

An Imbalance between Proteases and Endogenous Protease Inhibitors in Eosinophilic Airway Disease

Eosinophilic chronic rhinosinusitis (ECRS) and asthma were both long thought to be allergen-driven diseases given the prominent “Th2-type” inflammation in the majority of cases and association with other diseases of the “atopic march.” A major flaw with this scenario has always been that a substantial proportion of patients with these diseases, and in particular many of those with the most severe pathology, do not exhibit evidence of allergen sensitization on skin-prick testing or specific IgE testing (1, 2). Although this observation does not exclude a possible role for T-helper lymphocyte type 2 (Th2) cytokines and IgE, allergen-specific or otherwise, in both diseases, the past decade has seen a change in our mechanistic understanding of the immunopathology underlying eosinophilic airway disease from a paradigm in which allergen-specific, Th2 lymphocytes are the primary disease drivers to one in which production of cytokines by damaged respiratory epithelium is the primary driver for eosinophilic responses and possibly, but not inevitably, local IgE synthesis. These cytokines—in particular IL-25, IL-33, and thymic stromal lymphopoietin (TSLP)—act to stimulate production of Th2-type cytokines from innate lymphoid cells in an antigen-independent manner, as well as from Th2 lymphocytes (3, 4). In addition, there is evidence, at least from animal surrogates, that these cytokines can affect many of the aspects of airway remodeling deemed characteristic of asthma, such as neoangiogenesis (5). A range of both endogenous, local stimuli as well as external, environmental stimuli can elicit epithelial release of these cytokines—one such category of stimuli is endogenous and environmental proteases. It is of particular interest that certain common, inhaled aeroallergens exhibit such protease activity. As with other immune pathways, checkpoints and counterbalances exist to regulate these powerful proinflammatory pathways and prevent pathological inflammation. Of particular importance in this regard appear to be endogenous protease inhibitors (EPIs). In ECRS and certain asthma endotypes it appears that a failure to regulate these pathways, leading to an imbalance between airway proteases and protease inhibitors, may be central to the disease pathology.

The study presented by Kouzaki and colleagues in this issue of the *Journal* (pp. 737–747) advances our understanding of the significance of this imbalance between inhaled proteases and EPIs in patients with ECRS, using a comprehensive series of experiments with *ex vivo* patient samples and a range of immunological models (6). In particular, they studied the EPIs cystatin A, a cysteine protease inhibitor, and serine protease inhibitor Kazal type 5 (SPINK5). They discovered lower expression of these EPIs in nasal tissue samples from patients with ECRS as compared with those from patients with noneosinophilic chronic rhinosinusitis or healthy control subjects. In cell culture experiments using cultured, human bronchial epithelial cells they went on to show that recombinant EPIs reduced the release of IL-25, IL-33, and TSLP by these cells after exposure to two common aeroallergens with protease activity,

Alternaria and house dust mite, and protease from *Staphylococcus aureus*, a microbe commonly carried in the respiratory tract. Conversely, small interfering RNA knockdown of SPINK5 and/or cystatin A augmented the release of these cytokines in response to the protease stimuli.

In addition, they studied a murine surrogate of acute and chronic protease exposure involving intranasal instillation of a protease cocktail of both allergens and the staphylococcal protease. Treatment of the animals with recombinant EPIs reduced nasal tissue concentrations of TSLP, IL-25, and IL-33 produced in response to a single acute challenge with the protease cocktail. On chronic regular exposure of the animals to this protease cocktail, addition of recombinant EPIs again reduced multiple indices of Th2-type airway inflammation. In the absence of recombinant EPIs, nasal concentrations of the endogenous EPIs cystatin A and SPINK5 initially appropriately increased after exposure to the cocktail of exogenous proteases, but more prolonged exposure resulted in a significant decrease in the concentrations of both endogenous EPIs compared with control animals. This suggests an environmental scenario in which excessive exposure to proteases may in certain individuals exhaust innate defenses, resulting in the triggering of local inflammation and remodeling at least initially through innate, antigen-independent mechanisms.

Fascinating though these findings are, they beg but do not address the more fundamental question of which problem comes first: impaired EPI expression resulting in greater susceptibility to environmental proteases or excessive exposure to such proteases eventually exhausting “normal” EPI expression. Both scenarios might of course coexist. Cellular regulation of EPI expression itself is poorly understood. Netherton syndrome, due to inheritance of loss-of-function mutations in both copies of *SPINK5*, causes dermatological pathology and also elevated IgE levels, often with eosinophilia; however, such mutations are rare (7). Importantly, there are associations between polymorphisms in EPIs such as *SPINK5* and asthma, and these polymorphisms may provide a small contribution to the etiology of these eosinophilic airway diseases (8, 9). Nevertheless, it seems more likely that excessive exposure to environmental factors explains marked reduction in airway concentrations of EPIs in patients with chronic, eosinophilic airway diseases. In particular, chronic exposure to proteases appears to cause aberrant decreased concentrations of airway EPIs through an unknown mechanism that may be sensitive to further manipulation and be amenable to future pharmacotherapy.

Although some inhaled aeroallergens have protease activity, it is notable that the authors’ cocktail included a bacterial protease. There is a well-recognized association between nasal carriage of *S. aureus* and both severe asthma and severe rhinitis, and suggestive evidence that staphylococcal proteases may underpin this association (10, 11). Many other microbes have been shown to be capable of producing proteases, for example, *Aspergillus*

fumigatus (4). Interestingly, nasal inflammatory responses to *Alternaria* have been described in individuals with negative skin-prick test results (12); such responses might be mediated by proteases, rather than IgE. One might speculate that microbes colonizing the airways can chronically produce proteases that drive “Th2-type” inflammation rather than causing conventional clinical infection. If this is the case then topical antimicrobials might have a role in treating diseases such as asthma and ECRS even in patients without evidence of conventional infection.

Many of the individuals with ECRS in the study by Kouzaki and colleagues were indeed nonatopic, and it would have been interesting to know whether either atopic status or nasal carriage of *S. aureus* influenced nasal concentrations of EPIs within their ECRS subgroup.

Ultimately, one may envisage a future in which manipulation of EPIs and/or protease inhibition may provide a novel therapeutic option in eosinophilic airway diseases (13), perhaps with measurement of sputum protease activity used as a biomarker to titrate therapy. ■

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Finding the Missing Millions: Can a New Questionnaire Help to Detect Undiagnosed Chronic Obstructive Pulmonary Disease?

In this issue of the *Journal*, Martinez and colleagues (pp. 748–756) report the development of a new and simple case-finding methodology for chronic obstructive pulmonary disease (COPD) using a short questionnaire and peak expiratory flow (PEF) measurement (1). The authors propose that this new methodology will support clinicians in finding COPD cases in clinical practice who could benefit from a COPD diagnosis and the current available treatments. Case finding is recommended in the literature and by the U.S. Preventive Services Task Force, in contrast to population-based screening (2).

This work is based on discussions at an NHLBI workshop on the topic of COPD case finding that suggested efforts are needed to identify undiagnosed patients who are most likely to have improved outcomes from currently available therapies (3). In this cross-sectional case-control study, which was performed in a number of sites in the United States, cases were defined as COPD when they had obstruction according to guidelines and either one exacerbation in the past year or an FEV₁ less than 60% predicted or both. This means that the authors concentrate on the more severe cases, on the assumption that patients with mild obstruction and

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