

Mite allergens: significance of enzymatic activity

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Ten years ago, the cloning and sequencing of a cDNA encoding the group I allergen of house-dust mites unequivocally determined that protein allergens may have biochemical functions in addition to their ability to bind IgE. Since this discovery, several groups have speculated that the biochemical activities of allergens, or substances associated with allergens, may be involved in their immunogenicity or allergenicity. This paper will focus on just one biochemical function, proteolytic activity, and will be illustrated by examples of our own work that we believe support the hypothesis that this category of molecules are endowed with the properties of proallergic adjuvants.

Key words: adjuvant; Der p 1; enzyme; IgE; proteolytic.

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Scientists and clinicians view allergens as immunogenic IgE-binding proteins that provoke unwanted symptoms. From the viewpoint of the organisms that actually produce allergens, however, these proteins exist only to function as enzymes and structural or regulatory proteins (1; reviewed in Ref. 2). The exception is the allergens of parasites that may modulate, and thus subvert, host protective immune effector mechanisms. The biochemical functions of allergens have been extensively catalogued and researched by Stewart & Thompson (2). They and others, including ourselves, have considered the role and function of allergens from the viewpoint of both the allergen-producing organism and the scientist, and speculated that biochemical function, and proteolytic activity in particular, may be related to allergenicity.

Proteinases as proallergic adjuvants

Not all allergens are proteinases. In a recent review, only a small number of environmental

allergens were unequivocally identified as proteinases (2). Surely, therefore, the rarity of proteinase allergens brings into question the hypothesis that proteolytic activity may be an important feature of allergenicity?

With the exception of workplace or therapeutic exposures, allergens are never naturally encountered in a pure form. Instead, allergens are delivered to a target organ along with many other nonallergenic substances that are associated with the allergen-producing organism. Accordingly, allergen-producing organisms including the higher plants, bacteria, fungi, insects, mites, and nematodes release proteinases alongside their allergens. Therefore, it is almost inconceivable to imagine instances in which environmentally encountered allergens are delivered to the target organ of an individual in the absence of proteinases. This suggests that for risk assessment purposes, in addition to the IgE-binding characteristics of an allergen, the potential *pro-allergic adjuvancy* of the nonallergenic substances

associated with allergens should be taken into account.

Der p 1, a proallergic adjuvant of house-dust mites

At least four of the major *Dermatophagoides pteronyssinus* house-dust mite allergens are proteinases: Der p 1 is a cysteine proteinase and Der p 3, 6, and 9 are serine proteinases (2). These proteins thus fulfil the criteria of both allergens and proallergic adjuvants.

For our studies, we have immunoaffinity-purified Der p 1 from cultured mites and characterized its activity and cleavage site preference (3, 4). Contrary to expectations, we found that Der p 1 exhibited a mixed cysteine-serine proteinase activity. Active site purification of the proteinase confirmed this finding, and led us to suggest that Der p 1 may either exist in more than one mechanistic form, or possess an unconventional binding site. Given the predicted structure of the Der p 1 proteinase (5), we considered the latter option unlikely, and sought to demonstrate the existence of proteolytically distinct, but immunologically related Der p 1 proteinases by mapping the cleavage site preferences of the two activities in the insulin B chain, a model peptide substrate (3). These experiments concluded that the cysteine and serine proteinase activities cleaved at distinct and unrelated sites in the insulin B chain, supporting our contention that more than one mechanistic isoform of the proteinase allergen exists. It is tempting to speculate that the polymorphisms identified by others in Der p 1 (6) may account for our observations of heterogeneity in the activity and cleavage site preference of Der p 1.

Potential targets of proallergic proteolytic adjuvants

We have used purified Der p 1 to illustrate three points of an allergic immune response at which we believe a proteolytic, proallergic adjuvant may promote allergic immunity:

- 1) disturbance of the IgE network
- 2) interaction with viral confounders of allergy
- 3) polarization of T-helper cells to a dominant Th2 phenotype.

Disturbance of the IgE network

The IgE network describes the interactions between surface membrane and released IgE, and IgE receptors and their counterreceptors that

regulate the production of IgE (reviewed in Ref. 7). Central to this regulatory network are the activities of the low-affinity receptor for IgE, CD23. CD23 is expressed constitutively by B cells and is inducible on many other cell types. The CD23 monomer is a 45-kDa type II integral membrane protein, atypical of Ig receptors in that it belongs to the C-type lectin, asialoglycoprotein receptor family. Membrane CD23 (mCD23) probably exists on the cell surface as a homotrimer, with each monomer composed of a single extracellular carbohydrate recognition domain, supported by a coiled-coil stalk. Its expression is linked with inflammatory disease, and it is released from the cell surface, possibly by a metalloproteinase, to yield a soluble CD23 molecule (sCD23). Subsequent proteolytic events break sCD23 into smaller fragments, the larger of which probably retains an oligomeric structure. In addition to IgE, human CD23 also binds to CD21 and CD11b/c. Cell-surface CD23 has been proposed to control IgE synthesis by mediating feedback inhibition after binding IgE. In contrast, the cytokine-like soluble fragments of CD23 may enhance IgE synthesis by binding CD21 and membrane-bound IgE (7).

Knowing that CD23 is released from the cell surface by a proteolytic mechanism, and that the released fragment may enhance IgE synthesis, we have demonstrated that the proteolytic activity of Der p 1 cleaves CD23 from the cell surface of an Epstein-Barr virus-transformed B cell line (3). As before, we found that the proteolytic activity leading to the cleavage of CD23 was sensitive to inhibitors of both serine and cysteine proteinases, but not inhibitors of metallo- and aspartic proteinase inhibitors. Cleavage of CD23 by Der p 1 was highly selective, as no other cell-surface molecule tested, including HLA-DR, HLA-DP, HLA-DQ, surface membrane Ig, or CD20, was cleaved from the cell surface by Der p 1. This suggests that one aspect of the proallergic adjuvancy of Der p 1 may be its ability to disregulate the IgE network by removing a cell-surface transducer of IgE feedback inhibition while simultaneously generating soluble CD23 that may enhance IgE synthesis (3). This hypothesis, however, is difficult to analyse further *in vitro*, as current IgE-synthesis assays cannot unequivocally assess the role of additional sCD23 or mCD23 agonists and antagonists. This is due to the culture conditions required for the assays that have to include CD23-inducing substances such as interleukin (IL)-4 and CD40L throughout an 18-day culture period. Furthermore, the possibility that the cultured human B cells may immunologically recognize the proteolytic allergen could seriously confound the interpretation of results.

Interaction of proteinases with viral confounders of allergy

Several common cold viruses are associated with severe exacerbations of asthma. There is increasing evidence, however, that certain common cold viruses, such as respiratory syncytial virus (RSV), may also be important in the *development* of asthma.

Sigurs et al. (8) have examined the contribution of a family history of allergic asthma and RSV bronchiolitis to the risk of infants developing allergic asthma by the age of 2 years. None of the subjects without a family history of asthma who had not suffered from bronchiolitis went on to develop asthma. Just over 10% of those with no family history of asthma, but who had suffered an episode of bronchiolitis, developed asthma. However, over 50% of subjects with both a family history of asthma and a medical history of bronchiolitis, went on to develop asthma by 2 years of age. However, it is not yet known whether an association between allergic asthma and virus infection can be attributed to the proallergic adjuvancy of allergens on sensitization events.

To answer this question, we have examined the effect of Der p 1 upon viral replication and infectivity in human airway epithelial cells. We have found that a brief treatment of the cells with Der p 1 followed by infection with RSV causes a significant increase in the infectivity or replication of the virus. We are currently analysing this phenomenon to determine which of the above mechanisms accounts for this effect. Potential mechanisms include the hypothesis that damage or activation of epithelial cells by respiratory virus infection or proteolytic allergens may enhance the paracellular permeability of the airway epithelium to allergens (see Ref. 9 for an excellent review; 10). Activation of epithelial cell cytokine production, or the induction of virus receptors on cells by viral infection may also induce inflammation and enhance viral replication, thus increasing the permeability of the epithelium to allergens (10). Alternatively, the mechanism may involve the processing of the viral fusion protein by proteolytic allergens, increasing viral infectivity and subsequent activation and permeability changes (11).

Polarization of T-helper cells to a dominant Th2 phenotype

For several years, evidence has been accumulating to support the hypothesis that the type of antigen a memory T cell responds to, and the cytokines in the environment surrounding developing naïve T-helper cells are critical for determination of the

cytokine profile that a T cell expresses as an end-stage effector cell (reviewed in Ref. 12). Bacterial antigens and allergens thus appear to promote the development of Th1 and Th2 cells, respectively; and cytokines such as macrophage IL-12 promote the development of Th1 cells, while IL-4 promotes the development of Th2 cells. More recent analysis suggests that development of polarized Th cells may be dependent not on the *antigenicity* of a bacterial protein or allergen, but upon factors related to the *adjuvancy* of the antigen, or substances delivered with the antigens. In the case of a Th1-dominated response to bacterial infection, potential adjuvants could include macrophage activators such as bacterial lipopolysaccharides or bacterial DNA (13). Potential adjuvants important in the Th2-dominated responses to allergens include proteinases (14). The cellular target of proteinases in this activity may be mast cells and basophils that can be triggered by a number of natural, non-IgE-dependent stimuli to release the proallergic cytokines IL-4 and IL-13. Therefore, we and others (15) have proposed that this type of event creates an IL-4-rich milieu in which allergen-specific naïve T-cell populations may be skewed toward Th2 dominance. To examine this proposal, we have examined the proteinase allergen-induced, non-IgE-dependent release of IL-4 and IL-13 from a human basophil cell line KU812, and shown that proteinases induce the transcription of the IL-4 and IL-13 genes and the synthesis of IL-4 and IL-13 protein. This effect is inhibited by proteinase inhibitors, demonstrating that the cytokine release was caused by a proteolytic event.

Summary

The above examples illustrate how the nonantigenic properties of an allergen, and nonallergenic substances delivered with allergens may determine the quality of immune response generated. The adjuvancy of allergens, or substances associated with them is therefore an essential factor that should be taken into account when making risk assessments.

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