

OANO₂ Increases the Likelihood of Survival and Resolution Signaling in Response to Bleomycin-Mediated Lung Injury

Author Block: M. L. Wilkinson, E. Abramova, C. Guo, and A. J. Gow. *Rutgers, The State University of New Jersey, Piscataway, NJ.*

Nitro-oleic fatty acid (OANO₂), a potent electrophile, is endogenously found in humans and modifies cysteine residues via Michael addition to alter protein function. It reduces inflammatory activation in the cardiovascular system, and potentially the lung. Intratracheal bleomycin (ITB) is a model of pulmonary inflammation, resolution, and fibrosis. Earlier work showed a loss of resident alveolar macrophages (AMs) and activation of interstitial macrophages (IMs) in the early phase of the response to ITB (7 days) that was abrogated by OANO₂. Here, we examined the effect of OANO₂ on the resolution phase of the ITB response. ITB (3 U/kg) was administered to animals while OANO₂ was delivered via osmotic pump (1 nmole/g/hr). Bronchoalveolar lavage (BAL) and lung tissue were collected at 17 days post ITB. Our prior studies have shown that OANO₂ alters macrophage activation at 7 days post ITB. Therefore, we examined both BAL and tissue macrophage phenotypes in response to ITB with and without OANO₂ treatment. In the BAL, resident AMs (CD45+, SiglecF+, F4/80+, CD11c+) were lost 7 days post ITB (95 ± 3.9% vs 49 ± 3.9%*) but recovered by 17 days (68 ± 3.5%*). OANO₂ preserved resident AMs at 7 days (69 ± 3.9%*) and increased recovery at 17 days (73 ± 3.8%*). IMs show a similar pattern of activation and resolution. ITB increases the proportion of mature IMs (CD11c+) expressing both Ly6C (43 ± 3.7% vs 13 ± 3.4%*) and mannose receptor (MR) (28 ± 3.6% vs 5 ± 3.4%*) at 7 days; returning closer to control at 17 days (Ly6C+ 16 ± 2.4%; and MR+ 9 ± 1.2%). OANO₂ reduces activation at 7 days post ITB (Ly6C+ 27 ± 2.4%#; and MR+ 13 ± 3.9%#), but perpetuates activation at 17 days (Ly6C+ 28 ± 5.1%#; and MR+ 10 ± 2.3%). These observations are consistent with OANO₂ driving an increased level of resolution. This is confirmed within mesenchymal stem cells (CD45-, CD31-, Sca1+), where ITB increases fibrotic signaling (CD44+) at 7 days (70 ± 4.7% vs 26 ± 4.5%*); while the addition of OANO₂ reduces the early expression of CD44 (58 ± 4.3%#) but potentiates activation at 17 days (CD44+ 69 ± 5.3%#; and CD90+ 23 ± 1.7%#). Mice administered ITB lost a significant amount of bodyweight compared to controls; OANO₂ mitigated this loss at 7 days (-4.0 ± 0.3% vs 0 ± 0.5%#); and this weight loss continued to 10 days post ITB. At this time point mice either stabilized in weight or did not survive. Importantly survival rates were higher in OANO₂ treated animals (80% vs 35% #) at 17 days post ITB. Histological findings in animals sacrificed revealed signs of inflammation, epithelial destruction, and proteinaceous deposits. These findings suggest that OANO₂ administration increases the likelihood of resolution and survival (* p<0.05 vs control; # p<0.5 vs ITB).