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Letting It All Out: Neutrophils in Early Cystic Fibrosis Airway Inflammation

Cystic fibrosis (CF) lung disease is characterized by a vicious cycle of mucus secretion, airway infection, and inflammation. Neutrophils are the primary inflammatory cell involved in this process and are recruited from the blood into the airway lumen early in the disease process, as demonstrated by BAL studies performed in infants with CF (1, 2). These neutrophils contain an array of inflammatory mediators, oxidants, and proteases that are critical for antimicrobial defense. Large amounts of these enzymes escape from neutrophils in cell death and during phagocytosis, and directly damage the airway epithelium. Another mechanism is the release of enzyme through exocytosis, but the mechanisms that control the degranulation and release of these enzymes are less well understood (2). One particular enzyme, neutrophil elastase (NE), is capable of digesting diverse proteins and contributes to the progression of structural lung disease (3, 4). Higher levels of free extracellular NE in sputum have been shown to predict subsequent lung function decline (5). The antiprotease defenses in the airways are designed to neutralize free proteases such as NE and prevent their damaging effects. However, these defenses are eventually overwhelmed and degraded by the protease burden in the lung (6).

In this issue of the *Journal*, Margaroli and colleagues (pp. 873–881) advance our understanding of neutrophilic inflammation in the early CF lung (7). This cross-sectional study included children with CF under 3 years of age with evidence of early changes of CF lung disease as measured by the computed tomography (CT) Perth-Rotterdam annotated grid morphometric analysis for CF (PRAGMA-CF) scoring method, but virtually no bronchiectasis. As expected, BAL fluid (BALF) samples obtained the same day the CT was performed showed neutrophilic inflammation. Findings in the CF cohort were compared with disease control subjects recruited from the Aerodigestive Clinic who were undergoing bronchoscopy for a clinical indication and also showed evidence of neutrophilic inflammation, albeit less marked than that observed in the CF group. The airway neutrophils in individuals with CF demonstrated increased expression of surface markers reflecting hyperexocytosis of NE-rich granules into the airway lumen. This phenotype was seen in the airway neutrophils but not in blood neutrophils, and was not observed in control patients. This suggests that the inflammatory milieu of the CF airway recruits neutrophils from the blood and stimulates them to adopt an activated state. This neutrophil phenotype with hyperactive exocytosis of NE-rich granules correlated with early structural lung damage by CT coupled with the PRAGMA-CF scoring system. Therefore, this distinguishing feature of neutrophilic airway inflammation in CF could potentially be a key process in early CF lung disease.

By contrast, other biomarkers of neutrophilic inflammation, such as the BAL neutrophil percentage and free extracellular NE activity (measured by a sensitive Förster resonance energy transfer–based assay) did not correlate with structural changes on CT. This is contrary to previously reported work of AREST-CF (Australian Respiratory Early Surveillance Team for Cystic Fibrosis) group regarding the role of free NE, which predicted the subsequent development of bronchiectasis (4, 8). Potential reasons for this discrepancy may be the use of a more sensitive assay for free NE in the current study compared with the group's previous studies (4, 8), and the comparison of free NE with the sensitive PRAGMA-CF score, which has been reported to be more sensitive for detecting early structural lung abnormalities (9). In addition, the cross-sectional design of the current study, with structural changes detected at a single time point, may not be reflective of the impact of released NE and the dynamics of airway inflammation over time.

Although the data on neutrophil exocytosis are novel, this study has limitations. First, this was not a specifically designed prospective study; patients were drawn from different cohorts with different inclusion criteria. This may have resulted in a more heterogeneous study population and introduced variability into the data. Second, the control group was small and contained subjects with a mixture of underlying diagnoses. Ideally, individuals with CF would be compared with patients with a disease process also characterized by neutrophilic airway inflammation and structural lung damage over time, such as primary ciliary dyskinesia. Third, clinical data such as the temporal relationship between testing and episodes of increased respiratory symptoms, antibiotic use, respiratory microbiology, or functional measures of lung function (e.g., the lung clearance index) are not available. Fourth, BALF only reflects the inflammatory process the airway lumen and not in the airway wall, where structural remodeling takes place. Finally, surface expression assessed by flow cytometry may not be a direct representation of exocytosis, and a functional assay may be better suited to reflect the impact of exocytosis on the inflammatory process in the airways.

Biomarkers to detect neutrophilic inflammation could be useful for tracking the progression of airway pathology over time and be included as outcome measures in interventional trials. The results of this study raise the question as to whether neutrophil exocytosis could serve as a biomarker for airway inflammation in CF. However, bronchoscopy for BALF is an invasive procedure and impractical for

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repeated measurements in the clinical setting. A noninvasive test of neutrophil exocytosis would be required before this biomarker could be translated to the clinical setting.

Neutrophil exocytosis–specific inhibitors with antiinflammatory activity have been developed and tested in animal models (10). Could these small-molecule drugs have therapeutic potential in CF lung disease? Although this is an enticing prospect, our understanding of the process of neutrophil degranulation is still in its infancy; therefore, it is difficult to predict whether the neutrophil activation process would be an appropriate therapeutic target. If the major driver for enzyme release is cell death rather than exocytosis from live neutrophils, then pursuing the antiprotease shield mechanism would likely be a more successful approach. Ultimately, longitudinal studies directly assessing neutrophil degranulation will be required to determine exactly how NE and other neutrophil-derived products damage the airways, to better define the best targets for antiinflammatory treatment in patients with CF. ■

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Associations between Ozone and Fine Particulate Matter and Respiratory Illness Found to Vary between Children and Adults

Implications for U.S. Air Quality Policy

Exposure to particulate matter and ozone has severe repercussions on public health in the United States and globally. Fine particulate matter (or $PM_{2.5}$, particulate matter with aerodynamic diameter of 2.5 μm or less) has been associated with exacerbations of asthma and chronic obstructive pulmonary disease, cardiovascular disease mortality, and lung cancer (1–4). Ozone exposure has been linked to increased risks for asthma exacerbations and other respiratory illnesses, as well as to cardiovascular disease mortality (5, 6). Fossil fuel combustion–related emissions of particulate matter and precursors of ozone have been estimated to cause approximately 210,000 premature deaths annually in the United States (7). Further, combustion-related emissions that affect air quality also are the major drivers of climate change. There is new evidence that the effect of climate change on wildfires could double the

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