were assessed by western blot and DNA-binding activity of Nrf2, respectively. Our results showed that Nrf2 stabilization and activation in response to DNCB were exacerbated by each wavelength used 3 hours after light exposure. Regarding Nrf2-regulated genes, DNCB increased the expression of *HMOX1*, *NQO1*, and *GCLC* mRNAs, while PBM further enhanced the expression of these Nrf2-target genes after illumination of DNCB-treated KCs with both wavelengths. To investigate the role of Nrf2 in controlling the PBM-mediated pro-inflammatory response, a small interfering RNA-mediated Nrf2 knockdown model has been used. In the absence of Nrf2, illumination of KCs with red light did not reduce the *TNF-α, IL-6*, and *IL-8* mRNAs expression in response to DNCB, nor did it suppress the production of these cytokines measured with a multiplex assay. These findings implied that light's anti-inflammatory depends, at least in part, on Nrf2. This study is the first to report the involvement of Nrf2 in the PBM-induced anti-inflammatory response in KCs. To further demonstrate the role of PBM in cutaneous immunomodulation, skin explants will be exposed to light under inflammatory DNCB-induced conditions, and the Nrf2 pathway will be addressed.

PS 4101 Lysosomal Cholesterol Attenuates Silica-Induced Membrane Disruption

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The ability of inhaled crystalline silica to cause chronic inflammatory disease has been well established. When airborne silica is inhaled, the particles can deposit into the lungs and depending on the size, into the alveolar spaces. These particles can be encountered and phagocytosed by alveolar macrophages. Once phagocytosed, silica contained in the phagolysosome, has been reported to cause an event known as phagolysosomal membrane permeability (LMP). This disruption of the lysosome can release degradative enzymes into the cytosol, causing cell death and NLRP3 inflammasome activation. The NLRP3 inflammasome activates caspase-1, which cleaves IL-1ß and IL-18 to their active forms for secretion. While there is substantial detail surrounding this inflammatory pathway, not much is known about the mechanisms of LMP and potential therapeutic measures. This work involved examining the effects of lowered lysosomal cholesterol on silica-induced LMP. Bone marrow derived macrophages (BMdM) were used as a macrophage model and were treated with 18:1 phosphatidylglycerol (DOPG) to reduce lysosomal cholesterol. Pre-treatment with DOPG prior to BMdM silica exposure caused increased cell death and IL-1ß release compared to cells with no DOPG pre-treatment. Increasing lysosomal cholesterol with the compound, U18666A, produced an opposite effect to that of DOPG by reducing cell death and IL-1ß release. Silica-induced LMP was measured by examining n-acetyl-ß-glucosaminidase (NAG) activity in the cytosol. Cells treated with DOPG and silica had increased cytosolic NAG activity compared to cells that only received silica treatment. Filipin staining was used to assess cellular and lysosomal cholesterol. Treatment with U18666A produced increased lysosomal cholesterol that was visualized with confocal microscopy as distinct puncta. When treating with both U18666A and DOPG, the amount of lysosomal cholesterol puncta in the BMdM was reduced compared to U18666A treatment, indicating the ability of DOPG to reduce lysosomal cholesterol. The ability of cholesterol to attenuate silica-induced membrane disruption was examined in 100-nm 18:1 phosphatidylcholine (DOPC) liposomes with and without cholesterol. Lipid order was measured by time-resolved anisotropy of the membrane probe, Di-4-ANEPPDHQ. Cholesterol containing liposomes showed no significant change to lipid order after silica incubation. However, DOPC liposomes without cholesterol had a significant increase to lipid order after silica treatment. These results demonstrate that increased membrane cholesterol can mitigate silica-induced membrane disruption while reducing lysosomal cholesterol enhances the ability of silica to cause LMP and inflammation. *This work was funded by National Institute for Environmental Health Sciences [grant numbers, R01ES033533, F31ES033562, S10OD021806]. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the views of the NIH.*

PS 4102 Lysosomal Big-Conductance Potassium Ion Channel Activity Implicated in Susceptibility to Silica-Induced Inflammation in Macrophages

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Lysosomal dysfunction plays a contributing role in many inflammatory diseases. In particle-induced inflammatory diseases such as silicosis, lysosomal dysfunction occurs as lysosomal membrane permeabilization (LMP). LMP promotes cell death and perpetuates a cycle of NLRP3 inflammasome driven inflammation in macrophages, increasing the likelihood of fibrotic disease onset. Prevention of LMP then, is key to increasing the resolution of crystalline silica-induced inflammation thereby reducing the likelihood of disease onset. Because NLRP3 activation has been linked to a decrease in cytosolic K^+, lysosomal ion channel activity could play a critical role in maintaining lysosomal function. There is a large concentration gradient of K^+ across the lysosomal membrane in part due to the activity of the large conductance Ca^{2+}-activated potassium channel (BK channel) within the lysosomal membrane. Here, we confirm the importance of BK channel function to silica-induced LMP by treating BMdM with paxilline to inhibit BK channel activity. Paxilline pre-treatment significantly reduced LMP and IL-1β release in silica treated macrophages. Conversely, treatment with the BK channel activator NS1619 or NS11021 resulted in a significant increase in silica-induced LMP and IL-1β release. We confirmed that silica caused a decrease in cytosolic K^+ and assessed the impact of paxilline and NS1619/NS11021 treatments on the change in cytosolic K^+. Because lysosomal ion channel function can easily impact the acidification and subsequently cholesterol trafficking within the lysosomal environment, the impact of paxilline on lysosomal cholesterol was assessed with filipin staining in treated macrophages. Paxilline treatment resulted in increased filipin-stained puncta within the lysosomes, indicating an increase in lysosomal free cholesterol. In comparison, U18666A treated macrophages were treated with NS1619 or NS11021 to assess the impact of BK channel activity on lysosomal cholesterol accumulation. Because increased membrane cholesterol has been demonstrated to alter silica interactions with phosphatidylcholine phospholipids, the increase in lysosomal cholesterol is important to prevent LMP in silica-exposed macrophages. Here, we demonstrate that modification of lysosomal BK potassium channel activity can reduce or increase susceptibility of silica-exposed macrophages to LMP and IL-1β release and does so in a cholesterol-dependent manner.*This work was supported by funding from the National Institute of Environmental Health Sciences [grant numbers R21ES033511, F31ES033562]. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the views of the National Institute of Health.*

PS 4103 Lung Response to Coal Dust and Crystalline Silica Exposure in Rats

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The role of crystalline silica in the re-emergence of coal workers pneumoconiosis reported in certain U.S. Appalachian states was investigated by employing an experimental rat model of lung toxicity. A computer-controlled, automated aerosol generation system was custom-built and employed to generate aerosols containing crystalline silica (Min-U-Sil 5) or coal dust (Keystone Mineral Black 325BA). Determination of the particle size distribution in the aerosol samples generated, using a micro-orifice uniform deposit impactor (MOUDI), showed a mass median aerodynamic diameter of 1.6 µm [geometric standard deviation (σ_g) 1.6] and 1.36 µm (σ_g 2.3), respectively, for the crystalline silica and coal dust particles. Male Fisher 344 rats (n=4/group) weighing approximately 200 g were used in the whole-body inhalation exposure lung toxicity study. The four exposure groups were: 1. Filtered-Air (6 hours/day, 5 days/week during week 1 followed by 6 hours/day, 4 days/week during weeks 2-5), 2. Min-U-Sil 5 (15 mg/m^3, 6 hours/day, 5 days during week 1 followed by filtered air for 6 hours/day, 4 days/week during weeks 2-5), 3. Coal dust (filtered air for 6 hours/day, 5 days during week one followed by coal dust, 10 mg/m^3, 6 hours/day, 4 days/week during weeks 2-5), and 4. Min-U-Sil 5 + coal dust (Min-U-Sil 15 mg/m^3, 6 hours/day, 5 days during week 1 followed by coal dust, 10 mg/m^3, 6 hours/day, 4 days/week during weeks 2-5). At the end of the fifth week, since the initiation of the first exposure, the rats were euthanized, and bronchoalveolar lavage (BAL) was performed to determine the induction of lung toxicity. Exposure of rats to Min-U-Sil 5 or coal dust alone did not change lactate dehydrogenase (LDH) activity in bronchoalveolar lavage (BAL) fluid at the post-exposure time interval when the analysis was performed. On the other hand, combined exposure of the rats to Min-U-Sil 5 and coal dust, at the same post-exposure time interval, resulted in a 1.3-fold increase in LDH activity suggesting a modest induction of lung toxicity in the rats, compared to the individual agents. Similarly, the number of PMNs detected in the Min-U-Sil 5 alone, coal dust alone, or Min-U-Sil 5 + coal dust exposed rats were 1.41-, 1.13-, and 4.24-fold higher than air controls, respectively, confirming mild, but enhanced lung toxicity of the combined exposure. A similar trend in the generation of oxidants by the lung phagocytes was detected in the rats exposed to the test agents alone or in combination. Collectively, these results indicate that the combined exposure to Min-U-Sil 5 (crystalline silica) and coal dust results in lung toxicity in the rats whereas exposure of either of the agents separately, under the conditions employed in the current study, did not result in significant lung toxicity. This data is consistent with the theory of potential involvement of crystalline silica in the re-emergence of coal workers pneumoconiosis reported in the US.

PS 4104 ACAT-1 Inhibition Limits iNOS in an *In Vitro* Model of Macrophage Activation

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Each year in the United States, there are 190,600 cases of acute lung injury (ALI), associated with over 74,000 deaths. Data shows acyl-coenzyme A acetyltransferase-1 (ACAT-1) inhibition improves pulmonary inflammation in an *in vivo* murine model of ALI. We hypothesize that ACAT-1 inhibition has anti-inflammatory effects beyond its intended use to reduce cholesterol esterification. The purpose of this study is to establish an *in vitro* bone marrow-derived macrophage (BMDM) model to investigate the effect of ACAT-1 inhibition on macrophage activation. Monocytes were harvested from the bone marrow of 6-8 week old C57BL/6J (wild-type) mice. Cells were stimulated with M-CSF on d0, 3, and 7 to induce macrophage

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