2393 Pulmonary Fibrosis and Fibroblast Apoptosis Resistance: Combined Effects of Fas Death Receptor Loss and Toll-Like Receptor 4 Signaling

K. M. Hanson, A. M. Groves, C. J. Johnston, J. P. Williams, and J. N. Finkelstein. *University of Rochester Medical Center, Rochester, NY.*

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 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 1) Lung lavage fluid from rt mouse lungs activates TLR4 while control lavage fluid does not and 2) Endogenous TLR4 agonist HMGB1, known to be increased in damaged lungs, can activate TLR4 to the same extent as canonical ligand LPS. 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.

2394 Effect of Diet-Induced Obesity on Silica-Induced Pulmonary Toxicity in Rats

T. M. Sager, C. M. Umbright, B. L. Frye, W. McKinney, B. T. Chen, and P. Joseph. *NIOSH, Morgantown, WV.*

Millions of workers worldwide are exposed to silica, a causative agent for potentially fatal diseases such as silicosis. In addition, whether avoidable lifestyle factors, such as diet-induced obesity, may modify the likelihood of silicosis development is unknown. Therefore, the objective of this study was to determine the effect of diet-induced obesity, if any, on silica-induced pulmonary toxicity in a rat model. Throughout the study, Fischer 344 rats were continuously fed either a regular fat (RFD; 18% kcal as fat) or a high fat (HFD; 60% kcal as fat) diet. Two weeks after starting the study, the rats were exposed to either air or crystalline silica (15 mg/m^3, 6 hours/day, 5 days). Body weights and serum triglyceride levels, indicators of diet-induced obesity, in the HFD rats were higher compared to the RFD rats. At 1 and 3 months post-silica exposure, bronchoalveolar lavage was done and pulmonary toxicity assessed. Pulmonary toxicity parameters including, lactate dehydrogenase (LDH) activity, oxidant production, and cell counts (including infiltrating neutrophils and alveolar macrophages), were assessed. Inflammatory cytokine levels (IL-1β, IL-10, TNF-α, MCP-1, and MIP-2) were also measured. Expression profiling of a panel of ten genes (CCL2, CCL3, CXCL-1, CEACAM 10, LCN 2, MTIA, MMP-12, SLC13A2, SLC26A4, and SOD2), known to be involved in silica-induced pulmonary toxicity, were assessed by real-time PCR. The results showed that silica inhalation resulted in pulmonary toxicity, at both 1 and 3 months post-exposure, as evidenced by enhanced neutrophil infiltration, increased LDH levels, enhanced oxidant production, increased inflammatory cytokine levels, and overexpression of the genes in both RFD and HFD groups. Silica-induced pulmonary toxicity was more pronounced at 3 months post-exposure than 1 month. In addition, the effect of silica inhalation on gene expression and inflammatory cytokine levels was further enhanced, (up to a 21-fold increase in gene expression and 6-fold increase in cytokine levels) especially at 3 months post-exposure in the HFD group compared to the RFD group. In summary, our results indicated that certain early pulmonary toxicity parameters (inflammatory cytokine levels and gene expression) induced by silica inhalation were enhanced by diet-induced obesity in rats.

2395 Aerosol Characterization and Pulmonary Responses after Short-Term Inhalation of Fumes Generated during Resistance Spot Welding of Galvanized Steel

A. Afshari, P. C. Zeidler-Erdely, T. Meighan, W. McKinney, M. Jackson, D. Schwegler-Berry, D. Burns, R. F. LeBouf, B. T. Chen, and J. M. Antonini. *NIOSH, Morgantown, WV.*

Resistance spot welding is a common process to join metals in the automotive industry. Adhesives are often used as sealers to seams of metals that are joined. Anti-spatter compounds sometimes are sprayed onto metals to be welded to improve weldability. Spot welding produces complex aerosols composed of metal and volatile organic compounds (VOCs) which can cause lung disease in workers. Sprague-Dawley rats were exposed by inhalation to 25 mg/m^3 of aerosol for 4 h/d x 8 d during spot welding of galvanized zinc-coated steel in the presence or absence of an adhesive or anti-spatter spray. Controls were exposed to filtered air. Particle size distribution and chemical composition of the generated aerosol were determined. At 1 and 7 d after exposure, bronchoalveolar lavage (BAL) was performed to assess lung toxicity. The generated particles mostly were in the submicron size range with a significant number of ultrafine particles formed. The primary metals present in the fumes were iron (72.5 %) and zinc (26.3 %). The addition of the anti-spatter spray and adhesive did affect particle size distribution when spot welding galvanized steel, whereas they had no effect on metal composition. Multiple VOCs (e.g., methyl methacrylate, acetaldehyde, ethanol, acetone, benzene, xylene) were identified when spot welding using either the adhesive or the anti-spatter spray that were not present when welding alone. Markers of lung injury (BAL lactate dehydrogenase) and inflammation (total BAL cells and neutrophils) were significantly elevated compared to controls 1 d after exposure to the spot welding fumes. The elevated pulmonary response was transient as lung toxicity mostly returned to control values by 7 d. The VOCs or the concentrations that were generated during the animal exposures had no measurable effect on the pulmonary responses. Inhalation of galvanized spot welding fumes caused acute lung toxicity most likely due to the presence of zinc.

2396 Measuring the Bioenergetic Effects of 1,2-Naphthoquinone Exposure on Human Lung Macrophages Using Seahorse Extracellular Flux Analyses

K. S. Lavrich[1], N. E. Alexis[1], and J. M. Samet[2]. *[1]University of North Carolina at Chapel Hill, Chapel Hill, NC; and [2]US EPA, Research Triangle Park, NC.*

Exposure to ambient particulate matter (PM) is one of the leading causes of morbidity and mortality in humans. Quinones are organic PM components that induce inflammatory responses through redox cycling and electrophilic attack. 1,2-naphthoquinone (1,2-NQ) has previously been shown to cause bioenergetic effects in airway epithelial cells, but the effects in macrophages (Mac) are unknown. Induced sputum and bronchoalveolar lavage (BAL) are procedures that recover Mac from the central and peripheral airways, respectively and can be used to assess the impact of environmental agents on Mac function. In this study, we sought to characterize the bioenergetic effects of *ex vivo* exposure to 1,2-NQ on Mac derived from induced sputum or BAL in healthy human volunteers. We optimized techniques for analyzing primary airway samples on the Seahorse XFe24 Analyzer and assessed mitochondrial respiration and glycolytic activity in response to 1,2-NQ exposure. In sputum samples, the oxygen consumption rate (OCR) increase induced by 1,2-NQ was very small when compared to the robust oxidative burst response to the protein kinase C activator phorbol 12-myristate 13-acetate (PMA). In contrast, 1,2-NQ produced a greater OCR increase than that of PMA in BAL Mac. Both BAL and sputum Mac had lower 1,2-NQ induced OCR increases than bronchial epithelial cells, suggesting that Mac may have a lower capacity for quinone redox cycling. Differential responses in response to 1,2-NQ between BAL and sputum samples show Mac from lower airways, and to a greater extent, epithelial cells, may be more susceptible to oxidative damage by quinones. To our knowledge, this is a novel application of extracellular flux methodology to lung Mac acquired from human volunteers. Bioenergetic effects of *ex vivo* exposure to an environmental agent were successfully examined. The observed inter-lung compartment variability indicates that certain human Mac populations may be more susceptible to PM-associated quinones based on their anatomic location in the lung. This further elucidates the mechanisms underlying PM-induced health effects. *This abstract does not necessarily reflect US EPA policy.*


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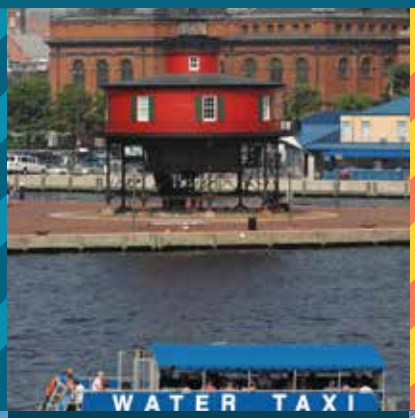

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