

REVIEW

Allergens from house dust and storage mites: similarities and differences, with emphasis on the storage mite *Lepidoglyphus destructor*

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Introduction

Allergic diseases are increasing worldwide and mites are one of the most common causes of allergy. Mites are normal inhabitants in our environment and play an important role in the biological recycling process by breaking down waste products of organic materials. They frequently occur in the indoor environment in house dust as well as other environments such as barns and grain stores. Mites, belonging to the subclass of acari (mites and ticks), form an important division of the great arthropodan class Arachnida (Fig. 1). About 40 000 different species are thought to exist [1]. Mites can broadly be divided into two categories: the pyroglyphid mites, referred to as house dust mites, and the nonpyroglyphid mites, referred to as storage mites.

Storage mites are predominantly found in agricultural environments where they can cause occupational allergy in farmers and grain handlers, but are now also being recognized as important contributors to the allergen content in house dust from indoor urban dwellings [2,3]. Therefore, the term dust mites is now commonly used to describe mites in the indoor environment, including both house dust mites and storage mites. Mites feed on a variety of protein-rich substances, house dust mites primarily on shed human skin scales, while the storage mites feed on plants and micro-organisms. The existence of storage mites in house dust is often connected with damp housing conditions [3], which also favours the growth of moulds that provide an important source of food.

In order to make correct diagnosis and provide proper treatment to patients with dust allergy it is important to also consider storage mites. Therefore we need to further study and characterize these mite species to obtain well standardized allergenic preparations. Furthermore, pure allergens, whether purified from the natural source or produced as recombinant

allergens, are needed to evaluate aspects of specific sensitization and cross-reactivity as well as to develop new approaches for treatment.

Storage mite allergy

Since the mid 1960s, when it was established that the pyroglyphid mites are the main source responsible for house dust allergy [4,5], these species have been extensively studied regarding their important role in allergic disease. The potential allergenic importance of storage mites was indicated by Maunsell already in 1968 [5]. Furthermore, a study by Spieksma in 1969 showed that out of 200 house dust allergic patients, 83 were also sensitized to *Lepidoglyphus destructor* (*L. destructor*), 68 to *Tyrophagus putrescentiae* (*T. putrescentiae*) and 68 to *Acarus siro* (*A. siro*) [6]. However, it was not until 10–15 years later that these mites were acknowledged as important sources of allergens in different parts of the world.

Studies in different parts of Europe [7–11] as well as in the United States [12,13] have shown that storage mites are found in stored hay and may cause respiratory allergy among farm workers. Since these mite species can infest grain, also grain elevator workers and bakers are occupationally exposed to storage mites and can become sensitized [14,15].

Many reports have shown that sensitization to storage mites also occurs among urban populations, in both sub-tropical and tropical countries as well as in temperate climates, indicating that this is a worldwide problem [2,3,6,16–20]. The occurrence and relative importance of different storage mites in these studies varies in different geographical areas, e.g. *L. destructor* seems the dominating species in Europe, whereas *Blomia tropicalis* (*B. tropicalis*) is most prevalent in South America. Furthermore, in these studies, a combination of high IgE-reactivities to both house dust mites and storage mites are often reported, reflecting either cosensitization, cross-reactivity or a combination of

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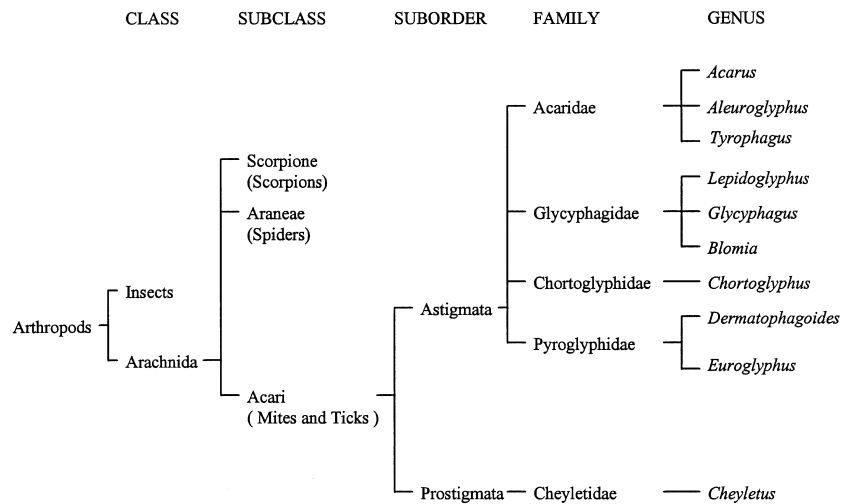


Fig. 1. The taxonomic position of important dust mites

both. This particular aspect needs further investigation in order to make correct conclusions regarding the importance and prevalence of storage mite sensitization *per se*.

As mentioned, *L. destructor* (Fig. 2) is one of the most abundant storage mite species in Europe. Two extensive epidemiological studies have been made regarding the IgE response to this mite in a farming population on the Swedish island of Gotland in the Baltic Sea [8,21]. Approximately 7% of the farming population had, in 1984 as well as in 1996, positive RAST values to *L. destructor* and this mite constituted an important cause of allergic symptoms. The total prevalence of positive RAST to storage mites in 1996 was 8.1% and to house dust mites only 7.3%. Remarkably, even though information on health hazards associated with farming as well as advice on allergen avoidance measures was provided in 1984, storage mites remained a dominant cause of allergy in 1996.

In this study population we have recently also investigated the IgG1, IgG4 and IgE responses to the major allergen of *L. destructor* (Lep d 2) and made comparisons with nonallergic and/or nonexposed individuals (unpublished data). Preliminary results show that the IgG4 response is very similar to that of IgE regarding epitope specificity, and furthermore, only *L. destructor* allergic subjects had an IgG4 response to Lep d 2. Allergen specific IgG1 however, was produced in both the allergic and the nonallergic individuals.

Mite allergens

The allergenic composition of the house dust mite *Dermatophagoides* has been extensively studied. It has resulted in the identification of over 30 different IgE-binding components and of at least 10 different groups of allergens that



Fig. 2. *L. destructor* mite prepared in lactic acid and assessed in a light microscope at $\times 150$ magnification. The photograph is published with the permission of Blackwell Scientific Ltd. It originally appeared in *Clinical and Experimental Allergy* 1995; 25:536–42.

Table 1. A summary of cloned dust mite allergens and their known biological functions

Allergen groups	Cloned SM* allergens	Appr. Mw	Appr. IgE-reactivity	Function
1	Lep d 2, Tyr p 2	25 kDa	80%	Cystein protease
2		14–18 kDa	80%	Unknown
3		30 kDa	60%	Serine protease
4		60 kDa	40%	Amylase
5	Blo t 5	14 kDa	40%	Unknown
6		25 kDa	40%	Chymotrypsine
7		22–28 kDa	50%	Unknown
8		26 kDa	40%	Glutathione-S-transferase
9	Blo t 12	24 kDa	60%	Collagenolytic serine protease
10		36 kDa	80%	Tropomyosin
11		98 kDa	?	Paramyosin
12		14 kDa	50%	Unknown
13	Aca s 13, Blo t 13	15 kDa	10%	Fatty acid-binding protein

*Storage mite

seem to account for most of the IgE-reactivity to the *Dermatophagoides* mite species (Table 1).

Regarding *L. destructor*, 21 IgE-binding components with molecular weights of 14–70 kDa have been demonstrated by SDS-PAGE and immunoblotting [22,23], and by using monoclonal antibodies two additional allergens (39 kDa and 79/93 kDa) were identified [24,25].

The major allergen Lep d 2 is a protein of approximately 15 kDa. As determined from the amino acid sequence the molecular weight of Lep d 2 is 13.2 kDa but according to SDS-PAGE analysis it ranges from 14 to 18 kDa [23,26]. This allergen was formerly designated Lep d 1, however, cloning and sequencing of the protein revealed a 40%

identity and over 50% homology with the group 2 house dust mite allergens (Fig. 3) and it was consequently renamed, Lep d 2 [27–29].

Using immunohistochemistry and confocal laser scanning microscopy we have shown that Lep d 2 has a quite widespread distribution in the mite body and that it is also present in faecal pellets [30].

In two different studies, approximately 90% of the individuals who were RAST positive to *L. destructor* reacted to Lep d 2 [23,26] making it the most important allergen known in *L. destructor*. Another allergen in the molecular weight range 53–57 kDa was detected by 50% of the *L. destructor*-sensitized subjects in the same studies,

	M . . K . . L . . L V A D C . . . E G C . G	Consensus
1	M- - K F L I L F A L V A V A A A G Q V K F T D C G K K E I A S V A V D G C E G	Tyr p 2
1	MM- K F I A L F A L V A V A S A G K M T F K D C G H G E V T E L D I T G C S G	Lep d 2
1	M I S K I L C L S L L V A A V V A D Q V D V K D C A N N E I K K V M V D G C H G	Der f 2
1	M M Y K I L C L S L L V A A V A R D Q V D V K D C A N H E I K K V L V P G C H G	Der p 2
	. . . C . I H A . F . A N Q . T G . . .	Consensus
39	- D L C V I H K S K P V H V I A E F T A N Q D T S K I E V K V T G Q L N G L E V	Tyr p 2
40	- D T C V I H R G E K M T L E A K F A A N Q D T A K V T I K V L A K V A G T T I	Lep d 2
41	S D P C I I H R G K P F T L E A L F D A N Q N T K T A K I E I K A S L D G L E I	Der f 2
41	S E P C I I H R G K P F Q L E A V F E A N Q N T K T A K I E I K A S I D G L E V	Der p 2
	. . P G C . . . K C P . . K G Y P . . . P . . .	Consensus
78	P I P G I E T D G C K V L K C P L K K G T K Y T M N Y S V N V P S V V P N I - K	Tyr p 2
79	Q V P G L E T D G C K F I K C P V K K G E A L D F I Y S G T I P A I T P K V - K	Lep d 2
81	D V P G I D T N A C H F M K C P L V K G Q Q Y D I K Y T W N V P K I A P K S E N	Der f 2
81	D V P G I D P N A C H Y M K C P L V K G Q Q Y D I K Y T W N V P K I A P K S E N	Der p 2
	. . V G . . G V . A C	Consensus
117	T V V K L L A T G E H G V L A C G A V N T D V K P .	Tyr p 2
118	A D V T A E L I G D H G V M A C G T V H G Q V - - E	Lep d 2
121	V V V T V K L I G D N G V L A C A I A T H G K I R D	Der f 2
121	V V V T V K V M G D D G V L A C A I A T H A K I R D	Der p 2

Fig. 3. Protein sequence alignment of the major group 2 dust mite allergens.

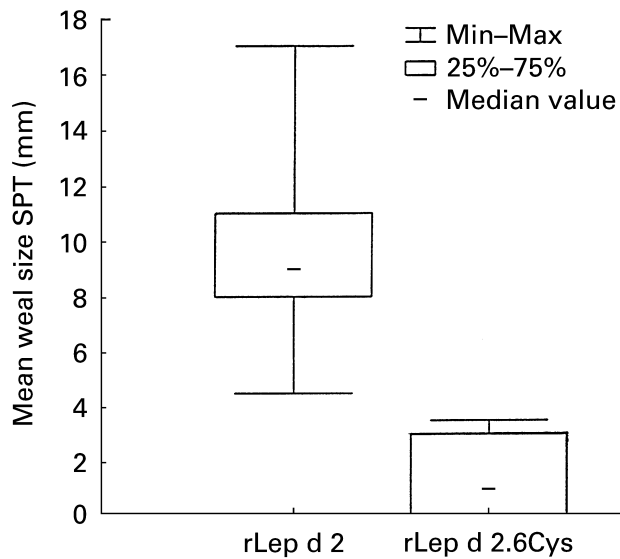


Fig. 4. Skin prick test results from 18 mite-sensitized farmers tested against recombinant Lep d 2 and the recombinant mutant Lep d 2.6Cys (100 µg/mL). The results are presented as mean of the weal diameter, calculated from duplicate tests performed on the volar forearm of the patient. $P < 0.001$.

and Ventas *et al.* also found that 50% reacted to a 30-kDa allergen.

Studies indicate that *L. destructor* has both unique as well as cross-reacting allergens [16,23,31,32]. Cross-reactivity has been shown to exist with allergens from the house dust mite species *Dermatophagoides* [18,20,23] and with the storage mites, *Glycyphagus domesticus* (*G. domesticus*) [31], *A. siro* and *T. putrescentiae* [33], *B. kulagini* [34] and *B. tropicalis* [35].

Recombinant allergens

Mite extracts that are used today in *in vitro* and *in vivo* tests contain many unspecified components which makes them difficult to standardize and may even cause unwanted allergic reactions when used in patients. The variability between different batches of extracts makes it difficult to compare the extracts and provide consistent diagnosis and treatment.

The use of molecular biology techniques has been a major breakthrough in allergen characterization and standardization. The genes coding for different allergens have been cloned and their DNA sequences determined, contributing a tremendous amount of new information concerning their relationships as well as their potential biological activities.

Moreover, the following production of recombinant allergens, has offered the possibility to produce specific, well-characterized allergens in large amounts. Many different expression systems have become available, each with their

different advantages and drawbacks. The system of choice has to reflect the nature and demands of the particular protein to be produced, such as specific post-translational modifications, production yields and purification requirements.

As many recombinant allergens seem to have preserved immunogenicity, comparable to that of the native counterparts, their possible applications are numerous. Several studies have now shown the diagnostic value of recombinant allergens and there are indications that only a few allergens from an allergenic source might be enough for diagnosis of sensitized subjects [36,37]. There seems to be a great potential for recombinant allergens in improving specific immunotherapy since they provide such a well-defined source of allergens. It would be possible to design 'tailor-made' treatment for individual patients according to their pattern of allergen-reactivity and thereby avoid the risk of additional, new sensitizations.

Concerning storage mites only a few allergens have been cloned and expressed as recombinant proteins (Table 1) [38–42]. Recombinant Lep d 2 has been extensively studied [38]. Two different expression systems have been applied, a eukaryotic (baculovirus in insect cells) and a prokaryotic (*E. coli*) system, in order to compare the immunoreactivity of the expressed allergens. Both systems were shown to produce recombinant Lep d 2 with comparable IgE-reactivity as the natural counterpart and since the prokaryotic system is more feasible to use it was concluded that no higher expression systems are needed for this particular allergen. Furthermore, recent studies using recombinant Lep d 2 and Tyr p 2 (the major allergen of the storage mite *T. putrescentiae*) in the CAP-RAST system and in skin prick tests, have proved the applicability of these recombinants [43,44]. By using these recombinant allergens it is possible to detect about two thirds of *L. destructor* and/or *T. putrescentiae* allergic subjects in RAST. These recombinant allergens have also been used *in vivo*, in skin prick tests. We found that 59% of the allergic subjects developed positive skin reactions to one of these allergens and there was a significant correlation between the weal sizes and the corresponding RAST values [44]. These results further substantiate the potential of recombinant allergens as diagnostic tools.

Isoallergens

Based on the cloning of allergens it has been shown that many allergens in nature exist in several isoforms. These isoforms may be as different in their sequence and structure as allergens from different species. Consequently, their immunogenicity could differ and they might have to be considered as separate allergens. This is of importance when recombinant allergens are to be used in immunotherapy, since all isoforms must be represented in the evaluation and treatment of patients [45].

House dust mite allergens from groups 1, 2, 3 and 5 have been shown to comprise several isoforms [46–49]. When comparing the isoform content in mites obtained from several areas in Australia and the UK, variations could clearly be shown [50] and differences were also seen when different commercial extracts were compared. This variation in isoform content has important implications both for the standardization of extracts and the use of recombinant allergens.

The cloning of Lep d 2 revealed a rather high degree of polymorphism in the coding gene, leading to the identification of two isoforms (Lep d 2.01 and Lep d 2.02) plus two variants (Lep d 2.0101 and Lep d 2.0102) [28]. Both these isoforms were expressed as recombinant allergens and although their sequence differed at 13 amino acid positions no apparent difference was found in their IgE-reactivity [38]. However, when assessing monoclonal antibodies raised against the natural Lep d 2, clear differences in reactivities were shown between the isoforms indicating that their epitope structures are not identical. Another study on the polymorphism of Der p 2 [47] showed three existing isoforms which differed in 5 amino acids on the cDNA level. However, when two-dimensional electrophoresis was performed on native Der p 2, at least 10 different isoforms emerged. Interestingly, the polymorphic sites on Der p 2 are in the same regions as those previously shown to be involved in T-cell responses to this allergen [51]. This indicates that the existence of allergenic isoforms can have profound effects on the allergic response.

Functions of mite allergens

Many house dust mite allergens have shown to be enzymes [52] as presented in Table 1. Two studies have evaluated the enzymatic content of the storage mites *Aleuroglyphus ovatus* and *L. destructor*, concluding that they possess the same repertoire of enzymes as the house dust mites [53,54].

These enzymatic activities might have a specific function in the sensitization process as well as the pathology of mite allergy. There are facts supporting such a possible role. For instance, the major allergen Der p 1, a cysteine protease, has been shown to specifically cleave the low affinity receptor for IgE, CD23, on cultured B cells [55] resulting in a loss of IgE-binding ability in B cells as well as the release of an IgE-binding fragment of CD23 [56]. Another study showed that Der p 3, a serine protease, could cleave the complement factors C3 and C5 and thereby possibly contribute to the pathogenesis of allergic disease [57].

A direct action of allergens with enzymatic activity on the epithelial cell barrier has also been suggested, with loss of adhesion molecules, due to proteolytic cleavage and increased permeability of the mucosa facilitating the entry of molecules [58]. These direct actions could have particular importance

in heavily exposed populations, such as agriculture workers, and contribute to the sensitization process as well as the maintenance of the allergic disease.

Modification of allergens

The major birch pollen allergen Bet v 1, has been demonstrated to consist of many isoforms of which some have reduced allergenicity but conserved T-cell antigenicity [58]. This concept has been employed by purposely modifying allergens in order to reduce their IgE-binding capacities. This has successfully been achieved for Bet v 1 by dividing the allergen into nonimmunogenic fragments or oligomers [60–62] and for the group 2 mite allergens by site-directed mutagenesis [63–65].

Some reports have now evaluated the possible clinical use of mutant mite allergens. Using intradermal skin tests in patients allergic to *D. pteronyssinus* it was shown that a mutant variant of the major allergen Der p 2, with a disrupted disulphide bond, had 10–100-fold reduced IgE-reactivity compared to wild-type Der p 2 and still retained a T-cell response [66]. Similar results were obtained with a Der f 2 mutant, which showed negative skin prick tests, a 100-fold reduction in IgE-reactivity according to histamine release experiments and positive T-cell responses in mite allergic patients [67].

We have recently produced recombinant mutants of Lep d 2 with site-specific mutagenesis [65]. These mutants are lacking one or more of three naturally occurring disulphide bonds, leading to structural changes in the allergen. The IgE-reactivity was evaluated and found to be markedly reduced in two of the mutants, one lacking an internal disulphide bond (Lep d 2.Cys72–77) and one with all three disulphide bonds lacking (Lep d 2.6Cys). We are now performing extensive studies concerning antibody responses and T-cell-reactivity to Lep d 2.6Cys. In both *in vitro* (ELISA, immunoblotting, RAST-inhibition) [65, Olsson S *et al.* in manuscript] and *in vivo* (skin prick tests) (Fig. 4) [Kronqvist M *et al.* in manuscript], the IgE response is highly reduced or nondetectable. However, most of the T-cell-reactivity seem to be preserved (unpublished data).

The purpose of developing modified allergens with reduced IgE-reactivity and retained T-cell-reactivity is to assess their potential use for improved immunotherapy. The underlying mechanism for successful immunotherapy are not fully understood but the nature of the T cell response plays a central role affecting many other events. It has been shown that high allergen doses improves the efficacy of immunotherapy resulting in clinical improvements [68] but this also involves important risks of anaphylactic reactions.

Taken together, this points to a potential role for modified allergens as tools in improving specific immunotherapy. They would offer the possibility of applying high doses with

greatly reduced risks of anaphylactic reactions. They also offer the advantage of having the complete T-cell epitope repertoire intact, making them useful in all patients no matter which specific epitopes the individual patient reacts to.

In summary, there is an increased awareness of the importance of storage mite sensitization, not only as an occupational hazard, but also as a cause of house dust allergy. However, these nonpyroglyphid mite species have not as yet been studied as extensively as the pyroglyphid mites. The prevalence of storage mite sensitization needs to be further evaluated in different populations, as does their relative importance compared to the pyroglyphid mite species. Therefore, it is now important to further identify and characterize storage mite allergens, investigate their structures and functions, and develop more and better assays for assessment of these allergens.

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