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Cross-Reactivity between *Dermatophagoides siboney* and Other Domestic Mites

II. Analysis of Individual Cross-Reacting Allergens after SDS-PAGE and Western Blotting Inhibition

Key Words

Mite allergens
Cross-Reactivity
Dermatophagoides siboney
SDS-PAGE
Immunoblotting
Inhibition
N-terminal sequence

Abstract

Background: *Dermatophagoides siboney* (*Ds*) cross-reacted with other mite allergens in mite-allergic patients. The aim of this study was to investigate the cross-reactivity between individual allergens responsible for this cross-reactivity. **Methods:** The inhibition of IgE binding to *Ds* allergens was investigated by non-reducing SDS-PAGE and Western blotting. Nitrocellulose membranes were incubated with a pool of sera from mite-sensitive asthmatics, after the addition of serial dilutions of *D. farinae* (*Df*), *D. pteronyssinus* (*Dp*), *D. microceras* (*Dm*), *Lepidoglyphus destructor* (*Ld*), *Tyrophagus putrescentiae* (*Tp*), *Acarus siro* (*As*) and *Blomia tropicalis* (*Bt*). N-terminal amino acid sequences and amino acid analysis of the purified major allergens, Der s 1, 2 and 3, were performed after transfer to polyvinylidene difluoride membranes. **Results:** The inhibition was higher with *Df* (86%), *Dp* (54%) and *Dm* (49%) extracts than with *Ld* (20%), *Tp* (11%), *As* (18%) and *Bt* (6%). The dose-response inhibition showed a diverse pattern for the individual allergens. Despite the high cross-reactivity between the pyroglyphid mites, some proteins of *Ds* were less inhibited, e.g. by the *Df* and *Dp* 80-kD protein, and by the *Dm* and *Dp* 52-, 37-, 30- and 14-kD allergens. The 65-, 62-, 37- and 30-kD proteins were always inhibited more than 50% by all the mite extracts at the maximum concentration used. The 80-, 52-, 43-, 27- and 14-kD proteins cross-reacted to a lesser extent. Individual allergens of *Ds* were much less inhibited by non-pyroglyphid mites. However, at the highest concentration, *Ld* also inhibited most of the *Ds* allergens. All the ten selected allergens were inhibited to some extent by the heterologous mite extracts. The N-terminal sequences of Der s 1, 2 and 3 allergens showed higher homology to *Df* and *Dm* than to *Dp*. The homology of the group 2 allergens was higher than that of the group 1 allergens. **Conclusion:** The individual allergens of *Ds* were more similar to *Df* and *Dm* than to *Dp*. There was a limited and variable cross-reactivity with non-pyroglyphid mites. No single allergen was unique for *Ds*.

Introduction

A diverse mite fauna is part of the indoor environment. These mites are important sensitizers and triggers of allergic symptoms [1]. Understanding of the cross-reactivity between prevalent mite species is essential to settle appropriate strategies for diagnosis and immunotherapy. Cross-reactivity between allergens from mites of the genus *Dermatophagoides* [2–5] and between allergens from less taxonomically related mites [6–13] has been reported. Moreover, intra-species cross-reactivity between different major allergens has been demonstrated within the genus *Dermatophagoides* [14].

Dermatophagoides siboney is found together with other domestic mite species in tropical environments [15, 16]. *D. siboney*, *D. pteronyssinus* and *Blomia tropicalis* are the most common mites causing sensitization [17]. Cross-reactivity between *D. siboney* and other domestic mite allergens, particularly with *D. farinae*, has previously been reported using IgE inhibition [18]. Thirteen IgE binding proteins have been identified in *D. siboney* extracts by Western blotting [19] and the major allergens Der s 1, Der s 2 [19] and Der s 3 [20] have been purified.

The aim of this study was to investigate the cross-reactivity between individual IgE binding components from *D. siboney* and other allergenic mite species.

Materials and Methods

Allergens. *D. siboney* and *B. tropicalis* extracts were obtained as previously reported [19]. Freeze-dried extracts of *D. farinae*, *D. microceras*, *D. pteronyssinus*, *Tyrophagus putrescentiae*, *Acarus siro* and *Lepidoglyphus destructor* were obtained from ALK (Hørsholm, Denmark).

Patients and Sera. Sera from 148 asthmatic patients, sensitized to several mite allergens [17], were collected and processed as previously described [18]. Ten of these sera were selected and pooled based on their high concentration of specific IgE to *D. siboney* (MagicLite®, ALK), as well as their broad individual band recognition in immunoblots [19]. The inhibition of *D. farinae* allergens was performed with a pool of 5 sera from mite-sensitized Swedish patients with high levels of IgE antibodies to *D. farinae*.

SDS-PAGE and Immunoblot Inhibition. SDS-PAGE under non-reducing conditions was performed in 12.5% acrylamide gels. Freeze-dried extracts of *D. siboney* and *D. farinae* were reconstituted in sample buffer without 2-mercaptoethanol (3 mg/ml), i.e. 14 µg protein/lane, and boiled during 5 min. After electrophoresis, the proteins were transferred to nitrocellulose membranes (0.2 µm, Pharmacia, Uppsala). One membrane strip containing molecular weight markers (Pharmacia) was cut and silver stained. The rest of the membrane was blocked for 1 h at 37°C in phosphate saline buffer, pH 7.4, containing 0.5% Tween-20. After blocking, the membrane was cut into strips and each strip was individually incubated overnight with 500 µl of a mix-

ture (1:1) of the serum pool with serial dilutions of the allergen tested, i.e. a serum dilution previously found to give a good resolution of the IgE binding to *D. siboney* allergens [19]. The dilutions used for such inhibition were 0.4, 1.56, 6.25, 25.0 and 100.0 µg of protein/ml. Furthermore, a concentration 20-fold higher (2 mg/ml) was included to investigate the maximum inhibition for each allergen. The same concentration of bovine serum albumin (Sigma) was used as negative control in each experiment. The next day, the membrane strips were extensively washed, incubated with ¹²⁵I-anti IgE (80,000 cpm/strip, Phadebas RAST, Pharmacia) and autoradiographed as previously described [19].

The autoradiography films were recorded by a BioImage analyzer, and the images were processed by Visage 4.6 software (Millipore, USA). Quantification of the inhibition of individual bands was performed according to Johansson et al. [10]. The inhibition of each individual selected peak was calculated, in each experiment, by comparison with the corresponding peak of the negative control.

N-Terminal Sequence and Amino Acid Analysis. Purified Der s 1, Der s 2 [19] and Der s 3 [20] allergens were electrophoresed by SDS-PAGE under non-reducing conditions using the Protean II (BioRad, USA) apparatus. After electrophoresis, the proteins were transferred to polyvinylidene difluoride membranes. The strips along the membranes, shown to contain the proteins by Coomassie blue staining, were cut into 2×2 mm pieces, dried under nitrogen and frozen. The amino-terminal sequence of Der s major allergens was determined using automated Edman degradation [21], and quantitative amino acid analysis was performed according to Barkholt and Jensen [22].

Results

The IgE binding inhibition of *D. siboney* allergens by different mite species is shown in figure 1. Ten allergens from *D. siboney* corresponding to 14, 25, 27, 30, 37, 43, 52, 62, 65 and 80 kD and 6 allergens from *D. farinae* corresponding to 14, 25, 27, 37, 43 and 65 kD were well defined on autoradiographs and selected for quantification. The coefficient of variation between all the non-inhibited strips for the 10 allergens selected was 25.6% (15.6–30.0%).

The highest concentration of *D. farinae*, *D. pteronyssinus* and *D. microceras* extracts inhibited most *D. siboney* allergens to more than 80% when tested with the pool of Cuban sera, indicating a high degree of cross-reactivity between these species (fig. 2). Similarly, *D. farinae* allergens were also inhibited to more than 75% by the *D. siboney* extract when tested with the pool of sera from 5 Swedish patients who were not exposed to this species. Despite the high cross-reactivity between the pyroglyphid mites, some proteins of *D. siboney* were less inhibited. Thus the 80-kD protein was less inhibited by *D. farinae*, the 52-, 37-, 30- and 14-kD proteins by *D. microceras* and the 80-, 52-, 37-, 30- and 14-kD proteins by *D. pteronyssinus* extracts. The 65-, 62-, 37- and 30-kD proteins were inhibited by all extracts more than 50% at the maximum concentration used

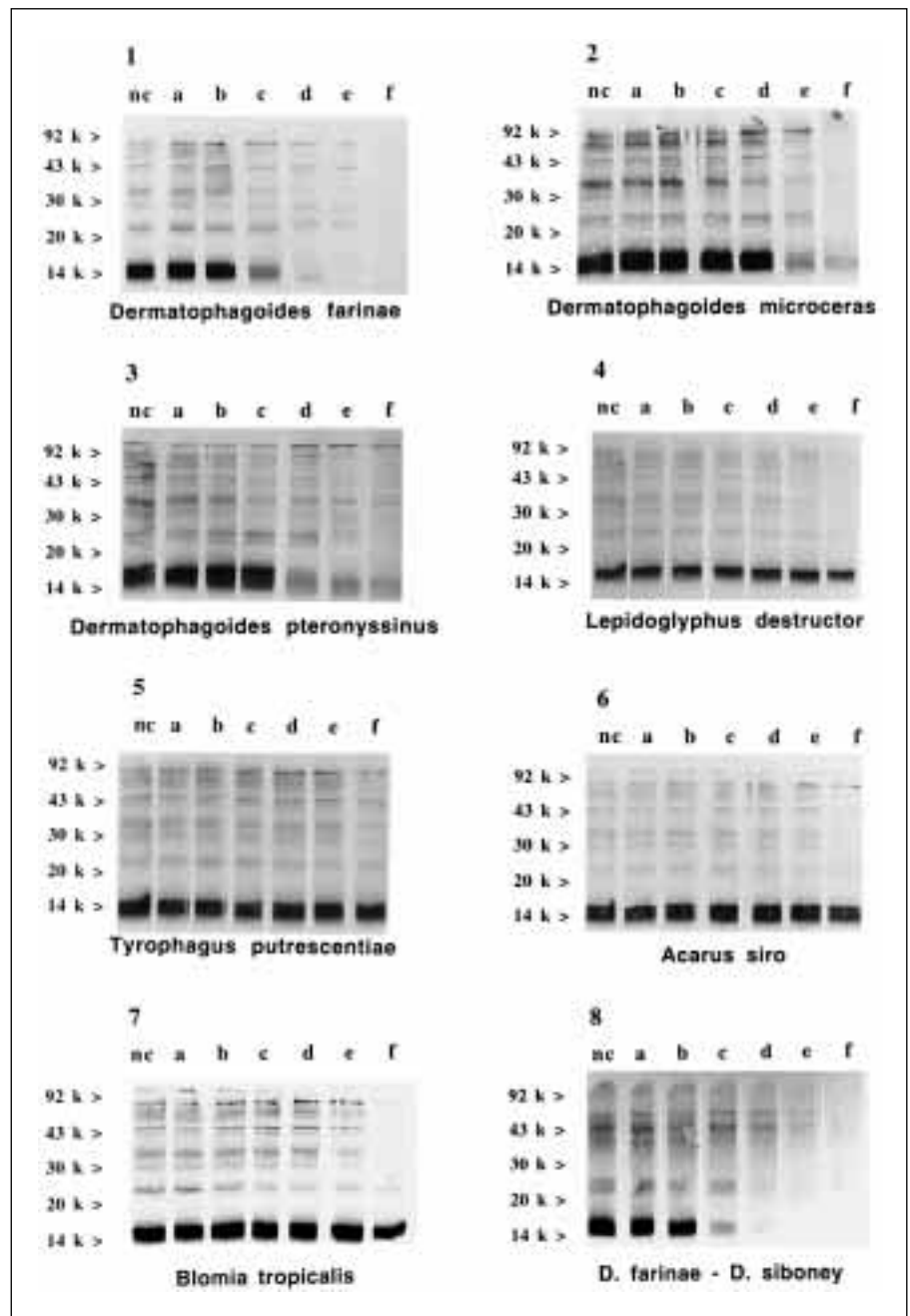


Fig. 1. Inhibition of the IgE binding to allergens of *D. siboney* after SDS-PAGE and Western blotting. Lane nc = Not inhibited negative control; lane a–f = dilutions (0.4, 1.56, 6.25, 25, 100 and 2,000 µg protein/ml) of the inhibiting mite extracts. The inhibition of IgE binding allergens in *D. farinae* by dilutions of *D. siboney* extract in a Swedish serum pool is also showed.

(2 g/l) and the 80-, 52-, 43-, 27- and 14-kD proteins were inhibited to a lower degree by some extracts. All the ten selected allergens were inhibited to some extent by all mite extracts. Most of the individual allergens of *D. siboney* were much less inhibited by non-pyroglyphid mites. However, at the highest concentration, *L. destructor* also inhibited most of the *D. siboney* allergens.

The dose-response inhibition for the allergens varied (fig. 3). The 80-kD allergen, which was inhibited to more

than 50% only by *D. farinae*, *D. microceras* and *L. destructor*, seemed to be the least cross-reacting allergen from *D. siboney*. At the highest concentration, this protein was completely inhibited only by *D. microceras* and was less inhibited by *D. pteronyssinus*. Der s 1 was equally inhibited by *D. farinae* and *D. pteronyssinus*, but poorly inhibited by *D. microceras* until the highest concentration of this extract was reached. Der s 2 was much more easily inhibited by *D. farinae* than by *D. pteronyssinus* or *D. microceras*, and Der

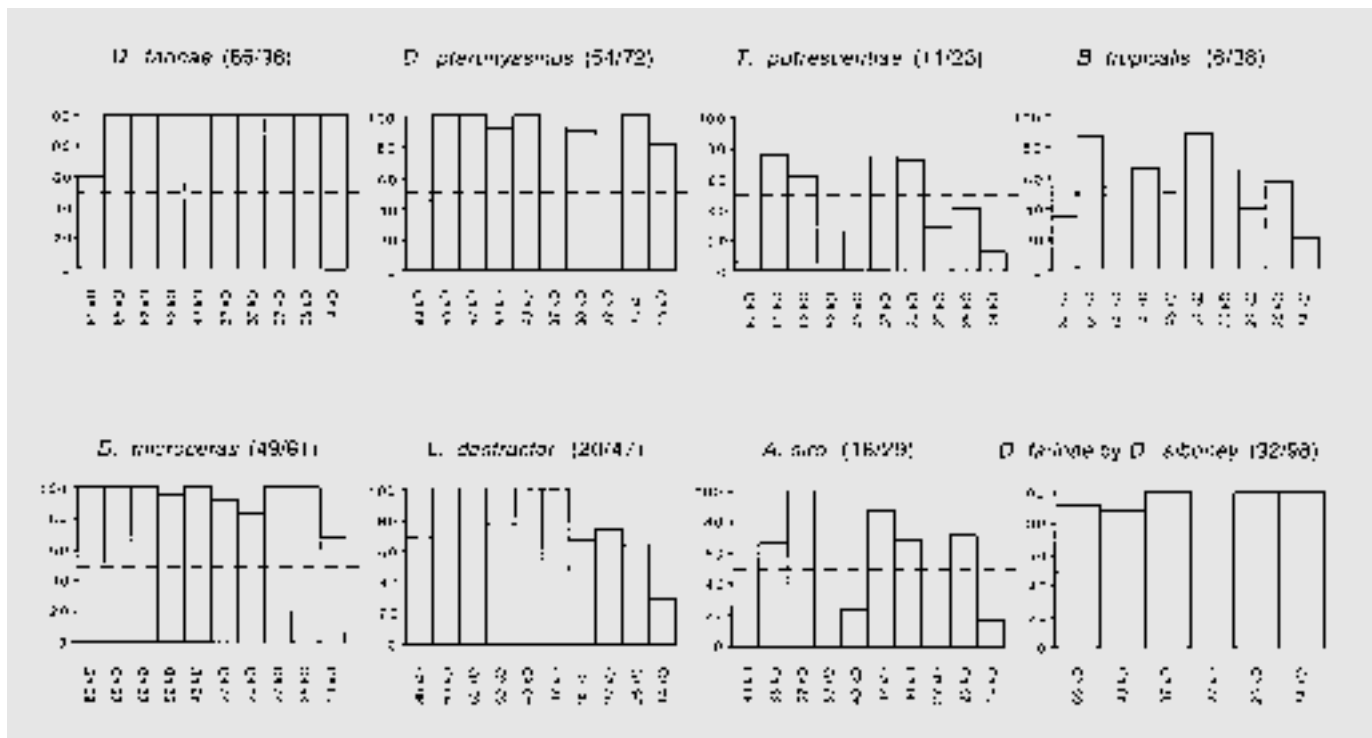


Fig. 2. Inhibition of individual allergens of *D. siboney* by 2,000 µg/ml of different mite extracts and the inhibition of *D. farinae* by *D. siboney* extract. The percentage of total integrated inhibition with 100 and 2,000 µg/ml is given in parentheses.

s 3 was inhibited to a similar extent by *D. pteronyssinus* and *D. microceras* but not by *D. farinae*, except for the highest concentration. The 27-kD allergen seemed to cross-react completely only with the most closely related species *D. microceras*, *D. farinae* and *D. pteronyssinus*, and was also inhibited to more than 50% by *L. destructor*.

The analysis of the N-terminal amino acid sequence (table 1) showed different degrees of homology between different species. The homology of Der s 1 and Der s 2 was higher with Der f 1 and 2 than with the group 1 and 2 of *D. pteronyssinus*, *D. microceras* and *E. maynei*. Der s 1 and Der s 2 seemed to be closely related, i.e. up to 95% of homology, with *D. farinae* group 1 and 2 allergens, respectively. The homologies between Der s 3 and Der f 3 and Der p 3 were lower, i.e. 50 and 59%, respectively, than those between group 3 allergens of *D. pteronyssinus* and *D. farinae*, i.e. 78%.

The amino acid analysis of Der s 1, 2 and 3 allergens indicated more similarities of Der s 1 and Der s 2 with Der p 1 and Der p 2 than with the group 1 and 2 *D. farinae* allergens (table 2). However, group 3 allergens seemed to be more similar between *D. siboney* and *D. farinae* than be-

tween *D. siboney* and *D. pteronyssinus* or between *D. farinae* and *D. pteronyssinus* which obviously reflects a high difference in Der p 3 amino acid composition, particularly demonstrated for aspartic and glutamic acids.

Discussion

The degree of inhibition of *D. siboney* individual allergens by *D. farinae* and *D. pteronyssinus* in Western blotting agrees with the results of previous studies comparing whole extract [18]. The results indicate a closer relationship between *D. siboney* and *D. farinae* than with *D. pteronyssinus*. The cross-reactivity of *D. siboney* with *D. microceras*, was also higher than that of *D. pteronyssinus*, which agrees with the taxonomic relationship between these species [23–25].

The cross-reactivity of *D. siboney* with *A. siro*, *T. putrescentiae*, *L. destructor* and *B. tropicalis* was somewhat lower than previously reported [18]. The 65-, 62-, 37- and 30-kD proteins were inhibited by more than 50% by these four mite species, and hence they seem to be responsible for most of the observed cross-reactivity.

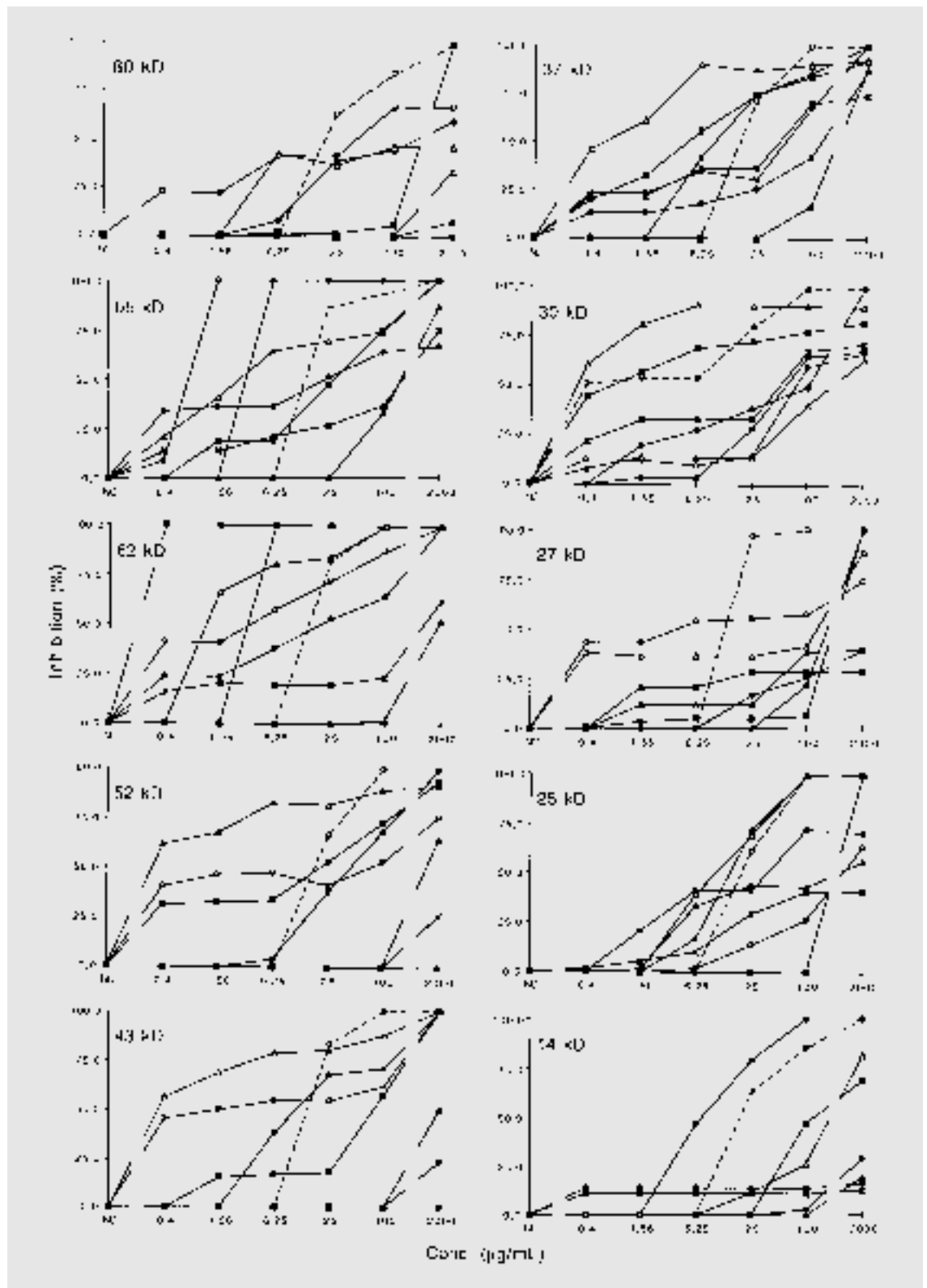


Fig. 3. Inhibition curves of the individual allergens of *D. siboney* by increasing concentrations of different mite species: —◆— = *D. farinae*; —■— = *D. microceras*; —▲— = *D. pteronyssinus*; —○— = *L. destructor*; —△— = *B. tropicalis*; —*— = *T. putrescentiae*; —●— = *A. siro*. The homologous inhibition by *D. siboney* extract is indicated as —○—.

Table 1. N-terminal sequences of *D. siboney* allergens and their homology with the group 1, 2 and 3 allergens.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Der s 1	T	S	A	?	R	I	N	P	V	N	V	P	A	E	L	D	?	?	?	L
Der f 1 [43]	T	S	A	C	R	I	N	S	V	N	V	P	S	E	L	D	L	R	S	L
Homology: 88%																				
Der p 1 [40, 41]	T	N	A	C	S	I	N	G	*	N	A	P	A	E	I	D	L	R	Q	M
Homology: 56%																				
Der m 1 [41]	T	Q	A	C	R	I	N	S	G	N	V	P	S	E	L	D	R	S	L	R
Homology: 75%																				
Eur m 1 [44]	T	Y	A	C	S	I	N	S	V	S	L	P	S	E	L	D	L	R	S	L
Homology: 75%																				
Der s 2	K	Q	V	D	V	K	D	?	A	N	N	E	I	K	K	V	?	V	D	G
Der f 2 [3]	D	Q	V	D	V	K	D	?	A	N	N	E	I	K	K	V	M	V	D	G
Homology: 95%																				
Der p 2 [3]	D	Q	V	D	V	K	D	?	A	N	H	E	I	K	K	V	L	V	P	G
Homology: 85%																				
Der s 3	V	V	G	G	Q	D	A	D	L	A	E	A	P	F	Q	I	S	L		
Der f 3 [3]	I	V	G	G	V	K	A	L	A	G	D	?	P	Y	E	I	S	L	E	V
Homology: 50%																				
Der p 3 [45]	I	V	G	G	E	K	A	L	A	G	E	?	P	Y	Q	I	S			
Homology: 59%																				
Asterisk indicates a deletion in position 9 for Der p 1.																				

Table 2. Amino acid composition (residues per 100 residues) of *D. siboney* and those of *D. farinae* and *D. pteronyssinus* major allergens.

		Der s 1	Der f 1 [3]	Der p 1 [46]	Der s 2	Der f 2 [3]	Der p 2 [3]	Der s 3	Der f 3 [3]	Der p 3 SwissProt
Asp	N	7.7	12.1	9.5	8.2	13.5	11.3	9.6	11.6	4.7
Thr	T	4.0	5.9	5.3	3.6	6.1	4.0	4.7	5.9	2.2
Ser	S	7.0	6.8	6.8	7.3	4.9	4.6	7.3	8.8	9.9
Gln	Q	18.5	7.9	18.0	15.5	11.8	12.4	14.3	9.7	3.9
Pro	P	5.5	5.9	9.8	4.3	6.1	7.4	5.2	5.2	2.6
Gly	G	11.6	11.0	8.9	13.7	8.5	8.9	12.8	11.4	11.6
Ala	A	7.1	7.4	8.1	7.9	8.1	8.6	5.8	6.6	7.3
1/2 Cys	C	1.7	0.7	3.4	N/A	N/A	N/A	1.7	N/A	3.0
Val	V	7.3	6.9	5.4	7.6	8.7	10.6	6.8	6.7	9.1
Met	M	0.9	1.5	1.1	1.2	1.7	1.4	1.2	2.1	0.9
Ile	I	7.1	8.7	3.6	5.6	7.4	8.0	5.7	6.3	6.5
Leu	L	6.98	5.4	5.6	8.4	8.8	7.3	8.2	7.4	6.5
Tyr	Y	4.2	7.8	2.0	2.3	1.7	2.0	3.3	3.8	4.7
Phe	F	2.6	1.4	2.2	3.2	3.6	2.8	2.5	2.3	1.3
His	H	2.0	2.2	1.9	2.5	2.9	3.5	2.8	2.2	1.7
Lys	K	2.3	1.4	4.6	5.3	3.4	3.7	5.0	6.6	8.2
Arg	R	3.6	6.9	3.8	3.2	2.6	2.4	3.0	3.4	3.4

Low-molecular weight allergens from *A. siro* and *T. putrescentiae* have been reported to cross-react poorly with *D. pteronyssinus*. A 16-kD component from *D. pteronyssinus* was also found to cross-react to some degree with *A. siro* and *T. putrescentiae* [10, 12]. In our study, the 14-kD component of *D. siboney*, Der s 2, was poorly inhibited by *L. destructor*, *A. siro*, *T. putrescentiae* and *B. tropicalis*. The 25-kD allergen, Der s 1, was also inhibited by these mites, but not as much as previously reported for the 25-kD allergen from *D. pteronyssinus*, which was inhibited to the same degree by *L. destructor* as by homologous extract [10].

Minimal or no cross-reactivity between *Blomia* and *Dermatophagoides* spp. has been reported by some authors [7, 11, 26–28], but others have demonstrated some cross-reactivity [29, 30]. Moreover, up to 47% inhibition of *D. siboney* whole extract was achieved by *Blomia* extract [18] and up till 38% of inhibition of IgE binding to individual allergens was observed in this paper. These results agree with the inhibition of IgE antibodies to *D. pteronyssinus* by *B. tropicalis* extracts as previously reported [31]. Cross-reactivity and sequence homology between group 5 allergens (13–14 kD) of *Blomia* and *Dermatophagoides* have been reported [32]. The group 5 content in *D. siboney* extract should be further investigated. According to the present results, the cross-reactivity observed between these two species seems to be explained by other components, i.e. the 65-, 52-, 37- and 30-kD proteins.

A 80-kD IgE binding protein of *D. siboney* was one of the least cross-reacting allergens. A component of 79 kD from *L. destructor*, which could be a degradation product of 93-kD protein, showed 60% of IgE binding in a group of RAST-positive sera to this mite and 25% in a group of sera positive to *D. pteronyssinus* [33]. It seems attractive to consider this component similar to the 80 kD of *D. siboney*, which was inhibited more than 65% by *L. destructor*. The use of specific monoclonal antibodies directed to this *L. destructor* protein on *D. siboney* extract could elucidate their partial identity.

Similar amounts of homologous extract was needed for 50% inhibition, i.e. about 10 µg in most cases, and 100% inhibition was achieved with 100 µg. The amount of heterologous extracts needed to achieve 50% of inhibition was different for each species, even when the inhibition with the highest concentration was quite similar. These results could reflect a partial identity between allergens of the different species tested, but also a variable amount of cross-reacting allergens in the different extracts used in these experiments. For some mites the inhibition curves did not follow a typical dose-response pattern. This could reflect the existence of different subpopulations of IgE with different affinity to

different proteins and/or the presence of several isoforms of each allergenic protein with different epitopes showing different IgE affinity. Furthermore, the possibility of more than one single allergen in each of these bands reactive to the IgE could not be excluded. It has been proposed that the response to group 1 allergens is directed against both cross-reactive and species-specific determinants whereas IgE antibodies to group 2 allergens were demonstrated to react almost completely to cross-reactive determinants [34]. However, our results could indicate a closer IgE cross-reactivity of Der s 1 than of Der s 2 with the corresponding group allergens, although the amino acid composition and n-terminal sequences of Der s 2 showed higher similarity with the corresponding group of *D. farinae* and *D. pteronyssinus* than did Der s 1. The possibility that some epitopes may have been destroyed during SDS-PAGE and/or that the inhibition of IgE binding to Der s 2 is diminished by an oversaturation effect of the autoradiography, cannot be excluded.

The results of cross-reactivity studies will largely depend on the allergenic extracts used for the inhibition and on the patient selection criteria. Large variations in the relative amounts of individual allergens between extracts of the same species is not an unusual phenomenon. Moreover, the relative amount of IgE antibodies directed to individual allergens will vary in the source of serum pools. In our study we have used well-characterized allergens, and a large number of allergic individuals have been screened in order to select a pool of sera. However, absolute data on the cross-reactivity between allergenic extracts are difficult to obtain. Not only the partial identity between some individual proteins of *D. siboney* with those of other mites, but also the effect of multiple sensitization to several mite species [35–37] should be considered, since over-exposure to other mite species can influence the IgE antibody response to the mite studied, i.e. patients who are sensitive to house dust mite allergens may produce IgE that binds to the allergens of mite species to present in their environment [38].

Cuban patients have not been exposed to *D. farinae* [15] and Swedish patients have not been exposed to *D. siboney*. Despite the lack of exposure, Cuban patients have IgE binding and reacted by skin test to *D. farinae* and Swedish did to *D. siboney* due to the pronounced cross-reactivity observed between these species [Casas et al., submitted]. Therefore the high percentage of inhibition of the individual allergens of *D. farinae* by *D. siboney* extract in a Swedish serum pool was not surprising.

The high degree of N-terminal amino acid sequence homology between Der s 1 and the group 1 allergens Der f 1, Der m 1 and Eur m 1 agreed with those previously reported

for group 1 allergens [39–41] and with the related taxonomy [24]. The low homology found between Der s 1 and Der p 1 reflects a dissimilarity in the N-terminal sequence of Der p 1, which is also evident when the homology of this allergen with the other species, i.e. 25% with Der f 1 and 35% with Der m 1, were calculated. Even higher similarity was observed between Der s 2 and Der f 2. However, when the relationship between the amino acid composition was estimated, Der s 1 and Der s 2 seemed to be more related to Der p 1 and Der p 2, respectively, than to Der f 1 and Der f 2. Der s 3 and Der f 3 showed the highest similarity in amino acid composition despite their poor homology at the N-terminal sequences. These contradictory results can reflect a more pronounced difference in the amino acid sequence than those observed from the first 20 N-terminal amino acids. Der s 3 was purified using monoclonal antibodies directed against Der f 3 allergen, showed tryptic-like activity and reacted with anti-Der 3 antibodies [20]. However, the N-terminal sequence homology with the group 6 allergens reported in 1993 by Yasueda et al. [42] was higher than that of group 3, possibly indicating a cross-reactivity between group 3 and 6 allergens, a question to be further investigated.

Our present findings confirm the close relationship between *D. siboney* and *D. farinae* and indicate that under

those experimental conditions selected, the individual allergens of *D. siboney* were more similar to those of *D. farinae* and *D. microceras* than to *D. pteronyssinus* and that despite limited, there is a variable cross-reactivity with non-pyroglyphid mites. Even through no single individual allergen was found to be unique for *D. siboney*, the presence and characterization of non-cross-reacting, as well as similar epitopes on its allergen molecules, should be further investigated.

Acknowledgments

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References

- 1 Spieksma FT: Domestic mites: Their role in respiratory allergy. *Clin Exp Allergy* 1991;21: 655–660.
- 2 Chapman MD, Platts Mills TA: Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980;125:587–592.
- 3 Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts Mills TA: Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989;83:1055–1067.
- 4 Thomas WR, Chua KY, Smith WA: Molecular polymorphisms of house dust mite allergens. *Exp Appl Acarol* 1992;16:153–164.
- 5 Johansson E, Schmidt M, Johansson SGO, Machado L, Olsson S, van Hage-Hamsten M: Allergenic crossreactivity between *Lepidoglyphus destructor* and *Blomia tropicalis*. *Clin Exp Allergy* 1997;27:691–699.
- 6 Miyamoto T, Oshima S, Misuno K, Sasa M, Ishizaki T: Cross-antigenicity among six species of dust mites and house dust antigens. *J Allergy* 1969;44:228–238.
- 7 Griffin P, Ford AW, Alterman L, Thompson J, Parkinson C, Blainey AD, Davies RJ, Topping MD: Allergenic and antigenic relationship between three species of storage mite and the house dust mite, *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1989;84:108–117.
- 8 Luczynska CM, Griffin P, Davies RJ, Topping MD: Prevalence of specific IgE to storage mites (*A. siro*, *L. destructor* and *T. longior*) in an urban population and cross-reactivity with the house dust mite (*D. pteronyssinus*). *Clin Exp Allergy* 1990;20:403–406.
- 9 van Hage-Hamsten M, Machado L, Barros MT, Johansson SGO: Comparison of clinical significance and allergenic cross-reactivity of storage mites *Blomia kulagini* and *Lepidoglyphus destructor* in Sweden and Brazil. *Allergy* 1990;45:409–417.
- 10 Johansson E, Borga A, Johansson SGO, van Hage-Hamsten M: Immunoblot multi-allergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1991;21:511–518.
- 11 Puerta L, Fernandez-Caldas E, Lockey RF, Caraballo LR: Sensitization to *Chortoglyphus arcuatus* and *Aleuroglyphus ovatus* in *Dermatophagoides* spp. allergic individuals. *Clin Exp Allergy* 1993;23:117–123.
- 12 Johansson E, Johansson SGO, van Hage-Hamsten M: Allergenic characterization of *Acarus siro* and *Tyrophagus putrescentiae* and their crossreactivity with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1994;24:743–751.
- 13 Ebner C, Feldner H, Ebner H, Kraft D: Sensitization to storage mites in house dust mite (*Dermatophagoides pteronyssinus*) allergic patients. Comparison of a rural and an urban population. *Clin Exp Allergy* 1994;24:347–352.
- 14 O'Neill GM, Baldo BA: Intra-species cross-reactivity of house dust mite allergens separated by protein blotting and detected by selective elution of mite components and IgE antibodies. *Electrophoresis* 1993;14:923–925.
- 15 Cuervo N, Dusábek F, de la Cruz J, Abreu R: Los ácaros (Acarina: Pyroglyphidae, Cheyletidae, Saprogllyphidae y Glyciphagidae) de los polvos domésticos en Cuba. *Rev Cubana Med Trop* 1983;35:83–103.

- 16 Fernández-Caldas E, Sepulveda A, Montealegre F: Identification of the domestic mite fauna in Puerto Rico. *Allergy* 1995;50(suppl. 26):72.
- 17 Ferrándiz R, Casas R, Dreborg S: Sensitization to *Dermatophagoides siboney*, *Blomia tropicalis* and other domestic mites in asthmatic patients. *Allergy* 1996;51:501–505.
- 18 Ferrándiz R, Casas R, Dreborg S, Einarsson R, Fernandez B: Crossreactivity between *Dermatophagoides siboney* and other house dust mite allergens in sensitized asthmatic patients. *Clin Exp Allergy* 1995;25:929–934.
- 19 Ferrándiz R, Casas R, Dreborg S, Einarsson R, Bonachea I, Chapman M: Characterization of allergenic components from house dust mite *Dermatophagoides siboney*. Purification of Der s 1 and Der s 2 allergens. *Clin Exp Allergy* 1995;25:922–928.
- 20 Ferrándiz R, Casas R, Dreborg S: Purification and IgE binding capacity of Der s 3, a major allergen from *Dermatophagoides siboney*. *Clin Exp Allergy* 1997;27:700–704.
- 21 Ipsen H, Hansen OC: NH₂-terminal amino acid sequence of the immunochemically partial identical major allergens of alder (*Alnus glutinosa*) Aln g I, birch (*Betula verrucosa*) Bet v I, hornbeam (*Carpinus betulus*) Car b I and oak (*Quercus alba*) Que a I pollens. *Mol Immunol* 1991;28:1279–1288.
- 22 Barkholt V, Jensen A: Aminoacid analysis. Determination of cysteine and half-cysteine in proteins after hydrochloric acid hydrolysis with a disulfide compound as additive. *Anal Biochem* 1988;177:318–322.
- 23 Cunningham AM: House-dust mites and respiratory allergy: A note on the identity of *Dermatophagoides farinae* Hughes, 1961. *Clin Allergy* 1971;1:447–449.
- 24 Dusbábek F, Cuervo N, de la Cruz J: *Dermatophagoides siboney* sp. n. (Acarina: Pyroglyphidae). A new house dust mite from Cuba. *Acarologia* 1982;23:55–62.
- 25 Colloff MJ, Spieskma FTM: Pictorial keys for the identification of domestic mites. *Clin Exp Allergy* 1992;22:823–830.
- 26 van Hage-Hamsten M, Johansson SG, Johansson E, Wiren A: Lack of allergenic cross-reactivity between storage mites and *Dermatophagoides pteronyssinus*. *Clin Allergy* 1987;17:23–31.
- 27 Puerta L, Fernandez-Caldas E, Caraballo LR, Lockey RF: Sensitization to *Blomia tropicalis* and *Lepidoglyphus destructor* in *Dermatophagoides* spp-allergic individuals. *J Allergy Clin Immunol* 1991;88:943–950.
- 28 Arlian LG, Vyszenski-Moher DL, Fernandez-Caldas E: Allergenicity of the mite, *Blomia tropicalis*. *J Allergy Clin Immunol* 1993;91:1042–1050.
- 29 Vailes LD, Rizzo MC, Naspitz CK, Montealegre F, Fernández-Caldas E, Platts-Mills TAE, Chapman MD, Arruda LK: Analysis of IgE antibody responses to *D. pteronyssinus* and *Blomia tropicalis* using recombinant group 5 allergens. *J Allergy Clin Immunol* 1996;97:213.
- 30 Smith A, Green R, Custovic A, Woodcock A, Arruda K, Chapman M: In vivo cross reactivity between IgE Ab to *Blomia* and *Dermatophagoides* spp and to recombinant group 5 allergens among mite allergic patients living in the UK. *J Allergy Clin Immunol* 1996;97:420.
- 31 Arruda LK, Rizzo MC, Chapman MD, Fernandez-Caldas E, Baggio D, Platts-Mills TAE, Naspitz CK: Exposure and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil. *Clin Exp Allergy* 1991;21:433–439.
- 32 Arruda LK, Fernández-Caldas E, Naspitz CK, Montealegre F, Chapman MD: *Blomia tropicalis* allergen Blo t 5: Homology to *D. pteronyssinus* Der p 5 (abstract). *ACI News* 1994; suppl 2:34.
- 33 Olsson S, Harfast B, Johansson SGO, van Hage-Hamsten M: Detection of at least one high-molecular-mass, IgE-binding component of the dust mite *Lepidoglyphus destructor*. *Allergy* 1994;49:620–625.
- 34 Yasueda H, Mita H, Yui Y, Shida T: Comparative analysis of physicochemical and immunochemical properties of the two major allergens from *Dermatophagoides pteronyssinus* and the corresponding allergens from *Dermatophagoides farinae*. *Int Arch Allergy Appl Immunol* 1989;88:402–427.
- 35 Fernández-Caldas E, Puerta L, Lockey RF, Caraballo LR: Mite allergy in the tropics: Sensitization to six domestic mite species in Cartagena. *Clin Exp Allergy* 1993;23:292–297.
- 36 Fernández-Caldas E, Puerta L: Sensitization to various mite species; in Johansson SGO (ed): *Progress in Allergy and Clinical Immunology*. Seattle, Hogrefe & Huber, 1995; vol 3, pp 317–322.
- 37 Bernd LAG, Ambrozio LC, Baggio D: Multiple sensitivity to mite allergens in urban atopics (abstract). *J Allergy Clin Immunol* 1995; 95:159.
- 38 Young RP, Hart BJ, Faux JA, Hopkin JM: House dust mite sensitivity: A comparison of immune response with exposure to four species of Pyroglyphidae. *Clin Exp Allergy* 1990;20:319–325.
- 39 Lind P, Hansen OC, Horn N: The binding of mouse hybridoma and human IgE antibodies to the major fecal allergen, Der p I, of *Dermatophagoides pteronyssinus*. Relative binding site location and species specificity studied by solid-phase inhibition assays with radiolabeled antigen. *J Immunol* 1988;140:4256–4262.
- 40 Dilworth RJ, Chua KY, Thomas WR: Sequence analysis of cDNA coding for a major house dust mite allergen, Der f I. *Clin Exp Allergy* 1991;21:25–32.
- 41 Chua KY, Kehal PK, Thomas WR: Sequence polymorphisms of cDNA clones encoding the mite allergen Der p I. *Int Arch Allergy Immunol* 1993;101:364–368.
- 42 Yasueda H, Mita H, Akiyama K, Shida T, Ando T, Sugiyama S, Yamakawa H: Allergens from *Dermatophagoides* mites with chymotryptic activity (see comments). *Clin Exp Allergy* 1993;23:384–390.
- 43 Dandeu JP, Le Mao J, Lux M, Rabillon J, David B: Antigens and allergens in *Dermatophagoides farinae* mite. II. Purification of AG f1, a major allergen in *Dermatophagoides farinae*. *Immunology* 1982;46:679–687.
- 44 Kent NA, Hill MR, Keen JN, Holland PW, Hart BJ: Molecular characterisation of group I allergen Eur m I from house dust mite *Euroglyphus maynei*. *Int Arch Allergy Immunol* 1992;99:150–152.
- 45 Stewart GA, Kollinger MR, King CM, Thompson PJ: A comparative study of three serine proteases from *Dermatophagoides pteronyssinus* and *D. farinae*. *Allergy* 1994;49:553–560.
- 46 Dandeu JP, Rabillon J, Lux M, David B, Guillaume JL, Camoin L: Isolation of Der p I, the *Dermatophagoides pteronyssinus* major mite allergen, from a crude mite culture extract, purification by ion-chromatography, and comparison between the material obtained and a cDNA-coded Der p I. *J Chromatogr* 1992;599: 105–111.

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