so, significantly decreases the risk of mutations, because it is not 3-NBA that is particularly harmful, but the process the human body takes once it enters lung tissue. This metabolism pathway could be replicated in the exhaust systems of diesel-powered automobiles to abate the production of this compound, similar to how urea is sprayed into the exhaust system to limit the production of NOx compounds. Further experimentation would investigate the practicality of this application.

2015 Single Cell Transcriptomics Identifies Key Cellular Players in an Animal Model of Asbestos-Induced Pulmonary Fibrosis

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Occupational and indoor exposure to asbestos can lead to the development of pulmonary fibrosis years after exposure has ceased, leading to significant morbidity and mortality. Asbestos fibers can lodge within the bronchoalveolar duct junctions and small airways of humans and mice respectively, persisting for years. Although multiple cell types have been implicated as important participants in the development and progression of asbestos-induced lung fibrosis, the specific mechanisms and key cellular players involved are not known. Using a comprehensive combination of unbiased single cell transcriptomic profiling (scRNA-Seq), genetic lineage-tracing, flow cytometry and *in situ* RNA hybridization, we tested the hypothesis that monocyte-derived alveolar macrophages are key drivers of asbestos-induced pulmonary fibrosis via epithelial cell injury and fibroblast proliferation. C57Bl6 mice were exposed to TiO_2 (control) or asbestos fibers intratracheally. Lungs were harvested 14 days later to capture the early stages of pulmonary fibrosis and scRNA-Seq libraries were prepared from cell suspensions using the 10X Chromium platform. Profiling 24,060 cells identified 24 known cellular populations represented in all experimental conditions. All populations exhibited transcriptional changes during the development of fibrosis. Importantly, the emergence of a new distinct subpopulation of alveolar macrophages was observed in asbestos-exposed animals. This subpopulation was characterized by an immature phenotype and elevated expression of genes known to be causally associated with fibrosis such as *Mmp12*, *Retnla, Chia1* and *Pdgfa* (involved in fibroblast proliferation). Furthermore, these cells expressed *Itgam* and *Cx3cr1*, suggesting a monocyte origin. Remarkably, this new subpopulation was represented only by cells from asbestos-exposed mice and was absent in control conditions. Flow cytometry, lineage-tracing analyses and immunohistochemistry confirmed this subpopulation to be monocyte-derived alveolar macrophages. Immunofluorescent microscopy confirmed that *Pdgfa*-expressing cells were specifically recruited to the areas of fibrosis and were located in the proximity of *Pdgfra*-expressing fibroblasts. Cre/lox-mediated genetic deletion of this population by targeting Casp8 prevented the development of pulmonary fibrosis. Collectively, these studies are the first to show a causal association between asbestos-induced epithelial lung injury, localized recruitment of monocyte-derived alveolar macrophages and subsequent development of spatially restricted lung fibrosis.

2016 Lack of Lung Tumor Promotion after Inhalation of a Copper-Nickel Welding Fume in A/J Mice

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The International Agency for Research on Cancer classified welding fumes as a Group 1 carcinogen (*carcinogenic to humans*) in 2017. The process of stainless steel welding creates fumes rich in carcinogenic metals such as chromium (Cr). Our lab has previously demonstrated that stainless steel welding fumes promote lung tumors in tumor-susceptible A/J mice. Consumables devoid of Cr are being produced in an attempt to limit worker exposures to potentially carcinogenic metals. The aim of this study was to characterize a new copper-nickel (Cu-Ni) fume and then investigate if inhalation of this fume would promote lung tumors in mice using a two-stage (initiation-promotion) model. To determine particle mass size distribution, a Micro-Orifice Uniform Deposit Impactor (MOUDI, model 110; MSP corp., Shoreview, Minn.) with additional Nano-MOUDI stages (MSP model 115) was used. Characterization of the fume indicated that most of the particles were between 0.1 and 1 µm in diameter, with a mass median aerodynamic diameter of 0.43 µm. Male A/J mice (4 - 5 weeks old) were initiated with 3-methylcholanthrene (MCA; 10 µg/g IP) or corn oil and, beginning 1 week later, were exposed to air or Cu-Ni welding fumes for 4 hours/day, 4 days/week, for 9 weeks. At 30 weeks, mice were sacrificed and lung tumor multiplicity and incidence were evaluated. MCA/Cu-Ni welding fume exposure significantly decreased tumor number and tumor size compared to MCA/air controls (7.11 ± 0.93 tumors *vs.* 15.57 ± 0.75 tumors and 0.57 ± 0.01 mm in diameter vs. 1.15 ± 0.02 mm in diameter, respectively). Future studies are planned to investigate the pneumotoxicity of Cu-Ni fume in A/J mice.

2017 Functional Significance of the SLC26A4 Gene in Silica-Induced Pulmonary Toxicity

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Occupational exposure to silica may result in potentially fatal diseases such as silicosis and cancer. Understanding molecular mechanisms responsible for silica-induced pulmonary toxicity is of great importance in preventing silicosis and other effects associated with occupational silica exposure. Previous studies in our laboratory identified a correlation between silica-induced pulmonary toxicity and SLC26A4 gene overexpression in the lungs of rats. However, the functional significance of this gene in silica induced pulmonary toxicity is not understood. To determine the role of the SLC26A4 gene in silica-induced pulmonary toxicity, SLC26A4 wild type (WT) and knockout (KO) mice were employed. All mice were exposed to either air or crystalline silica (15 mg/m^3, 6 hours/day, 4 days) and pulmonary toxicity was assessed at 1 day, 3 months, 6 months, and 9 months post-exposure. Pulmonary response parameters including, lactate dehydrogenase (LDH) activity, oxidant production, cell counts (including infiltrating neutrophils and alveolar macrophages), and gene expression changes were assessed. Silica exposure resulted in the induction of pulmonary toxicity and inflammation in both the WT and KO mouse strains, compared to corresponding air exposed controls. However, there were significant differences ($p<0.05$) in the measured pulmonary toxicity parameters between silica exposed WT and KO groups. For example, induction of pulmonary inflammation in the silica exposed mice was hallmarked by a significant increase in infiltration of neutrophils in the lung. This infiltration was vastly different between the WT and KO groups. Specifically, at 3 months post-exposure neutrophil infiltration in the WT mice was 480 fold higher compared to air exposed controls while being 205 fold higher in the KO mice. At 6 months post-exposure, neutrophil infiltration in the WT mice was 192 times higher than air controls while the KO mice had, a significantly lower, 54 fold increase in PMN number, compared to air controls. At 9 months post-exposure neutrophil number was 45 fold higher in WT mice and only 9 fold higher in KO mice compared to air controls. In conclusion, both the WT and KO mice presented with an enhancement in pulmonary toxicity parameters measured, however, the severity of silica induced pulmonary toxicity was more in the WT mice compared to the KO mice. These findings support the hypothesis that the SLC26A4 gene does, in fact, play a role in silica induced pulmonary toxicity.

2018 Understanding the Lung-Gut Axis by Modeling the Influence of Welding Fume Inhalation Exposure and Lifestyle on the Profile of Gut Microbiome and Systemic Immune Cells

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The gut microbiome has a regulatory influence on various systemic organs, and altered microbiome diversity correlates with various diseases and pathological conditions. The goal of the current work was to profile and correlate the influence of occupational pulmonary exposure (welding fume), lifestyle (high fat diet) and age on the gut microbiome and immune cell phenotype populations in blood, lung lymph nodes and spleen. Male Sprague-Dawley rats were maintained on a regular chow (RG) or high fat (HF) diet for 24 wk. At wk 7, groups of rats maintained on each diet were exposed by inhalation to stainless steel welding fume (WF; 20 mg/m3 x 3 hr/d x 4 d/wk x 5 wk) or filtered air until wk 12, at which time some animals from each group were euthanized. A separate set of rats from each group were allowed to recover from WF exposure until wk 24. At these three time points, immune cells from various systemic locations were profiled using flow cytometry. The DNA from the lower gut feces was extracted and sequenced for 16s. The ratio of firmicutes to bacteroidetes consistently decreased in RG-fed rats and increased for HF-fed rats over the 24 wk period. This was further exacerbated in WF-exposed animals. Random forest classifiers were used to distinctly identify specific alterations at genus and species level for the various treatments. There was no change in total leukocyte number but there was a significant increase in neutrophils recovered from the blood of rats fed the HF vs the RG diet. In the lungs, there was no change in the leukocyte profile between rats with various diets after WF exposure; however, following a recovery period, lung neutrophil and lymphocyte numbers, in addition to percent of pulmonary macrophages, remained significantly elevated in rats maintained on

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