An imbalance between proteases and endogenous protease inhibitors in eosinophilic airways disease

Editorial for: "Endogenous Protease Inhibitor in Airway Epithelial Cells Contribute to Eosinophilic Chronic Rhinosinusitis" manuscript number Blue-201603-0529OC.R2

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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 most severe pathology, do not exhibit evidence of allergen sensitisation on skin-prick testing or specific IgE testing (1) (2). While this observation does not exclude a possible role for 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 where allergen-specific, T helper type-2 (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 TSLP - act to stimulate production of “Th2-type” cytokines from innate lymphoid cells (ILCs) 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 effect many of the aspects of airways remodelling 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 counter-balances exist to regulate

these powerful pro-inflammatory 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 airways proteases and protease inhibitors, may be central to the disease pathology.

Kouzaki and colleagues in their article in this issue advance 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 SPINK5, a serine protease inhibitor. They discovered lower expression of these EPIs in nasal tissue samples from patients with ECRS as compared to those with non-eosinophilic chronic rhinosinusitis (NECRS) or healthy controls. 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 following 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, siRNA knockdown of SPINK5 and/or cystatin A augmented the release of these cytokines in response to the protease stimuli. They additionally 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. Upon chronic regular exposure of the animals to this protease cocktail, addition of recombinant EPIs again reduced multiple indices of Th2-type airways inflammation. In the absence of recombinant EPIs, nasal concentrations of the endogenous EPIs cystatin A and SPINK5 initially appropriately increased following 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 controls. This suggests an environmental scenario in which excessive exposure to proteases may in certain individuals exhaust innate defences, resulting in the triggering of local inflammation and remodelling 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 co-exist. 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 an 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 aetiology of these

eosinophilic airways 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 airways 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-recognised association between nasal carriage of *Staph. 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 tests (12); such responses might be protease-, rather than IgE-mediated. One might speculate that microbes colonising 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 of Kouzaki and colleagues were indeed non-atopic and it would have been interesting to know whether

either atopic status or nasal carriage of *Staph. 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 airways diseases (13), perhaps with measurement of sputum protease activity used as a biomarker to titrate therapy.

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