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Mite allergens: an overview

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Summary

Mite allergens from the Pyroglyphidae family are the most frequent and potent sources of perennial asthma and rhinitis. Since 1988 molecular knowledge has considerably increased and structures and functions have been determined for most of them.
Of the 22 denominated allergens, Der p 1 and Der p 2 are major allergens recognized by more than 80% of IgE from Dpt allergic patients in Europe. Der p 4, Der p 5 and Der p 7 appeared to be intermediate allergens. The binding of IgE to groups 3, 6, 8, 9, 10 and 20 is constantly low. Most of the allergens can be identified by amino-acid sequences and the tertiary structure of the major allergens has been solved. Most Dpt mite allergens are proteolytic enzymes: Der p 1 for instance is a cysteine protease. Der p 2 has structural homology with MD-2, a co-receptor of the Toll-like receptor (TLR4) whose ligand is LPS. Knowledge of the mite allergens structure has allowed a better interpretation of cross reactions between allergens from the same family or from more distant families. From a practical point of view molecular epidemiology has allowed a better choice of allergen molecules useful for diagnosis. Finally, new concepts of immunotherapy based on genetically engineered hypoallergenic variants of major allergens, used alone or in combination, can be considered.

Introduction

The biological activities of mite allergens with clinical relevance have been elucidated. Thanks to data banks, associations between sequences of allergens and proteins are known. For many allergens, the amino acid sequences and their three-dimensional structures, as well as T-cell epitopes have been determined. The International Union of Immunological Societies (IUIS) suggested that the naming of allergens should be standardized, using the first 3 letters of the sources of allergens (Der for Dermatophagoides for example), the first or second letter of the species (p for pteronyssinus), and finally an Arabic number corresponding to either the order in which the allergen was discovered or its clinical significance, or both. Allergens having significant homology, very close phylogenetically and with the same biochemical function, are included into specific groups. For example Der p 1, Der f 1, Eur m 1 are related to group 1 mite allergens. Recombinant allergens are preceded by the letter r, the natural allergens by the letter n. If iso-allergens have been identified, i.e. allergens with at least 67% sequence identity of amino acids (AA), a suffix from 01 to 99 is

used. The iso-allergen of Der p 1 are named Der p 1 .01, Der p 1.02, etc... The denomination "molecular variants or isoforms" applies to polymorphic variants of the same allergen having at least 90% sequence identity. They are identified by two supplementary numbers compared to iso-allergens ,for example Derp1 possesses 23 isoforms, i.e Der p 1.0101 to Der p 1.0123 (1, 2).

Mite allergens

Mite allergens have a molecular weight ranging from 11 to 190 kDa and are proteins or glycoproteins. 22 different allergens, identified from pyroglyphid mites, storage mites and Blomia tropicalis, received the "Imprimatur" of IUIS (3, 4). The identification of mite allergens is still not complete. For instance previously unrecognized allergens have been identified as homologues in groups 5 and 21 for allergens belonging to Tyrophagus putrescenciae, Suidasia medanensis and Aleuroglyphus ovatus in Singapore (5). Gao (6) identified a new major allergen of Blomia tropicalis, Blo t 21, which is likely a paralog of Blo t 5.

Origins of mite allergens

The major sources of mite allergens are faecal particles that accumulate in house dust, salivary secretions, whole body particles, debris of cuticles and cells.
Allergens from different mite species are either enzymes or proteins binding either to actin, to fatty acids ,to calcium or are proteins of unknown functions. Most pyroglyphid mite allergens are enzymes: cysteine proteases, trypsins and chymotrypsins, amylases, collagenases, chitinases... The proteolytic digestive enzymes secreted by cells lining the midgut (7, 8) are necessary for degradation and digestion of human skin flakes and food; they are found in the faeces of dust mites. Tovey (9) has shown that well-fed mites excrete between 20 and 40 particles per day, each containing Der p 1 allergen ranging from 0.1 ng to 10 ng. 90% of group 1 allergens derive from faecal particles. These faecal particles are surrounded by a chitinous peritrophic membrane preventing them from desegregating but allowing a rapid elution of allergens. The dead mites, eggs, larvae are also allergen sources. The major allergens Der p 1 and Der f 1 are destroyed at temperature of 60°C; in contrast group 2 allergens, are heat-resistant proteins that do not deteriorate at temperatures of 100°C (3, 4). Kort and Kniest (10) showed that even after mites are killed, textiles remained reservoirs of allergens and Der p 1 activity is unchanged for up to 47 months. The sizes of the particles carrying group 1 allergens from pyroglyphid mites are mostly equal or larger than 10 microns. These particles quickly fall down and are only measurable while they are in suspension during household activities (11, 12). Only 5 to 10% of particles with a 20 micron diameter can penetrate into the airways during normal mouth breathing (11). De Lucca et al. (13) showed that group 1 allergens can also come from salivary secretion, be carried by particles with a 5 micron aerodynamic diameter. Casset et al. (14) showed by inhalation tests that inhaled particles of Der p 1 with an aerodynamic diameter > 10 microns played a great role in the immediate bronchial response.

Group 1 allergens from pyroglyphid dust mites

Group 1 allergens are cysteine proteases, that is to says "papain-like" enzymes belonging to the same plant family as papain and cathepsin L-(15). Der p 1, with a molecular mass of 25 kDa, has a primary structure of 222 AA. The tertiary structure of Der p 1 has also be determined (16). The first part of the molecule is composed of three helices, while the second is composed of 5 β strands. The active center is located in the crevice between the two domains. Papain and caricaïne of papaya, and bromelain and ananaïne of pineapple, kiwi actinidin have very similar tertiary structures to group 1 allergens, but their sequence homology in AA is only 25 to 43.3%. This explains why there is no clinically significant cross-reactivity between Der p 1, Der f 1 and papain from plants. Among allergens from Dermatophagoides Der p 1 allergen was cloned first (17). It corresponds to a major allergen, as 50 to 70% of specific IgE of patients allergic to an overall Dpt extract or Df extract bind to Der p 1. These percentages can vary from one geographic population to another. The amino acid sequence identities are very close within specific mite families,for instance Der p 1 and Der f 1 have a AA sequence identity of 85%. Between Eur m 1 and Der p 1, the homology is about 80% (18). Areas of AA disparities are concentrated in the 20 N-terminal residues and at residues 80-130 of the central region (19).
All group 1 allergens have a N-glycosylation site at residues 53-55, which is consistent with the presence of carbohydrates in the purified natural extract. This also explains the initial difficulties of obtaining the recombinant allergen rDer p 1 when using bacterial expression systems. Another feature of Der p 1 is its high degree of polymorphism related to the allele of a single gene coding for

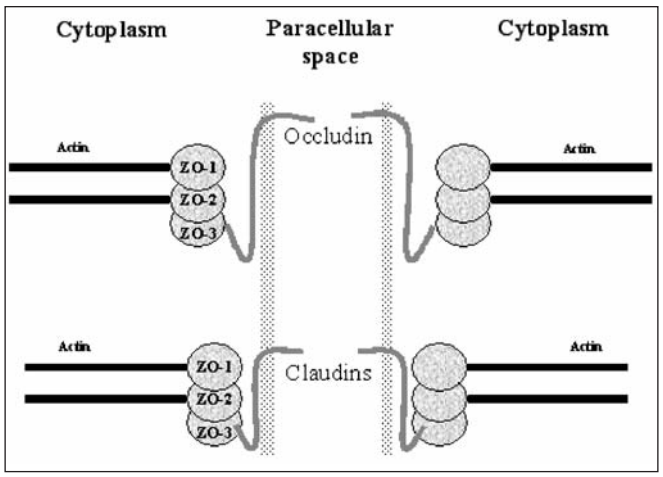
Der p 1. Twenty-three variants have been identified from Der p 1.0101 to Der p 1.0123. The sequences most often encountered are Der p 1.0102 and Der p 1.0105. The polymorphism of Der f 1 is lower. T cell epitopes are present at the central loop. Der p 1 and Der f 1 are abundant and frequently found at high levels in house dust samples. Regarding storage mites, it seems that they do not possess protease allergens belonging to group 1 allergens. Blot 1, a Blomia tropicalis allergen, was registered by the IUIS, however it has only a 35% AA homology with other group 1 allergens from Dermatophagoides.

Functions of group 1 allergens

The possible role of the enzymatic activity of group 1 allergens in mite allergenicity has been the subject of numerous studies since 1995 (20-24). Enzymatic activity of Der p 1 is related to its protease function. Mice injected with Der p 1 with enzyme activity, produce more IgE than mice injected with inactivated Der p1.

- Action on the permeability of bronchial epithelium and on tight junctions.
  The allergen Der p 1 is able to alter the permeability of the bronchial epithelial barrier; this has been shown in a culture of a monolayer of bronchial epithelial cells resulting in a an increased passage of protidic markers (20). Der p 1 can cleave the extracellular domains of occludin and claudins, which, combined with intracellular proteins ZO (zona occludens) constitute a protein macromolecular assembly regulating the para-cellular permeability (Fig. 1). The action of Der p 1 proteases has been demonstrated when using concentrations close to the estimated daily exposure to mite allergens (25, 26). Inhibition of protease activity of Der p 1 (by E-64 for example) decreases the transepithelial passage of labelled bovine serum albumin (14C-BSA) (27). Thus, the increase in transepithelial permeability due to the disruption of tight junctions facilitates the passage of allergens and increases their access to dendritic cells conferring to the bronchial epithelium a pivotal role in the pathophysiology of asthma (28).

Figure 1 - Simplified representation of the tight junctions (modified from Stewart et al. (24))



- Action on bronchial epithelial cells in culture
  Der p 1 induces the release of proinflammatory mediators: IL6, IL8, eotaxin and GM-CSF (granulocyte/macrophage colony-stimulating factor) (29, 30). Der p 1 also increases the production of chemokines receptors, CXC and CC family: CCL2, CCL5 (RANTES), CCL20 and CXCL10 (31, 32). These chemokines lead to the in vitro recruitment of dendritic cells, which is not observed in the presence of E64 or pro-Der p 1 (a pro-enzyme without any protease activity) (33). The activities of pro-inflammatory protease allergens have been attributed to the activation of PAR-2 (protease-activated receptors). Indeed, PAR-2 is overexpressed in the airway epithelium of asthmatic patients (34).In mice, PAR-2 is also involved in eosinophil infiltration and bronchial hyperresponsiveness (35). In PAR-2 deficient mice, eosinophilic inflammation is abrogated (36).
  In vitro studies using bronchial epithelial cell lines showed that dust mite allergens were able to induce an inflammatory response by directly stimulating the release of proinflammatory cytokines through activation of PAR-2 (37). Subsequently, it was shown that crude extracts of mites activated the transcription factors ERK1/2, MAPK in bronchial epithelial cells (38). Similarly, the involvement of the activity of ERK1/2 and NF-kappa B has been demonstrated in the production of IL-8 from epithelial cells challenged with Der p 1. But it was shown that the response mediated by ERK1/2 is independent of the activation of PAR-2 (39), which calls into question the activation of PAR-2.

- Action on mast cells and basophils
  Independently of IgE-dependent sensitization and direct stimulation of mast cells and basophils, Der p 1 provokes IL4 secretion (40).

- Action on eosinophils
  The allergen Der f 1-induced superoxide anion production by human eosinophils and their degranulation, results in the release of EDN (eosinophil derived neuro-

toxin) (41). These effects depend on cysteine protease activity of Der f 1, since they are absent when the enzyme activity is inhibited by E-64.

- Action on bronchial smooth muscle cells
  Der p 1 may act directly on rabbit bronchial smooth muscle cells, by increasing the acetylcholine induced contraction and decreasing the reversibility induced by isoproterenol (42).

- Action on B and T cells
  The in vitro observations suggested that the allergen Der p 1, through specific cleavage on the surface of human B lymphocytes of CD23(the low affinity receptor for IgE), could increase IgE production (43, 44, 45). CD23 and its soluble fragments actually possess immunoregulatory activity of IgE synthesis. Cleavage of CD23 could cause the deletion of a negative regulatory mechanism and enable the production of soluble CD23. However, this mechanism has not been confirmed by in vivo studies (46).
  In addition, Der p 1 can cleave the subunit of the IL-2 receptor (IL-2R or CD25) present on the surface of human T lymphocytes, which can induce a decreased secretion of IFN-gamma. IL-2R is essential for the proliferation of Th1 cells, its cleavage by Der p 1 could direct the immune response towards a Th2 profile (47). In vivo, in a mouse model, administration of Der p 1 enhances the Th2 response (48). Finally, Der p 1 is also able to cleave CD40, resulting in decreased production of IL-12 by dendritic cells (49). IL-12 plays a major role in driving T cells toward a Th1 profile. In addition, dendritic cells derived from monocytes of patients allergic to D. pteronyssinus, produce in the presence of Der p 1 more cytokines (IL-6, IL-10), chemokines (CCL-17 CCL-22), and less IL-12 than dendritic cells of non allergic donors (50).

Other Der p 1 activities

Alpha1-antitrypsin, which protects the respiratory inflammatory lesions induced by serine proteases is inactivated by Der p 1. Alpha1-antitrypsin is cleaved in its central part and near its NH2-terminal (51). This action is especially significant because it may potentiate the role of allergens with serine protease activity, such as Der p 3 and Der p 9. In addition, Der p 1 inactivates elastase inhibitors (52). This can exacerbate tissue damages caused by activation of neutrophils. Finally, Der p 1 also degrades proteins A and D of lung surfactant (53), which have a protective effect against allergic airway inflammation.

These effects were mainly observed in animals, particularly in mice or on cell cultures, using Der p 1 concentrations not always corresponding to realistic conditions. In addition, allergens with enzyme function represent only 40% of all allergens. Certain major allergens like Der p2 and Der f2 of Dermatophagoides pteronyssinus have no enzymatic functions.

Group 2 allergens

Group 2 allergens have a molecular mass of 14-18 kDa,and bind strongly to IgE (54). Der p 2 and Der f 2 have only a 12% disparity in their amino acid sequences; they are not glycosylated. Eur m 2 (55) has a 82% amino acid sequence identity with both Der p 2 and Der f 2. Group 2 allergens are major allergens just as group1. They are heat resistant and do not deteriorate at temperatures up to 100 ° C. They are found primarily in the mite faeces , but also in the cuticle. According to the study of O'Hehir et al. (56), the regions of Der p 2 most frequently recognized by T-cell epitopes are present at AA 61-86 and 78-104. Group 2 allergens are produced by single genes. Many variants especially Der p 2.0101 and Der p 2.0104 have been described. These variants are representative of natural variants of the Der p 2 allergen .Der p 2, because of its lack of glycosylation, was obtained more easily in recombinant form (57), the recombinant polypeptide having all the reactivity of the natural allergen. Crystallography studies conducted for Der p2 and Der f 2 showed an immunoglobulin structure folded around a hydrophobic cavity (58).The function of group 2 allergens remained hypothetical for a long time until it was discovered that its structure was also found in proteins with a ML lipid binding domain. Trompette et al. (59) have elucidated the major function of Der p2 showing structural homology with MD-2, which belongs to the superfamily of proteins with a domain ML lipid binding. MD-2 binds to LPS and activates the signalling pathway of TLR4 (Toll-Like Receptor 4) by binding to the ectodomain receptor (60, 61). Der p 2, like MD-2 is able in vitro to bind to LPS, to TLR4 , MD-2 and CD 14, suggesting that Der p 2 facilitates TLR4 aggregation. In experimentally sensitized mice intranasal provocation tests with recombinant Der p 2 associated to small amounts of LPS lead to experimental allergic asthma with Th2-type inflammation in mice deficient MD- 2, whereas this was not the case in mice deficient in TLR4 (59). Der p 2 could be a self-adjuvant in the development of adaptive immune responses by facilitating signalling through TLR4. Hammad et al. (62) showed that TLR4 was mainly expressed on lung epithelial cells, which, when acti-

vated, secrete chemokines and chimiomokines involved in recruitment and maturation of dendritic cells. As for Der p 2, it has been shown that Der f 2 was also able to bind to LPS (63). The fact that other members of the family possessing MD-2 with a lipid-binding domain, are aero-allergens, suggests that the intrinsic adjuvant activity of such proteins could be involved in allergenicity.
Group 2 allergens were described as major allergens from storage mites: Lep d 2, formerly Lep d 1 (Lepidoglyphus destructor), Gly d 2 (Glycyphagus domesticus), Tyr p 2 (Tyrophagus putrescenciae). The homologies between these allergens and those of group 2 Dermatophagoides allergens are variable and modest: Lep d 2 and Der p 2: 36%, Tyr p 2 and Der p 2: 41%, whereas homologies of group 2 allergens from storage mites are about 80%.

Group 3 allergens (trypsin-like allergen)

Group 3 allergens have a molecular weight of 30 kDa. Der p 3 and Der f 3 are serine proteases with trypsin-like function (64, 65).
The sequences of AA showed that Der p 3 had 233 residues and a molecular mass of 25 kDa. Der f 3 and Der p 3 have 81% sequence identity. These are major constituents of the dust mite faecal particles, but they are also present in low concentrations in mite bodies. Eur m 3 has 80% identity with Der p 3 and Der f 3. As for Der p 1, it has been suggested that proteolytic activity is related to allergenicity. Group 3 allergens produce anaphylatoxins C3a and C5a by cleavage of complement proteins. Der p 3 and Der p 9 can induce the formation of pro-inflammatory cytokines GM-CSF and eotaxin by activation of PAR-2 (protease activated receptor 2) (66). In addition, allergens having serine protease activity (Der p 3, 6 and 9), also present in faecal particles, may increase the permeability of the bronchial epithelium by disrupting tight junctions, and act on other cleavage sites than Der p 1 (25). The concentration of group 3 allergens in the environment is unknown. Their binding to IgE is low and they do not represent important allergens.

Group 4 allergens (amylase)

Der p 4 is an allergen of 60 kDa and one of the most important allergens besides Der p 1 and Der p 2. Its sequence is typically that of alpha-amylase and its modelized structure is that of the 13 glycosyl hydrolases family (67), i.e, a barrel of 8 strands alpha / beta containing active sites. The AA sequences of Der p 4 and Eur m 4 have a 90% identity. Der p 4 and Eur m 4 have a 50% identity with the amylases of insects, mammals and molluscs. Der p 4 and Eur m 4 both have glycosylation sites. The frequency of IgE binding to allergens of group 4 is 40% on an average (68), ranging from 25 to 46%.

Group 5 allergens (unknown function)

Der p 5 is a polypeptide with a molecular mass of 15 kDa, it binds 40% of Dpt IgE, thus ranking group 5 as allergens of middle importance. Its function has not yet been determined. Der p 5 does not seem abundant in house dust; in Taipei where pyroglyphid mites are abundant, Der p 5 is present at levels below 100 ng / g of dust (69). Isoforms of group 5 allergens may have important differences in their AA sequences. Der p 5 is secreted by epithelial cells from the intestinal tract (70).
Blo t 5 is a major allergen from Blomia tropicalis. It binds to 70% of serum IgE (71). The AA sequence identity is 43% with Der p 5 (72), however there is no cross-reactivity between Blo t 5 and Der p 5.
Blo t 5 has an AA sequence identity of 40% with Der p 21. The C-terminal region of Der p 5 has a similar sequence with Der p 21 (4).. Regardless of any protease activity, Der p 5 unlike Der p1 increases the production of IL-6 and IL-8 through pathways involving intracellular calcium mobilization (73).

Group 6 allergens (chymotrypsin-function)

Group 6 allergens are proteins of 25 kDa purified from extracts of D. pteronyssinus and D. farinae. Der p 6 contains the catalytic residues characteristic of serine proteases. A serine at position 189 determines the specificity of group 6 trypsin (74). There is no cross-reactivity with trypsins in group 3, which can be explained by the low AA sequence identity between the allergens of Group 6 and Group 3. Between Der p 6 and Der f 6, the AA sequence identity is 75%.

Group 7 allergens (unknown function)

In the hierarchy of pyroglyphid mite allergens Group 7 allergens have middle allergenicity. Der p 7 binds to 50% Dpt IgE. Its molecular weight is 22 kDa. Little is known about its structure. An important point is its high and variable glycosylation. The allergen binds in immunoblot to bands at 26, 30 and 31 kDa (75). Recent genomic analysis showed a similarity with prenylcysteine lyase domains found in insect proteins. Group 7 allergens are labile; they are found at low levels in mites extracts. (4).

Group 8 allergens (glutathione S-transferase)

Der p 8 is a polypeptide belonging to the glutathione S-transferase family. It contains 204 residues and reacts with 40% of IgE directed against overall Dpt extracts (76). Der p 8 is not very abundant in mite extracts. The reality of cross-reactivity with glutathione S-transferase of Blattella germanica, Bla t 5, has been discussed, but the AA sequence identity is only 25% (77). Cross-reactivity with the American cockroach would be more likely (78).

Group 9 allergens (serine protease with collagenolitic activity)

Der p 9 is a serine protease with collagenolitic activity. That makes it different from the serine proteases Der p 3 and Der p 6. Its AA sequence identity is 38% with Der p 3 trypsin and 40% with Der p 6 chymotrypsin. Its molecular mass is 28 kDa. Like Der p 3, Group 9 allergens are able to induce the liberation of GM-CSF and eotaxin by activation of PAR-2 receptors; they are able to increase the permeability of the bronchial epithelium by cleavage of tight junctions; they also act on sites different from those of Der p1.

Group 10: tropomyosin

Tropomyosin is involved in muscle contraction and in regulating morphology and cell motility. Because of its role in vital functions, tropomyosin has been highly conserved during evolution; it is also widely distributed among many invertebrates. Tropomyosin has a helical structure and is composed of two alpha-helices wound around each other. Twelve isoforms have been identified in rats (79). The high degree of different AA tropomyosine sequences confers to these molecules the role of a pan-allergen responsible for cross allergies within various invertebrates (80, 81). The prevalence of sensitization to tropomyosin in subjects allergic to dust mites in Europe is low, ranging from 5.6 to 18% (82), it is high in areas with endemic parasites such as Japan (83) or Africa. In fact nematodes (Anisakis simplex, Ascaris...) and trematodes have cross allergens with tropomyosin from dust mites. One of the characteristics of mite tropomyosin is its involvement in food allergies: tropomyosin being a major allergen of crustaceans (84, 85): rock lobster (Pan s 1), lobster (Hom a 1), crab (Cha f 1), crayfish ... It is also present in some shellfish such as squid (Tod p 1), oysters (Cra g 1), mussels (Myt e 1). Shrimp tropomyosin, considered as the single major allergen was compared to other tropomyosins (86). The recombinant shrimp tropomyosin is available in immunoCAP (rPen a 1).
The association between food allergy to snails and sensitization to mite tropomyosin, has been questioned (87). Snail tropomyosin has an AA sequence homology of 62 to 65% with shrimp tropomyosin. In several studies, analysis of sera from patients allergic to dust mites and allergic to snails failed to demonstrate tropomyosin fixation by immunoblotting.
Tropomyosin present in pyroglyphids (Der p 10, Der f 10) is also found in the storage mite (Lep d 10) and in Blomia tropicalis (Blo t 10). The AA sequence homologies of these different tropomyosins are major: 98% between Der p 10 and Der f 10, 94% between Der p 10 and Blo t 10 (88, 89).
Among insects, tropomyosin has been identified in cockroaches: Blattella germanica (rBla g 7) and Periplaneta americana (rPer a 7); they have a AA sequence homology of 96.5% and are considered as major allergens of cockroaches (90, 91). Tropomyosin is also an allergen in the order of Diptera (flies and non biting midges) (92), as well as an allergen of Thysanura order (silverfish:Lepisma saccharina, rLep s 1) (93).

Other mite allergens

- *Group 11 allergens (paramyosin)*
  The paramyosin, a high molecular weight protein (92-94 kDa) is a structural protein of invertebrate muscle. Studies performed in Taiwan and Singapore have shown high frequency of IgE binding to paramyosins of Dermatophagoides farinae, Dermatophagoides pteronyssinus and Blomia tropicalis (95). Other studies performed with sera from patients allergic to Blomia tropicalis showed 67% IgE binding to Blo t 11, (95, 96). The natural allergens are partly degraded and present in low doses in dust samples. Paramyosins are also present in parasites (Taenia, Schistosoma, Anisakis...), though cross-reactions seem unlikely because of low AA identity percentages (35%).

- *Group 12 allergens (unknown function)*
  Group 12 allergens have not been identified in pyroglyphid mites but have been detected in storage mites and in Blomia tropicalis. Blo t 12 has a molecular weight of 14 kDa and a 124 amino acid sequence. This protein binds to IgE of 50% of subjects allergic to Blomia tropicalis extracts. Group 12 allergens from Blomia tropicalis and Lepidoglyphus destructor have a very high unusual AA sequence identity of 93% (2).

- Group 13 allergens (protein binding to fatty acids)
  These proteins which bind and transport fatty acids are the 15th most abundant proteins found in mite extracts. They belong to proteins from the cuticle (97). Their lack of allergenicity may be their main characteristic. It is unusual that patients allergic to Glycyphagidae or Pyroglyphidae produce specific IgE against this protein. The tertiary structure of Der f 13 was solved by nuclear magnetic resonance, revealing the typical structure of cytosolic proteins from fatty acid binding families.
  The recombinant rBlo t 13 has a molecular weight of 14.8 kDa. It binds only to 10% of Blomia tropicalis specific IgE in allergic patients. Asa 13 from Acarus siro has a frequency of IgE binding of 23% (98).

- *Group 14 allergens (lipotransférase: LLTP).*
  Group 14 allergens are part of the family LLTP (large LTP) (lipid transfer protein). This family includes vitellogenins and apoliphorines, as well as other molecules of the defence system of insects and molecules involved in haematopoiesis. 250 amino acids were determined for group 14 allergens obtained from Euroglyphus maynei. The molecular weight is very high: 167 kDa. These allergens come from the body of mites and are disintegrated into fragments. Their sequences are very similar to that of vitellogenins from crustaceans (99). Specific antibodies to Der p 14 and Der f 14 react with both male and female mite extracts.
  The C-terminal regions of group 14 from Blomia tropicalis and Sarcoptes scabei show AA sequence identity of 60 and 44% respectively with Der p 14, which is much, taking into account the taxonomy distances between these species.

- *Group 15 allergens (chitinases)*
  Group 15 allergens are major allergens in dogs as IgE bind overwhelmingly to these chitinases (100, 101). In humans, approximately 70% of Dpt IgE also bind to a recombinant rDer p 15 (102). Their AA sequences of group 15 are exact reproductions of other chitinases and are glycosylated (100, 101). These allergens present in the mite intestine, do not pass into faecal particles (101, 102).

- *Groups 16 and 17 allergens (gelsolin and calcium-binding protein)*
  Designations 16 and 17 have been provided for gelsolin and calcium-binding protein, each binding 35 to 50% of specific Ig E present in sera of dust mite allergic patients (103).

- *Group 18 allergens (chitinases)*
  Der f 18 is a chitinase of 60 kDa molecular weight, distinct from Der f 15, with which it shares a 25% sequence identity (102). It is an important allergen for dogs. In humans, Der f 18 binds 54% of specific Dpt IgEs (104). Group 18 allergens are present in the upper intestine but not in faeces.

- *Group 19 allergens (antimicrobial peptide)*
  Group 19 allergen corresponds to an allergen of Blomia tropicalis, homologous to an antimicrobial peptide poorly defined, with a molecular mass of 7 kDa and low allergenicity (only 10% binding of specific Dpt IgE) .

- *Group 20 allergens (arginine kinases)*
  Group 20 allergen corresponds to arginine kinases of a 40kDa molecular mass. Its sequence is highly conserved in other invertebrates: 80% homology with shellfish, 75% with insects, 45% with mammalian enzymes. The frequency of IgE binding was overall 40% in patients allergic to dust mite extracts but Group 20 allergens induce only low IgE levels. (104).

- *Group 21 allergens (unknown function)*
  Group 21 allergens binds 30% of the sera of Austrian patients allergic to dust mites (105). Their sequences have similarities with those of group 5, but without any cross reactivity. Der p 21 is localized in epithelial intestinal lumen and in Dermatophagoides pteronyssinus faeces (105). Der p 21 could be a counterpart of Blo t 5, having progressively been detached from Blo t 5 during evolution. It is therefore a paralog of Blo t 5 (106).

- *Group 22 allergens*
  This group only includes Der f 22, which has 35% identity with Der f 2. It is an allergen of intestinal origin, which binds 50% of specific Df IgE of sera from patients allergic to Dermatophagoides farinae extracts. (106).

Blomia tropicalis allergens

Blomia tropicalis is now part of the Echimyopodidae family, it constitutes a genuine domestic allergen in tropical and sub-tropics regions. Twenty five protein bands binding to IgE of patients allergic to Blomia tropicalis were identified by immunoblotting (107). Ten allergens Blo t 1, Blo t 3, Blo t 4, Blo t 5, Blo t 6, Blo t 10, Blo t 11, Blo t 12, Blo t 13 and Blo t 19 have been officially named and placed in a database: www.allergen.org (108, 109). Blomia

tropicalis allergens have low or moderate specific cross-reactivity with other allergens from Dermatophagoides (110). Blo t 5 is a predominant allergen for patients in tropical and subtropical countries, the prevalence of IgE binding to Blo t 5 varying from 60 to 70% (110). It is followed by Blo t 12, Blo t 13 and Blo t 6,binding to Blo t4 and Blo t10 being less than 10%. Blo t 1 does not seem to be a major allergen contrary to what was originally suggested (108). Gao et al. (6) by using the EST (Expressed Sequence Tagging) have identified a new important allergen, Blo t 21 (6). Blo t 21 was recognized by the IUSS, it is a protein of 129 amino acids with 39% AA sequence homology with Blo t 1. Although over 75% of subjects allergic to Blomia tropicalis extracts are sensitized to both Blo t 5 and Blo t 21, the two allergens have moderate cross-reactivity. This is probably due to paralogous genes separated early in evolution, which induced proteins with low AA identity percentages.

Cross-reactions

The knowledge of the molecular structure of mite allergens, purified or cloned, has led to a better understanding of cross-reactions occurring in patients sensitized by allergens originating from various sources: Pyroglyphidae, Glycyphagidae, Acaridae (storage mites), and Echimyopodidae (Blomia tropicalis).
The cross-reactions can be classified into intra-species reactions, reactions in close species (e.g. Der p 1 and Der f 1) reactions between species belonging to more distant families (Pyroglyphidae and storage mites), reactions between phylogenetically distant species belonging to another order or another branch (shrimp and mites for example). It has been suggested that the AA sequence identities of two allergens above 70% are in favor of a significant crossreactivity between these allergens (111). The disparities between two homologous allergens are mainly due to their tertiary structure and the arrangement of amino acides at their surfaces.

Intraspecies reactions

Identity of proteins within a species is a molecular characteristic of the species. However, many variants or isoforms defined as having at least 90% AA sequence identity, have been described: 23 for Der p 1, 12 for Der p 2 (19). Some consider that the differences between these isoforms could be as large as those between allergens from different species, whereas other considers that polymorphisms, such as that of Der f 1 would not play a role in allergenicity (4).

Interspecies cross-reactions

The sequence homologies in AA are all the more important as species are phylogenetically close, with the exception of pan-allergens such as tropomyosin, which can be found in distant species. Each species has both common allergens shared with other species, and peculiar specific allergens. Table I reports the AA sequence homologies between allergens from pyroglyphid mites, and from storage mites and Blomia tropicalis.
The AA sequences of Dermatophagoïdes pteronyssinus and Dermatophagoïdes farinae allergens,of D.microceras and D.siboney allergens are quite similar, usually above 80%.(112).
Euroglyphus maynei is also part of Pyroglyphidae family; but from a taxonomically point of view, this mite is less close to Dermatophagoides species than are Dp and Df, and differences with Dermatophagoides allergens are also slightly more pronounced. Two hundred fifty subjects allergic to Euroglyphus maynei had simultaneously positive prick tests for Dermatophagoïdes pteronyssinus (113). By CIE (crossed immunoelectrophoresis)it has been shown that Euroglyphus maynei shared 4-6 common allergens with Dermatophagoïdes pteronyssinus (114). Inhibition of specific IgEs against E. maynei was obtained with Dermatophagoïdes pteronyssinus extracts, whereas inhibition of specific IgE against Dpt with E. maynei extract is less important (113). In immunoblotting, IgEs of patients sensitized to Dermatophagoides bind the majority of protein bands of an Euroglyphus maynei extract (115). It also was shown that Eur m 1 has a AA sequence identity of 89% with Der p 1 (116) while the homology of T epitopes of Euroglyphus and Dermatophagoïdes pteronyssinus was more modest (117).

Cross-reactivity between pyroglyphides mites and storage mites

The most representative and most frequently encountered storage mites are Lepidoglyphus destructor, Glycyphagus domesticus, Tyrophagus putrescenciae, Acarus siro, and more rarely Chortoglyphus. Lepidoglyphus destructor is considered as the predominant species in Europe (118). However, studies conducted in Spain (119) showed that Glycyphagus domesticus was the most common storage mite, followed in order by Lepidoglyphus destructor and Tyrophagus putrescenciae. In another study (120) Lepi-

Table 1 - AA (amino acids) homologies sequences between des pyroglyphidae, storage mites and Blomia tropicalis.

Mite allergens	AA sequences identities	Possible cross reactivity
Der p 1 et Der f 1	80-85 %	Yes
Der p 1, Der f 1 et Der m 1	80 %	Yes
Der p 1, Der f 1 et Eur m 1	76 %	Yes
Der p 1, Der f 1 et Blo t 1	36 %	No
Der p 2 et Der f 2	88 %	Yes
Der p 2, Der f 2, Eur m 2	82 %	Yes
Der p 2 et Lep d 2	36 %	No
Der p 2 et Tyr p 2	41 %	No
Der f 2 et Tyr p 2	43 %	No
Gly d 2 et Lep d 2	80 %	Yes
Der p 3 et Der f 3	81 %	Yes
Der p 3, Der f 3 et Eur m 3	81 %	Yes
Der p 3, Der f 3 et Blo t 3	48 %	No
Der p 4 et Eur m 4	90 %	Yes
Der p 5 et Blo t 5	43 %	No
Der p 5 et Lep d 5	30 %	No
Der p 6 et Der f 6	76 %	Yes
Der f 6 et Der p 3	37 %	No
Der p 6 et Blo t 6	58 %	No
Der p 7 et Der f 7	86 %	Yes
Der p 7 et Lep d 7	29 %	No
Der p 8 et Bla g 5	25 %	No
Der p 9 et Der f 9	72 %	Yes
Der p 9 et Blo t 9	56 %	No
Der p 9 et Der p 3	38 %	No
Der p 10 et Der f 10	98 %	Yes
Der p 10 et Lep d 10	94 %	Yes
Der p 10 et Tyr p 10	94 %	Yes
Der p 11 et Der f 11	89 %	Yes
Der p 11 et Blo t 11	78 %	Yes
Lep d 12 et Blo t 12	95 %	Yes
Der p 3 et Blo t 13	80 %	Yes
Der p 3 et Lep d 13	79 %	Yes

doglyphus destructor and Tyrophagus putrescenciae represent most sensitizations, regardless of age. Acarus siro, probably because of its nutritional requirements and food specificity (cheese, flour) seems less often encountered in a domestic environment. Several species can coexist in the same environment as long as it can satisfy the mites' three basic needs: protection, food and reproduction. The presence of D. pteronyssinus, even when Blomia tropicalis and storage mites are abundant (121) is almost constant. The question then arises whether the sensitization is due to species-specific allergen or cross-reacting allergens.

Arias-Irigoyen (119) insists that in his geographical environment, storage mites should be tested if allergy to dust mites is suspected. Most studies show inhibition of specific IgE against Dp and Df by storage mites extracts and vice versa (122-124). AA sequence identities between allergens from Dermatophagoides and storage mites are low, less than 50%, except for Der p 13 and Gly d 13, or Der p 13 and Lep d 13 sharing homology with numerous proteins binding to fatty acids (4). Usually, Dermatophagoides allergens are more powerful inhibitors of Acarus siro, Tyrophagus putrescenciae, Lepidoglyphus destructor extracts than storage mite allergens can be (123, 124). The AA sequence identities between group2 allergens: Tyr p 2, Gly d 2, Lep d 2 are high. The results obtained by CIE and CRIE suggest that Dermatophagoides and Tyrophagus putrescentiae have two common allergens whereas Dermatophagoides and Acarus siro have one common allergen only (123).

Regarding inhibition studies, it has been shown that Lepidoglyphus destructor could inhibit 80% of specific IgE binding Glycyphagus domesticus. Tyrophagus putrescentiae was able to partially inhibit IgE binding of Lepidoglyphus destructor while Glycyphagus domesticus and Lepidoglyphus destructor could completely inhibit binding to Tyrophagus putrescentiae IgEs. This indicates that Tyrophagus putrescentiae does not possess the complete repertoire of Glycyphagus domesticus and Lepidoglyphus destructor epitopes.

Molecular epidemiology

Molecular epidemiology is able to determine the prevalence of sensitization to different molecular allergens in terms of age and geographic locations of the populations studied.

Depending on age

When individuals were sensitized in childhood, reactivity to Der p 2 and to a lesser degree to Der p 1 prevails. IgE reactivity to one, two or three additional allergens appears in children older than 3. In adults, when sensitization to mites is

recent, the levels of specific IgEs for Der p 1 and Der p 2 are rather low, and significant levels of IgE against other allergens such as Der p 3 and Der p 4 are observed (125, 126).

Depending on location

In most reported studies, Der p 1 and Der p 2 are major allergens recognized by more than 82% of IgE from Dpt allergic patients (more than 82% in Japan and 89% in Spain) (127). Hales et al. (128) also showed that in Australia, specific anti-Der p 1 IgEs and anti-Der p 2 IgEs accounted for the majority of anti Dpt IgE. Der p 4, Der p 5 and Der p 7 appeared to be intermediate allergens; the amount of IgE binding to Der p 1, Der p 2, 4, 5 and 7 accounted for 80% of the mite IgE response while Der p 4, 5 and 7 accounted for only 30%. The binding of IgE to groups 3, 8, 10 and 20 was constantly low, as was IgE binding to groups 6, 9, 13 and 17.
In a population from Central Europe (129), the prevalence of sensitization was highest for nDer p1 and rDer p 2 (91 and 89% respectively), followed by Der p 4 and Der p 7 (74 and 27% respectively). Weghofer et al. (82) studied the prevalences of binding of IgE for 7 natural or recombinant allergens of Dermatophagoides pteronyssinus (n Der p 1, rDer p2, n Der p 4,r Der p 5, rDer p 8,r Der p 10 and rDer p 14), using dot-blots in different populations allergic to dust mites: Austria (n = 56), France (n = 55), Italy (n = 67) and Sweden (n = 65). In the four populations, nDer p 1 and rDer 2 appeared to be major allergens with prevalences of sensitization varying from 85 to 100% for nDer p 1 and 63 to 96% for rDer p 2. Differences were noted according to the populations studied: Der p7 was more often recognized by Italians and Der p 5 by French people. The Swedes responded less frequently to the major allergen rDer p 2 than did patients from France, Austria and Italy. In another study, the prevalences of sensitization for n Der p 1 varied between 60 and 75%, whereas sensitizations to rDer p2 differed slightly in Sydney, South Africa and Vienna. For Der p 9, prevalences above 50% were found in Sydney, Cape Town, but not in Vienna (130).

In Australia it was shown that Aborigines' sera reacted predominantly with Der p 4 and less frequently with Der p 1 and Der p 2 (131). Different profiles of mite allergens sensitizations were also identified in Europe and Zimbabwe (132), as well as in Colombia where the prevalences of sensitizations for groups 1 and 2 were 64 and 69% respectively (134).
The Blomia tropicalis major allergen ,Blot 5, was tested by skin tests and serum specific IgE in Brazil and Virginia: in Brazil, 45% of the sera from subjects allergic to Blomia tropicalis recognized Blo t 5; the percentage was only 25% in Virginia (134).
Differences regarding the prevalences of sensitization to specific molecular variants were also found. These studies have focused on Der p 2. Thus, the dominant allergen detected in inhabitants of Perth was Der p 2.0101 (2). In Korea, for half the clones recognized by specific IgE the variant was Der p 2.0104. Similar findings were observed in Bangkok (2), while no sensitizations were detected for the isoform Der p 2.0101. In Japan and Korea, the sequences most frequently recognized were 0101. All in all, the isoform most frequently encountered seems to be 0101 for Group 2 (4).

Application for dust mite allergy diagnosis

The aim of diagnosis studies is to associate the most representative mite molecular allergens in order to obtain the best diagnostis sensitivity.
Weghofer (82) has shown that the combination of Der p 1 + Der p 2 can diagnose at least 97% of patients allergic to Dermatophagoides pteronyssinus in Europe. Nevertheless, 50% of mite allergic patients also react to other allergens. The addition of Der p 5 and Der p 7 actually provides diagnostic sensitivity of 100%, at least in Austria, France and Sweden. Arruda et al. (134) propose a combination of Der p 1, Der p 2 and Der p 5. Hales and Thomas (4, 128) suggest a combination enlarged to Der p 1, Der p 2, Der p 4, Der p 5, Der p 7.
In conclusion, in most cases, a combination involving Der p 1 and Der p 2 or Der f 1 and Der f 2, seems sufficient for what Valenta called a "component resolved diagnosis". However, it was demonstrated that allergenic activity persists after depletion of groups 1 and 2 allergens from Dermatophagoides extracts (135). In some populations, other allergens should be associated, mainly Der p 4, Der p 5, Der p 7. One should also make sure that the clinical relevance of inhaled isolated allergens is proved, and that their actual presence in the indoor environment has been assessed. The possible role of non-allergenic components such as LPS should be taken into account, especially for group 2 allergens.

Application to the detection of mite allergens in the home environment

Two categories of tests have been developed in recent years, outside of counting and identifying mites by optical

methods: the immunochemical assay using monoclonal antibodies directed against Dermatophagoides major allergens and storage mites allergens, and the guanine detection test.

Guanine, the final catabolite of nitrogen metabolism of arachnids present in the mite faeces, is a marker used for detection of mite allergens present to a large extent in faeces. The Acarex® test, based on semi-quantitative determination of guanine, was developed by Bischoff and Schirmacher in 1984 and secondarily validated (136, 137, 138, 139). For 10 mg of allergen per gram of dust, the test has a specificity of 89% for class 2 and 98.5% for class 3 (138).

Immunochemical techniques were initially based on ELISA tests using monoclonal antibodies directed against murine epitopes of molecular allergens from dust mites. These assays were used to quantify Der p 1 and Der f 1 for Dermatophagoides species (140). Another assay was used for the detection of a common storage mite allergen of 39 kDa from Lepidoglyphus destructor, Glycyphagus domesticus, Tyrophagus putrescentiae and Acarus siro, together with an Elisa assay detecting specific allergens from Lepidoglyphus destructor and Blomia tropicalis (141, 142, 143).
ELISA techniques are however not suited for epidemiological studies. New techniques where specific antibodies are coupled to fluorescent microspheres (fluorescent multiplex array), were used to measure Der p 1 and Der f 1 allergens and group 2 allergens simultaneously, with a better detection limit (0.06 mg / ml versus 0.5 mg / ml for ELISA) (144). Correlation with ELISA assays is excellent.
Previously, immunochemical semiquantitative tests had been developed, using either monoclonal antibodies directed against group 2 allergens (Rapid-test * (145)), monoclonal antibodies against Der p 1 and Der f 1 (Dust Screen (146)), or polyclonal antibodies (Aclotest * (147)). The reading is based on the colour intensity of a stained band on the nitrocellulose membrane, varying according to the amount of bound antibodies.

Application to specific immunotherapy

The availability of recombinant molecules of major allergens offers many advantages. Recombinant peptides corresponding only to T-cell epitopes of allergens from Dermatophagoides pteronyssinus were used in mice (148, 149, 150). Hypoallergenic group 2 allergens were derived directly from the DNA of group 2 storage mite allergens (Lepidoglyphus destructor and Glycyphagus domesticus); it was shown that these isoforms of Lep d 2 and Gly d 2 induced blocking antibodies in mice (151). Reducing the allergenicity of Der p 2 was also obtained by using two fragments of Der p 2 and a hybrid molecule containing the entire T-cell epitopes of Der p 2 (152). Finally, two hybrid molecules were constructed, including two recombinant proteins corresponding to almost all sequences of Der p 1 and Der p 2, and further including a fragment of Der p 2 modified by point mutation at the disulfide bonds. The reduction of IgE reactivity of these hybrid molecules was studied in 106 patients allergic to Dermatophagoides. A significant decrease in skin reactions was obtained, as well as a decrease of specific IgE directed against hybrid molecules unobserved with natural allergens. T cell stimulation by hybrid molecules was confirmed in 25 patients, and induction of blocking IgG in mice was verified (153). However, none of these molecules has yet been used in desensitization trials that alone could provide evidence of their effectiveness.

Conclusions

In conclusion, the number of studies about molecular mite allergens increases from year to year. Some new allergens are awaiting recognition by the IUIS. Knowledge of molecular allergens has practical applications, offering better understanding of cross-reactions, and hopes of improvement in diagnosis and immunotherapy. At this stage, rather than using the words “dust mite allergy”, it would be more accurate to speak of allergies to different mite allergen molecules.

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