PGC-1β Regulates Inflammatory Macrophage Recruitment after Ozone Inhalation in Mice

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Ozone is a ubiquitous urban air pollutant that causes airway inflammation and hyperresponsiveness in both healthy and susceptible populations. Inflammatory macrophages play a key role in ozone-induced lung injury by regulating the acute initiation and later resolution phases of the inflammatory response. These distinct activities are mediated by subpopulations broadly classified as M1/pro-inflammatory and M2/anti-inflammatory which sequentially accumulate in injured tissues. Proper control of the inflammatory response requires a balance between M1 and M2 activity. The transcriptional coactivator PPARγ coactivator-1 β (PGC-1β) modulates the activity of transcription factors involved in M2 activation. We hypothesized that PGC-1β signaling attenuates ozone-induced lung injury by promoting M2 phenotype and resolution of inflammation. For these studies, we utilized a conditional Cre-lox mouse model in which PGC-1β is specifically knocked out in CX3CR1+ macrophages (PGC-1β KO). Wild-type and PGC-1β KO mice were exposed to air or ozone (0.8 ppm, 3 hr) and euthanized 72 hours post-exposure. $CX3CR1^+$ cells were enriched from lung tissue digests by magnetic activated cell sorting and specificity of knockdown confirmed by western blot for PGC-1β in $CX3CR^+$ and $CX3CR1^-$ populations. Phenotypes of $CX3CR1^+$ cells were then characterized using techniques in flow cytometry; results revealed that the majority of $CX3CR1^+$ cells were $Siglec\text{-}F^+/CD11c^+$ alveolar macrophages (69.2%) and $Siglec\text{-}F^-/CD11b^+$ inflammatory macrophages (28.5%). We observed an increase in the number of inflammatory macrophages in both male and female wild-type mice exposed to ozone compared to air controls; this response was diminished in male and female PGC-1β KO mice. We then performed immunohistochemical (IHC) staining of CD11b in lung tissue sections; efforts to semi-quantitate CD11b staining are currently underway. In conclusion, we confirmed that PGC-1β is specifically deleted in $CX3CR1^+$ macrophages, and that these are predominantly resident alveolar and to a lesser extent, inflammatory macrophages. Furthermore, these results suggest that knockdown of PGC-1β impairs recruitment of inflammatory macrophages. Future studies should investigate the mechanism by which PGC-1β regulates inflammatory macrophage recruitment and how this influences ozone-induced lung injury. Supported by R01ES004738, F32ES030984, K99ES032473, R25ES020721, and the ASPET SURF Program.