Exacerbation of Sepsis-Induced Acute Lung Injury (ALI) by Ozone Is Regulated by Macrophage Phenotypic Switching

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Ozone (O_3) is an urban air pollutant known to cause alveolar epithelial barrier damage and altered pulmonary functioning. EPA compliant levels of O_3 are linked to an increased incidence of acute respiratory distress syndrome, a severe form of acute lung injury (ALI). We previously showed that O_3 exacerbates sepsis-induced ALI by promoting neutrophil accumulation in the lung. In these studies, we analyzed the role of resident and inflammatory macrophages in this pathogenic response. Male C57Bl/6J mice were exposed to O_3 (0.8 ppm) or air for 3 h followed 24 h later by i.v. lipopolysaccharide (LPS) (2.5 mg/kg) to model bacterial sepsis or PBS control. Bronchoalveolar lavage (BAL) was collected 24 and 48 h later, and fluid analyzed for markers of lung damage (protein, IgM, phospholipids). BAL cells were magnetically separated based on Cd11b expression and subsets characterized by flow cytometry as resident alveolar macrophages ($CD11b^-$ $CD45^+SiglecF^+Ly6G^-CD11c^+F4/80^+$), inflammatory macrophages ($Cd11b^+CD45^+SiglecF^-$ $Ly6G^-$ $CD11c^-F4/80^+$) and neutrophils ($CD11b^+CD45^+SiglecF^-Ly6G^+CD11c^-F4/80^-$). Lung damage was significantly increased in mice exposed to O_3+LPS at 24 h relative to the other groups, a response correlated with increased numbers of neutrophils. At this time, *Nos2, Il1β, Cxcl1, Ccl2,* and *Ptgs2* mRNA expression was upregulated in resident macrophages. By 48 h, lung damage abated and resident macrophage expression of these genes decreased, while*Il4* and *Arg1* expression increased suggesting a switch from an M1 (proinflammatory) to an M2 (antiinflammatory) phenotype. In O_3+LPS treated mice, infiltrating macrophages increased in the lungs at 24 and 48 h post exposure. Whereas at 24 h, these cells were mainly *Nos2* and *Ptgs2*positive, by 48 h they were *Arg1* positive indicating a similar phenotypic switch from a pro- to an anti-inflammatory phenotype. These results suggest that the O_3 exacerbation of ALI is due to early pro-inflammatory activation of resident and infiltrating macrophages, which release reactive nitrogen species and recruit neutrophils into the lung. Subsequent resolution of injury occurs as a consequence of macrophage phenotypic switching. *Supported by NIH ES004738, ES005022, IRSS Donald E. Gardner Toxicology Education Award.*