PL 2420 Environmentally Prevalent Low Molecular Weight Polycyclic Aromatic Hydrocarbon Effects on Lung Cell Communication and Inflammation

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that are a human health concern due to exposures such as second and thirdhand smoke (SHS). High molecular weight (HMW) PAHs act as complete carcinogens, however, recent evidence suggests that the low molecular weight (LMW) PAHs, typically more prevalent than HMW species, may act through distinct mechanisms to contribute to lung injury, inflammation, and potentially tumor promotion. In C10 cells (a mouse, non-tumorigenic type II cell line), we evaluated two secondhand smoke PAHs *in vitro* (1-methylanthracene (1-MeA) and fluoranthene (Flthn)) for their ability to dysregulate gap junction intercellular communication (GJIC), decrease connexin 43 (Cx43; primary lung gap junction protein) expression and influence localization, activate MAP kinases, and induce several inflammatory markers. Novel pilot *in vivo* studies used Flnth that was oropharyngeally aspirated into C57BL/J (B6) and BALB/cJ mice to validate *in vivo* pulmonary inflammation 1 and 3 days following exposure using bronchoalveolar lavage analysis. *In vitro* methods used: scalpel-loaded/dye transfer assays (GJIC), immunoblotting (Cx43, MAPKs), qRT-PCR (inflammation), immunocytochemisty (Cx43 localization). 1-MeA and Flthn dysregulated GJIC and decreased Cx43 expression. Both p38 and ERK MAPK were activated in response to 1-MeA and Flthn and several inflammation pathways induced, however only p38 inhibition reversed the GJIC dysregulation and inflammation pathways. *In vivo*, Flthn induced significant inflammatory cell infiltration in both strains, including alveolar macrophages and PMNs, which supports a role for these PAHs in SHS-induced pulmonary inflammation. Future work will address the *in vivo* mechanisms of PAH induced lung injury, how these mechanisms may relate to our *in vitro* data, and the potential for tumor promotion.

PL 2421 Uranium and Environmental Metal Exposures from Legacy Mining Waste and Immune Dysfunction among Navajo DiNEH Study Participants

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Research on legacy mining waste by the Dine Network for Environmental Health (DiNEH) study (NIEHS 5R01ES014565, HRPO#: 03-059) used detailed health questionnaire and geospatial analysis showing exposures increased risk for hypertension, immune dysfunction, and autoimmune disease and risk for developing chronic diseases. We examined immune biomarkers to identify mechanisms of immune responses that occurred during active mining and those observed more recently. We used urine biomonitoring results of various heavy metals (U, Ni, V, and Cu) and total arsenic concentrations and applied exposure modeling based on the location information of participants and corresponding uranium mines. We applied multiplexing bead technique to measure 13 human serum cytokines of 268 DiNEH sera and established inflammatory status based on clusters of cytokines. Logistic regression and Bayesian modeling were performed on these immune measures. We presented cytokines for which urine metals and their interactions were 1) significant predictors for the cytokine values in reduced statistical models; and 2) accounted for more than 20% of the variance as measured by model R2 values corrected for the number of covariates in the model. Based on the percent of variance accounted for by these models, IL-1-β and IL-4 are the most sensitive cytokines, both significantly decreased by U, increased by Ni, and responsive interactions among U, As and V. Mining Era Exposures appeared as significant predictors in several models with increased IL-13, IL-1-β, and IL-5, while decreased IL-2 concentrations. Uranium excretion was associated with increased IL-13 production, and decreased IL-1-β, IL-2, and IL-4. Total urine As appears in significant interactions in all but the model for IL-2.

PL 2422 Proinflammatory Responses and Inflammasome Activation by Sintered Indium-Tin Oxide Particles

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Indium-tin oxide (ITO) is used to make transparent conductive coatings for touchscreen and liquid crystal display electronics. As the demand for consumer electronics continues to increase, so does the concern over occupational exposures to particles containing these potentially toxic metals. Epidemiologic studies have shown that workers exposed to ITO develop pulmonary alveolar proteinosis and fibrotic interstitial lung disease. Indium-containing compounds have been shown to be pro-inflammatory in animal models of indium lung disease, but how these particles initiate immune responses remains unknown. Our preliminary studies showed that sintered ITO (SITO) exposures caused significant cell death, NFκB activation, and cytokine production (IL-1β, IL-6, TNFα, and IL-8) within 24 h in RAW 264.7 macrophages and BEAS-2B bronchial epithelial cells, confirming pro-inflammatory responses. These results suggest NLRP3 inflammasome activation, which has been implicated in several immune-mediated diseases and pulmonary fibrosis via its ability to induce IL-1β release. This cytoplasmic, multi-protein complex responds to an array of different stressors but typically requires two signals. One involves toll-like receptor (TLR) priming through NFκB for the transcription of pro-IL-1β and the other activates caspase-1 for the processing and release of biologically-active IL-1β. When inflammasome components were blocked upstream or downstream of NLRP3, SITO-induced IL-1β production was significantly reduced. Further, SITO was able to cause significant caspase-1 activation. These results suggest that SITO particles were able to activate the inflammasome and subsequent cytokine production. Thus, it is possible that activation of the NLRP3 inflammasome by SITO plays a role in initiating and propagating indium lung disease. These findings will provide a better understanding of the mechanisms behind an emerging occupational health issue and will aid in the discovery of biomarkers for disease prevention.

PL 2423 IL-6 Deficiency Leads to a Specific Cytokine Profile during Irritant Contact Dermatitis

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Of reported occupational injury associated with workman's compensation, contact dermatitis ranks second most prevalent over all. Irritant contact dermatitis (ICD) is an acute inflammatory response that can vary between individuals. This difference between patients may be associated with varied modulation of the expression of specific inflammatory cytokines as a result of genetic or epigenetic alterations. This laboratory recently reported that mice deficient in the inflammatory cytokine interleukin 6 (IL-6KO) display worse ICD compared to WT in response to various chemical irritants. To further investigate this finding, denuded skin of IL-6KO and WT C57BL/6 mice was exposed to an irritant (benzalkonium chloride, BKC) or acetone (control) daily for 7 days. Histopathology revealed worse inflammation in the BKC exposed IL-6KO skin, primarily characterized by epidermal thickening and inflammatory cell infiltration. Multiplex protein analysis of skin inflammatory cytokine expression showed certain cytokines such as TNFα, IL-1β, CXCL2 were equally induced by irritancy in either mouse strain. However, G-CSF (2.5 fold), and IFNγ (4.3 fold) were significantly increased in IL-6KO skin, while other cytokines showed decreases, such as IL-12 (-8.4 fold), IL-18 (-2.4 fold), IL-22 (-2.1 fold), CXCL10 (-3.4 fold), CCL4 (-2.1 fold), CCL5 (-3.5 fold), and CCL7 (-2.1 fold). Cytokine mRNA expression also varied; however, it did not necessarily correspond with protein expression. Flow cytometric and mRNA analysis of the macrophage markers F4/80 and CD206 indicated that M1 macrophages dominate the response in IL-6KO skin. Thus, it appears that IL-6 deficiency exacerbates skin irritation, and characteristic inflammatory cytokine expression and possibly macrophage phenotypic changes may drive the altered response.

R 2424 Epigenetics and Chemical Safety Assessment: Are We Ready?

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Continuous exposure to certain natural and manmade chemicals might be a major cause of noncommunicable human diseases, including cancer. Evidence is accumulating indicating that some of the earliest events preceding the development of these pathological states involve perturbations of the cellular epigenome, including modifications at the 5-position of DNA-cytosine, histone modifications, and expression of noncoding RNAs. This suggests that despite a lack of conclusive information

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