

Potential Roles in Rhinitis for Protease and Other Enzymatic Activities of Allergens

Nita Sehgal, MD, Adnan Custovic, MD, and Ashley Woodcock, MD

Address

North West Lung Centre, Wythenshawe Hospital, Southmoor Road, Manchester M23 9LT, UK.

E-mail: nitasehgal@ukonline.co.uk

Current Allergy and Asthma Reports 2005, 5:221–226

Current Science Inc. ISSN 1529-7322

Copyright © 2005 by Current Science Inc.

Exposure to airborne pollen, fungal allergens, and dust mite allergens is associated with the development of allergic rhinitis. Biologic function of allergens is considered to be a key determinant for allergenicity, and many clinically important allergens have been shown to possess enzymatic activity. It is proposed that by enabling allergens to breach the integrity of the airway epithelial barrier, proteolytic activity plays an adjuvant pro-allergic role influencing immunogenicity. In this review, current evidence regarding enzymatic activity of aeroallergens is described, and the potential role of aeroallergens in allergic rhinitis is discussed.

Introduction

Characterization of allergens at the molecular level by cloning techniques has identified that many clinically important allergens are biochemically active. This has led to the suggestion that in addition to intrinsic properties, including the integrity of the allergenic protein, resistance to degradation, binding capacity, and the degree of sequence similarity to host proteins, biologic function may be a key determinant for allergenicity [1,2]. Based on biologic function, allergens can be divided into several diverse groups: enzymes; enzyme inhibitors; and binding, structural, and regulatory proteins [3•]. Many clinically significant airborne allergens are hydrolytic enzymes, including proteases from the house dust mite family and proteases, carbohydrases, and ribonucleases from fungi. Nonhydrolytic enzymes include mite glutathione-S-transferase and amylase; plant pectin lyase; and fungal enolase, alcohol dehydrogenase, and aldolase, few of which are recognized as major allergens [4,5].

It is proposed that protease activity leads to enhanced allergenicity, as proteolytically active allergens can compromise airway barrier function by reversibly disrupting inter-

cellular epithelial adhesion [6•,7]. Having facilitated their access to immunocompetent cells, several studies indicate that the proteolytic activity of allergens enables them to systemically perturb immunologic signaling, promoting IgE synthesis directly, through cleavage of CD23, the low affinity IgE receptor found on the surface of B cells, and indirectly, through cleavage of CD25 and CD40 [8–10].

In this article, we describe the enzymatic activity of pollen, fungal, and mite allergens and critically review their potential role in allergic rhinitis, focusing on their impact on airway epithelial function.

Pollen Allergens

Airborne allergens from plant pollens are important triggers for intermittent allergic rhinitis [11]. High local airway concentrations of pollen solutes (50 mg/mL) are achieved when pollen grains impact on the mucosal surface, as approximately 25% of the pollen mass is considered to be released following rapid hydration [12•]. Pollens contain a variety of enzymes, including proteases, but, to date, data regarding whether allergenic pollen proteins possess proteolytic activity is conflicting, and any putative role of pollen proteases in the disruption of epithelial barrier function remains unclear [12•,13–16].

Group 1 grass pollen allergens are major allergens, accounting for IgE reactivity in 95% of pollen-sensitized individuals. The primary sequence of these 30-kDa glycoproteins is highly conserved among various grass species, sharing between 85% and 95% homology. The group 1 grass allergens belong to the family of β -expansins and act as mediators of cell-wall loosening, which is essential for growth. Grobe *et al.* [14,17] have proposed that these highly immunogenic allergens are proteases. Phl p 1, a group 1 allergen from timothy grass (*Phleum pratense*), expressed in *Pichia pastoris*, along with affinity-chromatography purified natural allergen was shown to degrade a nitroanilide substrate in a colorimetric protease assay and to cleave a casein zymogram. Proteolysis was inhibited by the presence of iodoacetamide and phenylmethanesulfonyl fluoride, inhibitors of cysteine and serine proteases but not by specific metalloproteinase or aspartic peptidase inhibitors [14]. A literature search for conserved protease

sequences identified that Phl p 1 could be aligned with three functionally important motifs, including active-site cysteine and histidine residues, defining the C1 papain-like cysteine proteases. Further investigation by this group demonstrated that the β -expansins exhibit sequence similarity with the cysteine protease cathepsin B [17], and that, similar to C1 proteinases, the expansins are secreted and exported to the cell walls in an inactive proform, share a similar molecular mass and optimum functional pH, and require activation by reducing agents. The authors conclude that this intrinsic proteolytic activity of the β -expansins may mediate plant cell wall loosening and, theoretically, promote increased permeability of airway epithelium. These findings, however, have been directly contended by Li *et al.* [15]. In a series of experiments, three purified native group 1 allergens from timothy grass, ryegrass, and maize were studied; results of a protease assay, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and native PAGE zymography, and digestion of agarose gels containing casein, all demonstrated a lack of proteolytic activity. Additionally, amino acid analysis dismissed sequence similarity between the expansins and cysteine proteases. These results led to the proposal that the group 1 grass pollens are not proteases and that the proteinase activity observed by Grobe *et al.* was a result of trace contamination. However, further evidence in support of pollen proteinases stems from work conducted by Raftery *et al.* [12•]. Proteolytic activity of diffusates from grass pollens was assessed using a fluorogenic substrate and by zymography. In corroboration with findings from Widmer *et al.* [18], Bermuda grass diffusates demonstrated predominant serine protease activity, whereas Kentucky and rye-grass diffusates exhibited both cysteine and serine activity. High-performance liquid chromatography (HPLC) electrospray mass spectrometry/mass spectrometry (MS/MS) analysis of tryptic peptides derived from two-dimensional gel electrophoretically separated Bermuda grass diffusates, in conjunction with zymography, confirmed that the major proteolytically active protein observed was the group 1 allergen Cyn d 1.

In addition to group 1 grass allergens, group 5 and 13 allergens have also been shown to exhibit enzymatic activity. The crystal structure of the group 5 major allergen Phl p 5b has been elucidated and identified as a ribonuclease [19]. The role of such enzymatic activity in pollen is not fully understood, but may have a defensive function against viral infection. Group 13 grass pollen allergens bind IgE in the sera of 50% of sensitized subjects, and Phl p 13 has been identified as a polygalacturonase playing an important role in cell-wall lysis of stylus tissue [20].

The clinical relevance of these findings in allergic airways disease remains unknown. A limited number of studies have been undertaken to assess the potential role of these putative proteolytically active allergens in the disruption of epithelial barrier function in rhinitis. Hassim *et al.* [16] attempted to assess the effect of pollen diffusates on

cultured murine airway epithelial cells. Primary cell cultures were established from mouse tracheal explants and seeded onto a collagen matrix. Following exposure to pollen diffusates prepared using 100 mg/mL of pollen, cell detachment measured using a methylene blue dye binding assay was found to be between 30% and 90%. Detachment of cells from the substratum was inhibited by the addition of serum, leading to the conclusion that cell detachment was likely to be mediated by proteolytic activity of the diffusates, although the role of nonenzymatic components could not be excluded. Interestingly, in this study the cells were treated only 2 hours after they had been seeded. At this time, 95% or more had attached to the substratum; whether the cells had reached confluency is not stated, and transepithelial electrical resistance (TEER) was not measured. TEER is a measure of barrier function and correlates with the permeability of epithelial tight junctions to inorganic ions. It has been shown that cultured human bronchial epithelial cells take up to 6 to 8 days to develop maximum TEER [21], and, hence, after only 2 hours, exaggerated cell detachment in response to proteases may have been seen.

Fungal Allergens

A number of fungal allergens implicated in the pathogenesis of allergic rhinitis are enzymatically active. Allergens from *Alternaria alternata* and *Cladosporium herbarum* are known aldehyde dehydrogenases and enolases whereas allergens from *Aspergillus* species exhibit serine, aspartate, and metalloprotease activities [22,23]. However, studies investigating the effects of enzymatic activity of fungal proteases in rhinitis are limited. Kauffman *et al.* [24•] quantified proteolytic activity in various fungal extracts and assessed their ability to induce cell desquamation in an airway-derived cell line (A549) and in primary cultures of nasal epithelial cells, and to stimulate cytokine production. *Alternaria* and *Aspergillus* extracts induced cell shrinking and desquamation in the A549 but not in the primary nasal cell cultures, and, together with *Cladosporium*, they induced secretion of interleukin (IL)-6 and IL-8 in both cell lines. These findings were dependent on the proteolytic activity of the extracts and not seen in the presence of protease inhibitors. Cultured bronchial and nasal epithelial cells have been shown to be similar in their morphology, growth characteristics, and ciliary activity [25], and, as such, the reason for the difference in cell desquamation seen between the A549 and primary nasal cells is unclear.

Dust Mite Allergens

Exposure to allergens from the house dust mite family is associated with persistent allergic rhinitis [11]. To date, 19 different allergens from dust mite have been characterized and found to exhibit varying degrees of immunoglobulin E

(IgE) reactivity and T-cell proliferative responses [26]. The group 1 mite allergens are 25 kDa acidic to neutral allergenic proteins, synthesized by cells lining the mite gastrointestinal tract and secreted as preproteins, which function as digestive enzymes. Additionally, allergens from groups 3, 6, and 9 have also been identified as digestive enzymes exhibiting serine protease activity. These mite proteases are carried on fecal particles of respirable diameter, which can become airborne and deposited onto airway mucosa. They are classified as major allergens, and a mounting body of evidence links their allergenic potency to proteolytic activity.

Dermatophagoides pteronyssinus 1 (Der p 1), considered to be the most immunodominant allergen involved in the expression of IgE-mediated dust mite sensitivity, has been the focus of many studies in this field. Herbert *et al.* [27] observed that Der p 1 and unfractionated spent growth medium extract (SGME), from which it was purified, were able to detach cultured Madin-Darby canine kidney (MDCK) cells and canine tracheal epithelial cells grown on artificial and natural biopolymer substrata, causing visible disruption of the epithelial architecture. Both Der p 1 ($300 \mu\text{g} \cdot \text{mL}^{-1}$) and SGME also led to an increase in albumin flux across isolated sheets of bovine bronchial mucosa. Tomee *et al.* [28] applied *D. pteronyssinus* extract to human alveolar type II epithelial-like cells and primary nasal epithelial cultures; cell detachment occurred at concentrations of 40 and $400 \mu\text{g} \cdot \text{mL}^{-1}$, respectively, and could be inhibited by the presence of protease inhibitors. A subsequent study separated the cysteine and serine protease activities of mite fecal pellets using ammonium sulphate fractionation and demonstrated that both protease fractions led to a concentration-dependent increase in mannitol permeability across cultured epithelial cell monolayers [29]. These effects were negated by the presence of class-specific protease inhibitors. These early studies highlighted that proteolytic activity of mite allergens led to an increase in the permeability of epithelium in vitro. However, concentrations of purified allergen and extracts used were in the range of several hundred $\mu\text{g}/\text{mL}$. In contrast, exposure to Der p 1 during sleep has been measured to be only approximately $2 \mu\text{g}$ per year [30].

Roche *et al.* [31] demonstrated that proteolytically active Der p 1 altered epithelial permeability by increasing paracellular conductance as opposed to transcellular conductance in intact airway tissues. Paracellular channels are normally sealed by tight junctions (TJ), which form the most apical element of the junctional complex between polarized epithelial cells. Wan *et al.* [6,29,32,33] were the first to demonstrate that Der p 1 was able to disrupt barrier function through the degradation of TJ proteins, and further studies have shown that both cysteine and serine proteases from *D. pteronyssinus* can precipitate the disassembly of TJ. In these experiments, cultured MDCK, Calu-3, and 16HBE14o- cells (immortalized cell lines derived from human bronchial epithelial cells) were treated with pro-

teolytically active preparations of the mite allergens, and the morphology of TJs and desmosomes were studied by fluorescent antibody labeling. 2-Photon molecular excitation microscopy (2-PMEM), in conjunction with custom-written software, allowed quantification of breaks in fluorescence to be measured. Immunopurified Der p 1 and serine proteases from mite fecal pellets evoked a time-dependent decrease in TJ staining that could be detected within 30 minutes of exposure. Permeability to mannitol was measured following treatment with Der p 1 and mite serine proteases, and the observed increase paralleled the total number of breaks visualized by immunostaining. Immunoblotting confirmed that reduction in fluorescence intensity was a result of proteolysis of the transmembrane proteins occludin and claudin and of the cytoskeletal protein ZO-1. In these studies, degradation of the TJ proteins and the resulting increase in transepithelial permeability were dependent upon the protease activity of the allergens and could be inhibited by the presence of protease inhibitors. Wan *et al.* [33] have concluded that by accessing paracellular permeation routes and facilitating their delivery to dendritic antigen presenting cells, the opening up of TJ by environmental proteases may be an important early step in the development of sensitization and may contribute to ongoing symptoms of allergic airways disease.

Interpretation of such studies conducted in cell culture and extrapolation to in vivo scenarios with intact airway mucosa must be made with caution. In these studies, varying concentrations of allergen with different levels of proteolytic activity were applied to monolayers of cells. Comparable levels of activity in experiments yielded disparate levels of junctional breakdown [6,32]. In one study, mite serine proteases from fecal-pellet enriched *D. pteronyssinus* culture medium were applied to cell monolayers seeded on 13-mm coverslips with a protease activity quoted as equating to 1200 dust mite fecal pellets [33]. Using personal intranasal air samplers, it has been calculated that the average individual inhales approximately 60 fecal particles during sleep per night [30]. Therefore, treatment with activity of 1200 pellets seems excessive. These cell-culture experiments have recently been repeated in our laboratory using $\mu\text{g}/\text{mL}$ concentrations of allergen. Degradation of TJ was seen with immunopurified Der p 1 and commercially available *D. pteronyssinus* immunotherapy solution, but, surprisingly, proteolytically active extract from spent *D. pteronyssinus* culture had no effect on TJ morphology (Unpublished data). This crude extract, which was not toxic to the cells, provides the closest representation to natural exposure of all the allergen preparations used. Therefore, the lack of effect seen questions the clinical relevance of results obtained by applying laboratory-purified allergens to cultured cells.

MDCK cells are considered to be the gold standard epithelial cell line in the evaluation of junctional complexes. Parental MDCK cells form a heterogeneous line, and two subclones, type I and II cells, are now also available from the

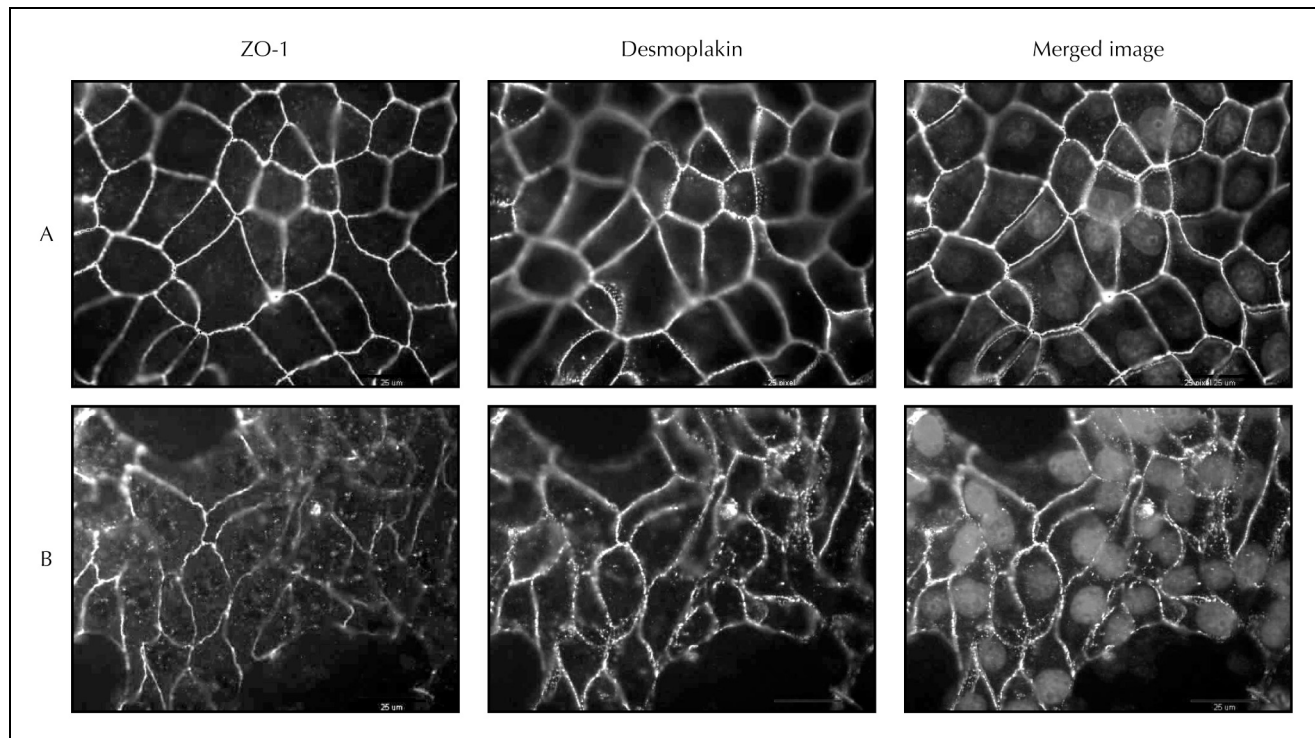


Figure 1. ZO-1 immunostaining in Der p 1-treated MDCK cells. Images **A** and **B** are taken from the same cell monolayer using 2-PMEM. In image **A**, no disruption in ZO-1 staining, which forms contiguous rings circumscribing each of the cells, is seen following exposure to the mite protease. Image **B** shows marked degradation of ZO-1 together with some disruption of the desmosomal protein desmoplakin. MDCK—Madin-Darby canine kidney; PMEM—photon molecular excitation microscopy.

European Collection of Cell Cultures. These cells have been shown to exhibit markedly different TEER. MDCK parental line cells develop a TEER of 225 to 250 Ωcm^2 , whilst type I cells are reported to display a high electrical resistance of more than 1000 Ωcm^2 and type II cells only 100 Ωcm^2 [34]. In comparison, TEER maintained by healthy tracheobronchial epithelium measures 200 to 700 Ωcm^2 [35]. Baseline TEER developed by cultured cells will influence the ability of proteases to permeate paracellular channels, and we have demonstrated differences in the effect of Der p 1 on TJ of these three MDCK cell lines. Wan *et al.* [21,36] have published data showing that the MDCK cells used in their work developed TEER of only 50 Ωcm^2 . Our own studies have also highlighted difficulties in quantifying changes in immunofluorescent staining. Loss of TJ-protein staining was found to be heterogeneous across cell monolayers, and Figure 1 demonstrates images obtained from the same monolayer depicting markedly different degrees of junctional protein degradation. 2-PMEM in conjunction with specific software enables changes in the intensity of fluorescence and detection of visible breaks in the contiguity of staining around a cell. However, the outcome of studies still critically depends on the apparently arbitrary selection of which cells in a given monolayer are imaged.

To evaluate the role of proteolytically active allergens in allergic rhinitis, it is essential that these studies examining tight junctions in MDCK cells and bronchial epithelial cells are replicated in primary nasal epithelial cell cultures

or in ex vivo biopsy samples. Several reports imply that, in view of the presence of coexisting inflammation, increased nonspecific bronchial hyperreactivity in both diseases, and the existence of the nasal-bronchial reflex, allergic rhinitis, and asthma should be viewed as a single syndrome [37–39]. However, Kauffman *et al.* [24•] demonstrated a differential response of bronchial cells and nasal cells to fungal proteases, and Tomee *et al.* [28] found that much higher concentrations of mite allergen were needed to increase permeability in primary nasal cells, compared with cultured bronchial cells. In addition, changes in the morphology of junctional proteins and airway permeability following allergen challenge in vivo will further assist in our understanding of the role of enzymatically active allergens in allergic rhinitis. Greiff *et al.* [40] examined nasal absorption of desmopressin in patients with persistent house dust mite allergic rhinitis and in healthy subjects in vivo. Desmopressin was instilled intranasally for a period of 15 minutes and the amount excreted in a 24-hour urine collection calculated. This study showed that absorption of desmopressin was not increased in allergic rhinitis compared with healthy nonatopics and that, in contradiction to the in vitro study data, the integrity of epithelial barrier function was maintained.

Future studies also need to take the possible cytoprotective effect of antiproteases into account. Hewitt *et al.* [41] demonstrated that $\alpha 1$ -antitrypsin was able to inhibit the protease activity of Der p 1, and our own studies support

inhibition of Der p 1 by secretory leukocyte protease inhibitor, the dominant antiprotease of the nose and upper airway.

Conclusions

Exposure to airborne pollen, fungal allergens, and dust mite allergens is associated with the development of atopic sensitization. Experimental evidence supports the view that continued exposure to these allergens in sensitized individuals contributes to the airway inflammation and symptoms seen in allergic rhinitis and asthma [11,42]. It has been proposed that protease activity of allergens enhances allergenicity. To date, however, evidence with regard to pollen allergens possessing proteolytic activity remains conflicting, and studies evaluating the protease activity of fungal allergens remains limited. Four proteases from the dust mite family have been well characterized and are recognized major allergens. Proteolytic activity of these allergens has been shown to enhance allergenicity, but experiments in cell culture show that their impact on epithelial cell monolayers in vitro is variable and, to date, in vivo intranasal effects have not been confirmed. Furthermore, it is difficult to imply clinical relevance from such experiments at current human exposure levels. Although enzymatic activity may play a role in modulating allergenicity, current evidence would suggest that it is not a pre-requisite for allergy, and this is supported by the finding that many allergens implicated in allergic rhinitis, including cat, birch pollen, and Der p 2, are non-enzymatically active.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Pomes A: **Intrinsic properties of allergens and environmental exposure as determinants of allergenicity.** *Allergy* 2002, 57:673–679.
2. Aalberse RC: **Structural biology of allergens.** *J Allergy Clin Immunol* 2000, 106:228–238.
3. Smith AM, Pomes A, Chapman MD: **Molecular biology of indoor allergens.** *Clin Rev Allergy Immunol* 2000, 18:265–283.
4. Thompson PJ: **Unique role of allergens and the epithelium in asthma.** *Clin Exp Allergy* 1998, 28(Suppl 5):110–116.
5. Stewart GA, Robinson C: **The immunobiology of allergenic peptidases.** *Clin Exp Allergy* 2003, 33:3–6.
6. Wan H, Winton HL, Soeller C, et al.: **Quantitative structural and biochemical analyses of tight junction dynamics following exposure of epithelial cells to house dust mite allergen Der p 1.** *Clin Exp Allergy* 2000, 30:685–698.

This review summarizes the biologic function of diverse groups of a number of allergens and their potential role in allergenicity.

7. Hewitt CR, Foster S, Phillips C, et al.: **Mite allergens: significance of enzymatic activity.** *Allergy* 1998, 53:60–63.
8. Schulz O, Sewell HF, Shakib F: **Proteolytic cleavage of CD25, the alpha subunit of the human T cell interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity.** *J Exp Med* 1998, 187:271–275.

9. Ghaemmaghami AM, Gough L, Sewell HF, Shakib F: **The proteolytic activity of the major dust mite allergen Der p 1 conditions dendritic cells to produce less interleukin-12: allergen-induced Th2 bias determined at the dendritic cell level.** *Clin Exp Allergy* 2002, 32:1468–1475.
 10. Gough L, Schulz O, Sewell HF, Shakib F: **The cysteine protease activity of the major dust mite allergen Der p 1 selectively enhances the immunoglobulin E antibody response.** *J Exp Med* 1999, 190:1897–1902.
 11. Nielsen GD, Hansen JS, Lund RM, et al.: **IgE-mediated asthma and rhinitis I: A role of allergen exposure?** *Pharmacol Toxicol* 2002, 90:231–242.
 12. Raftery MJ, Saldanha RG, Geczy CL, Kumar RK: **Mass spectrometric analysis of electrophoretically separated allergens and proteases in grass pollen diffusates.** *Respir Res* 2003, 4:10.
- Proteolytic activity of pollen diffusates is described and proteomics employed to determine the identities of the pollen proteins.
13. Petersen A, Grobe K, Schramm G, et al.: **Implications of the grass group I allergens on the sensitization and provocation process.** *Int Arch Allergy Immunol* 1999, 118:411–413.
 14. Grobe K, Becker WM, Schlaak M, Petersen A: **Grass group I allergens (beta-expansins) are novel, papain-related proteinases.** *Eur J Biochem* 1999, 263:33–40.
 15. Li LC, Cosgrove DJ: **Grass group I pollen allergens (beta-expansins) lack proteinase activity and do not cause wall loosening via proteolysis.** *Eur J Biochem* 2001, 268:4217–4226.
 16. Hassim Z, Maronese SE, Kumar RK: **Injury to murine airway epithelial cells by pollen enzymes.** *Thorax* 1998, 53:368–371.
 17. Grobe K, Poppelmann M, Becker WM, Petersen A: **Properties of group I allergens from grass pollen and their relation to cathepsin B, a member of the C1 family of cysteine proteinases.** *Eur J Biochem* 2002, 269:2083–2092.
 18. Widmer F, Hayes PJ, Whittaker RG, Kumar RK: **Substrate preference profiles of proteases released by allergenic pollens.** *Clin Exp Allergy* 2000, 30:571–576.
 19. Bufe A, Betzel C, Schramm G, et al.: **Crystallization and preliminary diffraction data of a major pollen allergen. Crystal growth separates a low molecular weight form with elevated biological activity.** *J Biol Chem* 1996, 271:27193–27196.
 20. Petersen A, Suck R, Hagen S, et al.: **Group 13 grass allergens: structural variability between different grass species and analysis of proteolytic stability.** *J Allergy Clin Immunol* 2001, 107:856–862.
 21. Wan H, Winton HL, Soeller C, et al.: **Tight junction properties of the immortalized human bronchial epithelial cell lines Calu-3 and 16HBE14o-.** *Eur Respir J* 2000, 15:1058–1068.
 22. Kurup VP, Shen HD, Banerjee B: **Respiratory fungal allergy.** *Microbes Infect* 2000, 2:1101–1110.
 23. Bush RK, Portnoy JM: **The role and abatement of fungal allergens in allergic diseases.** *J Allergy Clin Immunol* 2001, 107:S430–S440.
 24. Kauffman HF, Tomee JF, van de Riet MA, et al.: **Protease-dependent activation of epithelial cells by fungal allergens leads to morphologic changes and cytokine production.** *J Allergy Clin Immunol* 2000, 105:1185–1193.
- This study analyzes the proteolytic activity of fungal extracts and describes its effect on barrier function and cytokine production by nasal epithelial cells.
25. Devalia JL, Sapsford RJ, Wells CW, et al.: **Culture and comparison of human bronchial and nasal epithelial cells in vitro.** *Respir Med* 1990, 84:303–312.
 26. Thomas WR, Smith WA, Hales BJ, et al.: **Characterization and immunobiology of house dust mite allergens.** *Int Arch Allergy Immunol* 2002, 129:1–18.
 27. Herbert CA, King CM, Ring PC, et al.: **Augmentation of permeability in the bronchial epithelium by the house dust mite allergen Der p1.** *Am J Respir Cell Mol Biol* 1995, 12:369–378.
 28. Tomee JF, van Weissenbruch R, de Monchy JG, Kauffman HF: **Interactions between inhaled allergen extracts and airway epithelial cells: effect on cytokine production and cell detachment.** *J Allergy Clin Immunol* 1998, 102:75–85.

29. Winton HL, Wan H, Cannell MB, *et al.*: Class specific inhibition of house dust mite proteinases which cleave cell adhesion, induce cell death and which increase the permeability of lung epithelium. *Br J Pharmacol* 1998, 124:1048–1059.
30. Gore RB, Hadi EA, Craven M, *et al.*: Personal exposure to house dust mite allergen in bed: nasal air sampling and reservoir allergen levels. *Clin Exp Allergy* 2002, 32:856–859.
31. Roche N, Chinet TC, Belouchi NE, *et al.*: *Dermatophagoides pteronyssinus* and bioelectric properties of airway epithelium: role of cysteine proteases. *Eur Respir J* 2000, 16:309–315.
32. Wan H, Winton HL, Soeller C, *et al.*: **Der p 1** facilitates trans-epithelial allergen delivery by disruption of tight junctions. *J Clin Invest* 1999, 104:123–133.
33. Wan H, Winton HL, Soeller C, *et al.*: The transmembrane protein occludin of epithelial tight junctions is a functional target for serine peptidases from faecal pellets of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2001, 31:279–294.
34. Stevenson BR, Anderson JM, Goodenough DA, Mooseker MS: Tight junction structure and ZO-1 content are identical in two strains of Madin-Darby canine kidney cells which differ in transepithelial resistance. *J Cell Biol* 1988, 107:2401–2408.
35. Robinson C, Kalsheker NA, Srinivasan N, *et al.*: On the potential significance of the enzymatic activity of mite allergens to immunogenicity. Clues to structure and function revealed by molecular characterization. *Clin Exp Allergy* 1997, 27:10–21.
36. Winton HL, Wan H, Cannell MB, *et al.*: Cell lines of pulmonary and non-pulmonary origin as tools to study the effects of house dust mite proteinases on the regulation of epithelial permeability. *Clin Exp Allergy* 1998, 28:1273–1285.
37. Corren J: The impact of allergic rhinitis on bronchial asthma. *J Allergy Clin Immunol* 1998, 101:S352–S356.
38. Ciprandi G, Cirillo I, Tosca MA, Vizzaccaro A: Bronchial hyper-reactivity and spirometric impairment in polysensitized patients with allergic rhinitis. *Clin Mol Allergy* 2004, 2:3.
39. Downie SR, Andersson M, Rimmer J, *et al.*: Association between nasal and bronchial symptoms in subjects with persistent allergic rhinitis. *Allergy* 2004, 59:320–326.
40. Greiff L, Andersson M, Svensson J, *et al.*: Absorption across the nasal airway mucosa in house dust mite perennial allergic rhinitis. *Clin Physiol Funct Imaging* 2002, 22:55–57.
41. Hewitt CR, Brown AP, Hart BJ, Pritchard DI: A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. *J Exp Med* 1995, 182:1537–1544.
42. Sporik R, Platts-Mills TA: Allergen exposure and the development of asthma. *Thorax* 2001, 56(Suppl 2):ii58–ii63.