NK cells. These data are consistent with research demonstrating IFN-γ reduction and changes in TNF-α in both microplastic and toluene exposure studies (Fujimaki et. al., 2007, Bishop et. al., 2024). In combination with a growing body of research, this data suggests that workers who are routinely exposed to high levels of 3D printing emissions could potentially be at increased risk of respiratory infections or disease. References: Bishop CR, Yan K, Nguyen W, Rawle DJ, Tang B, Larcher T, Suhrbier A. Microplastics dysregulate innate immunity in the SARS-CoV-2 infected lung. Front Immunol. 2024 May 13;15:1382655. doi: 10.3389/fimmu.2024.1382655. PMID: 38803494; PMCID: PMC11128561. Fujimaki, H., Yamamoto, S., Tin Tin Win, S., Hojo, R., Sato, F., Kunugita, N., & Arashidani, K. (2007). Effect of long-term exposure to low-level toluene on airway inflammatory response in mice. *Toxicology Letters*, *168*(2), 132-139. https://doi.org/https://doi.org/10.1016/j.toxlet.2006.11.008

3474 TLR2 and TLR3 Exhibit Distinct Roles in the Inflammatory Response of Alveolar Macrophages to Organic Dust Exposure

E. O. Oyewole, T. Cunningham, L. S. Dean, *A. N. Threatt*, *M. Barahona*, K. Jones, and T. M. Nordgren. Colorado State University, Fort Collins, CO.

Background and Purpose: Occupational dust exposure is a significant health concern, ranking among the leading causes of common disease progressions like Chronic Obstructive Pulmonary Disease (COPD) and asthma. Agricultural workers are frequently exposed to organic dust from sources such as animal feed, droppings, and contaminated soil, which can contain endotoxins and other particles, leading to an increased risk of developing these lung diseases when inhaled. This risk extends not only to the workers but also to individuals living in the surrounding communities, including children and other adults. Toll-like receptors (TLRs) belong to a large family of pattern recognition receptors and are regarded as the ancient 'gatekeepers' of the immune system. Organic dust extracts (ODE) have been found to contain numerous TLR ligands. The role of TLRs in mediating inflammatory responses in the lung, particularly in alveolar macrophages, is crucial for understanding and managing lung inflammation caused by organic dust exposure. Our laboratory has discovered that alveolar macrophages are also producers of IL-22, challenging the traditional paradigm that this unique anti-inflammatory cytokine is exclusively produced by lymphocytes. However, the mechanisms underlying IL-22 induction in alveolar macrophages remain unknown, which forms the basis of our study to identify the role of TLR activation in alveolar macrophage activation and IL-22 production. **Methods:** To investigate, we treated MH-S cells (Immortalized murine alveolar macrophages) with known agonists for TLR2, TLR3, TLR4 and TLR9 for 6 and 24 hours, followed by the quantification of IL-6, IL-10, and IL-22 using sandwich ELISA. Additionally, we conducted inhibition studies by treating alveolar macrophages with known respective antagonists for 1 hour, followed by exposure to 1% ODE for 6 and 24 hours, with subsequent ELISA quantification of the cytokines. **Results:** Our results indicate that TLR2 agonist treatment led to the highest induction of IL-22 in supernates after 6 hours, suggesting a transient, rapid role for TLR2 in instigating inflammation resolution processes. Conversely, TLR3 agonism exhibited the least induction of IL-22. Despite the low induction of IL-6 and IL-10 following TLR3 activation in our inhibition studies, treatment with the TLR3 inhibitor CU-CPT and 1% ODE significantly restricted both IL-6 and IL-10 production, with cytokine levels comparable to baseline controls treated with DMSO. These findings suggest that a major component of ODE may act as a TLR3 ligand or that TLR3 exhibits slower kinetics. **Conclusions:** The significance of these studies lies in their potential to enhance our understanding of the distinct roles TLR2 and TLR3 play in lung inflammation and resolution. Future research directions include replicating these findings in human models, exploring the roles of other human TLRs, and conducting gene assays to further investigate the transcription kinetics and downstream events associated with TLR2 and TLR3. The resulting insights could help contribute to the development of targeted therapeutic strategies for lung inflammation induced by organic dust exposure.

3475 Elucidating the role of IL-5 in the pulmonary response to inhalation of *Aspergillus versicolor* and *Stachybotrys chartarum* using a murine IL-5 knockout model

K. L. Weaver. CDC/NIOSH, Morgantown, WV. Sponsor: *J. Roberts*

Background and Purpose: A changing climate has resulted in an increased frequency and severity of hurricanes and flooding that devastate communities and leave structures inundated with water. Water damage in buildings promotes growth of fungal species that can be harmful to human health. This is of concern given that humans spend over 90% of their time indoors - whether that be at home, work, or school. *Aspergillus versicolor* and *Stachybotrys chartarum* are two fungal species commonly found in damp indoor environments. Inhalation of fungal spores can lead to lung inflammation and development of allergic disease. Interleukin-5 (IL-5) is a mediator of the inflammatory allergic response and is a clinical target for treatment; however, further research is needed to fully understand the role of IL-5 in response to fungal exposure, how sex as a biological variable influences this process, and to ultimately develop tools to assess exposure and treatments to mitigate respiratory ailments. **Methods:** Male and female C57BL/6J or IL-5 genetic knockout mice were nose-only exposed to HEPA-filtered air, $3x10^5$ viable *A. versicolor* spores, or $1x10^4$ viable *S. chartarum* spores for up to one hour, twice weekly, for 13 weeks using an acoustical aerosol generator system. The dose for each fungal species was the estimated pulmonary deposition per exposure. Functional respiratory assessment was performed using whole body plethysmography at week 0, week 4, week 8, and week 13 allowing for repeat measures of individual mice over time. Mice were euthanized 24 hours following the final exposure and immunologically relevant tissues were collected to assess local and systemic immune responses. Pulmonary responses were further assessed by lung histopathology, quantification of immunoglobulins in sera, flow cytometry analysis of immune cell populations in murine tissues, and measurement of Th1 and Th2 associated genes using quantitative polymerase chain reaction. **Results:** Preliminary data suggests that sex is an important factor to consider in the response to the inhalation of *A. versicolor* and *S. chartarum* in this mouse model. Whole body plethysmography indicated that overall, females have lower respiratory function than males in wild-type mice exposed to air only at baseline. Exposure to *S. chartarum* led to a decrease in respiratory function as measured by tidal volume in IL-5 -/- female mice compared to male mice after 13 weeks of exposures. Histology and flow cytometry showed increases in immune cell infiltration into the lung and the development of adaptive immune responses in mice exposed to fungal spores compared to air only control mice. Preliminary analysis exhibited that the mechanisms by which these pulmonary responses develop differed following *A. versicolor* and *S. chartarum* exposure. **Conclusions:** IL-5 is a clinical target for treatment in allergic airway disease due to its role in the inflammatory response and differentiation of eosinophils. It is imperative to investigate the role of IL-5 in the response to fungal exposure given that anti-IL-5 therapies often inhibit the function of IL-5 in humans. The goal was to understand how the immune response differs following exposure to two common fungal contaminants, focusing specifically on the role of IL-5 and the influence of sex as a biological variable. Together, these data provided insights on the mechanisms of the immune response to fungal exposure that can be applied to the assessment of fungal-related respiratory disease in humans.

3476 Woodfire Smoke Induced Mucociliary Airway Injury is Preventable with MEK Inhibition

C. Cherry[1], A. Durra[1], E. Hou[1], C. Luo[1], T. Rickabaugh[1], K. Castillo[1], E. Vladar[2], M. Bouhaddou[1], and B. Gomperts[1]. [1]University of California, Los Angeles, Los Angeles, CA; and [2]University of Colarado, Aurora, CO. Sponsor: *O. Hankinson*

Background and Purpose: From the warmth of woodburning stoves or campfires to the devastation of wildfires, woodsmoke permeates both our ambient and household environment, but the health risks from these exposures are not known. Despite improvement in United States air quality through recent decades, wildfire emissions have now worsened the air we breathe. Furthermore, the frequency of wildfires is projected to increase due to climate change in the next several decades. The emissions from wildfires are highly variable and rely on differences in fuel, burning conditions, and environmental factors. In recent epidemiological studies, componentsof WFS have been shown to exacerbate respiratory and cardiovascular issues, although the mechanisms for this airway injury remain poorly understood. Mucociliary clearance (MCC) is the primary innate defense mechanism of the lung, and failure of this system results in a variety of respiratory diseases. Mucus secretion traps pathogens, particles and pollutants, and unilateral beating by cilia propels the mucus layer up and out of the airway to be expectorated or swallowed. Although the relationship between lung disease and ciliary dysfunction has been established, very little work has been done to identify the specific effects of environmental exposures on ciliary proteins, ciliary structure, and ciliary function. Some studies have reported environmental exposures to be associated with an activation of several cell signaling pathways. Post-translational modifications (PTM) regulate nearly all biological processes. Of note, protein phosphorylation is a regulatory mechanism that is important in nearly all cellular processes, such as protein synthesis, cell division, growth, or development. The Ras-Raf-MEK-ERK signaling pathway cross-talks with several other cellular signaling pathways and has been shown to be associated with ciliary motor behavior, ciliary gating, and cytoskeletal rearrangement. The MAPK pathways are well-known regulators of the cell cycle but have also been shown to control ciliary length in a wide variety of organisms. To date, several drugs have emerged as a rational therapeutic for treatment of Ras related cancers. Although these drugs, such as Pimasertib (AS703026), have only been studied in the context of cancer, the involvement of the Raf/MEK/ERK in regulating cell survival, cell cycle progression, and differentiation has been well established and could have other therapeutic potential. Therefore, this proposal aims to understand the mechanisms of ciliary injury following WFS exposure and identify potential therapeutic agents to mitigate this injury. **Methods:** Utilizing healthy human bronchial samples, our specialized air liquid interface (ALI) primary human airway culture approach allowed for mucociliary cells to be exposed to Woodfire Smoke (WFS) in a highly reproducible manor, relevant to ambient biomass burning. Pine wood was partially combusted and human mucociliary cells at air liquid interface were exposed to WFS for 3 minutes a day for 5 days. Extensive characterization of the particulate matter in our exposure system was assessed through SMPS, APS, mass quantification and excitation emission matrix. Airway Basal Cell injury following WFS exposure was assessed through bulk RNA sequencing, Proteomic and Phosphoproteomic analysis, and functional live beating ciliary video analysis. ERK inhibition was performed through addition of Pimasertib (100uM) 30 minutes prior to WFS exposure each day. Protection of ciliary injury through ERK inhibition was analyzed through live-beating ciliary video analysis and transmission electron microscopy and compared to WFS exposure alone. **Results:**

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