cow stable dust affects the integrity and inflammation of human airway tissue *in vitro.* In this study farm dust samples were collected from four different dairy farms (Northern Savonia region, Finland) and reference dust was collected from a city apartment (Kuopio, Finland). Lung tissue constructs differentiated from normal human bronchial epithelial cells were exposed to dust samples (150 µg/ml) in air-liquid interface for 24 hours at 37°C in 5% CO2. After stimulation the transepithelial resistance of the tissue as well as secreted proteins in the apical wash and a panel of pro-inflammatory cytokines, growth factors and chemokines in the cell culture medium were analysed. Of the studied cytokines, farm dust stimulation was associated mainly with increased production of IL-13, IL-15, IP-10 and IFN-γ. Only one of the farm dust samples induced a significant change in transepithelial resistance, whereas dust from two of the farms induced the secretion of proteins into the apical fluid. Overall, the exposure to farm dust affected cytokine responses of the respiratory tissue. The effect on protein secretion and tight junction dynamics was less pronounced, indicating that the effects of farm dust on airways could be mainly mediated through soluble factors.

### 3035 IL-18 Modulates Excitation Contraction Coupling in Human Airway Smooth Muscle Cells

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Obesity is causally associated with severe asthma that appears refractory to conventional treatments. Human airway smooth muscle (HASM) cells isolated from obese compared with lean donors showed amplified agonist-induced excitation contraction (EC) coupling and force generation. Serum IL-18 levels are elevated in obese individuals, and potentially plays a negative homeostatic role against obesity. It is unclear how elevated IL-18 modulate ASM cell function related to airway hyperresponsiveness (AHR). We tested a central hypothesis that IL-18 modulates EC coupling in HASM cells to amplify AHR. HASM cells were treated with IL-18 (10, or 100 ng/mL) or IL13-IL4 combination (50 ng/ml each) overnight, followed by 10 min treatment with contractile agonist carbachol (25 uM) or histamine (1 or 2.5 uM). Lysates were collected and immune-blotted for myosin light chain (MLC), myosin phosphatase target subunit 1 (MYPT1), and protein kinase B (Akt) phosphorylation. In parallel, cells were loaded with $Ca^{2+}$-binding dye fluo 8 and agonist-induced cytosolic $Ca^{2+}$ was measured by fluorescent microscopy. HASM cells exposed to IL-18 (overnight) showed increased MLC phosphorylation (fold change compared to vehicle: 100 ng/mL: 2.62±1.53; p=0.027; n=6 donors). However, overnight exposure to IL-18 had little effect on MYPT1 phosphorylation (100ng/mL: 0.052±0.009; baseline: 0.057±0.008; p=0.403; n=4 donors). Similarly, IL-18 (100 ng/mL, overnight treatment) increased Akt phosphorylation, a downstream effector for PI3K, (100 ng/mL: 0.365±0.206, baseline: 0.163±0.073, p=0.047; n=6 donors). Overnight treatment with IL-18 (10 or 100 ng/mL) also potentially increased histamine-induced cytosolic $Ca^{2+}$ (AUC baseline: 97.88±15.45; 10ng/mL: 118.75±11.17 100ng/mL: 113.35±4.67; 40 cells representing 2 donors). IL-18 itself, when used as an agonist, had little effect on cytosolic $Ca^{2+}$ in HASMC. Our findings suggest that IL18, although a homeostatic mediator increased in response to metabolic syndrome, amplifies EC coupling and potentially airway hyperreactivity and remodeling in obesity. IL18 signaling may provide a therapeutic target for obesity-associated airway dysfunction.

### 3036 Assessment of Respirable and Inhalable Indoor Microplastic Pollution and the Effects of Exposure on the Human Bronchial Epithelial Barrier

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Humans spend 70-90% of their time indoors, however, there is a significant lack of knowledge regarding human exposure to microplastic particles and fibers within the indoor environment. Studies have shown that nylon fibers inhibited the growth of human lung organoids, suggesting that exposure to these microplastic fibers could harm human lung development. Studies have also identified microplastic particles greater than 50 um in indoor dust, but these are unlikely to be respirable and little information is available regarding smaller airborne particles. Therefore, we are currently developing methods to identify respirable (<4 um aerodynamic diameter) and inhalable (4 - 10 um aerodynamic diameter) microplastic particles and fibers in indoor dust and air. We have also established methods to distinguish plastic from cellulosic, proteinaceous, and inorganic materials found in indoor air and dust samples using two different stains, Nile Red for plastics and Trypan Blue for cellulosic materials. Proteinaceous and inorganic materials remain unstained. Dust, from surfaces, and air collected using cascade impactors and, for the first time, via silicon nitride nanomembrane technology, were collected from various indoor environments. Microplastic particles and fibers were identified in all dust and air samples. Methods are currently underway to isolate and characterize microplastic particles and fibers in indoor dust and air, as well as determining human exposure. Reference microplastic particles from pure polymers were generated via cryomilling and microplastic fibers were generated as described by Cole 2016 and will be used to investigate the toxicological effects of respirable microplastics on the human bronchial epithelial barrier utilizing a lung-on-a-chip model.

### 3037 Lung miRNA Profiles Show a Time-of-Day Response in House Dust Mite (HDM)-Induced Allergic Asthma in Mice

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Allergic asthma is a chronic inflammatory disease that displays a time-of-day-dependent variation in clinical symptoms and severity. Recently, there is a lot of attention on the role of small non-coding RNAs such as microRNAs (miRNAs) as novel biomarkers in the pathobiology of allergic asthma due to their ability to modulate post-transcriptional regulation of mRNAs involved in cellular signaling and biochemical processes. However, whether the time-of-day response to house dust mite (HDM) exposure differentially affects miRNA-mRNA expression in the lung is not known. We performed miRNA and mRNA profiling and validation by NanoString that shows a time-of-day effect on the differential response to HDM exposure in mice. Here, we demonstrate the time-of-day difference in differential expression (DE) of miRNA-mRNA targets in an acute HDM-induced allergic asthma model *in vivo*. We found a significant time-dependent change in the DE miRNAs in HDM vs. PBS-exposed mouse lungs at Zeitgeber time 0 (ZT0: 6 am) and ZT12 (6 pm). DE miRNAs at ZT12 based Ingenuity pathway analysis predicted genes involved in cytokine/chemokine signaling, growth factor signaling and Th2 activation that was further validated by mouse myeloid innate immunity panel/qPCR analyses. To the best of our knowledge, this is the first study that shows a time-of-day effect on both miRNAs and their associated mRNA target genes in HDM-induced allergic asthma *in vivo*. Differentially expressed miRNA/mRNA predicted using Ingenuity Pathway Analysis and miR-Path analysis were linked to KEGG pathways directly associated with asthma and inflammation. Our findings have greater significance in the light of the time-of-day difference observed in asthma as it relates to differentially expressed miRNAs and suggests that there might be a possible role of core circadian clock genes that contributes to this phenotype. Future research will address how clock genes may affect the differential expression of both miRNA-mRNA targets that can be used to devise targeted therapies for severe asthma endotypes/phenotypes. *Supported by the NIH R01 HL142543 (IKS) and the University of Kansas Medical Center, School of Medicine, Internal Medicine Start-up Funds (IKS).*

### 3038 Differential Activation of Transient Receptor Potential Melastatin-8 (TRPM8) Variants and Associations with Asthma

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Coal Fly Ash (CFA) is a by-product of burning coal. It is found in building materials and is present in particulate air pollution which can cause adverse effects on human health. Transient receptor potential melastatin-8 (TRPM8) is activated by cold temperature, the chemical agonists menthol and icilin, and CFA. Activation of TRPM8 by CFA stimulates pro-inflammatory cytokine production by human bronchial epithelial cells, and these cytokines are relevant to the innate immune response and the acute exacerbation of asthma. Polymorphisms in TRP channels have been implicated in the development of childhood asthma. We hypothesized that single nucleotide polymorphisms (SNPs) in TRPM8 may affect the activation of TRPM8 by agonists and pollutants such as CFA, thereby affecting asthma symptom control. Activation of TRPM8 by canonical agonists and particulate materials was determined using a fluorometric calcium flux assay and HEK-293 cells either transiently transfected with or stably over-expressing the TRPM8 SNPs R247T (rs13004520), Y251C (rs17868387), S419N (rs7593557) or M462T (rs28902173). With the exception of Y251C, CFA-induced TRPM8 activation was decreased for all SNPs compared to the reference channel. The basis for these changes was attributed to diminished transcription and/or translation of the TRPM8 variants since lower levels of TRPM8 were detected by western blot for the variants exhibiting lower responses to agonists. Finally, assessment of the impact of the TRPM8 SNPs on asthma symptom control in children demonstrated that the presence of ≥1 allelic copy of the S419N variant associated with poorer asthma symptom control (mean ACS=4.8±3.2 vs. 3.96±3.2; n=227 and 893, respectively; p=0.008). Additionally, the I1016I (rs11563208) SNP correlated with slightly improved asthma control (mean ACS=3.8±3.3 vs. 4.5±3.5; n=565 and 625, respectively; p=0.017). Preliminarily, a decrease in TRPM8 expression/function may have a deleterious effect on asthma symptom control. *Support: ES017431, ES027015, GM121648.*

### 3039 Effect of Welding Fume Exposure on Silica-Induced Pulmonary Toxicity in Rats

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Mixed exposures to silica and welding fume are common in certain occupations. It is currently undetermined what health burden workers exposed to both silica and welding fume experience. The objective of this study was to determine the effect of welding fume on the lung response to silica exposure. Male Fischer 344 rats were exposed to air or silica (15 mg/m$^3$, 6 hrs/day, 5 days). At 5- and 11-months post-silica exposure, rats received welding fume (20 mg/m$^3$, 4 hrs/day, 4 days/week for

4 weeks) by inhalation. At 1 day post welding fume exposure bronchoalveolar lavage was conducted to assess pulmonary toxicity based on lactate dehydrogenase activity, oxidant production, cell counts, and cytokines. Lung gene expression changes were also assessed. The results showed that both silica and welding fume exposures resulted in lung toxicity, at both post-exposure time points. Both additive and synergistic lung responses were detected, based on the parameters analyzed. For example, silica or welding fume exposure, alone, resulted in neutrophil infiltration of 250 and 625 times, respectively, compared to the air-exposed controls at 12 months post-exposure. The combined exposure to silica and welding fume, however, caused neutrophil infiltration that was 1392 times higher compared to air controls. Silica or welding fume exposure alone resulted in significant increases in measured cytokine levels, compared to controls. However, the combined exposure of silica and welding fume caused a greater elevation of measured cytokine levels, at both post-exposure timepoints. For example, measured levels of MIP-2 were 37-fold and 35-fold higher in the silica alone and welding fume alone animals, respectively. However, the silica and welding fume combined exposure facilitated an additive effect, where the measured MIP-2 level was 45-fold higher. Furthermore, global gene expression changes in the lungs were detected in all exposure groups, at both 6- and 12-months post-exposure. For example, silica exposure alone resulted in 125 and 750 significantly differentially expressed genes (SDEGs), while the welding fume alone group had 1068 and 2255 SDEGs, and the silica-welding fume combined exposure had 1808 and 1910 SDEGs, at the 6- and 12-month time intervals, respectively. Together, these results suggest an additive/synergistic effect of the combined silica and welding fume exposure on the assessed lung toxicity parameters.

### 3040 Low-Dose Cadmium-Induced Alterations in miRNA/mRNA Interaction Regulate Chloride Efflux Channel in Airway Epithelial Cells

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Environmental heavy metal exposure leading to acute and chronic lung injury is a major health concern. Cadmium exposure through cigarette smoke (CS), diesel exhaust and other sources effects lung health by inducing mitochondrial and oxidative stress and cellular DNA damage. CS has an approximately 0.5-3ug of cadmium per cigarette and it is associated with increased number of mucus producing cells, impaired mucus clearance and increased mucosal permeability to allergens. The secretory epithelial cells are regulated by chloride channels like CFTR, ANO1 and SLC26A9. Increased expression of ANO1 in chronic airway conditions has been closely related to mucus hypersecretion and goblet cell metaplasia. Studies on cadmium responsive miRNAs suggest regulation of target genes with emphasis on critical role of miRNA-mRNA interaction for cellular homeostasis. miRNAs may be an effective target for regulating ANO1 expression in airways. In this study we evaluated the role of miRNA in cadmium induced expression of ANO1 in airway epithelial cells. Air liquid interface cultures of human bronchial epithelial cells were exposed to low dose cadmium and analyzed for ANO1 expression and interacting miRNA. QRT-PCR demonstrated downregulation of miR-381 which was negatively regulating ANO1 in these cells. ANO1 expression was confirmed through western blot analysis, florescence microscopy and qRT-PCR. Our results lead to the understanding that although cadmium is known to modulate ion uptake by acting as a calcium antagonist, it can induce ion imbalance by the epigenetic regulation of microRNAs acting as negative or positive regulators of gene expression at mRNA level. This opens up a paradigm of therapeutic targets for cadmium induced lung injury.

### 3041 Acute and Delayed Effects of H2S Poisoning on the Brainstem and Lungs

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Hydrogen sulfide (H2S) is a systemic toxicant targeting the cardiovascular, respiratory, and central nervous systems. Acute exposure to high H2S concentration causes acute death by inhibiting the breathing center in the brainstem. Pulmonary edema is also reported. We have previously shown that acute H2S exposure causes sequalae in the thalamus. However, there is a knowledge gap on the long-term consequences of acute H2S exposure in the brainstem and the respiratory tract. We hypothesized that acute H2S exposure causes delayed pathology in the brainstem where the breathing center is located as well as in the lungs. To test this hypothesis, male C57BL/6J mice were exposed to 1000 ppm H2S for 50 minutes. Surviving mice were euthanized at 5 min, 12hr, 24hr, 72hr, 7d, 14d, 21d, and 28d to track neurodegeneration in the brainstem and lung injury. Assessed endpoints included neurotransmitters, enzymatic activity, histology, immunohistochemistry, cytokine concentrations, and immunoblotting. In the brainstem a single H2S exposure caused an immediate increase in catecholamines and monoamine oxidase A activity. Glutamate concentrations significantly decreased starting at 72hr. Neurodegeneration in the pontine and medullary reticular formation was observed starting at 72hr post-exposure. In the lungs pulmonary edema was evident immediately following exposure and seems reversible within 24hr. Histology of the lung showed edema, arterial thrombus, eosinophilic infiltration, and immunoblotting showed increase in fibrin starting at 24hr post-exposure. Effects of H2S on the brainstem have long-term implications because of its role in regulating breathing, heart rate, sleeping and eating.

### 3042 Macrophage-Specific ARNT Signaling Mediates Acrolein-Induced Pulmonary Toxicity

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Acrolein is a reactive aldehyde and potent respiratory toxicant formed during combustion. Tobacco cigarette smoke and electronic cigarette aerosols pose the highest risks for human exposure to acrolein. However, the immunomodulatory effects of acrolein have yet to be fully elucidated. Given that pulmonary macrophages are important for initiating and resolving lung inflammation, we hypothesized that macrophages are likely involved in acrolein-associated lung pathologies. To test our hypothesis, we utilized a macrophage-specific transgenic mouse model that overexpresses the aryl hydrocarbon receptor nuclear translocator (*Arnt*) isoform-a (murine homolog of human ARNT isoform 1). ARNT is a crucial regulator of toxicant metabolism and immune homeostasis due to its interaction with the aryl hydrocarbon receptor and NF-kB transcription factors. Furthermore, ARNT is expressed as two isoforms (1 and 3) which exert opposing effects on immune regulation; isoform 1 supports pro-inflammatory activity and isoform 3 abrogates inflammation. We utilized young (12 weeks) and middle-aged (32 weeks) Arnt-a transgenic mice to determine the airway cell differential response to acrolein, and the influence of age on this response. As compared to non-carrier littermate controls, 12-week-old Arnt-a mice exposed to 2 mg/kg intratracheal acrolein exhibited an unchanged BAL differential cell count of >99% macrophages and negligible body weight change, whereas BAL from 32-week-old Arnt-a mice showed ~50% neutrophils, and an average decrease of 18% in body weight, over a 5-day post-exposure period. Our results suggest: 1) macrophage-specific Arnt-a overexpression can be associated with neutrophil recruitment in response to acrolein, and 2) increased age may be a factor in the susceptibility to acrolein toxicity, as indicated by increased neutrophil infiltration within the lung, and body weight loss. We conclude that the role of ARNT within macrophages is important during the initiation of acrolein toxicity, and may be crucial in the development of pharmacological interventions for prevention or reversal of toxicity. *Funding: T32ES007254, R01ES025809, the Gulf Coast Center for Precision Environmental Health (P30ES030285), and the Brown Foundation.*

### 3043 Effects of 1,3-butadiene Exposure on the Lung Metabolome in Collaborative Cross Mice

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Exposure to 1,3-Butadiene (BD) is associated with multiple negative health effects and has been shown to be carcinogenic in animals and humans. BD is metabolized within cells to form reactive epoxide intermediates, which are genotoxic. However, the effects of BD on general cellular metabolism are not known. We examined the effects of exposure to BD on the mouse lung metabolome in the genetically heterogeneous Collaborative Cross outbred mouse model. Mice were exposed to 3 concentrations of BD for 10 days (2, 20, and 200 ppm), and lung tissues were analyzed using high-resolution mass spectrometry-based metabolomics. As compared to controls (0 ppm BD), BD had extensive effects on lung metabolism at all concentrations of exposure, including the lowest concentration of 2ppm, as reflected by reprogramming of multiple metabolic pathways. Metabolites participating in energy metabolism, including glycolysis and the tricarboxylic acid cycle, were elevated, with 8 out of 10 metabolites demonstrating greater than a 2-fold increase. Furthermore, metabolites participating in lipid metabolism, glycosylation, steroid hormone metabolism, and signaling were altered. The observed metabolic alterations seen with BD exposure are similar to those found in lung cancer cells, and these altered metabolites may act to prevent or initiate adverse health effects and tumorigenesis.

### 3044 Face Mask Debris: Possible Risk to Face Mask Users?

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The COVID-19 pandemic along with wildfires have sparked an urgent need for improved guidelines regarding face mask use. The United States Centers for Disease Prevention and Control recommends reuse of filtering facepiece respirators or other alternatives such as surgical masks or home-made cloth masks to conserve supplies for essential workers. Although previous studies have demonstrated the protective nature of wearing masks to limit the dispersal of

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