavioral, positive, and negative behavioral experience groups. Following BE, post-translational histone modifications (PTHMs: H3K9ac, H3K4me3 H3K9me2 and H3K27me3) and gene expression of 8 histone modifying genes (Kat2a, Hdac1, Kmt2b, Kdm5d, Ehmt2, Kdm1a, Ezh2 and Kdm6a) were measured in frontal cortex (FC) and hippocampus (HIPP) in male and female control (no Pb or PS), Pb, PS, Pb+PS exposed adult mice that received different behavioral experiences (positive, negative and no behavior). Behavioral experience (BE) alone (either positive or negative) impacted expression of all PTHMs and most histone modifying genes examined, but the pattern of changes differed by brain region and sex. These BE-induced changes in PTHMs were both sex- and regionally- specific, indicating the need for such variables to be considered in relating epigenetic changes to brain and behavioral function. These findings also have significant implications for experimental designs, indicating the necessity of including non-behavioral control groups, as epigenetic markers are likely to differ pre- and post-behavioral experience. In addition, in some instances, the type of behavioral experience either positive or negative modified effects of Pb and PS when compared to the no behavioral experienced control group. Together, these data suggest that adult BE not only affects the brain epigenome but may also modulate expression of epigenetic effects associated with Pb+PS exposure, suggesting that not only can

Pb±PS alter adult behavior, but adult behavior can alter the effects of Pb±PS on adult CNS function.

[#5] **Carolyn R. Klocke, Ph.D.** (Postdoctoral Fellow)
Research Advisor: Deborah A. Cory-Slechta, Ph.D.

Gestational Particulate Matter Exposure Disrupts the Trajectory of White Matter Development

Carolyn Klocke, Joshua Allen, Marissa Sobolewski, JL Blum, D Lauterstein, JT Zelikoff, Deborah Cory-Slechta

Increasing evidence indicates that gestation is a critical window of neurodevelopmental susceptibility to air pollution. The particulate matter (PM) component of air pollution, specifically the fine (≤2.5µm; PM¬¬2.5), and ultrafine (≤100 nm; UFP) fractions, have been shown to induce neuropathology in mice and have been epidemiologically linked to neurodevelopmental disorders (NDs). Our previous studies in mice exposed postnatally (human 3rd trimester equivalent) to concentrated ambient UFPs using the U. of Rochester HUCAPS system, revealed white matter abnormalities and ventriculomegaly (enlarged lateral ventricles), which are poor prognostic indicators for neurodevelopment and characteristics of multiple disorders, including autism and schizophrenia. The current study sought to determine if the prenatal period in mice (human 1st and 2nd trimester equivalent) is a critical window of neurodevelopmental susceptibility to concentrated ambient particles (CAPs), which include both PM2.5 and UFP. Pregnant mice were exposed CAPs or filtered air control via whole-body inhalation for 6 hours daily from gestational days 0.5-16.5 using the VACES particle concentrator at NYU in Sterling Forest, NY. Offspring brains were collected at postnatal days (PNDs) 11-15 and 57-61 and analyzed histologically. Prenatal CAPs exposure induced perinatal ventriculomegaly in both sexes at PNDs 11-15 that did not persist to PNDs 57-61. In contrast, CAPs exposure induced early and persistent white matter overgrowth of the corpus callosum (CC) in both sexes, indicated by increased myelin basic protein density, with associated increase in CC area. Elemental iron, a critical driving factor of myelin biosynthesis, was elevated in the CC of females. Examination of CC oligodendrocytes (OLs) revealed a premature maturational shift in CAPs-exposed offspring, as indicated by decreased Olig2+ precursors and increased CC1+ mature OLs and no change in total OL cell number. These findings highlight the vulnerability of the brain and white matter during gestation to UFP/ PM¬¬2.5 exposures, and a corresponding potential for increased risk of NDs such as autism and

schizophrenia. The parallel findings from two different CAPs-generating systems (HUCAPS and VACES) suggest the importance of common exposure elements as opposed to regionally-specific constituents of air pollution as the basis of such effects.

[#6] **Claire E. McCarthy** (6th year student)
Research Advisor: Patricia J. Sime, M.D.

Aryl Hydrocarbon Receptor Inhibitors Rescue Impaired Anti-Viral Responses to Dung Biomass Smoke in Human Small Airway Epithelial Cells

Claire E. McCarthy, Parker F. Duffney, Thomas H. Thatcher, Richard P. Phipps, and Patricia J. Sime

The World Health Organization estimates that over four million deaths each year can be attributed to household air pollution from the burning of biomass fuels. Animal dung is the cheapest biomass fuel and is often used in the developing world; however, it is the least energy efficient and emits toxic compounds. Exposure to biomass smoke is epidemiologically associated with an increased risk for respiratory infections. We have previously shown that human small airway epithelial cells (SAECs) exposed to dung biomass smoke have impaired anti-viral mediator production in response to a viral mimetic. It has also been reported that activation of the aryl hydrocarbon receptor (AhR) attenuates innate anti-viral immune responses, and we have found that dung biomass smoke contains AhR ligands. Therefore, we hypothesized that inhibition of the AhR would rescue impaired anti-viral responses after dung biomass smoke exposure in human SAECs. To test our hypothesis, primary human SAECs were grown on inserts and moved to the air-liquid interface for 24 hours. The cells were pretreated with an AhR antagonist for 30 min (either CH223191 (50 μM) or TMF (1-10 μM)), exposed to dung biomass smoke or air (20 min), and post-treated with the AhR antagonist for 30 min. SAECs were then challenged with polyinosine-polycytidylic acid (Poly I:C), a viral mimetic. After 24 hours, cell supernatants were collected. Interferon-inducible protein (IP)-10 levels were measured by ELISA and type I interferons were assessed using interferon-sensitive response element (ISRE) reporter cells. We found that SAECs exposed to dung biomass smoke exhibited attenuated production of IP-10 and interferons ($p < 0.05$, two-way ANOVA) when stimulated with poly(I:C). However, SAECs treated with an AhR antagonist prior to dung biomass exposure had improved anti-viral responses to poly(I:C), including increased IP-10 secretion and

interferon reporter activity (p<0.05, two-way ANOVA). We show that impaired innate immune responses to a viral-like challenge in SAECs exposed to dung biomass smoke can be partially rescued by inhibition of the AhR. This data suggests that the AhR may be a potential therapeutic target to reduce the susceptibility to respiratory infections in vulnerable populations that burn biomass fuels. Research supported by NIH HL120908, NIH T32 ES007026, NIH P30 ES001247, NIH T32 HL066988

[#7] **Michael Rudy** (6th year student)
Research Advisor: Margot Mayer-Proschel, Ph.D.

Hyperoxia activates ATM independent from mitochondrial ROS and dysfunction
Michael Rudy*,* Margot Mayer-Proschel

Iron deficiency (ID) is the most common micronutrient deficiency, affecting more than nine percent of reproductive age women in the United States and over a billion women worldwide. When an iron deficient woman becomes pregnant, her body cannot provide the iron a developing fetus requires, and low iron during critical windows of development is associated with an increased risk of psychiatric disorders, behavioral problems, and learning difficulties. Despite an awareness of this risk, the developmental mechanism linking embryonic iron availability to these adult diseases, remains largely unknown. We established a mouse model of gestational ID where dam is given a diet with adequate iron to meet her daily needs but too little to support the increased iron requirement during pregnancy. As is often seen in humans, this model produces mild anemia during the third trimester which is then treated with postnatal iron supplementation. We found that iron normal, adult mice who had been gestationally iron deficient, had a significantly impaired response to both a GABA antagonist and a glycine agonist. These mice had an increased number of inhibitory GABAergic interneurons in the cerebral cortex, with no apparent decrease in excitatory pyramidal neurons. We also examined the region which gives rise to GABAergic interneurons during embryonic development and found an expansion of both Nkx2.1 and Gli1 signaling: two factors which are important for appropriate specification of GABAergic interneurons. Taken together, our data suggests that gestational ID increases inhibitory signaling in the adult brain by expanding the region which produces inhibitory GABAergic interneurons during development, thereby shifting the relative weight of synaptic activity toward increased inhibition in the adult brain; and that this effect remains long after iron

levels have returned to normal. Supported by the Toxicology training grant: T32 ES007026 and the NYSTEM training grant.

[#8] **Parker Duffney** (5th year student)
Research Advisors: Richard P. Phipps, Ph.D. and Patricia J. Sime, M.D.

Suppression of Anti-viral Responses in Lung Epithelial Cells Exposed to Tobacco Smoke is Mediated through the Aryl Hydrocarbon Receptor

Parker Duffney, Claire McCarthy, Thomas Thatcher, Richard P. Phipps, Patricia J. Sime

Cigarette smoking is the most common intentional insult to the lungs and is a risk factor for many diseases. Those exposed to tobacco smoke have an increased risk of viral infections in the lungs. We have previously shown that smoke exposure dampens the anti-viral response of small airway epithelial cells in response to influenza or to viral mimetics. Tobacco smoke contains multiple ligands for the aryl hydrocarbon receptor (AhR), and we and others have shown that AhR signaling plays a key role in the physiological response to tobacco smoke exposure. Thus, we hypothesize that AhR activation plays a role in smoke-induced anti-viral defects. Primary human small airway epithelial cells (SAECs) were grown on culture inserts and exposed to whole tobacco smoke or the AhR ligand 6-Formylindolo(3,2-b)carbazole (FICZ) for 60 min. In some experiments, tobacco smoke particulates were removed from the smoke stream by filtration prior to entering the exposure chamber. Following exposure, cells were treated with polyinosinic-polycytidylic acid (poly I:C). Supernatants were harvested and production of the interferon stimulated protein CXCL10 (IP-10) and interferon bioactivity were determined by ELISA and a luciferase reporter. In some experiments, cells were treated with 10µM of the AhR inhibitor 6,2',4'-trimethoxyflavone (TMF) for 1 hour prior as well as throughout smoke and poly I:C treatment. For infection experiments, SAECs were infected with an influenza A virus deficient in NS1 protein (IAVΔNS1) at an MOI of 5. SAECs that were exposed to tobacco smoke or FICZ prior to treatment with poly I:C produced lower levels of IP-10 and interferon bioactivity (two-way ANOVA $p<0.05$). Filtering of tobacco smoke did not rescue the immunosuppression of the anti-viral response. Inhibition of the AhR by TMF restored the IP-10 and interferon response to poly I:C in both smoke-exposed and FICZ treated cells. TMF treatment also rescued IAVΔNS1-induced IL-10 and interferon production in smoke and FICZ treated cells. These data show that

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tobacco smoke contains AhR ligands and that AhR activation is at least partially responsible for the suppressed anti-viral response in smoke-exposed epithelial cells. Targeting of AhR represents a novel therapeutic strategy to reduce respiratory infection in those exposed to tobacco smoke.

[#9] **Jennifer L. Judge** (5th year student)
Research advisor: Patricia J. Sime, M.D.

miR338-3p Negatively Regulates Lactate Dehydrogenase and Inhibits Myofibroblast Differentiation

Jennifer L. Judge, Collynn F. Woeller, Thomas H. Thatcher, Richard P. Phipps, R. Matthew Kottmann, and Patricia J. Sime

Rationale: Idiopathic pulmonary fibrosis (IPF) is a chronic and irreversible scarring disease. We recently reported that physiologic levels of the metabolite lactate promote fibrosis via pH-dependent activation of TGF-β to drive myofibroblast differentiation. We have also shown that TGF-β upregulates expression of lactate dehydrogenase-A (LDHA), the enzyme that produces lactate. Thus we propose a pro-fibrotic feed-forward loop that drives fibrogenesis via LDHA, lactate, and TGF-β. Interruption of this feed-forward loop by inhibiting LDHA may represent a novel therapeutic approach. We recently identified that miR338-3p can negatively regulate LDHA. We hypothesize that miR338-3p is anti-fibrotic by negatively regulating LDHA.

Methods: We examined published databases of dysregulated miRNAs in patients with IPF and identified miR338-3p as a predicted target for LDHA that is downregulated in IPF patients. miR338-3p levels were measured in the bleomycin and radiation mouse models of pulmonary fibrosis. Primary human lung fibroblasts were treated with TGF-β followed by transfection with either a control or miR-338-3p mimic. LDHA mRNA levels were measured by qRT-PCR. Extracellular acidification rates were measured using a Seahorse Bioanalyzer. Myofibroblast differentiation was assessed by α-smooth muscle actin (α-SMA) expression.

Results: miR338-3p reduced levels of LDHA expression, and prevented TGF-β induced increases in LDHA, extracellular acidification, and myofibroblast differentiation. miR338-3p levels were decreased 3-fold

in human IPF patients, and decreased 2-fold in mouse models of fibrosis.

<u>Conclusions:</u> miR338-3p is novel epigenetic regulator of LDHA expression. These results suggest that miR338-3p targets the LDHA-TGF-β feed-forward loop and may represent novel therapy for pulmonary fibrosis.

[#10] **Elissa L. Wong** (5th year student)
Research Advisor: Ania K. Majewska, Ph.D.

Live imaging of microglia, dendritic spines, and their interactions in adolescent mouse cortex after brain growth spurt alcohol exposure
<u>Elissa L. Wong</u>, Ania K. Majewska

Fetal alcohol spectrum disorder (FASD), caused by gestational ethanol (EtOH) exposure, remains the leading cause of non-heritable mental disability in the western world. High binge EtOH exposure during the brain growth spurt (BGS) is known to impair synaptic plasticity long-term. Specifically, in adolescent mice previously dosed with 3.6g/kg subcutaneous EtOH daily from postnatal day 4 through 9 (P4-P9), we show that visual cortex neurons are less able to remodel in response to changes in visual experience. However, the mechanism behind this plasticity deficit is not well understood. We investigated the potential effects of BGS EtOH on adolescent microglia, the brain's resident immune cells, which also have physiological roles critical for synaptic development and plasticity. We also probed the potential effects of BGS EtOH on excitatory synapses themselves, by focusing on dendritic spines. Using in vivo two-photon microscopy in mice with microglia, or a subset of layer V pyramidal neurons, or both, labeled transgenically, we assessed the effects of BGS EtOH on microglial process dynamics, microglial response to focal tissue injury, microglial interactions with dendritic spines, and the remodeling of dendritic spines over 7 days. Surprisingly, BGS EtOH did not affect microglial process motility in either adolescent somatosensory or visual cortex. It also did not change the ability of microglia to contact dendritic spines. Tracking the same dendritic spines at P28, P30, and P35 revealed that spine stability and remodeling were similar across EtOH and control groups. However, after BGS EtOH exposure, microglia in adolescent visual cortex tended to respond faster and more robustly to an induced focal tissue injury compared to the response in control mice, though the difference in

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responsiveness failed to reach significance. Interestingly, this trend was present in visual cortex but not in somatosensory cortex. Taken together, these data lead us to conclude that the observed EtOH-induced deficit in experience-dependent synaptic plasticity occurs independently of changes in the baseline physiology of either microglia or dendritic spines, but that early EtOH may impact how adolescent microglia respond to a secondary immune challenge in a region specific manner. Future studies will address whether inducing a change in experience may be necessary to uncover any lasting effects of BGS EtOH on microglial dynamics and dendritic spine remodeling, which could underlie the plasticity deficit observed.

[#11] **Denise Herr** (4th year student)
Research Advisor: Alison Elder, Ph.D.

The Effects of Ultrafine Particulates from Air Pollution on Microglia in a Mouse Model of Alzheimer's Disease
Denise Herr, Andrea Kennell, Katrina Jew, Robert Gelein, M. Kerry O'Banion, Alison Elder

Alzheimer's disease (AD) is a common neurodegenerative disease that affects over 30 million people worldwide. While genome wide association studies have identified some relevant genes, most cases of AD may best be attributed to complex gene-environment interactions, of which air pollution may be a contributor. Because AD is known to have a strong inflammatory component and air pollution is thought to cause neuroinflammation, microglia are being investigated as the cells that link the two. Microglia serve both pro-inflammatory and pro-resolution roles in the brain. We hypothesize that ultrafine particulates (UFP, <100nm) from air pollution interact with brain microglia, causing changes in activation state and AD progression. Ambient UFP were concentrated with the Harvard Ultrafine Concentrated Ambient Particle System (HUCAPS). 3xTgAD mice, a model for AD, and non-transgenic mice of the same genetic background (13 months) were exposed to aerosol for 4 hours a day, 4 days a week, for 2 weeks. The mean particle concentration was $7.43 \times 10^4 / cm^3$ and the count median diameter was 90.9 ± 6.1 nm (geometric standard deviation 1.5). Immediately after exposure, brains were removed, cryopreserved, and sectioned at 30 μm. Immunohistochemistry was performed to identify microglia (Iba1) and amyloid-β plaques (Congo Red), a pathological feature of the disease. Microglia morphology was assessed with Sholl analysis. The findings indicate that concentrated ambient UFP exposure affected the morphology of microglia in the subiculum of the 3xTgAD mice.

Unexpectedly, microglia in the HUCAPS-exposed 3xTgAD mice were more ramified and closer in morphology to the non-transgenic microglia. This morphological change suggests that UFP may alter the balance between inflammation and resolution in 3xTgAD mice and has implications for plaque clearance and other measures of AD progression.

[#12] **Christina M. Post** (4th year student)
Research Advisor: B. Paige Lawrence, Ph.D.

The ancestral environment shapes antiviral CD8+ T cell responses across generations
Christina M. Post, Jason Myers, and B. Paige Lawrence

Recent studies have linked health fates of great-grandchildren to environmental exposures of their great grandparents. However, few studies have considered whether ancestral exposures influence the immune system across generations. Here we report novel findings regarding transgenerational transmission of altered T cell responses resulting from maternal (F0) exposure to an environmentally relevant aryl hydrocarbon receptor (AHR) ligand. The AHR is a transcriptional regulator that plays diverse roles in cellular function, including modulating immune responses. AHR ligands comprise several classes of pollutants, such as dioxins and PCBs, as well as molecules from foods and other sources. AHR-binding pollutants cross the placenta and are excreted in breast milk. In animal models and human populations, early life exposure to dioxins and PCBs is associated with persistent defects in the offspring's immune function. Using a mouse model, maternal exposure to the AHR ligand and pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) results in a significantly reduced CD8+ T cell response to influenza A virus (IAV) in the adult offspring (F1), compared to the response of infected offspring of control-treated dams. Specifically, there are significantly fewer cytotoxic T lymphocytes (CTL; CD44hiCD62Llo), virus-specific CD8+ T cells, and CD8+ T cells that produce interferon gamma (IFNγ). Transcriptomic analyses using sorted CD8+ T cells from F1 offspring of TCDD and control dams support new evidence that triggering AHR during development changes programming of senescence or exhaustion regulatory pathways. Follow up studies show increased expression of proteins associated with hindered T cell responses, such as CTLA-4 and KLRG1 on CD8+ T cells. We next asked whether the diminished CD8+ T cell response in the F1 generation was observed in the F3 generation. We detected fewer CTL and virus-specific CD8+ T cells in the TCDD F3 lineage, as

well as increased expression of CTLA-4 and KLRG1 compared to control F3 lineage following IAV infection. These data indicate that F0 maternal exposure to AHR ligands is capable of disrupting immune function not only via direct activation of the AHR in the F1 generation, but also by reprogramming immune responses in subsequent generations. This has broad implications for understanding how the environment of prior generations shapes susceptibility to pathogens and antiviral immunity in later generations.

[#13] **Catherine Burke** (3rd year student)
Research Advisor: B. Paige Lawrence, Ph.D.

Triggering the aryl hydrocarbon receptor during development durably programs CD4+ T cell responses
Catherine G. Burke, Lisbeth A. Boule, Jason R. Myers, and B. Paige Lawrence

Emerging evidence shows that early life environmental exposures lead to lasting changes in immune function, including increased incidence and severity of respiratory infections. Among the environmental factors associated with altered immune function are ligands of the aryl hydrocarbon receptor (AHR). Yet, how inappropriate triggering of AHR during development changes immune responses later in life remains poorly understood. Recently, we showed that developmental activation of the AHR using the prototypical ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) results in an impaired response to influenza A virus infection (IAV) in adult offspring. Changes include significantly fewer conventional CD4+ T cell effector subsets in infected offspring of TCDD treated dams, compared to infected offspring of control-treated dams. Adoptive transfer experiments show that this poorer response reflects developmentally induced events intrinsic to the CD4+ T cell lineage. The activation and differentiation of naïve CD4+ T cells into functionally distinct conventional subsets involves the integration of multiple signaling pathways, providing many different genes and cellular processes that could be affected by AHR activation during development. To address this, we used an unbiased transcriptomic approach to identify differentially expressed genes (DEG) in naïve and activated CD4+ T cells from infected adult offspring of dams treated with TCDD or control. Specifically, we sorted CD44lo (naïve) and CD44hi (activated) CD4+ T cells, and used next generation RNA sequencing (RNA-seq). After alignment and mapping to the genome, we used DESeq2 to identify DEGs, and Ingenuity Pathways Analysis (IPA) to identify cellular pathways changed by developmental AHR activation. Developmental

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exposure altered several signaling pathways critical for CD4+ T cell differentiation, cell cycle regulation checkpoints, and metabolism. Activated CD4+ T cells had more DEGs compared to unactivated cells. In addition, many DEGs and pathways were unique to CD44hi cells, indicating that some developmentally programmed changes in gene expression are cryptic (i.e. only revealed after CD4+ T cells are activated). These results indicate that early life AHR activation influences several pathways critical for CD4+ T cells to mount appropriate responses to immune challenges later in life. As humans are regularly exposed to AHR ligands, this has broad spanning implications for human health, and gives insight into putative mechanisms by which early life environmental exposures result in long lasting deficits in immune function. Supported by: R01 ES023260, R01 ES017250, T32 ES007026, P30 ES001247.

[#14] **Kelly Hanson** (3rd year student)
Research Advisor: Jacob N. Finkelstein, Ph.D.

Pulmonary fibrosis and fibroblast apoptosis resistance: Combined effects of Fas death receptor loss and Toll-like Receptor 4 signaling

Kelly M. Hanson, Angela M. Groves, Carl J. Johnston, Jacqueline P. Williams, Jacob N. Finkelstein

Inhalation of environmental toxicants such as cigarette smoke, metal dust, wood dust, dung particulates, silica, asbestos, and microbial agents are associated with increased risk for pulmonary fibrosis (PF). This irreversible lung scarring adversely affects the quality of life of hundreds of thousands of patients worldwide. Due to ineffective treatments, patients experience difficulty breathing, dry cough, pain, and ultimately early death. These exposures cause lung epithelial damage, but the downstream mechanisms of IPF pathology are still unknown. However, recent studies suggest that TLR4 is a key element in the onset of IPF. TLR4 levels are elevated in IPF lung biopsies and mutations in TOLLIP (TLR4 inhibitor) are associated with increased risk for IPF. Moreover, TLR4 activation causes pro-survival signaling, helping cells evade apoptosis. Fibroblast apoptosis via Fas death receptor is essential for wound resolution, but in PF, fibroblasts become apoptosis resistant and proliferate excessively. We predicted that fibroblast apoptosis resistance in PF may result from not only loss of Fas expression but also pro-survival TLR4 signaling. To model IPF, we used radiation-induced PF because it very closely mimics the progressive and irreversible nature of IPF. Lungs of C57BL/6J mice

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receiving 0 Gy or 12.5 Gy thoracic radiation were collected at 3, 8, 16, and 26 wk post-irradiation (PI). Double staining for s100a4 (fibroblast marker) and Fas revealed that control and irradiated (rt) fibroblasts show similar Fas intensity until fibrosis develops at 26 wk PI. At this time, rt samples show reduced Fas staining on fibroblasts. This loss of Fas expression makes it less likely that these cells would undergo apoptosis, a phenotype that may be exacerbated by TLR4 signaling. We used HEK-Blue hTLR4 reporter cells to determine that lung lavage fluid from rt mouse lungs activates TLR4 while control lavage fluid does not. Additionally, pre-treating primary human lung fibroblasts with low doses of TLR4 ligand LPS for 24 hr (1 ng/mL, 10 ng/mL) resulted in less apoptosis after Fas death receptor stimulation as compared to cells not pre-treated with LPS. This is possibly due to increased transcription of pro-survival proteins downstream of TLR4-induced NF-κB activation. Our results suggest that the combined effects of TLR4 pro-survival signaling and loss of Fas death receptor may trigger fibroblast apoptosis resistance after lung damage, resulting in excessive fibroblast accumulation and therefore fibrosis.

[#15] **Katrina Jew** (3rd Year Student)
Research Advisor: Alison Elder, Ph.D.

Ultrafine particulate matter air pollution effects on learning and memory in an Alzheimer's disease mouse model

Katrina Jew, Denise Herr, Andrea Kennell, Robert Gelein, Deborah A. Cory-Slechta, M. Kerry O'Banion, Alison Elder

Alzheimer's Disease (AD) is a neurodegenerative disease with a complex etiology. In the most common sporadic form, environmental factors like air pollution may contribute to pathological development of the disease. Of concern is the inhaled ultrafine (UFP, <0.1 μm in aerodynamic diameter) fraction of particulate matter air pollution. These particles can deposit in all regions of the respiratory tract, evade macrophage mediated clearance, undergo translocation to secondary target organs like the brain, and induce inflammation. We hypothesize that exposure of a triple transgenic AD mouse model (3xTgAD) to concentrated UFPs can accelerate AD progression and cognitive decline in contrast to filtered air-exposed controls. The Harvard Ultrafine Concentrated Ambient Particle System (HUCAPS) was used to generate UFP-enriched aerosols for inhalation exposure to mice. Cohorts of male mice were exposed at 2.5-3 months of age for 2 weeks

(4 hours/day, 4 days/week) to filtered air or HUCAPS (5.28×10^4/cm3, count median diameter 89 nm, and geometric standard deviation 1.5). Starting at one month after exposure, mice underwent radial arm maze (RAM) testing for spatial learning and memory, novel object recognition (NOR) testing for recognition memory, and locomotor function testing. HUCAPS diminished the rate of learning independent of genotype across 43 sessions of testing in the RAM. NOR testing showed no effect of treatment or genotype on the recognition index, but was confounded by the inability for either genotype to show preference for the novel object. Locomotor activity testing showed a significant bin x genotype x treatment interaction for ambulatory distance ($F(11,47)=2.82$ $P=0.0065$). The results suggest the 3xTgAD mice have higher locomotor activity levels compared to NTg mice, and that HUCAPS exposure is diminishing exploratory activity specifically within the 3xTgAD mice. Mice analyzed for soluble mouse amyloid-β within the hippocampus found a significant genotype effect ($p<0.0001$) with elevated amyloid-β present within 3xTgAD mice. These findings suggest that HUCAPS exposure can diminish the rate of spatial learning and these alterations do not coincide with long lived changes in amyloid-β load within the hippocampus. Funding Sources: NIH R01 ES020332, T32 ES007026, P30 ES001247

[#16] **Keith Morris-Schaffer** (3rd year student)
Research Advisor: Deborah A. Cory-Slechta, Ph.D.

Neurotoxicological Consequences of Developmental Exposure to Hyperoxia and Air Pollution: Concurrent Risk Factors of Premature Birth

Keith Morris-Schaffer, Joshua L. Allen, Marissa Sobolewski, & Deborah A. Cory-Slechta

Advances in neonatal care have drastically increased the survival of preterm infants, who are exposed to a unique set of environmental conditions during a vulnerable stage of neurodevelopment. The etiology of CNS injuries in children born prematurely is multi-factorial, but one notable common risk factor is hyperoxia; preterm infants undergo an early transition from a low oxygen, hypoxic in utero environment to a high oxygen clinical environment. An environmental risk factor that is associated with an increase in preterm birth as well as with adverse neurodevelopmental outcomes is perinatal air pollution exposure. Our prior studies indicate that mice exposed postnatally (human 3rd trimester equivalent) to concentrated (10-20x) ambient ultrafine particles (CAPS; <100nm diameter) show ventriculomegaly, a common characteristic of prematurity, as well as increased impulsivity. In order to

assess the potential cumulative effects of hyperoxia and air pollution on neurodevelopment, we used a novel moKdel in which neonatal mice are exposed to 60% oxygen for the first four days of life, followed by CAPS exposure from postnatal days 4-7 and 10-13 for 4 hr/day, 5 days/week. We have generated preliminary data demonstrating that combined hyperoxia and CAPS can produce unique learning and performance alterations on a fixed-interval schedule of reinforcement. Specifically, females and males exposed to hyperoxia followed by CAPS displayed impairment of temporal discrimination suggestive of additive effects of both CAPS and hyperoxia. Additionally we show that only females exposed to both hyperoxia and CAPS display deficits on an extinction paradigm. Further elucidation of specific behavioral and pathological underpinnings of the unique effects of the combined insults will provide important insight into the potential for enhanced adverse outcomes in premature infants exposed to air pollution. Supported by: NIH ES025541

[#17] **Sarah J. Phelan** (3rd year student)
Research Advisor: Lisa A. DeLouise, Ph.D., M.P.D.

Effect of UVR and UVR Filters in Modulating AhR Dependent
Skin Immune Responses
Sarah J. Phelan, Lisa A. DeLouise

Ultraviolet radiation (UVR) is a ubiquitous environmental insult that has immunosuppressive effects in skin that have been connected to the development of various forms of skin cancer. This has led to the increasing use of commercial broad spectrum sunscreens, often marketed to infants particularly in high latitude countries, which contain nanoparticles (NPs) and/or organic UVR filters. Studies show that some organic filters are hormone disruptors and known to penetrate skin, and we have shown that UVR barrier disrupted skin is more prone to NP penetration. Hence, understanding if and how UVR filters (NP and organics) may alter skin immune responses following UVR exposure is an important emerging research area with significant health implications. Contact hypersensitivity studies have long showed that UVR skin exposure plays an important role in developing peripheral tolerance to both self and exogenous substances that topically contact and penetrate skin. UVR skin exposure generates antigen specific T regulatory (Treg) cells and activation of the aryl hydrocarbon receptor (AhR) is essential for this process. We hypothesized that dysfunction or alteration of AhR signaling pathway by environmental insults (e.g. UVR filters) could skew UVR induced skin immune responses toward

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generating proinflammatory effector T cells rather than Tregs, thereby causing allergic skin disorders or contributing to development of autoimmune disease. In fact, there are pathologies such as polymorphic light eruption (PLE) syndrome and cutaneous lupus erythematosus (CLE), where UVR skin exposure promotes proinflammatory responses. In this study we investigated the ability of several common UVR filters to affect AhR activity. We discovered that octinoxate potentiates the AhR response to 6-formylindolo[3,2-b]carbazole (FICZ), a tryptophan-derived AhR agonist produced in skin following UVR exposure. Further, we plan on testing the effect of oxtinoxate on the ability to alter development of the T cell compartment following skin UVR exposure.

[#18] **Candace Wong** (3rd year student)
Research Advisor: Alison Elder, Ph.D.

Aerosolized Gold Nanoparticle Suspensions and Spark-Generated Gold Nanoparticles Do Not Readily Enter the CNS Candace Wong, Bob Gelein, Andrea Kennell, Günter Oberdörster, Alison Elder

Studying the deposition of inhaled particles and their fate, especially in the central nervous system (CNS), is important to better understand the mechanisms by which engineered nanomaterials and ambient ultrafine particles (<100nm) found in air pollution affect health. It has been shown that some nanosized particles can enter the CNS through olfactory transport or the vasculature following Intratracheal instillation or inhalation of the particles. Fewer studies have examined biodistribution of particles from whole body inhalation exposures, particularly entry of these particles into the brain. Here, we compare the differences between the retained dose of spark-generated gold nanoparticles (AuNP) and colloidal AuNP in the brain and lungs. C57BL6/J mice were exposed via whole body inhalation to spark-generated AuNP (count median diameter (CMD), 20 nm; geometric standard deviation (GSD), 1.5; mass concentration, 0.97 mg/m3) for 6 hours a day for 8 days. In a separate cohort of mice colloidal AuNP (20nm) suspensions were aerosolized using a jet nebulizer to expose mice via whole body inhalation for 6 hours a day for 4 days (CMD, 157 nm; GSD, 1.4; mass concentration, 0.52 mg/m3). Brain tissues (olfactory bulb, hippocampus, striatum, frontal cortex, cerebellum) and whole lung were harvested 24 hours after the last exposure and the Au content analyzed by inductively-coupled mass spectrometry. The deposited dose in the lungs were 15.96 ± 5.7 μg for spark-generated AuNP exposed and 0.2 ± 0.02 μg for colloidal AuNP exposed. The retained dose in the various brain

regions analyzed for spark-generated AuNP exposed had very few tissue samples that had measurable amounts of Au while colloidal AuNP exposed brain tissues were variable (0.031 – 0.113 ng). From this study, we show that it is difficult to detect AuNP, spark-generated or aerosolized colloidal suspension, in the brain. For the gold we did detect in the brain, these particles most likely entered the CNS by vasculature accumulation instead of direct transport via the olfactory neurons. It is possible that direct transport via the olfactory route is material dependent, explaining why others have shown measurable amounts of other nano-materials in the brain.

[#19] **Jasmine Reed** (2nd year student)
Research Advisor: Martha Susiarjo, Ph.D.

Reproductive and Developmental Toxicity from Maternal Exposure to Estrogenic Endocrine Disrupting Chemicals
Jasmine M. Reed, Philip Spinelli, Martha Susiarjo

Humans are widely exposed to synthetic estrogen compounds, including bisphenol-A (BPA) and tetrabromobisphenol A (TBBPA). For BPA, sources of exposure include oral ingestion of food and drink packaged in BPA-containing products, although lower levels of exposure can come from air and dust. Like BPA, oral TBBPA exposure can result from its use as a plastic additive, however exposure can also occur from dermal absorption or inhalation of the aerosolized chemical used as a flame retardant in products such as electrical equipment and furniture. Increasing evidence has linked BPA exposure to recurrent miscarriages, spontaneous abortion, and pregnancy complications in humans as well as development of risk factors for diabetes and obesity in offspring exposed in utero, however, the precise mechanisms are not well elucidated. In contrast, the reproductive and developmental consequences of TBBPA are less understood, and work in the research area is ongoing. Recently, our laboratory found that gestational BPA exposure in mice resulted in elevated levels of fetal and maternal tryptophan. Interestingly, human and mouse studies have shown that tryptophan catabolism plays an important role in pregnancy health, and its perturbation, specifically by altering the levels of tryptophan and its catabolite (kynurenine), is associated with pregnancy losses and complications. Our preliminary findings therefore suggest that one potential mechanism underlying BPA-induced reproductive failure involves perturbation of tryptophan catabolism. In this study, we hypothesize that exposure to synthetic estrogens reduces gene

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expression of enzymes responsible for tryptophan catabolism resulting in increased accumulation of tryptophan. To test this hypothesis, we collected embryonic, placental, and maternal tissues from dams exposed to dietary BPA and TBBPA between embryonic day (E) 7.5 and 15.5 and measured gene activities of tryptophan-degrading enzymes including the Ido1 and Ido2 genes using real time quantitative PCR. Our preliminary data suggest that synthetic estrogen exposures decrease Ido1 and Ido2 gene activities and that reduced tryptophan degradation is a potential mechanism for elevated tryptophan levels in exposed mice. For future works, we will characterize how these genes are regulated during development and how environmental exposures affect their regulation. More importantly, we are interested in studying whether exposures to these chemicals induce reproductive and developmental abnormalities and the role of tryptophan catabolism in these phenotypes. Supported by EHSC Pilot Project 2015-2016 and NIEHS R00 ES022244

[#20] **Timothy R. Smyth** (2nd year)
Research Advisor: Steve Georas, M.D.

Diesel Exhaust Particles Weaken the Lung Epithelial Barrier through a Reduction of Tricellulin
Timothy R. Smyth, Timothy Chapman, Janelle Veazey, Zackery Knowlden, Sara Hillman, Steve Georas

Asthma is a chronic, life threatening disease which affects over 24 million Americans at a cost of over $56 billion per year. The prevalence of asthma has increased dramatically in the United States over the past 30 years, rising from approximately three percent of the population in 1980 to 9 percent in 2014. Reasons for the increase in asthma prevalence are complex, and include exposures to environmental pollutants such as diesel exhaust particles. Exciting new data has shown that the epithelial barrier in asthmatic patients is weakened through various mechanisms including downregulation of tight junction and adhesion junction proteins such as Occludin, ZO-1, and E-cadherin. Tricellulin is a recently discovered tight junction protein that localizes specifically to tricellular junctions in epithelial tissues. Tricellulin and has been suggested to regulate not only permeability to macromolecules, but also the movement of dendrites from intra-epithelial dendritic cells. Very little is known about the expression or function of Tricellulin in the airway. Using the human bronchial epithelial cell line 16HBEo- in vitro, I found that exposure to diesel exhaust particles (DEP) causes a reduction of Tricellulin with a corresponding increase in permeability to macromolecules. We hypothesize that

exposure to DEP elevates the risk of asthma development and exacerbation by disrupting epithelial barrier function. In future works, we will measure the impact of DEP on epithelial barrier function and Tricellulin expression in vivo after DEP inhalation by mice. We will also investigate the impact of Tricellulin on crosstalk between the epithelial barrier and innate immune cells such as dendritic cells and macrophages using Tricellulin knockout mice.

[#21] **Kadijah Abston** (1st year student)

Research Advisors: Martha Susiarjo, Ph.D., and Deborah Cory-Slechta, Ph.D.

Transgenerational Epigenetic Inheritance of Behavioral Alterations in Mice Exposed to Lead and Stress

Kadijah Abston, Martha Susiarjo; Marissa Sobolewski (Terry), Deborah Cory-Slechta

Environmental lead (Pb) exposure during development is a well-established neurotoxicant known to alter cognition in children and increase lifelong risk of behavioral deficits. Increasing studies of interactions between Pb and prenatal stress (PS), however, suggest that studying Pb in isolation may lead to underestimation of its toxicity. To build a more translatable exposure model we must consider that humans are concurrently exposed to multiple risk factors that can either attenuate or enhance the effects of other environmental agents linked to adverse neurobehavioral outcomes. Pb and prenatal stress are co-occurring risk factors for adverse neurobehavioral outcomes and cognitive impairment, particularly for low socioeconomic status communities. Pb and PS both impact the HPA Axis, glucocorticoid release, and specific tissues associated with brain mesocorticolimbic dopamine/glutamate circuits, including prefrontal cortex, nucleus accumbens, and hippocampus, in directly-exposed offspring (F1). I will investigate the role of developmental Pb and PS exposure on DNA methylation of target genes that are associated with stress and cognitive function, including glucocorticoid receptor (Nr3c1), Bdnf, Mecp2, Crfr2, Esr1, Igf2/H19, Peg3, and mineralocorticoid receptor. Recent studies in the Cory-Slechta lab have identified developmental and behavioral alterations in the F3 generation, purportedly offspring not directly exposed to Pb or PS. I therefore hypothesize that Pb and PS will alter DNA methylation patterns in target genes associated with stress and cognition in the hippocampus and frontal cortex, which may contribute to increased risk of cognitive impairments and behavioral alterations in multiple generations. I will investigate DNA methylation alterations at the

genes of interest described above in the F1 mice, with the ultimate goal to follow these alterations out to the F3 generation. DNA methylation changes in the F3 generation would provide evidence of a mechanism for the observed behavioral alterations.

[#22] **Ashley Fields** (1st year student)
Research Advisor: Martha Susiarjo, Ph.D.

Effects of vitamin B6 deficiency of epigenetic and genetic regulation in mice
Ashley Fields and Martha Susiarjo

Increasing evidence suggest that exposure to endocrine disrupting chemicals (EDCs) can cause aberrant DNA methylation changes to the genome. A recent study in our laboratory showed that gestational bisphenol A (BPA) exposure is linked to glucose intolerance in pregnant mice and to adult onset obesity in the offspring. Our metabolomics study revealed that BPA-exposed dams and pups had abnormal vitamin B6-dependent pathways. We demonstrated, for instance, that vitamin B6 supplementation can rescue the gestational diabetes phenotype in BPA-exposed mice. During my rotation, I hypothesized that a vitamin B6 deficient mouse model can recapitulate some of the molecular phenotypes of BPA-exposed mice, including gene-specific DNA methylation and expression changes. To test the hypothesis, I assessed the DNA methylation and gene expression levels of the Pon1, Arid4b, and Fabp5 genes in embryonic day (E) 9.5 embryos exposed in utero to a vitamin B6 deficient diet. These genes were selected because they showed changes in DNA methylation and/or gene expression in BPA-exposed embryos, and are genes related to metabolism. My results suggest that vitamin B6 deficiency alters gene expression of Fabp5 and Pon1 but not Arid4b, implying that vitamin B6 deficiency can directly alter some, but not all metabolism-related genes affected by BPA exposure. Ongoing studies are aimed to further investigate global DNA methylation changes and monitor gestational glucose intolerance in these vitamin B6 deficient mice. Supported by P30 ES001247 and R00 ES022244

[#23] **Ashley R. Rackow** (1st year student)
Research Advisor: Patricia J. Sime, M.D.

Metabolism and Pulmonary Fibrosis: A Potential Role for TG2

Ashley R. Rackow, Jennifer L. Judge, Thomas H. Thatcher, Richard P. Phipps, Patricia J. Sime

Idiopathic Pulmonary Fibrosis (IPF) is a chronic, interstitial lung disease characterized by increased deposition of extracellular matrix proteins in the lung. Approximately 50,000 individuals are diagnosed with IPF each year and, due to limited treatment options, half of IPF patients die within three years of diagnosis. One potential mechanism underlying the pathology of IPF is the dysregulation of metabolism. Our lab has shown that IPF lung tissue has elevated levels of lactate, a byproduct of glycolytic metabolism, and increased expression of lactate dehydrogenase (LDH), the enzyme that produces lactate. Lactate decreases the pH in the lung and contributes to the activation of TGFβ, a profibrotic cytokine and a master regulator of fibrosis. This promotes the differentiation of fibroblasts to myofibroblasts, increases deposition of extracellular matrix in the interstitium, and generates a fibrotic phenotype. A second mechanism of IPF pathogenesis is enhanced expression of the protein tissue transglutaminase (TG2). TG2 has several functions, including crosslinking of ECM proteins and signaling via NFκB, which are known to promote fibrosis in multiple organs. There is new evidence that TG2 upregulates HIF1α, a master regulator of metabolism that increases glycolytic enzymes such as LDH. We hypothesize that TG2 represents a novel link between the ECM crosslinking and metabolic pathways in fibrosis, by promoting a shift to aerobic glycolysis that results in increased lactate production and activation of TGF □ contributing to fibrosis. To test this hypothesis, TG2 was overexpressed or knocked down in human lung fibroblasts. Myofibroblast differentiation was stimulated via treatment with TGFβ. Cell lysates were examined for protein expression of metabolic enzymes and myofibroblast differentiation via Western blot. We demonstrate that overexpression of TG2 increases expression of LDH and αSMA, a marker of myofibroblasts. This suggests TG2 regulates the glycolytic shift observed in IPF. These results may help us understand the mechanism of action of TG2 in fibrosis, which could reveal novel information relevant to drug targets and development of new therapeutics for IPF.

[#24] **Keegan L. Vaughan** (1st year student)
Research Advisor: Paige B. Lawrence, Ph.D.

Exploring regulatory crosstalk between AHR and HIF-1α in hematopoiesis

Keegan L. Vaughan, Kameshwar Singh, Paige B. Lawrence

Hematopoiesis is the well-regulated generation of the cellular components of blood. This process occurs through the differentiation and proliferation of hematopoietic stem cells (HSCs), which give rise to pluripotent and lineage committed progenitors. In adults, hematopoiesis occurs in the relatively hypoxic environment of the bone marrow, where HSCs and progenitors further differentiate into cells of the lymphoid, myeloid or erythroid lineages. There is increasing evidence highlighting the importance of the aryl hydrocarbon receptor (AHR) and hypoxia-inducible factor-1α (HIF-1α) in the regulation of hematopoiesis. AHR and HIF-1α are important environmental sensing transcriptional regulators. HIF-1α is considered the master transcriptional regulator of cellular response to oxygen levels. The AHR mediates responses to numerous environmental factors, including exogenous and endogenous chemicals. AHR and HIF-1α have a common binding partner: ARNT/HIF-1β. Dimerization with HIF-1β is required for both AHR and HIF-1α, which suggests the possibility of regulatory crosstalk between these two signaling pathways. Likewise, AHR and HIF-1α signaling are critical in HSCs, regulating a finely tuned balance between quiescence and proliferation; however, whether and how HIF-1α and AHR interact in regulation of HSCs is unknown. Prior work indicated that pharmacological stabilization of HIF-1α with dimethyloxalyl glycine (DMOG) increased HSC quiescence. In this study, we used in vivo pharmacological stabilization of HIF-1α to further elucidate how HIF-1α and AHR regulatory crosstalk influences hematopoiesis, which we hypothesize alters hematopoietic lineage commitment. To begin to test this, C57Bl/6 mice were treated with DMOG to further characterize the effects in vivo stabilization of HIF-1α on hematopoiesis. In the treated mice, there was a significant increase in circulating red blood cells, but no significant change in the white blood cell population in the peripheral blood. In the bone marrow, DMOG treatment increased myeloid and decreased lymphoid potential, indicating a change in colony forming ability of bone marrow progenitor cells when HIF-1a is stabilized. Similar changes in lymphoid and myeloid progenitors are also observed following AHR activation. These findings provide further evidence of AHR and HIF-1α crosstalk in the regulation of lineage differentiation during hematopoiesis.