

Crossreactivity between *Dermatophagoides siboney* and other house dust mite allergens in sensitized asthmatic patients

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Summary

Background The role of *Pyroglyphid* mites in IgE-mediated sensitization has been well established. Although mites belonging to the genus *Dermatophagoides* dominate in the acarofauna of domestic dust, non-pyroglyphid mites might also be of clinical importance. In Cuba, *Dermatophagoides siboney* is found in dust coexisting with *D. pteronyssinus* and *Blomia tropicalis*. *Dermatophagoides farinae* is not found. Storage mites, such as *Acarus siro*, *Lepidoglyphus destructor* and *Tyrophagus putrescentiae*, might also be present.

Objectives The aim of this study was to investigate the allergenic relationships among the mites present in the environment of allergic patients in Cuba.

Methods The crossreactivity between an extract of *D. siboney* and the above mentioned six mites was studied by specific IgE inhibition, using sera of mite-sensitive patients after incubation with serial dilutions of *D. siboney*.

Results The inhibitory effect of *D. siboney* extract was more pronounced against *D. farinae*, 91%, than against *D. pteronyssinus*, 62%. Specific IgE to *B. tropicalis*, *A. siro*, *L. destructor* and *T. putrescentiae* were inhibited to a much lesser extent, i.e. between 47 and 58%. The correlation between IgE to *D. siboney* and IgE to *D. pteronyssinus* and *D. farinae* was of 0.92 and 0.90, respectively. A weaker correlation was found between *D. siboney* and *L. destructor* (0.7), *A. siro* (0.67), *B. tropicalis* (0.54) and *T. putrescentiae* (0.51).

Conclusion Thus, *D. siboney* seems to be most closely related to *D. farinae*.

Keywords: *Dermatophagoides siboney*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blomia tropicalis*, storage mites, crossreactivity, inhibition, IgE

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Introduction

Pyroglyphid mites of the genus *Dermatophagoides* are recognized in most parts of the world as a major source of allergens. Allergen extracts from *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* have been extensively studied. Their major allergens have been characterized and species-specific and crossreacting

epitopes identified [1]. There is an extensive cross-reactivity between these two mite species [2–5]. Cross-reactivity or parallel sensitization to mites present in the 'domestic' environment of allergic individuals has been studied during the last few years [6–11]. The results of these studies have contributed to the understanding of the role of mite allergen exposure. This knowledge has also been of value when designing programmes intended to reduce mite allergen levels to control allergic symptoms in mite-sensitive patients [12].

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Even though one mite species usually predominates in a certain location, several other species can coexist [13]. Therefore, mite-sensitive subjects are normally exposed to allergens from more than one mite species, which may or may not be antigenically related.

The existence of *Dermatophagoides siboney* was reported in Cuba in 1982 [14]. *D. pteronyssinus*, *D. siboney* and *B. tropicalis* are the most frequent mite species in domestic dust in Cuba, i.e. present in 100, 85 and 72% of homes, respectively [15]. Storage mites such as *A. siro*, *T. putrescentiae* and *L. destructor* were also found in dust from Cuban homes. So far, *D. farinae* has not been found in Cuba [9]. *D. siboney* has been found recently in Puerto Rico (Fernández-Caldas, pers. comm.) who also found *D. farinae* (less frequent in the Caribbean area).

Extracts of *D. siboney* show major components with characteristics of Group 1 and Group 2 *Dermatophagoides* allergens. Proteins identified as Der s 1 and Der s 2 have been shown to have the same molecular weights as the corresponding proteins from Der p and Der f, respectively. These proteins react with monoclonal antibodies against Group 1 and 2 *Dermatophagoides* allergens, respectively [16].

The aim of this study was to investigate the cross-reactivity, as measured by specific IgE-inhibition, between *D. siboney* and *Pyroglyphid* and storage mites, using sera from a group of mite-sensitive asthmatic patients from Cuba.

Materials and methods

Subjects

Sera were obtained from 35 mite-sensitive asthmatic patients, 25 women and 10 men, aged 17–49, mean 31·7 years. They were selected, during an epidemiological study, among 150 adult Cuban asthmatic subjects who showed continuous symptoms, indicating possible house dust mite allergy and who responded with a positive skin-prick test to one or several mite allergens (*D. pteronyssinus*, *D. farinae*, *D. siboney*, *A. siro*, *L. destructor* and *B. tropicalis*). Once the sera were obtained, they were aliquoted, freeze-dried, and stored at –20°C. Immediately before use they were reconstituted by adding distilled water.

Allergens

Allergen extracts of *D. siboney* and *B. tropicalis* were produced as described earlier [15]. Extracts were stored freeze-dried and reconstituted before use. Birch (*Betula*

verrucosa) pollen extract was obtained from Pharmacia (Uppsala, Sweden).

Determination of specific IgE

Specific IgE levels against mite allergens were determined by a luminescence immunoassay (MagicLite™, ALK, Denmark). Immobilized mite allergens from *D. pteronyssinus*, *D. farinae*, *A. siro*, *L. destructor* and *T. putrescentiae* were obtained from the manufacturer. *D. siboney* and *B. tropicalis* allergens (1 mg protein/mL) were also coupled to paramagnetic particles according to the procedure recommended by the manufacturer.

The patient sera were tested for specific IgE towards the respective allergens. Five sera which showed the highest specific IgE levels (MagicLite class 3 or higher, i.e. >20 SU/mL) for each of the seven mite allergen were pooled and used for inhibition experiment.

Inhibition experiments

Inhibition of specific IgE against each of six different mite allergens was performed by incubation the selected patient sera with *D. siboney* extract. The highest concentration of *D. siboney* allergen extract (100 µg protein per mL) was diluted fourfold six times in phosphate buffer pH 7·4 containing human serum albumin 0·03% (Sigma, USA). The lowest concentration was 0·1 µg of protein per mL, whereas 2 mg/mL, i.e. the concentration 20 times higher than the highest concentration used was also applied to investigate maximum inhibition. The results were expressed as percent of the inhibition of specific IgE in the sera incubated with *D. siboney* allergen in relation to the inhibition using the extract diluent, according to:

$$\text{Inhibition (\%)} = 100 - \frac{\text{IgE level after inhibition with extract}}{\text{IgE level after inhibition with diluent}} \times 100$$

Birch pollen extract and Bovine Serum Albumin (BSA), both at the same concentrations as *D. siboney*, were used to check the possibility of non-specific inhibitory effect of unrelated allergens by inhibition of *D. farinae* and *D. pteronyssinus*, respectively.

Statistical analysis

The grade of relationships between allergens from different species was estimated by calculation of correlation coefficients between Magic Lite scores. Correlation and regression analysis were calculated using StatView software (Abacus Concepts, USA) on Macintosh LCIII.

Results

The inhibition of specific IgE by different mite allergens by *D. siboney* extract was studied by mixing increasing dilutions of *D. siboney* with the pooled sera (Figs 1 and 2).

A higher degree of inhibition was observed by the *D. siboney* extract in *D. farinae* (61%) and *D. pteronyssinus* (62%) than with *L. destructor*, *T. putrescentiae* and *A. siro* (51–52%). *B. tropicalis* was inhibited less than 50% even at the highest point of the dilution series (Fig. 2).

To investigate the maximum inhibition, the effects of a higher protein concentration (20 × lower dilution, i.e. 2 mg/mL) of *D. siboney* extract was studied. Stronger inhibition was achieved of *D. farinae* (Fig. 1) whereas *D. pteronyssinus* (61%), *B. tropicalis* (47%), *A. siro* (58%), *L. destructor* (63%) and *T. putrescentiae* (56%) were inhibited to a lesser extent even at this concentration (Fig. 2).

The effect of homologous inhibition was studied in *D. siboney* which was reduced to 86% with 100 µg/mL, and to 96% (Fig. 1) with 2 mg/mL.

As shown in Fig. 2, there was no non-specific inhibition of *D. farinae* by birch nor of *D. pteronyssinus* by BSA even at a concentration of 2 mg of protein/mL.

The protein concentration of *D. siboney* inhibiting the IgE towards the other mite allergens to 50%, was lowest for *D. farinae* followed by *D. pteronyssinus*. *L. destructor* was the most inhibited storage mite, followed closely by *T. putrescentiae* and *A. siro*.

When the relationship between the levels of specific IgE for each combination of mite allergens was studied (Fig. 3), *D. farinae*, *D. pteronyssinus* and *L. destructor* showed the best correlation to *D. siboney*, whereas

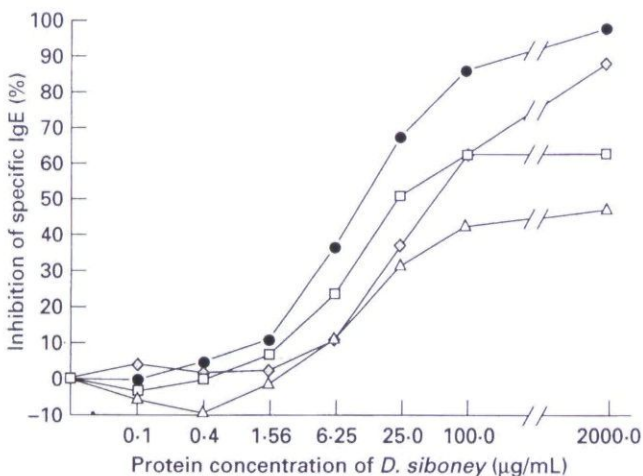


Fig. 1. Inhibition of specific IgE to (—□—) *D. pteronyssinus*, (—◇—) *D. farinae* and (—△—) *B. tropicalis* by increasing concentrations of *D. siboney* extract. Inhibition of homologous specific IgE to *D. siboney* (—●—) allergen is also shown.

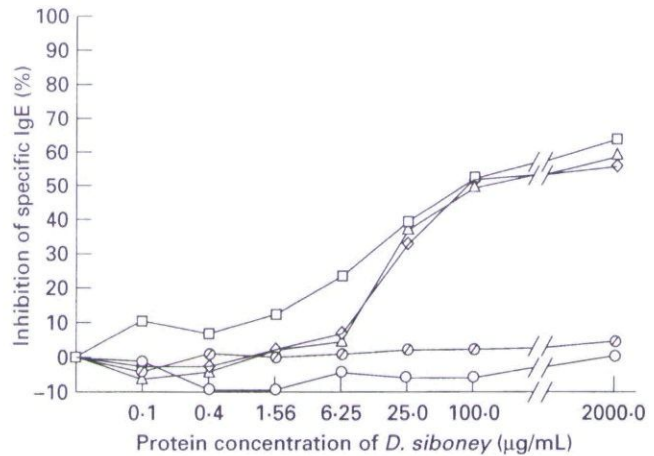


Fig. 2. Inhibition of specific IgE to (—□—) *L. destructor*, (—△—) *A. siro* and (—◇—) *T. putrescentiae* by increasing concentrations of *D. siboney* extract. The non-specific inhibition of *D. farinae* by birch pollen (—●—) and *D. pteronyssinus* by (—○—) Bovine Serum Albumin (BSA) are also shown.

specific IgE to *B. tropicalis*, *A. siro* and *T. putrescentiae*, did not show significant correlations with IgE to *D. siboney*.

Discussion

A close correlation was found between specific IgE in patient sera against *D. siboney* and *D. pteronyssinus* ($r = 0.92$) and *D. farinae* ($r = 0.90$), respectively, indicating a high degree of crossreactivity. Results of inhibition experiments agree with these findings. The close relationship between allergens from *Dermatophagoides* species has been well documented [1,4,5,17,18]. *D. siboney* showed not only a high degree of crossreactivity with *D. pteronyssinus* and *D. farinae* in this study but Der s1 and Der s2 were also purified [16] using crossreacting monoclonal antibodies to Group 1 and 2 allergens, indicating the presence of common epitopes on their structures. Especially in the case of *D. farinae*, which has not been reported in Cuba, the presence of both specific IgE levels (data not shown) and correlating specific IgE responses strongly indicate the presence of common IgE epitopes on allergenic components. The high degree of crossreactivity between *D. farinae* and *D. pteronyssinus*, which is the most abundant mite in house dust in Cuba, could further explain the high correlation found between levels of specific IgE to *D. siboney* and *D. farinae*. Inhibition studies using purified allergens from these species, as well as protein mapping of *D. siboney* allergens, would give further information on these relationships.

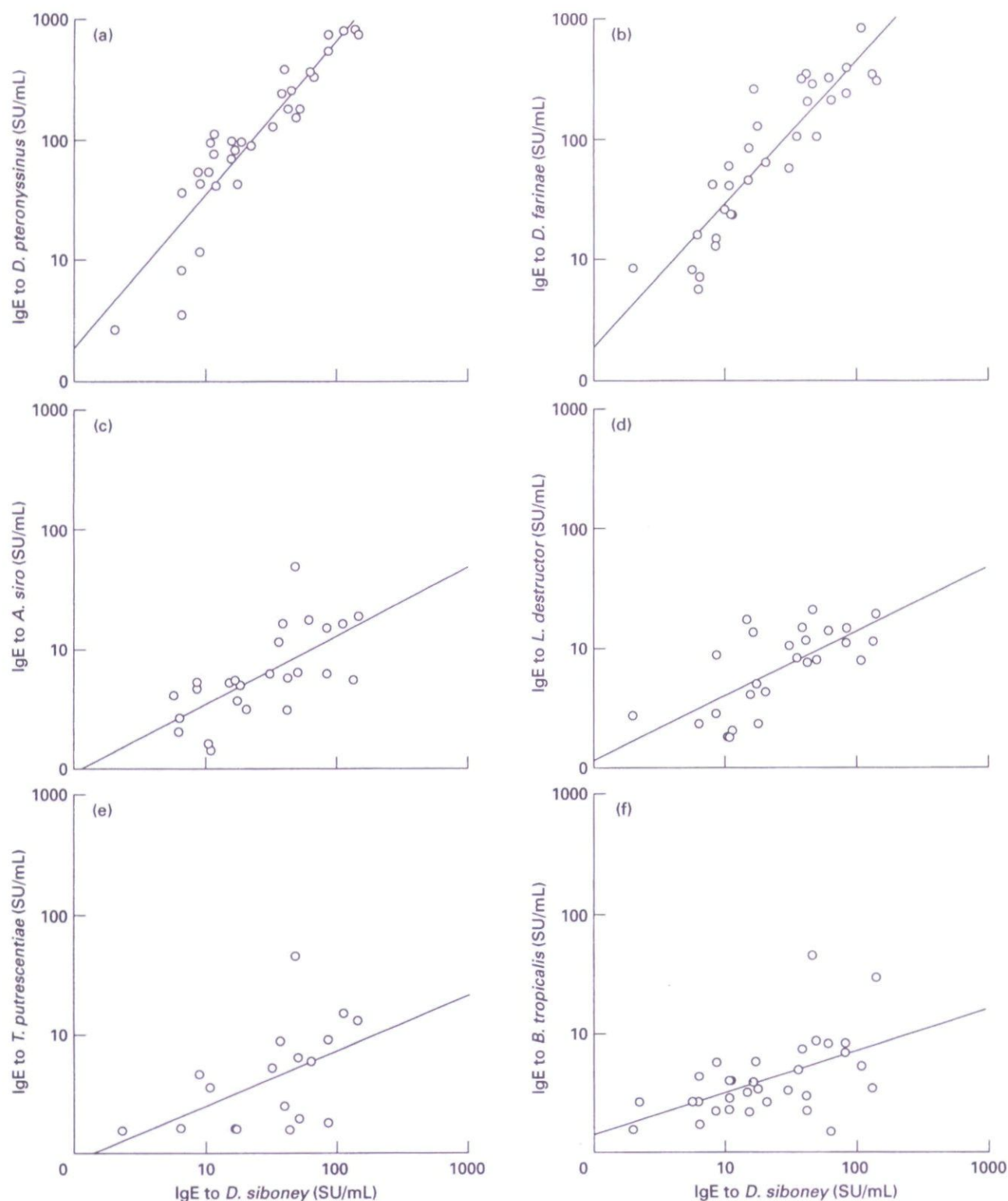


Fig. 3. Correlations between specific IgE levels (SU/mL) to *D. siboney* and different mite allergens in 35 patient sera from mite allergic individuals. (a) $R = 0.92$; $P < 0.0001$. (b) $R = 0.90$; $P < 0.0001$. (c) $R = 0.67$; $P = 0.0002$. (d) $R = 0.7$; $P < 0.0001$. (e) $R = 0.51$; $P = 0.034$. (f) $R = 0.54$; $P = 0.0015$.

Previous studies have focused on the role of *B. tropicalis* as sensitizing allergen in the tropics as well as the degree of crossreactivity between this mite and *Pyroglyphid* and storage mites [5,10,11,19]. We obtained a low correlation between IgE to *D. siboney* and *B. tropicalis*, and less than 50% of inhibition by *D. siboney* extract of the IgE response even at protein concentration of 2 mg/mL. Similar low crossreactivity has been shown to occur between *D. farinae* and *B. tropicalis* ($r = 0.59$) as measured by radioallergosorbent test (RAST) [10]. Arlian *et al.* [19] showed not only a limited amount of crossreactivity between *B. tropicalis* and *Dermatophagoides* species, but also that most of the multiple allergens found for this species are species-specific. Similar relationships were obtained between *D. pteronyssinus* and *B. kulagini* in Brazil [7], where unique allergens as well as common allergens were found in both species. The fact that the levels of specific IgE to *B. tropicalis* detected in our experiments are relatively low despite the frequency and abundance of this species in domestic dust in Cuba, requires further investigation.

Our results show that the relation between *D. siboney* and storage mites, *A. siro*, *L. destructor* and *T. putrescentiae*, was relatively low, even though some inhibition (about 50%) was obtained. Similar moderate crossreactivity was demonstrated between *D. pteronyssinus* and *L. destructor* [10], whereas in a group of farmers no significant correlation was found between *D. pteronyssinus* and *A. siro*, *T. putrescentiae*, *L. destructor* and other storage mites was found by others [20]. By immunoblotting inhibition technique, however, *L. destructor* inhibited the IgE antibodies to 25 kDa component of *D. pteronyssinus*, and to a lesser extent, the 16 kDa major allergen [8]. The IgE levels to *T. putrescentiae*, *A. siro* and *L. destructor* were relatively low even in the selected pool. The fact that we selected mite sensitive patients from a non-farmer population could explain this finding. However, others [21] have also shown that the sensitization rate to *L. destructor* and *T. putrescentiae* was high in city dwellers.

The complexity of allergen mixtures and the difference in reactivity to individual allergens by different patients might greatly influence the inhibition effect caused by an extract [6]. Predisposed individuals are exposed to different mite allergens. To study of crossreactions among these allergens and their role in mite allergic patients requires the use of purified allergens, i.e. Der s 1 and Der s 2.

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