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


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 Sftpδ which exhibit chronic pulmonary inflammation. C57Bl6/J (Wt) mice and Sftpδ −/− 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 Sftpδ −/− mice (75 ± 2.6%*) relative to Wt (91 ± 1.8%). O$_3$ exposure caused a greater loss of AM in Sftpδ −/− 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 Sftpδ −/− 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 Sftpδ −/− was increased relative to Wt (51 ± 11 µg vs 8.23 ± 18 µg*). Moreover, this was exaggerated following O$_3$ exposure (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 Sftpδ −/− 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 Sftpδ −/− 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.