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ABSTRACT

Ozone is a ubiquitous air pollutant that causes moderate parenchymal stress and monocyte/macrophage inflammation in healthy individuals. These effects are heightened in susceptible populations including the elderly and patients affected by interstitial lung disease (i.e., pulmonary fibrosis, PF). Pulmonary fibrosis is a degenerative disease characterized by progressive disruption of the alveolar architecture interspersed with episodes of acute inflammatory exacerbations. Mutations in the alveolar epithelial type-2 (AT2) cell-specific Surfactant Protein C (SP-C) gene (STFC) have been identified in a subset of PF patients, with the Ile73Thr substitution at position 73 (SP-C173T) as the most prevalent. To investigate the susceptibility of SP-C mutant populations to acute ozone exposure, we leveraged a novel inducible SP-C173T transgenic mouse. Low-level SP-C173T expression produces moderate enlargement of the alveolar septae, AT2 cell hyperplasia, and minor inflammation beginning at 16wk and deteriorating with time. Conversely, SP-C173T induction results in extensive polycellular inflammation, decline in respiratory function and lung remodeling, distinctive features of acute exacerbations. Population RNA-seq and targeted cytokine analysis of bronchoalveolar lavage fluid show that AT2 cells initiate monocyte/macrophage recruitment and activation via canonical (CCR2, CCL2, CX3CL1, and CCL17) pathways during SP-C173T acute exacerbations. Consistent with these findings, RNA-seq analysis of SingleCellFIDb1 CD64 Ly6C+ monocytes indicate highly inflammatory (Il6, Il1b) and pro-fibrotic (TGFb1, Colla2) phenotypes. Pharmacological (intravascular clodronate liposomes) and genetic (Ccr2 knockout mice) monocyte ablation resulted in reduced inflammatory burden and improved survival following SP-C173T exacerbations. Acute low-dose ozone exposure (0.8ppm, 3h) of SP-C173T mice resulted in heightened alveolar septal disruption, edema and perivascular immune cell infiltrate compared to SP-CWT cohorts. These responses were observed in mice undergoing acute exacerbations, as well as aged SP-C173T cohorts (52 eko) expressing low levels of mutant protein. Taken together, our findings highlight the intimate crosstalk between epithelial and inflammatory monocytes during acute exacerbations. In addition, these data support the notion that epithelial dysfunction augments respiratory symptoms induced by ozone exposure.

RESULTS

Figure 2. (A) Enrichment analysis using gene sets from Reactome and KEGG pathway, showing immune associated AT2 transmembrane 3 days post induction. (B) Differential expression of selected genes involved in recruitment and activation of monocyte/macrophages in SP-C173T (left panel) and SP-CWT (right panel) mice 3 days post tamoxifen induction. (C) BAL fluid cytokines involved in monocyte/macrophage recruitment and activation (CCL2, CX3CL1, and CCL17) in SP-CWT (grey dots) and SP-C173T (black dots) mice. All data represents time course post tamoxifen induction.

Figure 3. (A-C) Immunohistochemical staining of SP-C173T (CTL, left panels) and SP-CWT (right panels) mouse lungs stained for (A) CCR2, (B) CX3CR1, and (C) CCR4 3 days post tamoxifen induction. Arrowheads show prominent monocyte/macrophage staining.

Figure 4. (A) Flow cytometric analysis and quantification of BALF for Ly6C+ monocytes from SP-CWT and SP-C173T mice. Absolute numbers left y-axis and relative percentage right y-axis. (B-C) RNA-seq analysis of Ly6C+ monocytes collected from SP-CWT and SP-C173T mice 3 and 14 days post tamoxifen. (D) Top 5000 significant genes clustered by group. (E) Inflammatory and recruitment genes selected from top 19 differentially regulated. (F) Differential expression analysis of 50 selected genes involved in immune cell recruitment, inflammation, and fibrosis from Ly6C+ monocytes from SP-CWT and SP-C173T mice 3 and 14 days post tamoxifen induction.

Figure 5. (A) CCR2 staining of clodronate treated SP-CWT (CLOD), tamoxifen treated SP-CWT mice treated with vehicle (TAM) or clodronate liposome (TAM+CLOD) at 14 days. (B) Kaplan-Meier survival analysis from SP-CWT, TAM, or TAM+CLOD treated mice 14 days post tamoxifen induction. (C) Total BAL cell count recovered from SP-CWT (CTL), SP-CWT+CLOD, or SP-CWT+CLOD+TAM 14 days post tamoxifen induction. (D) HE-stained sections from SP-CWT (CTL), SP-CWT+CLOD, or SP-CWT+CLOD+TAM 14 days post tamoxifen induction.

Figure 6. (A) Dot plot with group mean ± SEM shown of total BAL cells counts from SP-CWT and SP-C173T mice exposed to air or ozone (O3) 5 days post induction BAL collection was performed 7 days post induction. (B) Dot plots with mean ± SEM of relative (%) numbers of neutrophils accumulating in the BAL of SP-CWT and SP-C173T mice exposed to air or ozone (O3) 7 days post tamoxifen induction, BAL collection was performed at 7 days post induction.

Figure 7. (A) H&E staining of SP-C173T mice exposed to air or ozone (O3) 42 days post tamoxifen induction. Tissue collection was performed 43 days post induction. (B) Immunohistochemical INOS staining of SP-C173T mice exposed to air or O3, 42 days after tamoxifen induction. Tissue collection was performed 43 days post induction. Arrowheads show prominent monocyte/macrophage staining in SP-C173T lungs.

CONCLUSION

- SP-CWT injury drives progressive injury and fibrosis, accompanied with lung functions decline (restrictive physiology).
- Epithelial cells release a complex mixture of chemokines associated with monocyte/macrophage recruitment and activation, matched with CCR2-, CCR4-, and CX3CR1- myeloid cells.
- Ly6C+ monocyte analysis reveals highly proinflammatory cell activation at 3 days post induction.
- Immunomodulatory (clodronate) and genetic (CCR2/-) depletion of monocytes is associated with reduced inflammation, tissue injury and improved survival.
- Ozone exposure at a time coordinated with initiation of acute exacerbation is associated with increased inflammation (neutrophilia).
- Ozone exposure at a time coordinated with fibrosis is associated with worsened tissue injury, and proinflammatory cell activation.