

Showcase on Research

Allergenic Peptidases: Enzymatic Activity and Immunogenicity

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Introduction

Allergic diseases such as asthma and allergy are particularly prevalent in Western societies and, because of this, much attention has been directed to the study of the many allergen sources known to be associated with their pathogenesis. Some of the most important allergen sources include pollens, animal danders, insects and mites, as well as a number of occupational sources. Each comprises many components but, generally, only the proteins contained therein (referred to as allergens), stimulate the production of immunoglobulin IgE. In allergic diseases, this IgE binds to specific receptors on mast cells present in target organs such as the lung and, on subsequent contact with allergen, induces mast cell degranulation and release of biologically active mediators which initiate disease in susceptible individuals.

The introduction of molecular biological techniques has had a profound impact on our knowledge of the proteins involved in allergic disease, and the structure and function of most of them have been delineated (1). We now know that allergens belong to previously recognised groups of proteins including enzymes, enzyme inhibitors, transport proteins and regulatory proteins. Of particular importance to this review are the group of allergens known to possess peptidase activity. These enzymes are produced by dust mites, cockroaches, fungi, and bacteria, and are of interest not only because of the frequency with which they are recognised by allergic individuals but also because of the possibility that peptidase activity contributes to the development of allergenicity (reviewed in refs 2 and 3). In this review, we briefly

describe the allergenic peptidases and discuss ways in which peptidase activity might influence allergenicity, based on findings from our laboratory and elsewhere.

Origin of allergenic peptidases

The majority, if not all, of the peptidases possessing allergenic potential are found in dusts generated in the home or work place. The function and origin of the allergenic peptidases in domestic dusts varies according to the source, but the majority of the clinically important allergens, for example, peptidases from arachnids (mites) and insects, are digestive enzymes secreted by epithelial cells derived from the gut wall. These enzymes become entrapped within a peritrophic membrane formed around both cells and food particles, and subsequently excreted as faecal pellets into the environment. Other peptidases such as those derived from fungi and bacteria, and possibly cat, are secreted into their environment due to their involvement in growth or spore germination. Occupational allergens may be similarly derived, for example, in industries where insects are reared, or contained within dusts where peptidases (e.g. papain and subtilisins) are used in the pharmaceutical and laundry detergent manufacturing industries (reviewed in refs 1 and 4).

Structure of allergenic peptidases

Clinically, the most important peptidases are derived from house dust mites such as *Dermatophagoides pteronyssinus*. They include several serine peptidases as well as a cysteine peptidase (reviewed in ref 1), and all belong to

previously described peptidase superfamilies on the basis of marked sequence homology and substrate preferences (Table 1). In the allergy literature, they are named in accordance with recommendations of the Allergen Nomenclature Sub-Committee of the IUIS, where the first 3 letters of the genus name is combined with the first letter of the species name and a number corresponding to either its importance or discovery sequence. For example, the cysteine peptidase allergen from *D. pteronyssinus* is denominated Der p 1 and is similar in structure to papain. The serine peptidases are denominated Der p 3, Der p 6 and Der p 9, and correspond to trypsin, chymotrypsin and a collagenase-like enzyme, respectively.

Other allergenic peptidases include the Bla g 2 allergen from German cockroach which shows sequence similarity to members of the aspartate peptidase family (Table 1) (reviewed in ref. 5). More specifically, it is related to the pregnancy-associated glycoproteins (PAG) found in cow, sheep and horse. Most do not appear to possess peptidase activity, including the cockroach allergen, but all possess the characteristic bilobed aspartate peptidase structure capable of binding peptide ligands including the aspartate peptidase inhibitor, pepstatin. Peptidase allergens have also been described in fungi (e.g. Asp f 13, Pen c 2), cat dander (Fel d 1) and bacteria (Table 1) (1, 6, 7). Although our understanding of the function of the cat allergen is limited, the fungal allergens are similar in structure to the bacterial subtilisin allergens, which represent important occupational allergens in the detergent manufacturing industry.

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Potential immunological consequences of peptidase activity

A major driver for characterising allergens has been to establish how and why they promote allergy, given that individuals are exposed to minute quantities annually (ng/year). Immunogenicity (equivalent to allergenicity) has previously been considered to be dependent upon properties such as size of antigen, dosage and route of exposure (1). However, more recently, the role of inherent biochemical activity mediated directly or via a group of receptors known as pattern recognition receptors (PRR) has received attention (1-3). Whilst there are a few studies describing the role of PRR (8), *in vitro* and *in vivo* data supporting a direct

role for peptidase activity in immunogenicity have been generated, particularly with regard to influencing epithelial permeability, pro-inflammatory mediator function and immune cell function.

The potential consequences of the interaction between peptidase allergens and the respiratory epithelium has been looked at in some detail as this tissue represents the first to encounter inhaled allergen. These cells have the potential to impede the interaction between allergens (as well as other antigens) and dendritic cells (DCs) beneath this barrier, thus inhibiting antigen presentation to cells of the adaptive immune system (3). In this regard, recent studies showed that house dust mite peptidase allergens, in concentrations reflecting natural

exposure, selectively cleaved the extracellular domains of the tight junction proteins, occludin and claudin which help hold cells together (3, 9) resulting in enhanced permeability and, potentially, enhanced allergen presentation. In addition to these studies, others have shown that mite peptidases may also influence permeability either by non-IgE mediated mast cell degranulation or kinin generation (10, 11).

Similarly, peptidase allergens have been shown to stimulate respiratory epithelial cells to release the pro-inflammatory cytokines IL-6, IL-8 and granulocyte monocyte-colony stimulating factor (GM-CSF), as well the prostanoid PGE₂ (12, 13). All of these high and low molecular weight mediators have the potential to modulate antigen-presenting cells, T cell and B cell

Table 1. Allergenic peptidases

Peptidase equivalent	Catalytic type	Trivial name family	EC Number	Peptidase
Arachnids				
House dust mites				
Group 1	Cysteine peptidase	Papain	EC 3.4.22.2	C1A
Group 3	Serine peptidase	Trypsin	EC 3.4.21.4	S1A
Group 6	Serine peptidase	Chymotrypsin	EC 3.4.21.1	S1A
Group 9	Serine peptidase	Collagenase-like	EC 3.4.21.-	S1A
LM1	Serine protease	?	EC 3.4.21.-	S1A
Bacteria				
<i>Bacillus subtilis</i>	Serine peptidase	Subtilisin	EC 3.4.21.62	S8A
Insects				
Cockroach				
Bla g 2	Aspartate peptidase-like		EC 3.4.21.-	-
Fungi				
<i>Aspergillus and Penicillium spp.</i>				
e.g. Asp f 13	Serine peptidase	Extracellular protease	EC 3.4.21.63	S8A
e.g. Pen c 2	Serine peptidase	Vacuolar protease	EC 3.4.21.48	S8A
Mammals				
Cat				
*Fel d 1	?	?	?	?

Peptidase nomenclature is according to ref. 21 (also see text). EC, Enzyme Commission nomenclature.

*Or substance immunologically indistinguishable from Fel d 1 (7).

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function in the induction of an adaptive immune response. In this regard, there is evidence for mite peptidases inducing Th2-type cytokine responses, thus augmenting IgE production perhaps by cleaving the IL-2 receptor (CD25) or the low affinity IgE receptor (CD23) (14-16). The mechanisms underlying cytokine and mediator release are unclear at present although we and others have evidence that the family of protease-activated receptors (discussed elsewhere in this *Showcase on Research*) is involved (13, 17).

That peptidase activity influences immunogenicity has also been suggested by studies performed *in vivo*. Here, it has been shown that both bacterial and mite peptidase activity enhances specific IgE or IgG production in animal models (18, 19). Finally, it has been shown that mite peptidases cleave complement components C3 and C5 to release pro-inflammatory anaphylotoxins (20) capable of chemoattracting inflammatory cells to sites of allergen deposition. As the lung is replete with a variety of protease inhibitors, it could be argued that peptidase activity would be modulated. However, there is evidence showing that some allergenic peptidases are capable of inactivating such inhibitors and, even if this occurred *in vivo*, enhanced immunogenicity might be facilitated by the selective endocytosis of antigen presenting cells (such as macrophages and monocytes) possessing receptors that bind protease-protease inhibitor complexes (2).

Conclusion

In summary, we have described several protease-dependent mechanisms by which peptidase allergens may enhance immunogenicity and thus provide important new insights into allergic sensitisation. They are not novel *per se* as such mechanisms, for example, epithelial permeability and mediator production, are all known to be initiated by endogenous proteases such as trypsin and thrombin. That peptidase activity may be important, however, does not indicate that it is the sole, or necessarily major, parameter. This is because not all allergens are peptidases, although some, for example the mite peptidases, are recognised by the majority of mite-

allergic individuals. However, most individuals come into contact with mixtures of proteins containing such enzymes which may, nevertheless, interact synergistically with them to promote allergen delivery or skew host adaptive responses towards the allergic phenotype (4, 18). Finally, in the animal model experiments, it has been found that peptidase activity is particularly influential at mucosal surfaces compared to other routes of entry to the body. These observations form the basis for further studies aimed at delineating the role of peptidase activity in allergenic sensitisation.

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References

1. Stewart, G.A., and Thompson, P.J. (1996) *Clin. Exp. Allergy* **26**, 1020-1044
2. Stewart, G.A., Thompson, P.J., and McWilliam, A.S. (1993) *Pediatr. Allergy Immunol.* **4**, 163-172
3. Robinson, C., Wan, H., Winton, H.L., Herbert, C.A., Ring, P.C., et al. (2000) In *Asthma & Rhinitis* (Holgate, S.T., and Busse, W.W., eds) pp. 1143-1156, Blackwell Science, Oxford
4. Schweigert, M.K., Mackenzie, D.P., and Sarlo, K. (2000) *Clin. Exp. Allergy* **30**, 1511-1518
5. Arruda, L.K., Vailes, L.D., Ferriani, V.P., Santos, A.B., Pomes, A., and Chapman, M.D. (2001) *J. Allergy Clin. Immunol.* **107**, 419-428
6. Shen, H.D., and Han, S.H. (1998) *J. Microbiol. Immunol. Infect.* **31**, 141-145
7. Ring, P.C., Wan, H., Schou, C., Kroll Kristensen, A., Roepstorff, P., and Robinson, C. (2000) *Clin. Exp. Allergy* **30**, 1085-1096
8. Currie, A.J., Stewart, G.A., and McWilliam, A.S. (2000) *J. Immunol.* **164**, 3878-3886
9. Wan, H., Winton, H.L., Soeller, C., Tovey, E.R., Gruenert, D.C., Thompson, P.J., Stewart, G.A., Taylor, G.W., Garrod, D.R., Cannell, M.B., and Robinson, C. (1999) *J. Clin. Invest.* **104**, 123-133
10. Stewart, G.A., Boyd, S.M., Bird, C.H., Kraska, K.D., Kollinger, M.R., and Thompson, P. (1994) *Am. J. Ind. Med.* **25**, 105-107
11. Maruo, K., Akaike, T., Inada, Y., Ohkubo, I., Ono, T., and Maeda, H. (1993) *J. Biol. Chem.* **268**, 17711-17715
12. Tomee, J.F., Wierenga, A.T., Hiemstra, P.S. and Kauffman, H.K. (1997) *J. Infect. Dis.* **176**, 300-303
13. King, C., Brennan, S., Thompson, P.J., and Stewart, G.A. (1998) *J. Immunol.* **161**, 3645-3651

14. Comoy, E.E., Pestel, J., Duez, C., Stewart, G.A., Vendeville, C., Fournier, C., Finkelman, F., Capron, A., and Thyphronitis, G. (1998) *J. Immunol.* **160**, 2456-2462
15. Shakib, F., Schulz, O., and Sewell, H. (1998) *Immunol. Today* **19**, 313-316
16. Ghaemmaghami, A.M., Robins, A., Gough, L., Sewell, H.F., and Shakib, F. (2001) *Eur. J. Immunol.* **31**, 1211-1216
17. Sun, G., Stacey, M.A., Schmidt, M., Mori, L., and Mattoli, S. (2001) *J. Immunol.* **167**, 1014-1021
18. Sarlo, K., Ritz, H.L., Fletcher, E.R., Schrotel, K.R., and Clark, E.D. (1997) *J. Allergy Clin. Immunol.* **100**, 480-487
19. Gough, L., Schulz, O., Sewell, H.F., and Shakib, F. (1999) *J. Exp. Med.* **190**, 1897-1902
20. Maruo, K., Akaike, T., Ono, T., Okamoto, T., and Maeda, H. (1997) *J. Allergy Clin. Immunol.* **100**, 253-260
21. Barrett, A.J., Rawlings, N.D., and O'Brien, E.A. (2001). *J. Struct. Biol.* **134**, 95-102

