

Immunoblot multi-allergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*

E. JOHANSSON, Å. BORGÅ, S. G. O. JOHANSSON and M. VAN HAGE-HAMSTEN

Department of Clinical Immunology, Karolinska Institute, Karolinska Hospital, Stockholm, Sweden

Summary

The allergenic similarity of the pyroglyphid mite *D. pteronyssinus* and the glycyphagid mite *L. destructor* was investigated with a new immunoblotting inhibition technique allowing simultaneous comparison of several allergens. Extracts of *D. pteronyssinus* and *L. destructor* were separated by SDS-PAGE and electroblotted to nitrocellulose (NC). A serum pool containing IgE specific to the major allergens in both mites was mixed with serially diluted extracts of *D. pteronyssinus* and *L. destructor* and incubated with the mite allergens of NC. The inhibition of the IgE binding to NC was evaluated by densitometric scanning and percentage inhibition was calculated. The IgE antibodies to the 25-kD component in *D. pteronyssinus*, were inhibited to the same degree by extracts of *D. pteronyssinus* and *L. destructor*. Another major allergen component in *D. pteronyssinus* (16 kD) was also inhibited by *L. destructor* extract but to a lesser degree: 400 times more of the heterologous than of the homologous extract was needed for 50% inhibition. To produce 50% of heterologous inhibition of the two major allergen components at 15 and 53 kD of *L. destructor*, 2000 and 10 000 times more, respectively, of *D. pteronyssinus* than of *L. destructor* extract were needed. Two minor allergen components of *L. destructor* showed some cross-reactivity with *D. pteronyssinus*. However, *L. destructor* was a stronger inhibitor of *D. pteronyssinus* than vice versa, probably because the sera were obtained from persons more sensitized to *L. destructor* than to *D. pteronyssinus*.

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Introduction

More than 20 years ago Voorhorst *et al.* [1] established that the pyroglyphid mite, *D. pteronyssinus*, was the major allergen source in house dust. Several reports have followed where the role of these mites in different allergic diseases has been investigated and their clinical importance confirmed [2–4].

Recent studies have reported allergy to glycyphagid mites belonging to the genus *Lepidoglyphus* and acarid mites of the genera *Tyrophagus* and *Acarus*, especially among farmers [5–10]. These mite species live in great

numbers in stored hay, grain and other cereal products but they are also found in dust of damp houses [10–12].

The allergenicity of *Dermatophagoides* spp. has been extensively studied and two groups of major allergens, the group I and the group II, common to the three species studied, have been purified [3]. Epitope mapping with monoclonal antibodies show the existence of both species-specific and cross-reacting epitopes among the group I allergens of *D. pteronyssinus*, *D. farinae* and *D. microceras* [13,14].

Whether any of these group allergens have analogies in storage mites is not yet known. Nevertheless, some allergens in storage mites have been identified and characterized with CRIE [15,16] and SDS-PAGE followed by immunoblotting and probing with human sera

Correspondence: Eva Johansson, BSc, Department of Clinical Immunology, Karolinska Hospital, S-104 01 Stockholm, Sweden.

[17] and immunological cross-reactivity between storage mites and *Dermatophagoides* spp. has been demonstrated by means of CRIE [15] and RAST inhibition [16,18,19]. A monoclonal antibody to *L. destructor* 14-kD component has recently been described [20].

Here we have used a new immunoblotting inhibition technique to further investigate allergenic similarities of separated and identified allergen components in *D. pteronyssinus* and *L. destructor* by SDS-PAGE using human IgE antibodies. This approach allows comparison of several allergenic proteins without isolation of individual components.

Materials and methods

Mite extracts

Extracts were prepared from whole cultures of the house dust mite *D. pteronyssinus* and the storage mite *L. destructor*, obtained from Allergon, Ängelholm, Sweden. Extraction was performed in 0.15 M NaCl containing 0.02% NaN₃ at 1/10 w/v, for 16–18 hr at 20°C. The extract suspensions were clarified by centrifugation (6600 g for 10 min) and the supernatants were desalted on prepacked Sephadex G-25 columns, PD 10 (Pharmacia, Uppsala, Sweden) and lyophilized. One milligram of each extract was redissolved in 1.0 ml of PBS and the protein content was determined with BCA protein assay reagent according to instructions of the manufacturer (Pierce Chemical Company, Rochford, IL). One milligram lyophilized extract of *D. pteronyssinus* contained 0.30 mg of protein and 1 mg lyophilized *L. destructor* extract contained 0.35 mg of protein.

Human sera

Sera from 440 farmers were screened for specific IgE antibodies to *D. pteronyssinus* and *L. destructor* with Phadebas RAST® (Pharmacia) as described previously [7]. Twenty of these sera, positive in RAST to both mites (RAST class ≥ 2) and with a total IgE range of 37–1600 kU/l, were analysed in an immunoblotting system using extracts of *D. pteronyssinus* and *L. destructor*.

Twenty sera with a negative RAST to *D. pteronyssinus* and *L. destructor* were used as negative controls. Ten sera were selected randomly from non-atopic farmers, originating from the group of 440 farmers mentioned above. The other 10 were consecutive samples from atopic patients sent to the Department of Clinical Immunology at the Karolinska Hospital for RAST analyses. Their range of total IgE was 1300–3000 kU/l.

Murine monoclonal antibodies

Two mouse monoclonal antibodies to *Der p* I (10B9 and 5H8) and one anti-group II reactive with both *Der p* II

and *Der f* II (7A1) were used in the immunoblotting system to identify group I and group II allergens. The monoclonal antibodies were kindly provided by Martin D. Chapman, University of Virginia, U.S.A.

SDS-PAGE followed by electroblotting and immunoprobings

SDS-PAGE, electroblotting to nitrocellulose (NC) and immunoprobings were performed as previously described [17]. Briefly, lyophilized extracts of *D. pteronyssinus* and *L. destructor* were separated by gradient (7.5–20%) SDS-PAGE. Before electrophoresis, the extracts were resuspended in distilled water, mixed with equal volumes of sample buffer without beta-mercaptoethanol and boiled for 5 min. Nine mg dry weight of *D. pteronyssinus* and 1.8 mg (dry weight) of *L. destructor* (the maximum amounts without overloading the gel) were applied to each gel. One well on each gel was used for low molecular weight proteins. After electrophoresis, the mite components were electroblotted to NC and the molecular weight protein markers to a polyvinylidene difluoride (PVDF) membrane, (Immobilon™ Transfer Membrane; pore size 0.45 μ m; Millipore, Bedford, MA). The PVDF membrane was stained with Coomassie brilliant blue R 250 and unstained with 90% methanol. The NC membrane was blocked, cut into strips and incubated with IgE-specific sera. After washing, the strips were incubated with a mixture of rabbit anti-human IgE and sheep anti-rabbit IgG beta-galactosidase. The antibodies were diluted 2000 and 200 times, respectively, and mixed immediately before the testing. The allergens were visualized by incubating the strips for 30 min on filter paper saturated with the enzyme substrate 2-N-beta-D-galactopyranosidemonohydrate and fast blue B salt.

Some of the strips with blotted mite allergens were incubated with mouse monoclonal antibodies and a mixture of rabbit anti-mouse immunoglobulin (1/1000) (Dakopatts, Glostrup, Denmark) and sheep antirabbit IgG beta-galactosidase (1/200). The developing procedure was performed as described above.

Inhibition of the immunoprobings

Twelve different amounts of *D. pteronyssinus* and *L. destructor* extracts, 0.0005, 0.0025, 0.005, 0.025, 0.05, 0.25, 0.5, 2.5, 5, 10, 20 and 40 mg (dry weight), were redissolved in 2 ml of a serum pool containing specific IgE to both mites. The dilution of the serum pool was 1/4. Inhibitions were made by incubating strips blotted with *D. pteronyssinus* or *L. destructor* with the different concentrations of mite extracts and serum pool. The reference strips used for evaluating the results were

incubated with the serum pool only. The results of the inhibition experiments were estimated by densitometric scanning of the strips (see below).

As a control for non-specific inhibition of IgE, strips with blotted mite extracts were treated as above except that 40 mg lyophilized bovine serum albumin was used as inhibitor instead of mite extract. No inhibition was detected.

Densitometric scanning and calculating of inhibition results

To evaluate the inhibition, the visualized bands were scanned for absorption at 633 nm with an UltroScan XL Laser Densitometer (LKB Products, Bromma, Sweden). A peak diagram with absorbance *vs* position was obtained for each strip. The majority of the stained bands visible on the strips were detected by the densitometer and the allergenic components of interest were easily identified.

The absorbance obtained from the peak diagram was used for calculating the degree of inhibition. The background was the absorbance of the straight horizontal line between the peaks which corresponded to the unstained parts of the blot. The background was subtracted from the maximum absorbance peak before calculating the degree of inhibition. Results were expressed as percentage of inhibition, using the following formula:

$$100 - 100 \times \frac{\text{Absorbance after inhibition with extract}}{\text{Absorbance after inhibition with diluent}}$$

Percentage inhibition was plotted against the increasing amount of mite extract used as inhibitor.

Results

IgE binding pattern of allergen components in *D. pteronyssinus* and *L. destructor*

In order to characterize and identify the most common IgE-binding components of *D. pteronyssinus* (Fig. 1) and *L. destructor* (Fig. 2), non-reduced extracts were analysed by SDS-PAGE, transferred to NC and immunoprobed with 20 sera positive in RAST to both mite species. The major allergens were identified as IgE-binding components detected by at least 50% of the 20 sera screened. In *D. pteronyssinus*, a total of eight allergenic components with mol. wts of 11, 15, 16, 22, 25, 43, 47 and 57 kD were distinguished (Fig. 1). Although the mol. wt values are approximate these figures will be used to designate the respective bands. Two of the components occurred with very high frequency. The component located at 16 kD was recognized by 16 of the 20 (80% sera) studied. The other frequent IgE-binding component was located at 25 kD and recognized by 50% of the 20 sera tested.

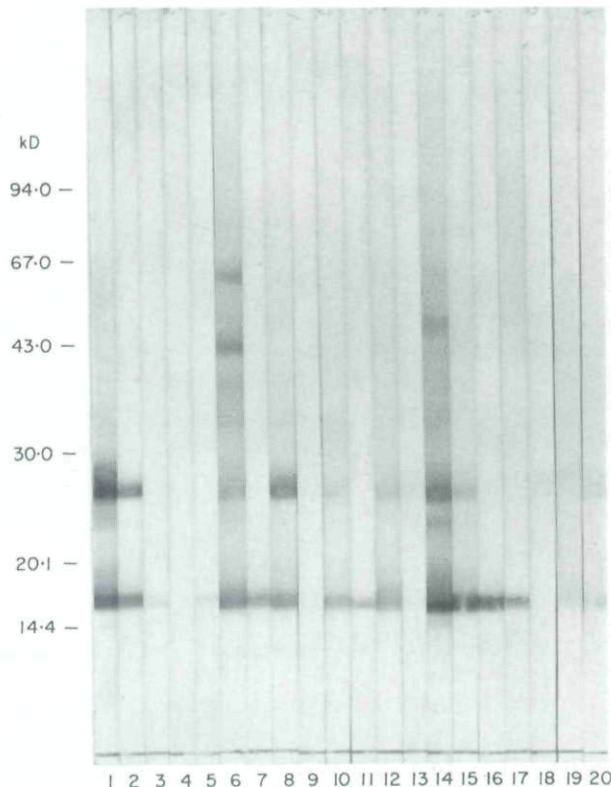


Fig. 1. Allergen components in *D. pteronyssinus* detected on immunoblots by 20 individual sera with positive RAST to both *L. destructor* and *D. pteronyssinus*.

In *L. destructor*, at least 21 IgE-binding components were identified in the approximate range of 13–53 kD (Fig. 2). The 15-kD component showed the highest IgE-binding frequency, being recognized by 18 of the 20 (90%) sera tested (when *L. destructor* extract is electrophoresed under reducing conditions [17], the 15-kD component seemed to split up in two components visualized at the 16-kD and 18-kD positions). The other major component was located at 53 kD and was recognized by 50% of the sera examined. Based on the immunoblotting pattern, a pool was made of sera numbers 1, 6 and 15 for use in cross-inhibition studies. The serum pool contained specific IgE to the allergen components at 16 kD and 25 kD in *D. pteronyssinus* and 15 kD and 53 kD in *L. destructor*. No non-specific binding of IgE and anti-IgE to blotted proteins of *D. pteronyssinus* and *L. destructor* was obtained with a panel of 20 sera with negative RAST to both mite species.

Monoclonal antibodies to Der p I and Der p II on NC blots

Blotted strips with *D. pteronyssinus* and *L. destructor* extracts were probed with two different monoclonal

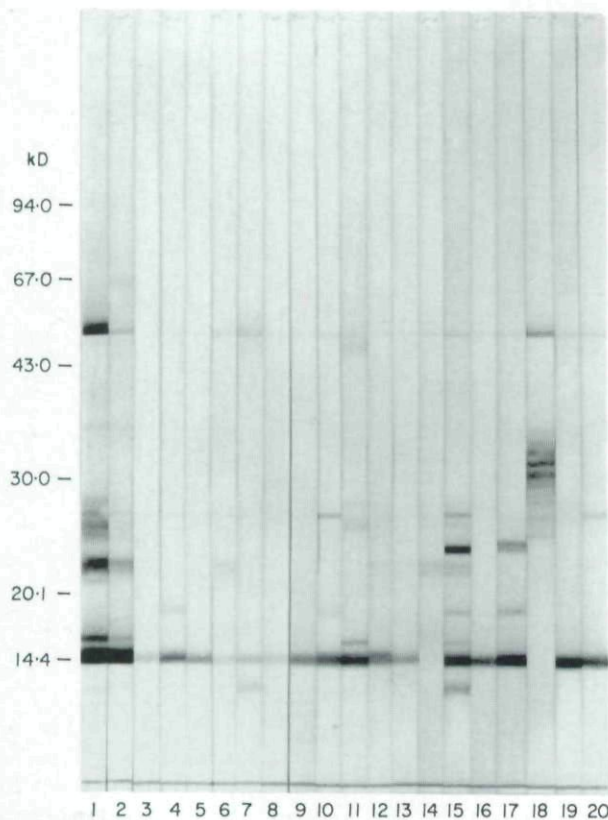


Fig. 2. Allergen components in *L. destructor* detected on immunoblots by 20 individual sera with positive RAST to both *L. destructor* and *D. pteronyssinus*.

antibodies to *Der p* I and one monoclonal antibody to *Der p* II. The *Der p* I allergen was not detected on either *D. pteronyssinus* or *L. destructor* blots. The *Der p* II allergen was identified as a single and strongly stained band on the *D. pteronyssinus* blot at the same position as the component at approximately 16 kD detected by 80% of the 20 screened sera. The *Der p* II allergen was not detected on the *L. destructor* blot.

Cross-inhibition of IgE-binding to allergen components in *D. pteronyssinus*

Figure 3 shows *D. pteronyssinus* allergen components on NC blots and the inhibition of the IgE-binding of the serum pool by *D. pteronyssinus* extract (Fig. 3a) and *L. destructor* extract (Fig. 3b). Homologous inhibition (Fig. 3a) required less than 0.5 mg of *D. pteronyssinus* extract for complete inhibition as no bands were visible on the corresponding blot. The two components at 16 kD and 25 kD were inhibited equally fast when comparing the decrease of the staining intensity. Heterologous inhibition (Fig. 3b) indicated cross-reactivity as the allergen components of *D. pteronyssinus*, after inhibition with

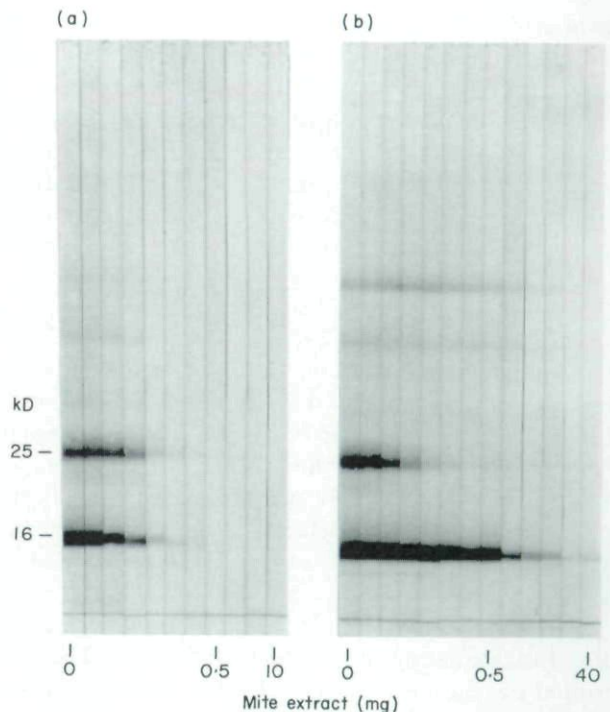


Fig. 3. Allergen components in *D. pteronyssinus* detected on immunoblots by a serum pool with positive RAST to *L. destructor* and *D. pteronyssinus* after inhibition with (a) *D. pteronyssinus* and (b) *L. destructor*. The blot to the left in (a) and (b), respectively, marked with 0 mg, shows the IgE binding to *D. pteronyssinus* without inhibition. The other blots are inhibited with increasing amounts of mite extract (dry weight) starting with (a) 0.0005–10 mg and (b) 0.0005–40 mg.

L. destructor extract, were more weakly stained than the uninhibited blot. Especially the 25-kD component seemed to cross-react with *L. destructor*, showing about the same inhibition pattern as in the homologous system (Fig. 3a). The 16-kD component also seemed to be inhibited but to a lesser degree than the 25-kD component.

The relative inhibition of the two allergen components was calculated from scanning data and plotted against the amount of extract used in the inhibition assay. Figure 4a shows the homologous inhibition curve and the heterologous inhibition curve of the 16-kD allergen component. To obtain 50% inhibition, it was necessary to add about 400 times more heterologous extract (1.7 mg) than homologous extract (4 µg). However, results indicating strong cross-reactivity between *D. pteronyssinus* and *L. destructor* were obtained when the IgE antibodies to the 25-kD component in *D. pteronyssinus* were inhibited (Fig. 4b): extracts of *D. pteronyssinus* and *L. destructor* gave superimposable inhibition curves. Five micrograms of homologous extract and 4 µg of heterologous extract were required for 50% inhibition.

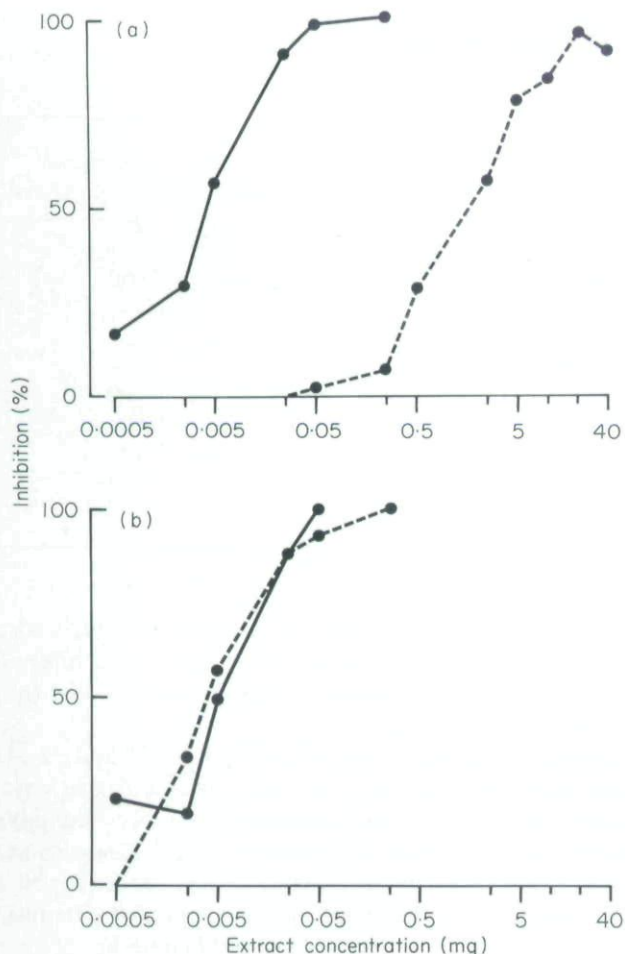


Fig. 4. The inhibiting effect on (a) the 16-kD and (b) the 25-kD allergen components in *D. pteronyssinus* immunoblots after probing with a mix of the serum pool and increasing amounts of *D. pteronyssinus* extract (solid line) and *L. destructor* extract (broken line).

IgE-binding to *D. pteronyssinus* components on blots in sera from 11 individual subjects with positive RAST to both mites were inhibited with 5 mg only of *D. pteronyssinus* and *L. destructor* extracts (data not shown). This amount of extract was chosen to ensure complete homologous inhibition. The degree of inhibition was only assessed visually and classified as none, partial or complete. All sera had IgE antibodies to the 16-kD allergen component. They were partially inhibited in nine sera and completely inhibited in the other two by *L. destructor* extract. These results were similar to the result with the serum pool, indicating limited cross-reactivity to the 16-kD component in *D. pteronyssinus* and *L. destructor* extract.

The finding of strong cross-reactivity between *D. pteronyssinus* and *L. destructor* extracts achieved with the

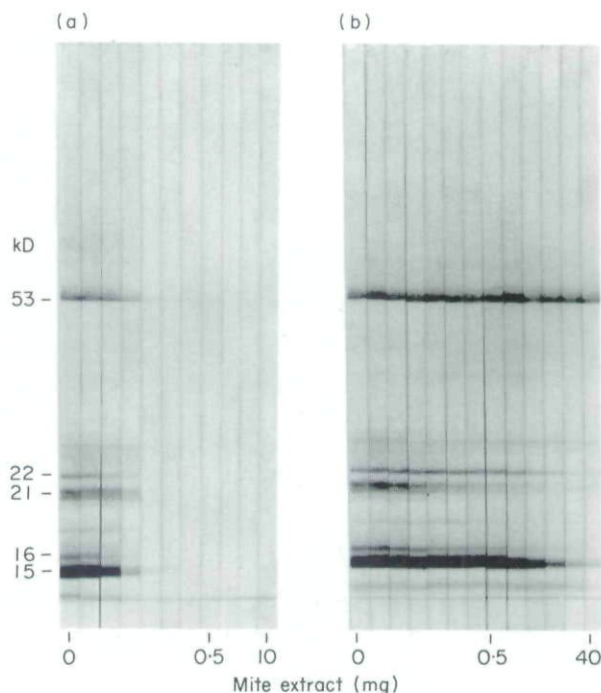


Fig. 5. Allergen components in *L. destructor* detected on immunoblots by a serum pool with positive RAST to *L. destructor* and *D. pteronyssinus* after inhibition with (a) *L. destructor* and (b) *D. pteronyssinus*. The blot to the left in (a) and (b) marked with 0 mg, shows the IgE binding to *L. destructor* without inhibition. The other blots are inhibited with increasing amounts of mite extract (dry weight) starting with (a) 0.0005–10 mg and (b) 0.0005–40 mg.

serum pool was also supported by this simplified inhibition. Six of the 11 sera had IgE antibodies to the 25-kD component in *D. pteronyssinus* and in four of them the IgE binding was completely inhibited by 5 mg of *L. destructor*. In the remaining two sera, the IgE binding was partially inhibited.

Cross-inhibition of IgE binding to allergen components in *L. destructor*

Figure 5 shows the *L. destructor* allergen components on NC blots and inhibition of the IgE-binding of the serum pool by *L. destructor* extract (Fig. 5a) and *D. pteronyssinus* extract (Fig. 5b). Homologous inhibition (Fig. 5a) required less than 0.5 mg of *L. destructor* extract for complete inhibition, and all components seemed to be equally inhibited. Heterologous inhibition (Fig. 5b) indicated only low cross-reactivity between *L. destructor* and *D. pteronyssinus*. Most components were still visible on the blot corresponding to 10 mg of the inhibiting extract, and the 53-kD component was strongly stained even when it was inhibited with 40 mg. However, the 21-kD

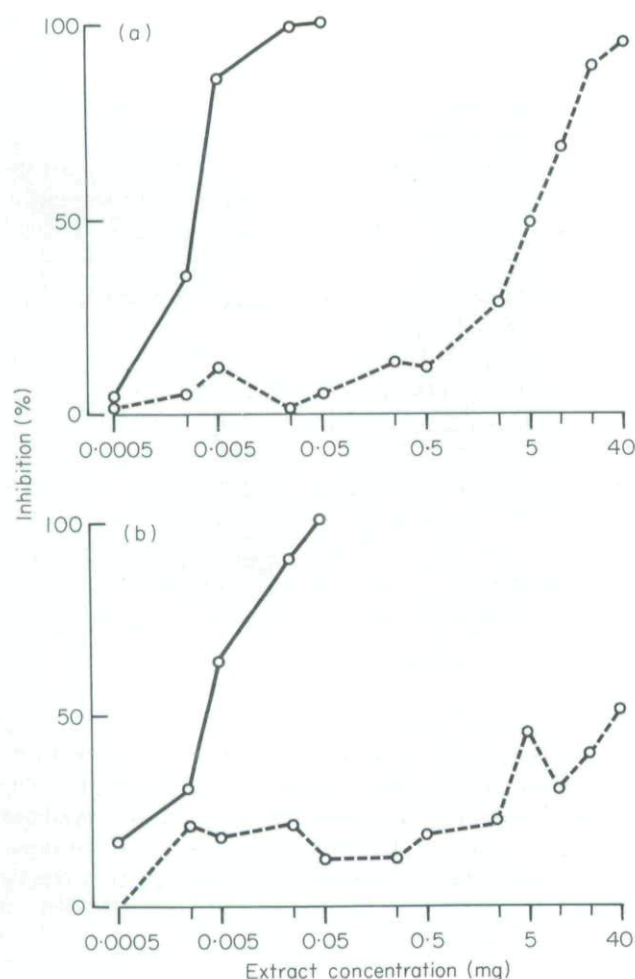


Fig. 6. The inhibiting effect on (a) the 15-kD and (b) the 53-kD allergen components in *L. destructor* immunoblots after probing with a mix of the serum pool and increasing amounts of *L. destructor* extract (solid line) and *D. pteronyssinus* extract (broken line).

component showed a somewhat higher degree of cross-reactivity with *D. pteronyssinus*.

The results of homologous and heterologous inhibition of the major allergen components in *L. destructor* were calculated from scanning data (Fig. 6). The homologous inhibitions of the *L. destructor* components were equal, requiring 3–4 µg of *L. destructor* extract for 50% of inhibition and about 50 µg to produce complete inhibition. To produce 50% of heterologous inhibition of the 15-kD component, about 2000 times more (5.3 mg) of *D. pteronyssinus* extract than of *L. destructor* extract was needed (Fig. 6a). To inhibit the 53-kD component to the same extent, 40 mg or 10 000 times more inhibiting extract was necessary (Fig. 6b).

Table 1. Variation in amounts of extract used for 50% of inhibition of allergen components in *D. pteronyssinus* and *L. destructor*

Allergen components on NC blot	Amounts of extract (homologous:heterologous)
<i>D. pteronyssinus</i> (kD)	
16	1:400
25	1:1
<i>L. destructor</i> (kD)	
15	1:2000
16	1:60
21	1:10
22	1:3000
53	1:10 000

Three minor components in *L. destructor* were also recognized on the blots by the serum pool, and inhibition of these was also calculated from the scanning data (not shown).

These components had mol. wts of 16, 21 and 22 kD and were detected only by four, five and one sera, respectively. The 22-kD component showed negligible cross-reactivity, 3000 times more of *D. pteronyssinus* extract than of *L. destructor* extract being needed for 50% inhibition, in contrast to the 21-kD component (10 times) and the 16-kD component (60 times) (Table 1).

Discussion

Mites of the genus *Dermatophagoides* have been established as the major source of allergens in house dust [1]. In particular, *D. pteronyssinus* and *D. farinae* have been studied extensively during the past 20 years. Other mites, especially those belonging to the genera *Lepidoglyphus*, *Acarus*, *Tyrophagus* and *Glycyphagus*, are numerous in dust from grain and hay and in all kinds of stored agricultural products [21]. These genera can also be found in house dust [11]. Several studies have indicated that storage mites are the most important allergens among farmers [5–7,9,10]. Although the allergenicity of dust mites is well documented, there have only been a few studies of allergenic cross-reactivity between different families of mites.

Our earlier RAST correlation and RAST inhibition studies [22] have shown that the storage mite *L. destructor* and the house dust mite *D. pteronyssinus* possess distinct allergenic determinants. Bronchial provocation studies [8] with extract of *L. destructor* and *D. pteronyssinus* on farmers with a positive RAST to *L. destructor* and on

patients with asthma caused solely by *D. pteronyssinus* support these findings.

Griffin *et al.* [16] recently demonstrated with RAST inhibition and affinity absorption experiments that *L. destructor* and *D. pteronyssinus* also possess common allergenic determinants. In the present study we confirm the existence of distinct as well as common allergenic determinants in *L. destructor* and *D. pteronyssinus*, using SDS-PAGE and an immunoblot inhibition technique. The advantage of the latter method compared with the RAST inhibition method is that multiple allergens in an extract can be studied individually.

We have focused on the major allergens of the two dust mites *D. pteronyssinus* and *L. destructor*. *Der p* II in *D. pteronyssinus* was identified as a component at approximately 16 kD by applying a monoclonal antibody to the blot. Two monoclonals specific to different epitopes on *Der p* I were also employed, but neither detected the allergen, probably because the extract was heated before electrophoresis. Lombardero *et al.* [23] have shown that heating *Der p* I reduced the binding of the allergen to monoclonal antibodies in a competitive inhibition assay on microtitre plates. However, we found in our immunoblot system that polyclonal IgE most likely detected *Der p* I, under non-reducing conditions, as a component at 25 kD. Other investigators have also identified *Der p* I at 25 kD by the same method [23,24], indicating differences in epitope specificity between monoclonal antibodies and human IgE.

The IgE antibodies to the 16-kD and 21-kD allergen components in *L. destructor* cross-reacted with *D. pteronyssinus* extract but were not completely inhibited, which indicates that these components also possess distinct allergenic determinants. However, the IgE antibodies to the 25-kD component in *D. pteronyssinus*, most likely *Der p* I, showed considerable cross-reactivity with *L. destructor* extract as they were inhibited to the same degree by extracts of *D. pteronyssinus* and *L. destructor*. However, these antibodies were not detecting a 25-kD component in *L. destructor* extract on blots. The reason for this is not clear. One explanation could be that the component in *L. destructor* extract is for some reason more sensitive to heat treatment than the component in *D. pteronyssinus* extract. Another interpretation could be that the determinants in *L. destructor* extract are hidden in the allergen and not available to the IgE antibodies. Nevertheless, our results show that the two mite species share at least one allergenic determinant, which with regard to *D. pteronyssinus*, is situated on the 25-kD allergen component; but they also seem to lack allergenic determinants in relation to each other. The fact that *L. destructor* inhibited *D. pteronyssinus* more strongly than vice versa is probably because our serum samples were obtained from a farming

population, which is more sensitized to *L. destructor* than to *D. pteronyssinus*. Different results might have been obtained with sera from an urban population. Indeed, Luczynska *et al.* [19] found with RAST inhibition studies in an urban population limited cross-reactivity between *L. destructor* and *D. pteronyssinus* and, furthermore, *D. pteronyssinus* was the strongest inhibitor of the two mite species.

The immunoblot multi-allergen inhibition technique described was very useful for the study of allergenic similarities between *D. pteronyssinus* and *L. destructor*. Quite detailed information about the cross-reactivity between individual allergens was obtained without isolation and purification of each component. However, further research on identification, and characterization of dust mite allergens and their cross-reactivity are required in order to understand the complexity of epitopes and allergens. This work will have to include the purification of major allergens and the production of monoclonal antibodies.

Acknowledgments

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