Loss of C/EBPα in Chronic Cigarette Smoke Exposure: A SAD Day for Chronic Obstructive Pulmonary Disease

Tobacco or cigarette smoke (CS) exposure is a main driver of chronic obstructive pulmonary disease (COPD), a progressive disease involving small airway disease (SAD) with airway remodeling and fibrosis, and loss of alveoli and terminal bronchioles (1, 2). Human studies have identified pathology of small airways <2 mm of diameter as the major contributor to increased airflow resistance in COPD (1). In addition, CS exposure is associated with development of centrilobular emphysema (CLE). CLE is distinct from panlobular and paraseptal emphysema (PSE), the latter being more common in alpha-1 antitrypsin deficiency (2). Emphysema pathology is defined by irreversible enlargement of the peripheral airspaces distal to the terminal bronchioles (3, 4). In smokers, alveolar attachments (the radial attachment of alveolar walls to the small airways) are decreased (5), promoting destabilization of the small conducting airways (6). Abnormalities in the small airways can occur before emphysema in COPD; for example, CLE in COPD was found to be preceded by narrowing and loss of terminal bronchioles (5). Unfortunately, significant progression of SAD typically occurs without clinically detectable impairments of lung function in individuals (7), with an estimated 75% loss of small conducting airway as a requirement before airflow limitations could be detected through routine pulmonary function assessments (8). Recent advances in imaging techniques have improved the ability to detect the loss of small conducting airways, and highlight the association of SAD to lung function decline in mild and moderate COPD (5, 9).

Lung destruction and pathogenesis of COPD and emphysema can be driven by disrupted balance in the levels of proteinases and antiproteinases, most clearly demonstrated by alpha-1 antitrypsin deficiency, which is responsible for protection from neutrophil-derived serine proteases, such as neutrophil elastase (10, 11). Although recent reviews have highlighted the role of inflammation-induced protease activity in eliciting extracellular matrix destruction (1, 2), the cellular and molecular mechanisms regulating the development of protease/antiprotease imbalance during SAD is not fully understood.

In this issue of the *Journal*, Uemasu and colleagues (pp. 67–78) bring us one step closer to understanding potential signaling pathways involved in CS-induced SAD by examining the role of the transcription factor, C/EBPα (CCAAT/enhancer binding protein-α) in maintaining distal airway homeostasis and response to CS exposure in mice (10). Previous work established C/EBPα as critical for alveolar type 2 cell maturation during lung development (4, 12, 13), and selective deletion of C/EBPα under the Scr promoter decreased alveolarization, resulting in pulmonary immaturity resembling bronchopulmonary dysplasia in infants (14). At maturity, the lung histopathology from these mice resembled COPD, including bronchiolitis, mucus plugging, and emphysema, suggesting both a developmental and destructive process (15). The potential involvement of C/EBPα in human COPD was previously defined by reduced C/EBPα activity in airway epithelial cells of smokers with COPD compared with smokers without COPD, suggesting that C/EBPα may confer a protective benefit (15).

The current study investigates how deletion of C/EBPα modifies CS-induced epithelial changes. The contribution of C/EBPα to cell differentiation in response to chronic CS exposure was examined with an inducible Cre recombinase system driven by the *Scgb1a* promoter to delete C/EBPα in club cells, and a lineage-tracing model to express enhanced GFP (eGFP) in both club and alveolar type 2 cells. CS exposure did not alter the number of club and ciliated cells in the distal airways of wild-type mice, but there was an increase in $eGFP^+$ ciliated cells, suggesting that CS exposure induced a compensatory regeneration of ciliated cells. In contrast, absence of C/EBPα reduced the number of ciliated cells in the distal airways of CS-exposed mice, with a reduced number of $eGFP^+$ ciliated cells and shortened cilia, suggesting a role for this transcription factor in ciliated cell regeneration. C/EBPα participation in maintaining epithelial barrier function in response to challenge with CS was discovered, as deficient mice had reduced levels of the adhesion molecule, Zo-1 (zonula occuldens-1) protein and greater protein leak into the alveolar compartment. Development of airspace enlargement in C/EBPα-deficient mice independent of CS exposure suggests that C/EBPα contributes to age-dependent emphysema, as previously reported (15). In addition, C/EBPα was important for the maintenance of alveolar attachment in response to CS exposure.

Protease activity was increased in CS-exposed C/EBPα-deficient mice compared with wild type, accompanied by reduced expression of the antiserine proteases, *Spink5* (serine peptidase inhibitor kazal type 5) and *SerpinD1* (serpin family D member 1), in distal airway epithelium. These results indicate that C/EBPα is an important transcription factor involved in maintaining the balance of serine proteases and their inhibitors in distal epithelium in response to CS. Importantly, the observed phenotypes of loss of alveolar attachments, barrier dysfunction, and ciliated cell regeneration in CS-exposed C/EBPα-deficient mice could be rescued by treatment with serine protease inhibitors.

To translate the murine results to human emphysema, lung biopsies were obtained from former smokers who were subtyped as nonemphysema, CLE, or PSE dominant based on computed tomographic scans. In these samples, C/EBPα was detected in both

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airway and alveolar epithelial cells, with significantly reduced *CEBPA* (CCAAT/enhancer binding protein α) mRNA levels in PSE-dominant disease and a trend for reduced mRNA levels in CLE-dominant disease compared with nonemphysema. *CEBPA* levels were significantly associated with *SPINK5*, but not *KLK13* or *SERPIND1*, in divergence from the murine model. Abundance of LEKTI (*SPINK5* protein) was reduced in the small airways of lungs from the CLE group compared with the PSE-dominant and nonemphysematous groups, suggesting that decreased C/EBPα may be associated with emphysema and decreased levels of the antiserine protease, LEKTI, in CLE-dominant disease. Whether these associations reflect regional differences in antiprotease expression that may contribute to COPD heterogeneity versus limitations due to regional sampling of tissue and small sample size among the groups remains uncertain. However, the murine studies link loss of epithelial C/EBPα to increased susceptibility to SAD in response to chronic CS that is dependent on increased serine proteinase activity. Future challenges for this area of investigation will be to use human tissue to further identify and validate changes in these pathways among nonsmokers without lung pathology and smokers with and without SAD and emphysema. These types of studies will provide more significantly relevant data on par with the findings presented by Uemasu and colleagues. Validated therapeutic targets that can be used before SAD has resulted in significant nonreversible lung damage will be of interest for future clinical development. Overall, this study advances the understanding of the pathogenesis of SAD in COPD, and provides new insight to potential strategies to alter susceptibility. ■

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