Effects of Chronic Pulmonary Inflammation on Ozone-Induced Alterations in the Lung Microbiome

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Ozone (O_3) is a ubiquitous urban air pollutant known to cause pulmonary inflammation and constriction of the airways. The effects of O_3 are particularly severe in those with pre-existing lung disease, potentially via a "2-hit" mechanism involving alterations in the pulmonary microbiome. In these studies, we examined the effects of O_3 exposure on the microbiome in mice lacking *Sftpd* which exhibit chronic pulmonary inflammation. C57Bl6/J (Wt) mice and *Sftpd -/-* mice were exposed to air or O_3 (0.8 ppm, 3 hr); bronchoalveolar lavage fluid (BAL) was collected 24 hr later. Flow cytometry was used to identify lung macrophages (CD45+/SiglecF+/F4/80+) as resident alveolar macrophages (AM) (Cd11b-/Cd11c+) or inflammatory macrophages (IM) (Cd11b+/Cd11c+), or neutrophils (PMNs) (CD45+/SiglecF-/Cd11b+/Ly6G+) in BAL. Significantly fewer AM were detected in *Sftpd -/-* mice (75 ± 2.6%*) relative to Wt (91 ± 1.8%). O_3 exposure caused a greater loss of AM in *Sftpd -/-*mice (62 ± 2.9%*#) than Wt (88 ± 3.2; this correlated with a greater percentage of IM (9.8± 0.9% vs 2.2 ± 1.0%*). However, O_3 had no effect on these responses. Significant neutrophilia was observed in *Sftpd -/-* mice (8.9 ± 0.6%*#) compared to Wt (4.3 ± 0.6%#) after O_3 exposure. This was associated with increased disruption of the lung lining fluid. Thus, the BAL phospholipid content in *Sftpd -/-* was increased relative to Wt (51 ± 11 µg vs 8.23 ± 18 µg*). Moreover, this was exaggerated following O_3exposure (89 ± 12 µg*) relative to Wt (31 ± 10 µg). As inflammatory cell activation is known to affect the pulmonary microbiome by regulating turnover, changes to the lung lining fluid alter the growth capacities of different microbes, we next profiled microbial diversity within the lung of these mouse strains and the impact of O_3 exposure. Baseline differences in microbial populations were observed between Wt and Sftpd -/- mice (3.8 vs 2.7 Simpson index). A loss in microbial diversity was observed in Wt mice (2.2) 48 hr following O_3 exposure, with an expansion in microbial species in *Sftpd -/-* mice (7.5). As the increase in diversity is proposed to occur as a result of the change in growth conditions, we investigated growing bacteria using stable isotope probing. A significant shift in the expanding microbial populations was detected in both strains following O_3 exposure. These data demonstrate that O_3 alters inflammatory cell activation and BAL lipid content and the pulmonary microbiome in a manner that is exaggerated by chronic inflammation. (*p<0.05 vs Wt, #p<0.05 vs air). *Supported by NIH Grants: HL086621, ES004738, ESO05022 and ES029254.*