

Unique role of allergens and the epithelium in asthma

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Summary

The respiratory epithelium has been regarded as an inert barrier separating the human airway from the external world. Equally allergens have been perceived to be environmental proteins which interact with immunocompetent cells, activate inflammatory cells via IgE and promote the allergic response. However many allergens are biochemically active and are able to increase epithelial permeability and to stimulate epithelial cells to produce cytokines, adhesion receptors and MHC Class II expression. Thus the biochemical properties of allergens are likely to be important in enhancing the allergic responses and disease.

Introduction

The respiratory system is repetitively exposed to a wide variety of airborne particles. These consist of an array of organic and inorganic dusts as well as larger particles in the form of pollen, fungi, bacteria and viruses. Some of these particles will be rapidly cleared from the lung, some will induce an irritant response, while others will cause recruitment and activation of immunocompetent and inflammatory cells. In susceptible individuals the response will be allergic in nature but why this should preferentially occur for some particles and how these particles might be interacting with the epithelium is the subject of this review.

What are allergens and what characteristics promote allergenicity?

Allergens are peptides (or haptens) that are capable of generating a host IgE response and manifesting as a variety of related disease states including asthma, rhinitis, conjunctivitis and urticaria. The development of an allergic response involves the two-stage process of sensitization and subsequent immune cell activation. In the context of asthma and allergic rhinitis, allergens need to be present in the atmosphere as particles small enough to be inhaled. Since a diversity of proteins are inhaled and only a relatively small number seem to generate allergic responses in the majority of allergic patients it is important to consider what factors might be influencing

this. In the context of the allergen these include the molecular complexity, size, solubility, stability, concentration, and 'foreignness' of the relevant peptide and in the host include the immunological milieu (Th1/Th2 cells, dendritic cells and macrophages) and genetic factors influencing recognition of the allergen. However, before interaction with the host can occur the allergen needs to have adequate access to antigen-presenting cells, mast cells and possibly other inflammatory cells in the respiratory tract. This primarily involves transgressing the epithelium and the tight junctions that bind epithelial cells together. This is likely to occur when the epithelium is immature or when it has been 'injured' (viruses, air pollutants, cigarette smoke). Alternatively some proteins (allergens) may be able to interact biochemically/pharmacologically with the epithelium and in so doing enhance their interaction with the host.

The major sources of allergens

House-dust mites

These are the most clinically important source of allergens worldwide with *D. pteronyssinus*, *D. farinae* and *E. maynei* being the most prevalent species in domestic dwellings. These have been studied in detail and many of the allergens have been extensively characterized (see Table 1 for a list of some of these) [1–7].

Pollens

Allergens from pollens are a common precipitant of rhinitis and asthma. The most characterized are pollens

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Table 1 Physicochemical properties and biochemical activity of some mite allergens

Allergen	Frequency of reactivity (%)	Molecular weight (kDa)	Biochemical identity
Der p 1	> 90	25	Cysteine protease
Der p 2	> 90	14	Unknown
Der p 3	51–90	25	Trypsin
Der p 4	25–46	56	Amylase
Der p 5	> 55	13	Unknown
Der p 6	39	30	Chymotrypsin
Der p 7	53	22	Unknown
Der p 8	?	26	Glutathione transferase
Der p 9	> 90	28	Collagenolytic, serine protease
Der f 10	81	33	Tropomyosin

from ragweed, birch and rye grass. Allergenic pollens tend to be wind-borne and of varying shape and size and exhibit considerable cross-reactivity between species. In some cases (rye grass) submicronic particles of the pollen grain have been shown to deliver more potent allergens [8–10].

Fungi

Fungal spores are the most abundant organic particles in the atmosphere. They are often less than 10 microns in diameter and are found both indoors and outdoors. Some of the more important species are *Aspergillus*, *Alternaria*, *Cladosporium* and *Bipolaris*. Cross-reactivity seems to be small [11,12].

Animals

Cats and dogs are the most common animal source of allergens, but a wide variety of other animals have been implicated. The allergens are derived from a variety of sites including epithelia, dander, saliva and urine [11,12].

Insects

Bee, wasp and hornet venoms all contain pro-inflammatory peptides as well as variety of enzymes [11,12]. Cockroaches, midges, locusts and other insects are a source of allergens both in the home and in specific work environments [11,12].

Occupational allergens

These range from simple chemicals to complex proteins and include a variety of enzymes. Common occupational triggers include isocyanates, vegetable dusts, baker's asthma, hydrolytic enzymes (Table 2), wood dusts and laboratory animals [11,12].

Food and drugs

The prevalence of food allergy and in some cases the allergens involved are difficult to determine.

The biochemical nature of allergens

The complete amino acid sequence of more than 50 allergens is now known and it has become increasingly apparent that many of these are biochemically active. The majority can be assigned to one of four broad groups: (a) hydrolytic and nonhydrolytic enzymes; (b) enzyme inhibitors; (c) transport proteins and (d) regulatory proteins [11,12].

A large number of clinically significant allergens (both occupational and domestic) are hydrolytic enzymes with most being derived from plants and dust mites. They include proteases (cysteine, serine, aspartate and metalloproteinases), carbohydrases and ribonucleases. Very few of the non-hydrolases are recognized as major allergens but include an array of allergens from fungi, plants, cockroach and mites. A number of occupational and domestic allergens are inhibitors of proteases or amylase and are derived from plant seeds (wheat, barley,

Table 2 Proteases causing occupational asthma

Enzyme	Source	Industry
Trypsin	Pancreas	Drug industry & medical
Chymotrypsin	Pancreas	Drug industry & medical
Lipase	Pancreas	Medical
Amylase	Pancreas	Medical, baking, drug industry
Cellulase	Fungi	Drug industry, baking
Alcalase	<i>B. subtilis</i>	Detergent
Esperase	<i>B. licheniform</i>	Detergent
Papain	Papaya	Food & drug industry
Bromelain	Pineapple	Food & drug industry
Pepsin	Stomach	Drug industry

castor bean), pollens (ragweed, poa, olive) and eggwhite (ovomucoid allergen Gal d1).

The most important allergenic transport proteins are the laboratory animal urinary proteins (e.g. Mus m1) that are similar to molecules such as α -1 microglobulin and are termed lipocalins [11,12]. They are responsible for carrying hydrophobic molecules through hydrophilic environments. A significant number of other transport proteins are also allergenic and are derived from midges, cockroach, ragweed and grass pollens. The regulatory proteins such as profilins (plant derived), calmodulin, tropomyosin (mites) and heat shock proteins (mites) seem to play a role in binding actin or calcium [11,12].

Some of the clinically significant allergens do not, as yet, have ascribed biochemical activity. These include the group I grass pollens which have a high probability of being enzymes and many of the birch tree pollen allergens (and similar allergens) which have homology with 'stress proteins' from other plants that appear to be enzymes or enzyme inhibitors. However, little is known about the structure and function of fungal allergens while the functional properties of Fel d 1 (? a transport protein), Der p 2, Der p 5 and Der p 7 remain uncertain. As such it is important to acknowledge that some allergens will not be biochemically active, however, many will be colocated with either other allergens or non-allergenic proteins that are enzymes. Both house-dust mite faecal pellets and pollens provide rich examples of this. In house dust alone the following enzyme activities can be detected: trypsin, chymotrypsin, collagenase, amylase, glucoamylase, chitinase, cellulase, lipase, elastase, lysozyme, alkaline phosphatase and lysozyme.

Examples of the differing enzyme activities of specific allergens are given in Table 3. For significant biochemical activity to occur the concentration may also be important, however, estimates of focal concentrations of mite and pollen allergens in the airway are of the order of mg/mL [13–16]. In some cases the allergen needs to be reduced to be active but the glutathione concentrations on the mucosal surface of the airway seem adequate. Furthermore both Der p 1 and Der p 9 can also induce glutathione production from epithelial cells and ensure an enhanced reductive state. Whether other allergens can do this is unknown.

How might the biochemical properties of allergens enhance immunogenicity and airway responses

There are several theoretical ways in which the biochemical activity of allergens could enhance the likelihood of allergic sensitization or activation of an inflammatory response and some of these have been investigated (Table 4). Realization of the genetic capability to generate an IgE response by necessity requires interaction with environmental allergens, however, exposure per se is not a guarantee that sensitization and activation will occur. The respiratory mucosa is an obvious site of interaction and any physicochemical property which facilitates access to immunocompetent cells such as MHC Class II dendritic cells is likely to enhance an IgE response [17]. Equally if antigen presentation can be promoted, or if inflammatory changes are induced, then immune responses are likely to be primed or amplified. Binding to lectins and enzymatically linked receptors on cells such as

Table 3 Examples of biochemically active allergens

Protein grouping	Specific enzyme examples	Allergen source and examples
Serine proteases	Trypsin Chymotrypsin Alcalase	Bovine, Mites (Der p3), Bovine, Pig, Mites (Der p6) <i>B subtilis</i> (Detergents)
Cysteine proteases	Papain like	Mites (Der p1), papaya
Aspartate proteases	Pepsin, renin	Pig, Cheese making, Cockroach (Bla g2)
Metalloproteinases	Collagenase	<i>Clostridium</i> (occupational)
Carbohydrases	Amylase Pectate lyase Lysozyme	Mites (Der p4), Baking & Drug industries Cedar, ragweed group 1, fungi Egg white,? mites
Ribonuclease		Timothy grass (Grp 5), <i>A. fumigatus</i> (Asp f1)
Non-hydrolytic enzymes	Glutathione transferases	Cockroach, mites (Der p8)
Enzyme inhibitors	Napins Trypsin/amylase inhibitors Cystatins	Castor Bean (Ric c2) Wheat allergens Ragweed
Transport proteins	Lipocalins	Urinary proteins (mus m1, Rat n2), cockroach (Bla g4), Ragweed (Amb a3, Amb a6, Amb a7)
Regulatory proteins	Profilins Tropomyosins Heat shock proteins	Grass, weed, tree pollens, fungi Mites (Der f10), Fungal and mite

Table 4 Ways in which the biochemical activity of allergens might help sensitization/activation

Possible mechanism	Confirmation/examples
Alter epithelium integrity	Yes; Der p 1
Alter airway permeability	Yes; Der p 1
Diminish normal epithelial function	Not studied
Induce cytokine release	Yes; Der p 1 & 9. Epithelium:IL1,IL6, IL 8, GM-CSF
Induce MHC Class II expression	Yes; Der p1 & 9. Epithelium
Induce mediator release	Yes; Mite extract. Epithelium: PGE2
Induce ICAM expression	Yes; Der p9. Epithelium
Cleave CD 23 from B cells	Yes; Der p 1.
Activate T cells	Yes; Der p 9. Epithelium dependent
Hydrolysis of IgG	Yes; Der p 1.
Activate inflammatory cells	Yes; Der p1. Mast cells
Neutralize/consume antiprotease system	Not studied
Activation of protease dependent receptors	Being assessed
Depot storage of allergen	Not studied
Enhanced allergen presentation via alveolar macrophages	Not studied

monocytes and macrophages may enhance antigen presentation and in such a setting, the amount of allergen required to induce an immune response may be several-fold less than would otherwise be required [18–20].

Assessment of the effect of biochemically active allergens on airway permeability and on the production of cytokines and inflammatory mediators seems warranted. In so doing it is important to conceptualize that allergens may be capable of activating inflammatory pathways independent of an IgE mediated response. Equally it is important to realize that allergens are often deposited in the airway in concert with other particles and proteins (pollen and mite faecal pellets) as whole or submicronic entities.

The role of the epithelium

The epithelium has traditionally been seen as a barrier between self and the external environment and in a healthy mucosa it is a tightly and highly regulated barrier offering intrinsically high resistance to passive penetration [21,22]. The tight junctions are made up of the proteins occludin and ZO-1. ZO-1 forms an almost continuous belt around each cell [23]. However, in asthma, airway permeability is increased and tight junction morphology abnormal [24,25]. Whether this is secondary to asthma pathology and/or is involved in the pathogenesis of the disease is unclear.

Although its role as a barrier is fundamental it is also important to recognize that the epithelium is an important metabolically active regulatory system in the human airway. It has the capacity to produce cytokines (IL-1 β , IL-6, IL-8, TGF- β , RANTES, eotaxin, and GM-CSF), prostaglandin E₂, nitric oxide, endothelin,

fibrinogen, glutathione, matrix metalloproteinases and protease inhibitors as well as many other pro and anti-inflammatory mediators [26–30]. There are also receptors and adhesion receptors for many of the molecules generated by other cells during inflammation and these help to make the epithelium a focus for inflammatory cell recruitment [31–34]. There seems little doubt that the epithelium plays an autocrine and paracrine role in regulating mediator production and epithelial repair processes through molecules such as iNOS and COX-2 and has a role in the degradation of mediators through molecules such as NEP. Furthermore there is evidence for MHC Class II molecules being expressed on the epithelium although the precise role of this is unclear [31].

Endogenous proteases from mast cells and neutrophils have been shown to initiate cytokine and mediator release from the epithelium [35,36] and to alter epithelial permeability [37]. Amongst these tryptase is likely to be of considerable significance [38,39] while the possibility of defective antiprotease activity in asthma has been given credence [40–42]. Furthermore neutrophil elastase and chymase are powerful secretagogues [43,44]. The possibility that enzymatically active allergens can mimic these effects, and others, needed to be tested.

The evidence for the biochemical activity of allergens interacting with the epithelium

Most of the data for this have been derived from studies assessing the effects of house-dust mite allergens. These have utilized crude mite extracts, specific physico-chemical fractions of crude mite extract and the specific allergens Der p 1 (cysteine protease), Der p 3 (trypsin), Der p 6 (chymotrypsin) and Der p 9 (collagenase) [2–7,45].

Fig. 1. Photomicrograph of canine tracheal epithelial cells in culture before and after incubation with Der p 9.

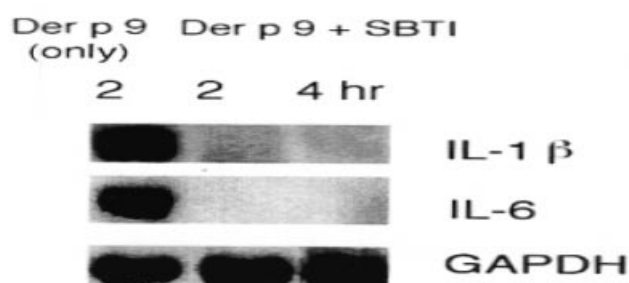
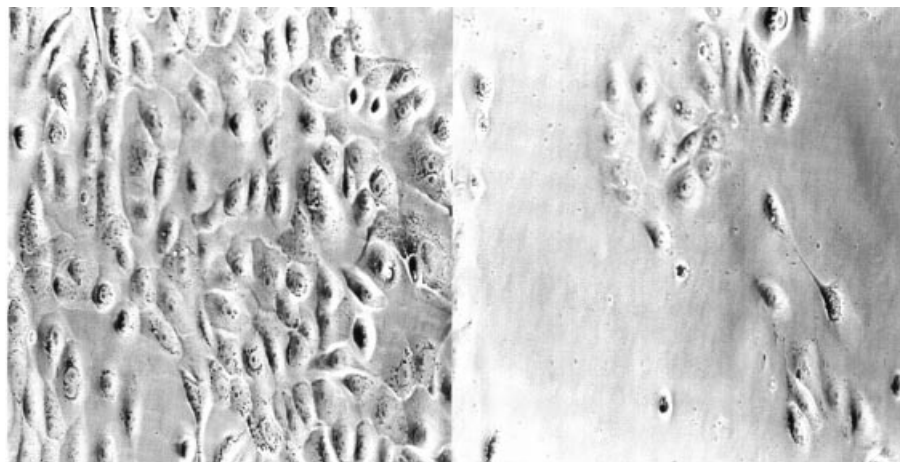


Fig. 2. Northern blot analysis for IL-6 and IL-1 β in BEAS2B cells following incubation with Der p 9 for 2 h, and following incubation with Der p 9 and the serine protease inhibitor Soy Bean Trypsin Inhibitor for 2 and 4 h. cDNA probe for GAPDH was used as an internal control.

Table 5 Epithelial cell cytokine production following stimulation with various allergens and proteases

BEAS 2B cells	GM-CSF*	IL-6*	IL-8*
Unstimulated	105 $\pm$ 19	108 $\pm$ 28	2980 $\pm$ 450
Der p 1	712 $\pm$ 95	108 $\pm$ 1289	18900 $\pm$ 3600
Der p 9	890 $\pm$ 135	1794 $\pm$ 324	17500 $\pm$ 2330
Thrombin	734 $\pm$ 82	1626 $\pm$ 297	12900 $\pm$ 2146
Bovine Trypsin	112 $\pm$ 34	986 $\pm$ 145	5620 $\pm$ 780

*pg/10⁶ cells

Der p 1 disrupts mucosal integrity [46] and does so by breaking down intercellular adhesion receptors. This is manifested by an increase in epithelial permeability and gives allergens such as Der p 1 a distinct 'advantage' in effecting interactions with dendritic cells. Significantly, markers of cell damage (LDH levels) or necrosis (trypan blue exclusion) are absent highlighting that the effect is likely to be specifically related to altering adhesion

receptor integrity. These results have been derived from studies using bovine tracheal epithelium for assessment of permeability [15] and canine tracheal epithelial cells, BEAS 2B and human primary cell cultures for detachment studies [46]. Use of specific enzyme inhibitors confirmed that these effects were mediated via protease specific pathways.

Der p 1 and Der p 9 induce IL-1 β , IL-6, IL-8 and GM-CSF release from primary epithelial and BEAS-2B cell cultures (Fig. 1, Fig. 2, Table 5) [47,48]. They also cause up-regulation of NF κ B activity [49] and have been shown to induce expression of ICAM 1 and HLA class II [47]. Similar findings have been described for proteases from *Aspergillus fumigatus* [50]. Der p 1 and Der p 9 also increase Ca flux into epithelial cell lines. Some effects of mite proteases are codependent as is seen with T-cell proliferation that occurs only in the presence of airway epithelial cells and the interaction between OSM and mite allergen on PGE₂ production. How these diverse effects occur is not clear although the increasing recognition of the role of tethered ligand receptors is likely to be important.

Other potential effects of allergens

Activation by mite allergen of other relevant cells has also been demonstrated including mast cells [51]. Furthermore it is of considerable interest that alveolar macrophages have binding sites for proteases, cytokines, transport proteins and carbohydrates and many other molecules [52]. This may allow alveolar macrophage generated antigen presentation to occur to a greater degree [18–20]. In contrast the mite allergens have also been shown to consume antiproteases such as α -1-antitrypsin and thereby diminish this particular defence system [53,54].

Summary

Many clinically important allergens have some form of biochemical activity. This biochemical activity is likely to play an important role in facilitating allergen recognition and in progressing inflammatory responses. Studies to date have shown mite allergens enhance airway permeability, cause epithelial cell detachment and induce cytokine release and ICAM1 and MHC Class II expression on epithelial cells. All of these effects are likely to be important and require further investigation to characterize the mechanisms and pathways involved.

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