

STRONG CHILDREN'S RESEARCH CENTER

Summer 2014 Research Scholar

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ABSTRACT

Title: SERPINE2 Deficiency Is Associated With Alterations In Lung Epithelial Cell Chemokine Expression And Lymphocyte Accumulation

Background: In 2011, it was estimated that 12.7 million adults in the United States have COPD. COPD, or Chronic Obstructive Pulmonary Disease, is one of the leading causes of death in the United States. COPD consists of chronic bronchitis and emphysema, both of which cause the individual to have breathing difficulties. A key feature of the disease is persistent inflammation in the lung, which often is a result of smoking tobacco. The accumulation of large numbers of lymphocytes within the lung, resulting in the pathological formation of bronchus-associated lymphatic tissue (BALT), is a recently-appreciated and poorly-understood feature of COPD. SerpinE2 is a protease inhibitor that inhibits thrombin, and was recently identified as a molecule involved in COPD. In the lung, SerpinE2 is expressed by epithelial cells that line the airways and blood vessel fibroblasts. Prior work by the laboratory found BALT formation in mice deficient in the SerpinE2 gene. The formation of BALT was preceded by increased expression of lymphocyte-recruiting chemokines in the lung.

Objective: To determine the cell type responsible for increased chemokine expression in the lungs of SerpinE2 deficient mice.

Methods: We prepared enriched populations of freshly-isolated epithelial cells or fibroblasts from adult SerpinE2 deficient (KO) (n=4) and control (SerpinE2 heterozygous; n=4) mouse lungs. We analyzed cell lineage-specific marker and chemokine expression at the steady-state mRNA level by quantitative PCR (qPCR). We tested for significant differences in gene expression levels using student's T-Tests and Mann-Whitney U tests.

Results: Analysis of general cell type-specific marker gene expression confirmed enrichment of epithelial and fibroblast cells; epithelial cell marker (Cdh-1 and Epcam) expression was higher in epithelial cells, and fibroblast marker (Vim and Acta2) expression was higher in fibroblasts. Enrichment was more notable when lung-specific cell type markers for epithelial (Scgb1a1 and Sftpc) and fibroblast (Pdgfra and Pdgfrb) cells were examined. When we tested epithelial- and fibroblast-enriched populations for expression of chemokines upregulated in SerpinE2 deficient lungs, we noted most were preferentially induced in SerpinE2 deficient epithelial cells; Cxcl9 (1.9 vs 29.8, P<0.05), Cxcl10 (2.6 vs 8.7, NS), Cxcl13 (1.2 vs 4.3, NS), Ccl20 (6.1 vs 20.6, P<0.05), Ccl21b (1.9 vs 7.3, P<0.05), Ccl19 (3.1 vs 18.4, NS) and Ccl17 (2.8 vs 5, NS). Chemokine expression in SerpinE2 deficient fibroblasts was either similar, or reduced, as compared to controls.

Summary: Our results suggests that SERPINE2 deficiency results in dysregulated lymphocyte attracting chemokine expression, and that epithelial cells play a major role in this process. This dysregulation might have contributed to the COPD-related BALT- like lesion formation in aged SerpinE2 deficient mice.