

Allergenic characterization of *Acarus siro* and *Tyrophagus putrescentiae* and their crossreactivity with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*

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Summary

Allergenic components in extracts of *Acarus siro* and *Tyrophagus putrescentiae* were identified by sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) and immunoblotting. Five and four allergenic components, respectively, were visualized with sera from farmers sensitized to storage mites. The highest frequency of IgE-binding was to a 15 kDa component of *A. siro* (7/9 sera) and a 16 kDa component of *T. putrescentiae* (23/29 sera). The allergenic crossreactivity of *A. siro* and *T. putrescentiae* with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus* was studied with an immunoblotting inhibition technique. Crossreactivity between *D. pteronyssinus* on the one hand and *A. siro* and *T. putrescentiae* on the other, was shown, as the IgE reactivity to a 25 kDa component of *D. pteronyssinus* was inhibited to the same degree by extracts of *A. siro*, *T. putrescentiae* and *D. pteronyssinus*. However, *D. pteronyssinus* was a poor inhibitor of the allergenic components of *A. siro* and *T. putrescentiae*. Strong cross-reactivity was also shown between *L. destructor* and the allergenic components of *A. siro* and *T. putrescentiae*, while the latter mite species only to a very low degree inhibited the allergenic components of *L. destructor*.

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Introduction

Acarus siro, *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* belong to the storage mites which are common inhabitants of dust of different stored agricultural products [1–4] and responsible for occupational allergy among farmers [5–7] as well as causing sensitization among grainworkers [8] and bakers [9,10]. They may also be a source of allergen exposure in dwellings [11–13], especially those with high humidity and mould growth as these mites are fungivorous [14]. Several studies report on sensitization to storage mites among urban populations [15–18].

The major allergen source in house dust is the mite *Dermatophagoides pteronyssinus* [19], the allergenic properties of which have extensively been studied [20]. The fact that house dust occasionally contains different

species of mites raises the question of whether the sensitizing mite allergens are species-specific or common and how far any common allergens may crossreact. Despite the extensive literature on allergenicity of different mite species, the degree of crossreactivity is still poorly investigated. Knowledge of allergenic crossreactivity between different species of dust mites is of considerable importance for accurate diagnosis as well as for effective immunotherapy for patients suffering from mite allergy.

The aims of this study were to identify IgE-binding components in extracts of *A. siro* and *T. putrescentiae* and to evaluate allergenic cross-reactivity between *A. siro*, *T. putrescentiae*, *L. destructor* and *D. pteronyssinus*.

Materials and methods

Mite extracts

Extracts of *A. siro*, *T. putrescentiae*, *L. destructor* and *D.*

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pteronyssinus were prepared from whole cultures obtained from Allergon, Ängelholm, Sweden. The cultures were extracted in 0.15 M NaCl containing 0.02% NaN₃ at 1/10 w/v for 16 h at room temperature. The extract suspensions were clarified by centrifugation at 6600 g for 10 min and the supernatants were desalted on prepacked Sephadex G-25 columns, PD 10 (Pharmacia, Uppsala, Sweden) and lyophilized. The protein content was determined with Pierce Bicinchoninic acid (BCA) Protein Assay Reagent [21] (Pierce Chemical Company, Rochford, IL., USA).

Serum samples

Sera from farmers who had previously participated in an epidemiological investigation of respiratory diseases were chosen for the study [6]. Nine of these sera, positive in Phadebas RAST[®] (Pharmacia AB, Uppsala, Sweden) to *A. siro*, were used for screening IgE-binding components in extract of *A. siro* with SDS-PAGE and immunoblotting. Twenty-nine sera (RAST positive to *T. putrescentiae*) were used for screening IgE-binding components in extract of *T. putrescentiae*. Seven sera had IgE antibodies to both *A. siro* and *T. putrescentiae*. According to the screening results six sera were pooled. Five of these sera contained IgE to the major allergenic components of both *A. siro* and *T. putrescentiae* and one serum contained IgE only to the major component in *T. putrescentiae*. These six sera also had IgE antibodies to the major allergenic components in extracts of *L. destructor* and *D. pteronyssinus* [22]. The serum pool was used at 1:4 in the studies of allergenic cross-reactivity of *A. siro*, *T. putrescentiae*, *L. destructor* and *D. pteronyssinus*. The RAST response of the serum pool diluted 1:4 was 4.2 PRU to *A. siro*, 5.5 PRU to *T. putrescentiae*, 18 PRU to *L. destructor* and 12 PRU to *D. pteronyssinus*. None of the sera mentioned above caused any visible bands in SDS-PAGE and immunoblotting when screened for IgE to mite culture medium.

SDS-PAGE and immunoblotting

SDS-PAGE was performed in a Mini-PROTEAN[®] II Dual Slab Cell (Bio-Rad, Richmond, California, USA) according to the operating instructions. The separation slab gel was cast as a gradient (7.5–20%), on top of which the stacking gel was moulded (6%). The mite extracts were each resuspended in a non-reducing sample buffer, and 1 mg of protein was loaded into a preparative well (68 × 1.0 mm). After electrophoresis, the proteins were transferred onto nitrocellulose (Trans-blot[®] NC membrane; pore size 0.45 µm; Bio-Rad) in a Mini Trans-Blot[®] Electrophoretic cell

(Bio-Rad) according to the manufacturer's instructions. The membrane was then blocked for 60 min at 37°C with 0.5% (v/v) Tween 20 in 0.05 M phosphate buffer, pH 7.4, also containing 0.15 M NaCl, 0.2% (w/v) bovine serum albumin and 0.02% (w/v) NaN₃. After blocking, the membrane was cut into narrow strips and incubated for 16 h at room temperature with 500 µl of serum diluted 1/4 in blocking buffer. After washing, the strips were first incubated for 2 h with 500 µl of affinity-purified rabbit anti-human IgE (Miab, Knivsta, Uppsala, Sweden) and then for another 2 h with 500 µl of affinity-purified ALP-conjugated goat anti-rabbit IgG (Bio-Rad). Both antibodies were diluted 1/2000 in blocking buffer. Finally, the IgE-binding components were visualized by incubating the strips with 1 ml of a chromogenical enzyme substrate, Alkaline Phosphatase Conjugate Substrate Kit (Bio-Rad) for 30 min. On the gel loaded with mite extract, a reference well (3 × 1.0 mm) was used for low-molecular-weight standard proteins (LMW, Electrophoresis Calibration kit, Pharmacia). After electroblotting, the membrane with LMW standard proteins was stained with AuroDye[®] forte (Janssen Biotech N.V., Olen, Belgium) and the molecular weight of each mite allergenic component was determined by comparing its relative mobility with that of LMW standard proteins. The molecular weights of the allergenic components should be considered as approximate.

Blotting inhibition

The cross-allergenicity of dust mites was studied by using different mite extracts to inhibit the IgE-binding to mite components on NC strips. A serum pool containing IgE to allergens in *A. siro*, *T. putrescentiae*, *L. destructor* and *D. pteronyssinus* was preincubated for 3 h at room temperature with a serially diluted extract of one of the four mite species. The final dilution of the serum pool was 1/4. The mixtures were then incubated with blotted mite proteins on NC strips and the allergenic components were visualized as described above. Four protein amounts, 0.0002, 0.002, 0.02 and 0.2 mg of the mite inhibitor, were used in the homologous pair system and at least three protein amounts between 0.00002 and 2 mg in the heterologous pair system. The reference strip used for evaluating the results was inhibited with the diluent only.

Densitometric scanning and calculating of inhibition results

To assess the inhibition of each IgE-binding component, the strips were scanned with a densitometer, Model 620 Video Densitometer (Bio-Rad). The intensity of the

immunoreactivity was measured in fluorescent cool white light in the reflectance mode. Data from the densitometer were transferred to a computer and analysed by 1-D Analyst Program (Bio-Rad). A strip with blotted mite proteins (treated as the sample strips except for incubation with serum and extract) was scanned and subtracted as background from each sample strip. A peak diagram with reflectance versus position was obtained for each strip, which made the allergenic components of interest easy to identify.

To calculate the degree of inhibition, the optical density (OD) values for a peak after inhibition with extract and after inhibition with diluent were used. The results were expressed as percentages of inhibition and plotted against the increasing amount of the inhibiting mite extract.

The strips obtained when screening the allergenic components in extracts of *A. siro* and *T. putrescentiae* with individual sera, were scanned in order to determine the degree of IgE-binding to each component. The sera were divided by OD value into three classes labelled as weak (< 0.1), moderate (0.1–0.5) and strong (> 0.5).

Results

Screening of IgE-binding components in *A. siro* and *T. putrescentiae*

The allergen pattern in extracts of *A. siro* and *T. putrescentiae* was investigated by SDS-PAGE, blotting and immunoprobings with human sera positive in RAST to these mite species. IgE-binding bands visualized by more than 50% of the sera were denoted as major allergenic components. Table 1 shows the classes and the number of sera which recognized five different components in *A. siro* extract. Seven of the nine sera detected a major allergenic component at 15 kDa, most

Table 2. Number of sera ($n = 29$) recognizing IgE-binding components in extract of *T. putrescentiae* measured by densitometric scanning

IgE-binding component (kDa)	Class		
	Weak	Moderate	Strong
16	12	10	1
17		1	
21	1	2	
33	1		

of them moderately. The strongest reaction was to a component at 22 kDa but it was recognized by only four of the sera. Minor allergenic components at 16, 17 and 20 kDa were also identified by some of the sera. Two sera revealed no bands at all.

The distribution of sera and IgE-binding components in extract of *T. putrescentiae* is demonstrated in Table 2.

Table 1. Number of sera ($n = 9$) recognizing IgE-binding components in extract of *A. siro* measured by densitometric scanning

IgE-binding component (kDa)	Class		
	Weak	Moderate	Strong
15	2	5	
16		2	
17	2	2	
20		1	
22	1	2	1

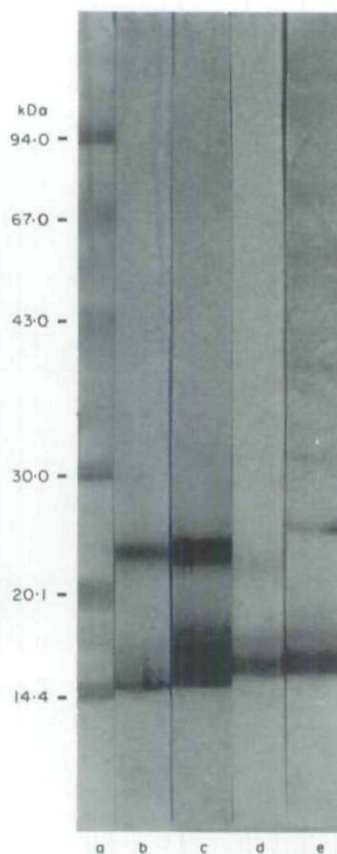


Fig. 1. IgE-binding components in dust mites detected on immunoblots by a serum pool. The blots are as follows: (a) AuroDyeTM staining of Pharmacia low mw markers; (b) *A. siro*; (c) *L. destructor*; (d) *T. putrescentiae*; (e) *D. pteronyssinus*.

The 16 kDa allergenic component showed the highest IgE-binding frequency, being recognized by 23 of the 29 (79%) sera tested and the reaction was moderate or weak in 22 of the sera. Some minor components at 17, 21 and 33 kDa were also detected by three different sera.

IgE-binding components detected by the serum pool

Based on the screening results above, a serum pool was made and used in crossinhibition studies of *A. siro*, *T. putrescentiae*, *L. destructor* and *D. pteronyssinus*. Figure 1 shows the IgE-binding of the pool to allergenic components in extracts of the four mite species. The major components in *A. siro* (15 kDa), *T. putrescentiae* (16 kDa), *L. destructor* (15 kDa) and *D. pteronyssinus* (16 and 25 kDa) along with a minor component at 22 kDa in extracts of both *A. siro* and *L. destructor* were strongly stained.

Inhibition pattern of IgE-binding components in A. siro and T. putrescentiae on immunoblots

The results of homologous and heterologous inhibition of IgE-binding in a serum pool to blotted *A. siro* and *T. putrescentiae* components are shown in Fig. 2(a and b). The inhibition was regarded as complete when no bands were visible on the blots. Complete homologous inhibition of the 15 kDa component of *A. siro* required 0.2 mg *A. siro* extract. The minimum amount of extract demanded for complete homologous inhibition of the 22 kDa and the 16 kDa component of *A. siro* and *T. putrescentiae*, respectively, was only 0.02 mg. The heterologous inhibitions with extract of *A. siro*, *T. putrescentiae*, *L. destructor* or *D. pteronyssinus* decreased the staining intensity and indicated cross-reactivity. Inhibition of IgE-binding components of *L. destructor* and *D. pteronyssinus* were performed with homologous extract and with extract of *A. siro* and *T. putrescentiae* (not shown).

Crossinhibition of IgE-binding to allergenic components in A. siro, T. putrescentiae, L. destructor and D. pteronyssinus, evaluated by densitometric scanning

Crossreactivity between *A. siro* and *T. putrescentiae*
Almost identical inhibition curves of the two allergenic components of 15 kDa and 22 kDa of *A. siro* (Fig. 3a and b) were revealed with *T. putrescentiae* extract and only 10-fold more of *T. putrescentiae* extract than of *A. siro* extract was necessary for 50% inhibition. However, to achieve 50% inhibition of the 16 kDa allergenic component of *T. putrescentiae*, about 1000-fold more of *A. siro* extract than of *T. putrescentiae* extract was required (Fig. 3c).

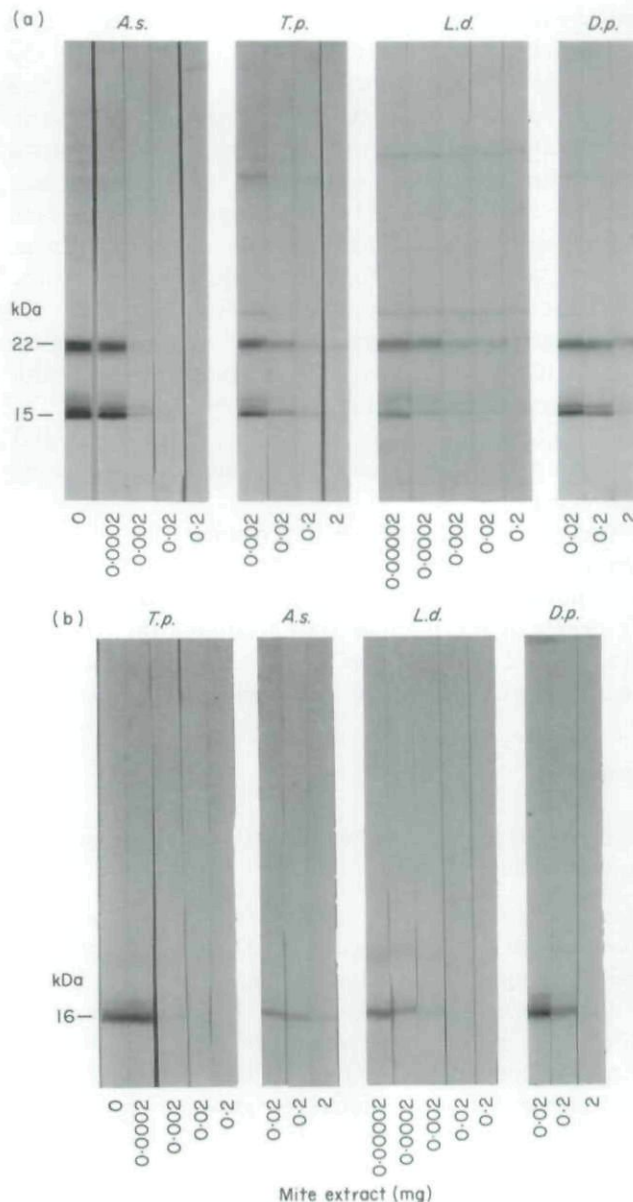


Fig. 2. Inhibition of IgE-binding to (a) *A. siro* allergenic components and (b) *T. putrescentiae* allergenic components on immunoblots by serial diluted extracts of *A. siro* (A.s.), *T. putrescentiae* (T.p.), *L. destructor* (L.d.) and *D. pteronyssinus* (D.p.).

Crossreactivity between *A. siro* and *L. destructor*
The strongest crossreactivity of the 15 kDa allergenic component of *A. siro* was shown with extract of *L. destructor* as even less *L. destructor* extract than *A. siro* extract sufficed for 50% inhibition (Fig. 3a). However, the major allergenic component of *L. destructor* (15 kDa) was not inhibited at all by extract of *A. siro* (Fig. 3d). In fact, 50% inhibition was not achieved even when 2 mg of *A. siro* proteins was added. Extract of *A. siro* was also a poor

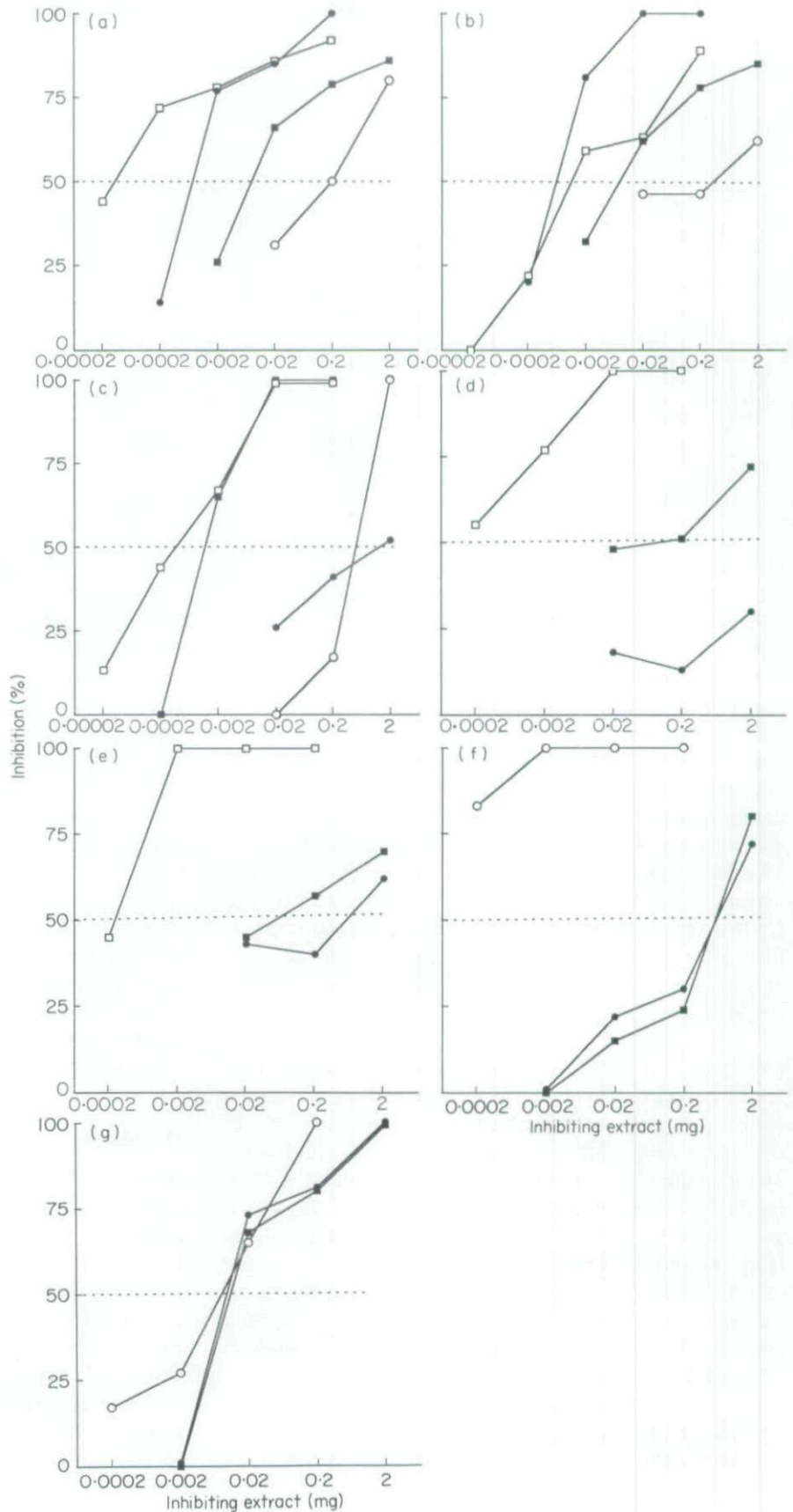


Fig. 3. The inhibiting effect on mite IgE-binding components on immunoblots a–g, after probing with a mix of a serum pool and increasing amounts of homologous and heterologous mite extracts: (●) *A. siro*, (■) *T. putrescentiae*, (□) *L. destructor*, (○) *D. pteronyssinus*. The inhibition figures were calculated from peak diagrams received by densitometric scanning of immunoblots. (a) *A. siro* 15 kDa. (b) *A. siro* 22 kDa. (c) *T. putrescentiae* 16 kDa. (d) *L. destructor* 15 kDa. (e) *L. destructor* 22 kDa. (f) *D. pteronyssinus* 25 kDa. (g) *D. pteronyssinus* 16 kDa.

Table 3. Variation in amounts of extract used for 50% inhibition of IgE-binding components in *A. siro*, *T. putrescentiae*, *L. destructor* and *D. pteronyssinus*

IgE-binding components on NC blot	Amounts of extract (homologous : heterologous)			
	<i>A. siro</i>	<i>T. putrescentiae</i>	<i>L. destructor</i>	<i>D. pteronyssinus</i>
<i>A. siro</i> (kDa)				
15		1 : 10	1 : < 1	1 : 300
22		1 : 10	1 : 2	1 : 600
<i>T. putrescentiae</i> (kDa)				
16	1 : 1000		1 : < 1	1 : 400
<i>L. destructor</i> (kDa)				
15	1 : > 10000	1 : > 400		
22	1 : 2000	1 : 200		
<i>D. pteronyssinus</i> (kDa)				
16	1 : > 3000	1 : > 3000		
25	1 : 1	1 : 1		

inhibitor of the 22 kDa component of *L. destructor* (Fig. 3e). About 2000-fold more of *A. siro* extract than of *L. destructor* extract was needed to obtain 50% inhibition. In contrast, extract of *L. destructor* was a strong inhibitor of the 22 kDa component of *A. siro* (Fig. 3b), only twice as much of the *L. destructor* was needed.

Crossreactivity between *A. siro* and *D. pteronyssinus* The crossreactivity between the 15 and 22 kDa allergenic components of *A. siro* and extract of *D. pteronyssinus* was very low (Fig. 3a and b), as was that between the 16 kDa allergenic component of *D. pteronyssinus* and extract of *A. siro* (Fig. 3f). In contrast, extract of *A. siro* crossreacted strongly with the 25 kDa allergenic component of *D. pteronyssinus* (Fig. 3g) since about the same amounts of *A. siro* and *D. pteronyssinus* extracts inhibited the component to 50%.

Crossreactivity between *T. putrescentiae* and *L. destructor* The 16 kDa allergenic component of *T. putrescentiae* showed strong crossreactivity with extract of *L. destructor* (Fig. 3c) since even less of *L. destructor* extract than of *T. putrescentiae* extract caused a 50% inhibition. However, extract of *T. putrescentiae* was a poor inhibitor of *L. destructor* allergenic components (Fig. 3d and e). About 400-fold more *T. putrescentiae* extract than *L. destructor* extract was necessary to achieve a 50% inhibition of the major allergenic component of *L. destructor* at 15 kDa and 200-fold more at 22 kDa.

Crossreactivity between *T. putrescentiae* and *D. pteronyssinus* The pattern of crossreactivity between *T.*

putrescentiae and *D. pteronyssinus* was almost identical with that of *A. siro* and of *D. pteronyssinus*. The two 16 kDa allergenic components of *T. putrescentiae* and of *D. pteronyssinus* showed poor crossreactivity to the heterologous species (Fig. 3c and f). The 25 kDa allergenic component of *D. pteronyssinus*, on the other hand, crossreacted strongly with extract of *T. putrescentiae* (Fig. 3g), as about the same amounts of *D. pteronyssinus* and *T. putrescentiae* extracts produced 50% inhibition of the *D. pteronyssinus* allergenic component.

Crossinhibition summary

The inhibition results are summarized in Table 3. The relative amount of heterologous extract in comparison with the amount of homologous extract, necessary to produce 50% inhibition, is given for each IgE-binding component.

Discussion

Although dust mites are well established as provokers of asthma and IgE-mediated allergy [23], there have been relatively few studies of allergenic crossreactivity between different species of mites. A great difficulty in exploring crossreactivity is the lack of well characterized and purified mite allergens. The allergenicity of house dust mites, especially *D. pteronyssinus*, has been extensively studied, but although 49 antigens [24] and 28–32 allergens [25,26] have been identified, only a few allergens have been purified and well characterized [27,28; reviews]. In contrast, there are only a small

number of reports on characterization of storage mite allergens. Most of the work has focused on the storage mite *L. destructor*. We have in *L. destructor* identified a total of 11 allergenic components in the molecular weight range of 14–54 kDa [29] and about 90% of sera RAST positive to this mite species have IgE antibodies to the principal allergenic component of 15 kDa [22]. The major allergen *Lep d* I of *L. destructor* has been purified [30] and its N-terminal amino acid sequence showed no homology with other proteins in the Swissprot sequence database (release 21) with the FASTA program [31]. Ansotegui *et al.* [32] have reported on an allergenic component with a molecular weight of 39 kDa, and we have demonstrated that this allergen was localized to the exoskeleton of *L. destructor* as well as the faecal pellets [33]. Two high-molecular-weight components of *L. destructor*, of which at least one binds IgE, have also been identified [34]. Regarding other storage mite species, crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE) studies have revealed 14 antigens and three allergens in extract of *A. siro* [35] and 20 antigens and five allergens in extract of *T. putrescentiae* [36]. In this study we have with SDS-PAGE and immunoblotting characterized *A. siro* and *T. putrescentiae* and shown that these two mite species contain at least five and four allergenic components, respectively. The 15 kDa component of *A. siro* and the 16 kDa component of *T. putrescentiae* were shown to be major allergenic components as they were detected by more than 50% of the sera studied. That two sera RAST positive to *A. siro* did not reveal bands on *A. siro* immunoblots, could be because the concentration of specific IgE was too low, since the sera were diluted.

The 50% inhibition of the major allergenic components of *A. siro* (15 kDa), *T. putrescentiae* (16 kDa) and *L. destructor* (15 kDa) was only achieved in one direction, which indicates that they are not identical. However, the result could be influenced by the fact that the immune response of the serum pool is primarily against *L. destructor* [4,6] and its IgE antibodies cross-react with components of *A. siro* and *T. putrescentiae*, but with a lower affinity than with the *L. destructor* allergens. As regards the allergenicity of *T. putrescentiae*, it seems that this mite species is more closely related to *L. destructor* than to *A. siro*. The hypothesis of species-specific allergens in *A. siro* and *T. putrescentiae* has support from a mite inventory of 30 farms on Gotland (to be published), which shows that these farmers are exposed to *T. putrescentiae* as well as *A. siro* and thus these mites could be responsible for IgE-mediated sensitization. We have demonstrated considerable cross-reactivity of IgE between components of the dust mites *A. siro*, *L. destructor*, *T. putrescentiae* and *D. pteronyssinus*.

The 25 kDa component of *D. pteronyssinus* was strongly inhibited by extracts of *A. siro* and *T. putrescentiae*. However, the IgE antibodies which were so strongly inhibited by the two extracts were not able to detect a 25 kDa component in either *A. siro* or *T. putrescentiae* on immunoblots. The reason for this is not known. We have earlier reported the same observation in a study of crossreactivity between *D. pteronyssinus* and *L. destructor* [22]. The IgE binding to the 25 kDa component, most likely *Der p* I, in extract of *D. pteronyssinus* on immunoblots was completely inhibited by extract of *L. destructor* while the same IgE antibodies failed to detect a group I allergen equivalent in extract of *L. destructor* on immunoblots. One explanation why a 25 kDa component is detected in extract of *D. pteronyssinus* but not in extracts of *L. destructor*, *A. siro* or *T. putrescentiae* could be that the determinants in the storage mite extracts for some reason are blocked or destroyed during electrophoresis and/or electroblotting and therefore unavailable to the IgE antibodies. In spite of this unexplained observation, our results in the present study show that *A. siro* and *D. pteronyssinus* on the one hand and *T. putrescentiae* and *D. pteronyssinus* on the other share at least one allergenic determinant. Further, this determinant is located on the 25 kDa component, most likely *Der p* I, the major allergen of *D. pteronyssinus*.

Earlier results on crossreactivity of *A. siro*, *T. putrescentiae* and *D. pteronyssinus* have been comparable [15,37]. Several investigators using RAST correlation [38–40] have also found significant correlation between *A. siro* and *T. putrescentiae* but not between *A. siro* or *T. putrescentiae* and *D. pteronyssinus*. Blainey *et al.* [8,41], on the other hand, found a significant correlation between *D. pteronyssinus* and *A. siro*, and *D. pteronyssinus* and *T. putrescentiae* using skin-prick test and RAST correlation, but this could be due to multi-sensitization as well as to crossreactivity. In a previous investigation of the allergenic relationship between dust mites [38] we found no crossreactivity between *A. siro* and *D. pteronyssinus* or *T. putrescentiae* and *D. pteronyssinus* using the RAST inhibition method, although sera from the same farming population as in the present study were used. The discrepancies between the two studies are probably due to the higher sensitivity of the blotting inhibition method in which the inhibition of each individual allergenic component is revealed. We have shown the complexity of studies regarding allergenic crossreactivity between different dust mite species. The results obtained are dependent on several variables such as the properties of the allergen extracts and of the sera used, i.e. the principal mite species in the environment giving rise to sensitization. Furthermore, future studies should

include purified allergens, once these are available for all the storage mite species studied. However, crossreactivity studies are prerequisites for obtaining high-quality extracts for diagnosis and hyposensitization treatment.

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