

REVIEW

Uneasy breather: the implications of dust mite allergens

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Epidemiological studies have demonstrated that there is a relationship between exposure to dust mite allergens and the incidence of allergic asthma. Surveys on the impact of the indoor environment on children and adults showed that this link between the potent allergens from house dust mite (HDM) *Dermatophagoides pteronyssinus* (Der p) or *Dermatophagoides farinae* (Der f) and the occurrence of allergic asthma in patients was unmistakable. Der p 1 is a major allergen from the house dust mite and nine different Der p antigens (Der p 1–9) have been identified to date. Three of these, including Der p 1 are cysteine proteases while others are serine proteases. Der p 1 is a 30-kDa protein which is produced in the mid gut of the mite, where its role is to serve in the digestion of food substrates including human keratin [1].

Homes of patients who are HDM-sensitized contain high levels of Der p 1, Der p 2 and Der f 1 [2], although this is probably also the case in the homes of normal subjects. House dust mite faeces have been shown to contain high concentrations of Der p 1 (up to 10 mg/mL) and there exists a significant association between the concentration of Der p 1 in house dust and the prevalence of sensitivity to HDM extracts and the IgE antibody levels [3]. The majority of adult asthmatics skin test positive to HDM and over 90% of dust mite allergic individuals have IgE antibody directed against Der p 1. As well as elevated IgE levels, these patients also have anti-Der p IgA and IgG, while normal subjects have only low titres of anti-Der p IgG [4].

It has recently been suggested that allergens such as Der p 1 are not only capable of provoking asthmatic attacks in sensitized patients, but are also causally linked to the origin of asthma in some individuals. It has been suggested that exposure to HDM allergens and asthma follows a two-step process, firstly the early exposure leading to sensitization and then repeated exposure leading to airway inflammation and bronchial hyper-reactivity. Several investigators have found that the relative risk of sensitization to HDM increases when levels of HDM increase [5, 6]. Increased levels of exposure result in a lower age of onset of the disease [7]. In asthmatic patients, seasonal variation in HDM levels has also been associated with variations in bronchial hyper-responsiveness, where the levels of HDM seem to peak during late summer and autumn [8].

Despite the wealth of epidemiological data that implicates Der p 1 in the development of bronchial allergic inflammation, there are only limited data to address the exact molecular mechanisms by which this might occur. Four main concepts for the action of Der p 1 have been suggested to account for the biological effects observed with Der p 1 in experimental *in vitro* and *in vivo* models (Fig. 1). There exists considerable evidence to

show that Der p 1 acts as a potent allergen. In such cases, Der p 1 induces a Th2 lymphocyte-mediated immune response and the downstream consequences of an allergic inflammatory response. In humans, Der p 1 and other allergens from HDM induce local and systemic immunological and inflammatory changes in sensitized subjects. Bronchial challenge with these allergens results in the recruitment of helper and suppressor T lymphocytes [9], neutrophils, eosinophils and mast cells [10]. Activation of mast cells and eosinophils results in the release of potent pro-inflammatory mediators, such as histamine, prostaglandin D₂, cysteinyl leukotrienes, proteases, heparin, eosinophil cationic protein and major basic protein [11]. Der p 1 and other HDM allergens may also influence inflammatory processes through stimulating the secretion of mediators such as cytokines and enzymes, and up-regulation of pro-inflammatory adhesion molecules [12]. Der p 1 has been shown to bias human CD4⁺ and CD8⁺ T lymphocytes towards a type 2 cytokine profile thus impacting on immunological processes such as IgE up-regulation [13].

Der p 1 also affects immunological processes directly. CD23, the low-affinity IgE Fc receptor, is a cell surface receptor found on B lymphocytes, eosinophils and dendritic cells. Whilst the precise role of CD23 on eosinophils and dendritic cells remains unclear, considerable evidence suggests that binding of IgE to CD23 on the surface of B lymphocytes provides a negative feedback signal to IgE synthesis. As the human CD23 molecule has been shown to be sensitive to cleavage by cysteinyl proteases such as Der p 1, it is reasonable to postulate that this will result in the enhancement of IgE synthesis through the inhibition of the normal suppressive feedback mechanism to the IgE-producing B lymphocytes [14]. In this scenario, synthesis of Der p 1-specific IgE and IgE against other allergens will be elevated, resulting in a non-specific enhancement of allergic responses.

Recent studies have found that Der p 1 also cleaves the α sub-unit of the IL-2 receptor (CD25) from peripheral blood T lymphocytes. It has been shown that in doing so these cells show a markedly diminished proliferation and IFN- γ production, suggesting that CD25 cleavage by Der p 1 may bias the immune response towards a Th2 pathway [15]. The importance of the cysteine protease activity of Der p 1 has been further demonstrated through its ability to enhance both total IgE and Der p 1-specific IgE synthesis *in vivo*, actions inhibited by the irreversible actions of the protease inhibitor E-64 [16]. Most recently, Ghaemmaghani and Shakib [17] have demonstrated that isolated human T lymphocytes can be conditioned through exposure to proteolytically active Der p 1 allowing them to trigger enhanced IgE synthesis from B lymphocytes. This is most likely due to a shift in the cytokine profile, with an elevation in IL-4 production and a concurrent reduction in IFN- γ .

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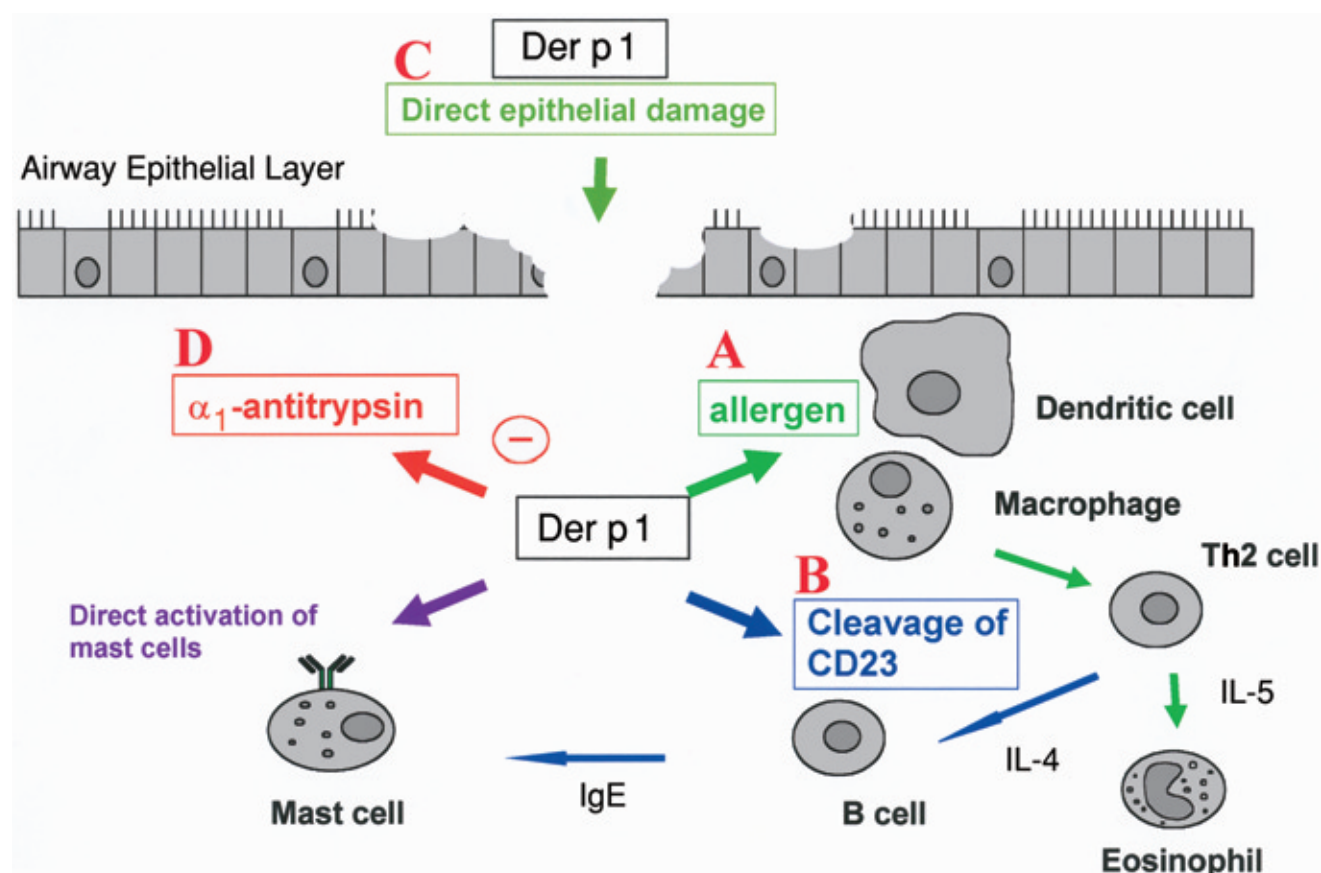


Fig. 1. Diagrammatic summary showing four key actions of Der p 1 as an allergen (a), by cleavage of CD23 (b), direct damage to epithelium (c) and inhibition of α_1 -antitrypsin (d).

In addition to the immunological changes brought about by HDM allergens, biologically active allergens, such as Der p 1 may induce direct damage to the airway epithelium through its cysteinyl protease activity. It has been shown that Der p 1 increases bovine bronchial mucosal permeability to serum albumin as well as causing cellular detachment of cultured cells [18]. Wan et al. [19, 20], have demonstrated that Der p 1 causes disruption of epithelial cell tight junctions by cleaving occludin as shown by the release of soluble cleavage products [19] thus facilitating transepithelial allergen movement. The Der p 1-induced cellular detachment may also promote inflammatory processes in its own right [21]. Exposure of human bronchial epithelial cells *in vitro* to Der p 1 decreased electrical resistance across the cellular boundary as a result of increased permeability [22]. These observations could be inhibited through the actions of a highly potent and selective irreversible inhibitor of Der p 1 PTL11028 [23]. Roche et al. [24], have observed similar findings in tracheal fragments from Lewis rats in an Ussing-type chamber system, showing that a low dose of crude Der p extract was able to increase epithelial conductance in the absence of cell detachment or death. These effects could be abolished in the presence of the cysteine protease inhibitor E-64.

α_1 -antitrypsin represents one of the major antiproteases involved in the homeostatic regulation of the airway environment. It inhibits the activity of other proteases in the airways [25], such as neutrophil elastase and bacterial proteases, which would otherwise cause cellular damage [26]. Through its

cysteinyl protease activity, Der p 1 may also inhibit the activity of α_1 -antitrypsin. Down-regulation of α_1 -antitrypsin activity could result in the loss of host defence against epithelial tissue damage by other proteases, thus potentiating the damage to airway tissue.

The prevalence and severity of allergic asthma has been increasing worldwide. Research into the effects of HDM allergens has predominately been carried out in asthmatic patients and *in vitro* systems. This has been perpetuated by the lack of success in developing *in vivo* animal models. Recently Ishii et al. [27] have successfully sensitized guinea-pigs to HDM allergens to produce a useful model of respiratory sensitization to HDM [27]. More recently it has been shown that inhalation of a crude HDM extract aerosol in mite-sensitized guinea-pigs induced eosinophilia in bronchoalveolar lavage fluid which persisted for at least 7 days [28]. Further work using HDM extracts has focused on different routes of sensitization in order to determine the role of IgE in allergen-induced lung inflammation [29]. Extending this, we have described a rat model of allergic bronchial hyper-responsiveness, driven by intratracheal exposure to HDM extract, to reveal that Der p 1 itself plays a pivotal role in the acquisition of airway hyper-responsiveness and inflammation [30]. We have used a highly potent and selective irreversible inhibitor of Der p 1 PTL11028 to inhibit both airway hyper-responsiveness and inflammation, specifically eosinophilia. Reduced HDM specific IgE levels in blood with PTL11028 treatment parallel the reduced inflammatory response. Whilst

many groups have developed novel transgenic or SCID mouse models to study various aspects of asthma, only a few have used HDM allergens as the sensitizing agent and yet this is the dominant allergen responsible for a major allergic component of asthma in humans. Tournoy et al. [31], demonstrated that SCID mice with resident human peripheral blood mononuclear cells and after intraperitoneal injection with Der p 1, once exposed to HDM allergens produce bronchial hyper-responsiveness mediated by Th2 cytokines IL-4 and IL-5 although the role of IgE and eosinophils in this response was unclear.

There is clearly a need for more relevant animal models of human asthma, including the use of allergens relevant to the human disease (rather than egg albumin which humans do not normally inhale!). Increasingly, there is also evidence that the intrinsic biological activity of allergens provide additional stimuli that contribute to asthma pathogenesis. This provides compelling reasons to investigate some of these actions further, recognizing that asthma is a unique disease of humans.

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