Review

House dust mite allergens in asthma and allergy

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IgE antibodies in house dust mite (HDM) allergy follow a predictable pattern. Half are directed against two dominant allergens and the remainder largely against four midpotency allergens. This hierarchical pattern is not changed by the titre of the IgE response or severity of disease. The structures of these allergens are known and they can be produced as authentic recombinant allergens. There is also evidence that the allergenicity is augmented by the biological activity of the key allergens, validating them as targets for vaccination. Collectively, these developments should facilitate the development of new diagnostics, improve immunotherapy and allow vaccination with defined reagents. Highly purified recombinant polypeptides representing the important mite allergens are now available so that informative and reproducible experiments can be performed with mite allergens in place of poorly defined and variable extracts.

HDMs in allergic disease

HDM allergy is the most prevalent cause of allergic sensitisation that afflicts asthmatics. Although there are geographical differences, up to 85% of asthmatics of highly populated centres of North and South America, Europe, south east Asia and Australasia are typically HDM allergic [1]. Altogether, 10–15% of individuals in most western populations have asthma; about 25% of asthmatics experience weekly symptoms and 15% daily symptoms [2]. The current diagnosis and immunological treatment of HDM allergy are conducted with HDM extracts made from the bodies, excrement and other emanations of mites. For safety reasons, extracts are standardised by their ability to produce skin prick test reactions in allergic volunteers rather than by the allergen content [3]. Understanding the molecular profile of the HDM allergens will allow the replacement of extracts with well-defined antigens in known and effective concentrations and the development of new types of immunotherapy based on the defined allergens, their sequences and their structures. Presently, immunotherapy consists of an initial series of 20–30 injections of HDM extract followed by maintenance treatment for three years, so pharmacological amelioration of the symptoms is usually preferred by patients. This is not a good long-term solution because compliance with medication is frequently not maintained, and without adequate preventative medication asthmatics progress from mild to severe persistent disease [2].

The predominant *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* mites coexist in most geographical regions with notable exceptions being Australasia and the UK where *D. pteronyssinus* predominates [4]. *D. pteronyssinus* prefers temperate/tropical coastal regions, whereas *D. farinae* is more abundant in continental climates. There are, however, many microenvironmental variations. The allergens of *D. pteronyssinus* and *D. farinae* typically have 15–20% amino acid sequence disparity and, although they are immunologically cross-reactive, they also have unique epitopes [5]. The importance of using extracts tailored to the species prevalent in the local environment is not known and is difficult to ascertain because of the variation in extracts made from the same species. Mites from storage mite families can also be important HDMs; however, because their allergens have 70% amino acid sequence disparity with *Dermatophagoides* spp., there is minimal IgE cross-reactivity. *Blomia tropicalis,* found in South America, the Caribbean and Asia, is the most important of the storage mites [6,7] and usually occurs in conjunction with *D. pteronyssinus*.

Allergen nomenclature denominates allergens with the first three letters of the genus, the first letter of the species and then a number representing the order in which they were recorded [8]. The first allergen of *D. pteronyssinus* is accordingly Der p 1. Homologous allergens from related species are given the same number, for example Blo t 1, and collectively allergens with the same number are called a group. The introduction of cDNA technology began a discovery phase during which some of the known allergens were characterised biochemically. Using the criterion of IgE binding for allergenicity (Box 1), many other allergens were also identified. A new phase of allergen research described here has now replaced the fragmentary IgE-binding information with quantitative studies on allergenicity and the structural evaluation of the important allergens. These developments have transformed the concept that HDM allergy is caused by a variable immune response to the components of a complex mixture of unknown allergens to that of largely predictable immune responses to a small number of well-defined allergens (Table 1). There is evidence that the biochemical properties of the dominant allergens are important for allergenicity and that these proteins could be the best targets for immunotherapy and vaccination.

Dermatophagoides spp. allergen hierarchy

Absolute IgE-binding measurements have been used to establish the hierarchy of a panel of nine isolated

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Box 1. Allergenicity

The term allergy was introduced in 1906 by Clemens von Pirquet to describe the change in the reactivity of the immune system that occurs following an initial (sensitising) exposure to an antigen. It was derived from the Greek words *allos* ('other') and *ergon* ('work'). In the intended use of the word, an allergy could either be protective and lead to immunity or be harmful and lead to tissue damage and the adverse reactions that characterise immunological hypersensitivity. Workers have, however, since used allergy only to mean the potential to produce harmful hypersensitivity reactions and it has retained this meaning. Von Pirquet introduced the word 'allergen' to describe an agent that induces the changed reactivity. Herein, allergens are proteins of the HDM. Allergenicity is the property of being an allergen or being able to induce an allergic sensitisation. Like the word allergy, it is now used to describe the ability to cause immune responses that can lead to immunological hypersensitivity, particularly type 1 hypersensitivity mediated by the IgE antibody. The ability of an allergen to induce the IgE antibody is a measure of allergenicity that shows the immune system has been altered to the allergic state. Although this usually correlates with the ability of an allergen to induce clinically relevant hypersensitivity, there are circumstances where it does not. The induction of the IgE antibody that only reacts with a single epitope and cannot cross-link the IgE receptors on mast cells to stimulate the release of inflammatory mediators is an example. The allergic wheal and flare reactions produced by the skin prick tests with allergen provides a measure of a hypersensitivity response, although better correlations are found with end organ provocation such as nasal or ocular conjunctival challenge. An *in vitro* test can also measure allergen-induced histamine release from IgE antibody-armed basophils in tissue culture. As well as mediating hypersensitivity via the IgE antibodies, allergens induce Th2-type T cells that contribute to allergic reactions by inducing the infiltration of harmful inflammatory cells. Th2 reactivity can be measured directly in assays of cultured blood lymphocytes but the IgE antibody, which requires Th2 T-cell help for its production, is also a measure of this activity.

D. pteronyssinus allergens [9]. The panel included purified and well-folded recombinant allergens that absorb nearly all of the IgE binding to HDM extracts, as measured by solid phase immunoassay and immunoblotting [10]. For all sera from a large series of allergic subjects, approximately 50% of the IgE binding was accounted for by Der p 1 and 2 and a further 30% was equally contributed by Der p 4, 5 and 7. The binding of the other allergens was weak albeit detectable. The weak IgE-binding proteins included glutathione S-transferase and, as reported originally for Der f 3 [11], the group 3 trypsin allergen, which is the highest IgE binder of the three serine protease allergens. The low IgE binding of the serine proteases and glutathione S-transferase is of note because of speculation that proteolytic activity or similarity to parasite allergens might be linked to allergenicity. IgE binding to the group 10 tropomyosin allergens, with its highly conserved amino acid sequence and, therefore, high potential for cross-reactivity, was also infrequent and low. Although determined in Australia, this hierarchy has now been confirmed with skin test reactivity in several European countries [12]. A newly identified allergen designated Der p 21, which has sequence homology with Der p 5, has the same degree of IgE binding as Der p 4, 5 and 7 [13], so a large proportion of the IgE binding to extracts can be accounted for by this small number of allergens. This hierarchical pattern is consistent in individuals with high and low anti-HDM IgE titres, in children and adults, and in children with asthma exacerbations recruited from an emergency department [9]. Furthermore, no differences in IgE binding have been detected between children with frequent asthma exacerbation and those with intermittent episodes [14]. Accordingly, individuals with HDM allergy do not have sporadic responses to a wide range of different HDM allergens and do not respond to more allergens if they are more allergic.

Some allergens have not been adequately investigated. Highly prevalent IgE binding has been found for the chitinase group 15 allergens and to a lesser extent the chitin-binding group 18 allergens [5]. Chitin-binding proteins are of particular interest because they are the dominant allergen for HDM-allergic dogs [15] and might interact with chitin, which can be an adjuvant [16]. Other potentially high IgE-binding allergens are the group 11 paramyosin and group 14 vitellogenin-like proteins [5,7]. Several studies in Asia have reported a high prevalence (80%) of IgE binding to paramyosin [7] but the degree of IgE binding has not been quantified. IgE binding to the group 14 allergen called M-177 is highest against frag-

Table 1. Important and cross-reactive HDM allergens

Nomenclature	Biochemical property	Allergenicity *Dermatophagoides* (quantitative)	Allergenicity *Blomia* (quantitative)	Structure (allergens examined)	Recombinant host (structure authenticated)
Group 1	Cysteine protease	Dominant	Unknown	Type c peptidase by X-ray crystallography (Der p 1, Der f 1)	*P. pastoris* (Yes) *E. coli* (No)
Group 2	ML domain lipid-binding protein	Dominant	Low	ML domain by X-ray crystallography (Der p 2, Der f 2)	*E. coli* *P. pastoris* (Yes)
Group 4	α-amylase	Midpotency (a)	Low	Enzymatic activity (Der p 4)	*P. pastoris* (active enzyme)
Group 5	Unknown	Midpotency	Dominant	NMR anti-parallel α-helix bundle (Blot 5)	*E. coli* (Yes)
Group 7	Lipid-binding protein	Midpotency	Uncertain	α-helix wrapped in curved anti-parallel β-sheets	*E. coli* *P. pastoris* (Yes)
Group 21	Unknown	Midpotency	Dominant	Similar to group 5 (Der p 21)	*E. coli* (Yes)
Group 10 (a)	Tropomyosin	Low (a)	Low (a)	Predicted coiled coil	*E. coli* (incomplete)

(a) IgE binding high in some populations

ments of the degraded allergen. IgE binding to both of these proteins is an important and neglected area of investigation because they are degraded in extracts [5]. In summary, IgE-binding studies have shown that over half of the allergenicity found in HDM extracts can be attributed to the dominant group 1 and 2 allergens and, with some reservations about high molecular weight allergens, most of the rest to the group 4, 5, 7 and 21 allergens.

Regional variation and cross-reactivity

Allergic disease is increasing in rural cultures that adopt urbanised lifestyles [17]; therefore, the diagnostic potential of HDM extracts needs to be assessed within new environments.

In Japan, HDM tropomyosin, denominated Der p 10, is a frequent and high IgE-binding protein; similar results have also been obtained in Africa [5]. These anti-Der p 10 responses are not simple cross-reactivities to another antigen in the environment because they are only found in subjects with IgE antibodies to the dominant allergens. By contrast, large studies in Australia [9] and Europe [12] have shown that IgE antibodies to Der p 10 are rare. It seems, therefore, that the IgE antibody repertoire is influenced by environment or diet.

The Der p 4 amylase allergen has unusually high titre binding to IgE in the sera of aboriginals from a tropical region of Australia [18]. The aboriginals do not have IgE antibodies to the Der p 1 or 2 allergens or to Der p 10, which might have been expected from cross-reactive antibodies. IgE binding is restricted to subjects with skin test reactions to HDM extracts, but this IgE reaction is probably attributable to the amylase allergen. The reason for the anti-amylase response has not been elucidated, but it is likely that this phenomenon (i.e. a dominant reaction to an allergen other than the group 1 and 2 allergens) accounts for the lower-than-expected prevalence of asthma. Recent results from the Chengdu province in China are similar; 25% of asthmatics showed IgE binding to Blo t 4 from *B. tropicalis* and half of these individuals were not allergic to the dominant Blo t 5 allergen [19]. In Singapore, *B. tropicalis* is the dominant HDM species and asthma can be produced by nasal provocation with *B. tropicalis* extracts [20]; 80% of subjects react to Blo t 5 and 4% to Blo t 4 in skin tests. These results show that HDM extracts developed for urbanised populations of temperate regions are not necessarily suitable for allergy diagnosis in other regions and can produce misleading results.

Allergen hierarchy of *B. tropicalis*

The dominant allergen of *B. tropicalis* is Blo t 5 [7], and the related Blo t 21 has the same high reactivity [21]. The roles of their group 1 and 2 allergens are unresolved. Two proteins with 60% sequence identity to the group 1 cysteine protease allergens are Blo t 1.0101 [22] and Blo t 1.0201 [23]; both recombinant allergens bind IgE with high frequency without cross-reacting with antibodies to Der p 1. Several group 2 allergens from *B. tropicalis* are listed in GenBank, but, in keeping with the low reactivity observed in immunoblots of extracts, where only 20% of sera contain IgE that binds to a Blo t 2-like band [24], none of these sequences have been linked to allergenic proteins or polypeptides.

B. tropicalis produces a seemingly potent allergen, denominated Blo t 12, which has no counterpart in *Dermatophagoides* spp. Zakzuk *et al.* [25], however, have recently shown that the IgE binding by Blo t 12 might not be as important as once thought. The authors have published a new sequence showing high amino acid identity with the carboxy-terminal region of the group 15 chitinases of *Dermatophagoides* spp. Because none of the published Blo t 12 cDNA sequences show a 5′ untranslated region, it is possible that the putative allergen is a fragment from a yet undiscovered Blo t 15.

Structure of the important HDM allergens

The group 1 allergens are typical cysteine proteases [5], and X-ray crystallography confirms their structural similarity to papain [26–28]. Group 1 allergens contain equally sized amino- and carboxy-terminal globular domains that juxtapose to produce a cleft containing the catalytic site. The possibility that some group 1 allergens form a unique subgroup of cysteine proteases is indicated by the presence of a magnesium-binding site in Der p 1 that is absent in Der f 1 [28]. The proenzyme sequences of these allergens differ from those of other members of the C1A cysteine protease family because they are short and lack elements of a consensus (ERFNIN) sequence [29]. The proenzyme sequence of Blo t 1.0201, however, contains an ERFNIN consensus sequence and is longer, suggesting that it is not equivalent to Der p 1. A contentious issue in the field is whether the group 1 allergens are dimers, as reported for recombinant Der p 1 [27], or monomers, as reported for natural Der f 1 [28]. It is noteworthy that the Der f 1 structure is the first for a natural allergen.

The group 2 allergens are the founding members of the ML (MD-2-related lipid-recognition) domain lipid-binding family. They consist of two planes of beta sheets that fold to form a large internal hydrophobic cavity [30,31]. This fits with the known function of other ML domain proteins, such as the Niemann–Pick type C2 protein that transports cholesterol and the MD-2 protein that loads lipopolysaccharide (LPS) onto Toll-like receptor 4 (TLR4). Der f 2 binds LPS with nanomolar affinity by inserting acyl chains between the two large β-sheets in a clam-like action similar to MD-2 [32]. A modelled structure of the Der f 2–LPS complex with TLR4 (based on the structure of the complex of TLR4, LPS and MD-2) shows a plausible interface for interaction with TLR4. Another important consideration for the group 2 allergens is that they have an evolutionary pattern of sequence variations in, for Der p 2, four key amino acids [33–35]. The distribution of the sequence variants differs in different countries, and three independent reports [34,36,37] have shown that the IgE–binding ability is altered by these sequence changes; the recombinant Der p 2.0101 variant binds only half as well as that of the Der p 2.0104 type. Given the size of the IgE-binding disparity, it is important to determine if the natural variants have the same difference as the recombinant proteins. Knowledge of the structural changes that reduce IgE binding might assist the production of hypoallergens for immunotherapy.

The group 4 allergens are typical α-amylases [5]. Given the possible cross reactivity with other environmental

antigens and the potential of IgE-binding carbohydrate structures, cross-reactive carbohydrate moieties should be considered. Der p 4 does have an N-glycosylation site, but its molecular mass measured by SDS-PAGE indicates that it is not glycosylated and the site is absent in Eur m 4 and Blo t 4 [19]. The sequence identity between Der p 4 and amylases from cockroaches, shellfish and humans is approximately 50%, which is not normally enough to produce high cross-reactivity.

Sequences similar to the group 5 and 21 allergens have only been found in mites. They are clearly homologous proteins and the work of Gao and colleagues presents evidence for a third paralogous gene in *B. tropicalis* [21]. Cross comparisons of all combinations of the sequences of Der p 5, Der p 21, Blo t 5 and Blo t 21 show about 40% sequence identity without any regions of similarity that would distinguish the group 5 from the group 21 sequences. Accordingly, it is not clear if the historical designations of 5 and 21 are correct; Der p 5 could, for example, be the equivalent of Blo t 21. The solution structure of Blo t 5 shows a flexible 17 residue amino terminus followed by three similarly sized α-helices, which are tightly packed into an anti-parallel bundle [38,39]. This unique structure is surprising because the peptide contains well-defined heptad repeats that generally predict a coiled coil. There are, however, interactions between the helices; the hydrophobic side chains are tightly packed and, interestingly, the exchange of these packing interactions confers flexibility to the bundle. Short-angled X-ray scattering analysis of Der p 21 also shows a shape consistent with the three-helix bundle [13] but, in contrast to Blo t 5, this allergen is a dimer. Further structural analysis might, therefore, reveal defining differences between the group 5 and 21 allergens.

The X-ray crystallographic structure of Der p 7 is similar to that of LPS-binding protein (LBP) and bactericidal permeability increasing protein [40]. Der p 7 has a superwrap fold with an α-helix wrapped in curved anti-parallel β-sheets, which is also shared by odorant-binding proteins. Der p 7 does not specifically bind LPS but can be a ligand for other bacterial lipids. Because LBP complexed to CD14 binds to TLRs after lipid binding, there is potential for another important allergen to interact with the innate immune system.

Based on their sequences, the group 10 and 11 allergens are typical tropomyosins and paramyosins, respectively [5]. Tropomyosins are highly conserved and antibodies cross-react to allergens from disparate species, whereas paramyosins are not as well conserved and would not be expected to be as cross-reactive, for example, with the anti-paramyosin antibodies induced by helminthic parasite infections.

The large lipid transfer protein group 14 allergens can be either apolipophorin or vitellogenin-like proteins. The possibility that these antigens are derived from an egg vitellogenin suggests that they are dispersed into the environment in eggs, which usually constitute the most abundant stage of the mite life cycle. This would not only result in a plentiful supply of allergens but also produce differences in dispersal and environmental repositories compared with faecal allergens.

Based on their sequences, the group 15 allergens are typical family 18 glycosyl hydrolases. They have characteristic O-glycosylation domains such that the natural glycosylated allergens exist in 98 and 105 kDa forms compared with their 60 kDa polypeptides [5,15]. This is the most heavily glycosylated HDM allergen and apart from its influences on antigenicity glycosylation will be important to consider when constructing recombinant allergens.

Determinants of allergenicity and innate immunity

The hierarchical responses to allergens might result from their abundance, aerobiology and stability in the environment. There is, however, little information about these features. Allergen abundance in fresh mite extracts does not show concordance with their allergenicity [41]; Der p 1 and 2 are the 31st and 41st most abundant proteins, followed closely by Der p 7, whereas the poorly reactive allergens Der p 10, 13 and 20 are more abundant. Group 1 [42], 2 [43], 4 [44], 5 [45] and 21 allergens [13,21] associate with the dung ball, which is believed to be important, but the chitinase and chitinase-like allergens [15,46] are found in the gut but not the faeces. The origin of the group 7 allergens is unknown.

Biochemical properties such as enzymatic activity and interaction with the elements of the innate immune system (Box 2) could help determine allergenicity (Figure 1). The cysteine protease activity of Der p 1 suggests that it might break mucosal barriers or otherwise enhance immune responses. A broad range of such activities have been demonstrated, including altering barrier functions and activating dendritic cells [47], but it is difficult to judge their physiological importance. For example, potential immunoenhancing effects such the *in vitro* induction of cytokine release from cultured cells has been reported for

Box 2. Innate immunity

The adaptive immune system is dependent on the highly specific recognition of antigen by the antigen receptors of antibodies and T cells, namely the V regions of immunoglobulins and T-cell receptors. This causes the activation and clonal expansion of antigen-specific T and B cells from a repertoire of millions of lymphocytes with different and often exquisite antigen-specific reactivity. Vaccination and infection activate the adaptive immune responses to produce lasting protection and immunological memory, which are features of both immunity and allergy. The induction of adaptive responses is, however, slow and dependent on the rapid production of danger signals from the innate immune system. The innate system is mediated by a variety of cells and molecular mechanisms that generically recognise molecules or biochemical activities produced by potential pathogens. Pathogen recognition elements can be lectins that bind microbial polysaccharides and direct them to phagocytic cells, intracellular receptors of microbial products or protease-sensitive receptors that can be activated by microbial proteases. An especially important and interesting family of innate immune receptors are the TLRs. They are transmembrane proteins found on the cell surface and on endosomes of antigen-presenting dendritic cells that interact with microbial products to upregulate the production of proteins for the inflammatory responses via MyD88 signalling. Humans have 10 TLRs and each shows a characteristic but broad binding specificity for microbial products. These include bacterial lipopeptides, LPS, agellin, single stranded RNA and unmethylated (CpG) DNA. Antigens that activate TLRs induce more effective immune responses. TLR4, which binds ubiquitous LPS, is central to the induction of allergic responses as well as immunity.

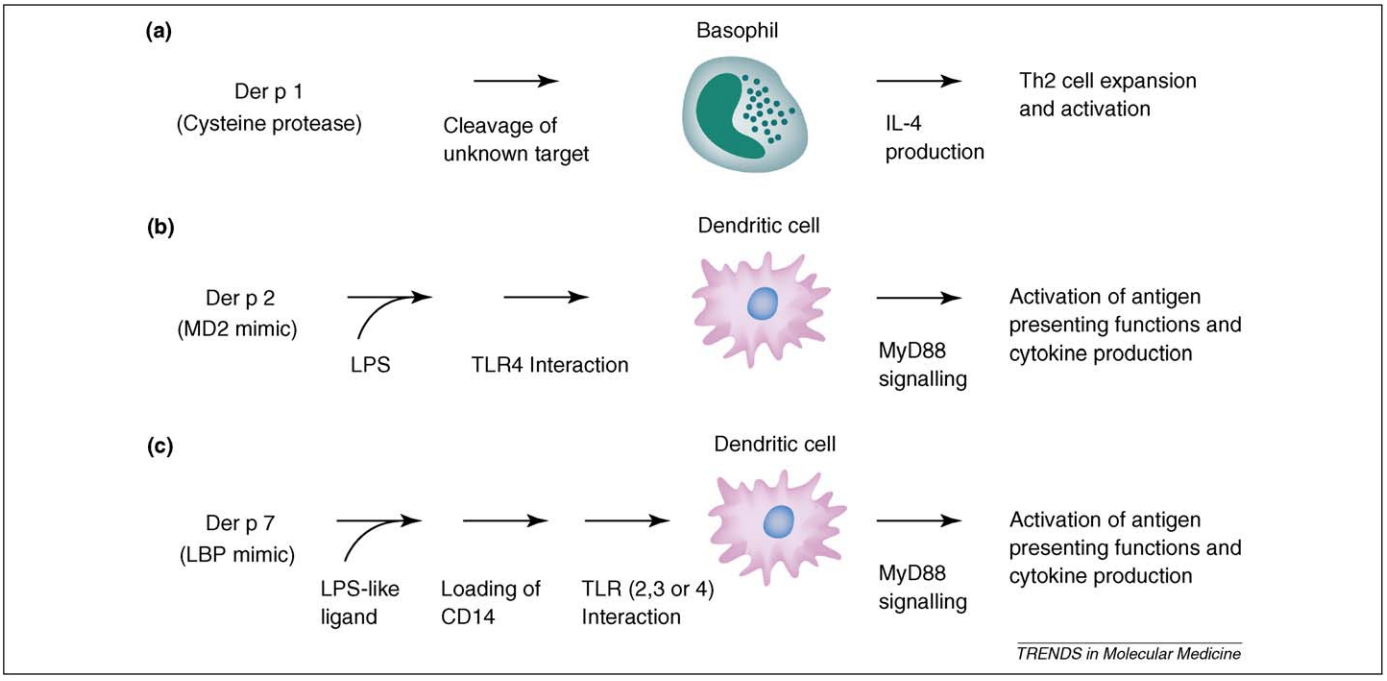

Figure 1. Pathways for HDM allergen interaction with the innate immune system.

group 3 trypsin allergens, but these are weak allergens as shown by IgE binding. Additionally, the IgE binding of Der f 1 shows that it is not a dominant allergen for dogs [15]. Cysteine proteases have catalytic thiols that are sensitive to oxidative deactivation [48] and in the absence of a reducing agent, little proteolytic activity is attributed to Der p 1 in HDM extracts [49]. In addition, body fluids have high concentrations of thiol-protease inhibitors, so extracellular protease activity is rare [48]. Lysosomes, however, have special transport mechanisms for cysteine; therefore, the enzymatic activity of allergens could be activated if they reached this subcellular compartment [48]. Notwithstanding these reservations, enzymatically active Der p 1 and Der f 1 induce more robust IgE antibody responses in mice than inactive enzymes [47,50]. It is, however, not known if the Th2 bias in these experiments is directed by proteolytic activity because the Th2-inducing alum adjuvant was used. In a related experiment, enzymatically active Der p 1 induces higher lung inflammatory responses in allergic mice than enzymatically inactivated Der p 1, but the increase is in the Th1-type macrophage response and not in the Th2-driven eosinophilia [47] as would be expected for the promotion of Th2 immunity. Evidence for a Th2 adjuvant activity of the cysteine protease activity comes from a study on the homologous protease papain [51]; the injection of papain into the footpads of mice induces Th2 and IgE antibody responses that depend on the cysteine protease activity. In a more physiological setting, the intranasal administration of low doses of papain induces high and persistent IgE and Th2 inflammatory responses without the need for adjuvant [52]. This avenue of investigation can be readily pursued because purified papain is commercially available at a low cost. Two crucial results already derived from this model system are that trypsin and other non-cysteine protease proteolytic enzymes are not active and that the adjuvant activity of papain is mediated via basophils [51].

The discovery that MD-2 loads LPS onto TLR4 suggests that group 2 allergenicity might be enhanced by a similar interaction [53]. Der p 2 complexed with LPS indeed induces Th2 responses in mice and this occurs in MD-2- but not TLR4-deficient animals [54]. Der f 2 binds LPS with a nanomolar affinity and, from NMR, in a manner similar to the binding by MD-2 [32]. The hydrophobic LPS-binding residues are similar in all of the group 2 allergen sequences but the potential TLR4-binding interface might be of more interest. Der f 2 displays a triangle of basic residues, Lys-48, Lys-77 and Lys-82, which occupy the same positions as Lys-72, Arg-106 and Lys-109 in the TLR4 interface of MD-2. These residues are conserved or similar for other HDM and storage mite homologues except for *B. tropicalis*, which has Glu-48, Thr-77 and Lys 82. The loss of these basic residues, especially the acidic Glu-48 substitution, associates with the lower allergenicity of Blo t 2 [24]. In general, comparing the relative ability of the group 2 allergens from different mite species to present LPS to TLR4 with their ability to induce IgE antibody and Th2 T-cell responses could test the importance of the TLR4 interaction. Because group 7 allergens are homologous to LBPs [40], they could have a similar connection with the innate immune system. LBP is required for the efficient loading of LPS onto CD14 and the activation of TLR2, TLR3 or TLR4. LPS does not seem to be the ligand for Der p 7 [40], so it is likely to bind a different microbial or mite phospholipid, to the MD-2 mimic Der p 2 and activate via a different TLR.

In summary, there is *in vivo* evidence for the activation of innate immunity by the two dominant HDM allergens via the pathways summarised in Figure 1 and recent results show a plausible pathway for the important Der p 7 allergen.

Production of recombinant allergens

The production of a recombinant group 1 allergen in high yield was initially accomplished by the introduction of the

proenzyme form of Der f 1 by transfection in *Pichia pastoris,* a yeast system commonly used for the expression of heterologous proteins. *P. pastoris* produces and secretes mature protein with enzymatic activity [55]. Pro-Der f 1 has also been produced in *Escherichia coli* and can be converted to active enzyme by self-cleavage under acidic conditions [56]. Secretion and acid cleavage of the proenzyme is also used to produce Der p 1 in *P. pastoris* [57–59]. Hyperglycosylation of the N-glycosylation site in mature Der p 1 and Der f 1 can occur but this can be prevented by site-directed mutagenesis, which does not have a large effect on the enzymatic or antigenic activity [59,60]. Der p 1 has an additional N-glycosylation site in the pro-region and, although authentic processing of hyperglycosylated pro-Der p 1 occurs, the production of Der p 1 from a mutant lacking this site is more efficient [59]. Careful control of the pH is also important for the cleavage process because, although the acidic conditions unfold the zymogen, they can inactivate the enzymatic activity [61].

High levels of Der p 2 [62] and Der f 2 [63] can be produced in *E. coli* inclusion bodies, solubilised and refolded into natural structures. They can also be secreted from *P. pastoris* [31,64]. The antigenic and structural comparison of recombinant Der p 2 produced in *E. coli* and *P. pastoris*, however, reveals differences in folding and ligand binding between allergens produced by these two methods. Importantly, even though Der p 2 secreted from *P. pastoris* has high IgE-binding activity, circular dichroism analysis shows that this peptide is a random coil. A structured molecule with even better IgE-binding activity is obtained following precipitation and refolding [64]. Thus, it is important not to assume a natural structure solely on the basis of IgE binding, and the natural structure might be important for experiments requiring antigen presentation and T-cell stimulation.

Recombinant polypeptides with amylase activity representing Der p 4 allergens have been produced from *P. pastoris* [65]. Group 5 [39,45] and group 21 [13] allergens can be expressed from *E. coli* without fusion partners as soluble, highly allergenic molecules, and these proteins have been used for structural determinations. Structured group 7 allergens can be produced in *E. coli* and *P. pastoris* and they have excellent IgE-binding activity [40,66]. HDM tropomyosin produced in *E. coli* has structural characteristics of the natural protein [67]. Recombinant allergens with authenticated structures of full-length paramyosin, chitinase and the large lipid-binding protein allergens have not been reported. The successful production of these proteins would help the assessment of their importance.

Commercially available allergens

Purified and recombinant group 1 and 2 allergens are now available from a commercial source and are being used in cellular assays [68–70]. The availability of these proteins for laboratories that do not specialise in the production of allergens heralds the possibility of a new era of HDM allergy research with an emphasis on purified allergens. The conclusions on allergenicity in this review have largely been drawn from analyses of IgE-binding activity and need to be further developed with more biological and clinical measures. Precursor frequencies of T cells that specifically react to the different allergens and produce Th2 and possibly regulatory cytokines can now be measured, not only with informative dose response analyses, but also in the absence of proinflammatory stimulants artifactually found in allergen extracts. Nasal challenges with pure allergens can be performed to determine the allergens with the best ability to induce inhalation allergy. It is also important to establish the amount of allergens that are inhaled in relation to their ability to induce allergic reactions on allergen provocation. The allergens can also be used to monitor immunotherapy to ascertain if the current treatments reduce the responses to all the important allergens and devise new treatments that are highly effective at this. The hierarchy of allergens described here can now be used to formulate defined diagnostic reagents or arrays of allergens for IgE-binding tests to distinguish patients with HDM allergy from people producing cross-reactive antibodies to other antigens. Clearly, the most important allergens would be the main targets for new types of immunotherapy and this information is now available. Simple formulations containing known, adequate, balanced and standardisable concentrations of these proteins using the regimens currently practised with extracts would be an obvious point to start clinical trials. There are, however, many other strategies that could enhance these treatments; the use recombinant allergens engineered to reduce IgE binding, for example, would reduce adverse anaphylactic reactions during treatment and allow the rapid updosing of the amount of allergen injected, thereby enhancing patient compliance [71]. It might be an advantage to engineer the structures that stimulate the innate immune system, although the reverse might also be true.

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