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Analysis of the Cross-Reactivity between BtM and Der p 5, Two Group 5 Recombinant Allergens from *Blomia tropicalis* and *Dermatophagoides pteronyssinus*

Key Words

Cross-reactivity
Recombinant allergens
Blomia tropicalis
Dermatophagoides pteronyssinus
IgE-binding
Epitope
RAST inhibition
Immunoblot inhibition

Abstract

Background: In tropical climates, sensitization to *Blomia tropicalis* and *Dermatophagoides pteronyssinus* is high and mainly directed to species-specific allergens. There is some cross-reactivity between extracts of these mites, probably due to the group 5 allergens that have high sequence homology. **Objective and Methods:** We used the radioallergosorbent test (RAST), RAST inhibition and immunoblotting inhibition experiments to investigate the cross-reactivity between the recombinant allergens BtM and Der p 5, expressed as glutathione S-transferase fusion proteins, to detect the epitopes involved and to analyze the importance of this cross-reactivity. **Results:** Seventy-nine percent of 48 patients sera were RAST positive to both recombinants, with a strong correlation ($r = 0.8$, $p < 0.0001$). BtM inhibited 25 and 21.1% of IgE-binding to *B. tropicalis* and *D. pteronyssinus* extracts respectively and Der p 5 inhibited 22 and 24% of IgE-binding to *D. pteronyssinus* and *B. tropicalis* extracts. Furthermore, BtM inhibited 74.5% of IgE binding to Der p 5 and Der p 5 inhibited 72.4% of IgE-binding to BtM. RAST inhibition with BtM-derived synthetic peptides showed that peptide 4 (residues 35–50) and peptide 5 (residues 46–61) inhibited 37 and 16% of IgE-binding to BtM while peptides 5 and 2 (residues 14–30) were able to inhibit the IgE binding (32 and 28%, respectively) to Der p 5. **Conclusion:** There is cross-reactivity between BtM and Der p 5, which explains almost all the cross-reactivity between the two mite extracts. This cross-reactivity seems to be related to epitope(s) at the C-terminal segment of these allergens.

Introduction

Blomia tropicalis and *Dermatophagoides pteronyssinus* are the most prevalent domestic mites in house dust in tropical and subtropical climates [1, 2]. Sensitization to these mites is high and mainly directed to species-specific allergens [3–6], suggesting that they contribute to the increase in the allergenic potential of house dust and that allergens

from both mites should be included as reagents for the diagnosis and treatment of allergic respiratory diseases in these areas. However, several lines of evidence have shown some cross-reactivity between *D. pteronyssinus* and *B. tropicalis*. Knowledge of the extent and specificity of this cross-reactivity is important to evaluate its clinical role and the basic immune response to allergens. In 1994, Stanaland et al. [4] showed that a *D. pteronyssinus* extract could in-

hibit 33% of IgE binding to a *B. tropicalis* extract and *B. tropicalis* inhibited 13% of IgE-binding to a *D. pteronyssinus* extract. Arlian et al. [5], using crossed radioimmuno-electrophoresis, identified two cross-reacting allergens from *B. tropicalis* and *D. pteronyssinus*, but with this methodology it was not possible to further characterize these molecules. Analysis of the IgE responses patterns to *D. pteronyssinus* and *B. tropicalis* extracts among patients from tropical regions and patients unlikely to be exposed to *B. tropicalis* suggests that only 20–30% of *B. tropicalis* allergens may be shared with *D. pteronyssinus* [7]. The availability of purified mite allergens and the corresponding monoclonal antibodies have contributed to the clarification of this point. Arruda et al. [8] found no cross-reactivity between group 1 and 2 allergens of *D. pteronyssinus* and allergens from *B. tropicalis*. However, group 5 allergens from these mites have been cloned and expressed recently [7, 9–11]. The amino acid sequence homology between Der p 5 (132 residues, 13,585 D) and both BtM, a partial clone of Blo t 5 [11], and the full-length clone Blo t 5 [7] is 43 and 40%, respectively, suggesting that this homology may have an equivalence at the epitope level. In fact, sero-epidemiological data indicate that cross-reactivity between Der p 5 and Blo t 5 (134 residues, 13,876 D) may exist [12]. BtM is the C-terminal (72 residues, 8,373 D) segment of Blo t 5 and previous studies of immunoblotting inhibition suggest that this molecule has almost all the allergenic epitopes of the natural allergen in the *B. tropicalis* extract [11]. Therefore, defining the cross-reactivity between BtM and Der p 5 will provide additional information about their immunological properties and potential clinical uses. In this study, we used the radioallergosorbent test (RAST), RAST inhibition and immunoblot inhibition experiments to directly evaluate the existence of cross-reactivity between the recombinant allergens Der p 5 from *D. pteronyssinus* and BtM from *B. tropicalis* and to analyze the importance of this cross-reactivity to that observed between the extracts from the two mites. Here we report that there is cross-reactivity between the two allergens and that they are major allergens with important IgE antibody binding relative to the whole mite extracts.

Materials and Methods

B. tropicalis and *D. pteronyssinus* Extracts

B. tropicalis mites isolated from dust samples from homes of allergic patients living in Cartagena (Colombia) were cultured in tetramin fish food/brewer's yeast medium and kept at 27°C and 85% relative humidity. Adult mites were separated from the medium using a modified Tullgren apparatus, immediately frozen in liquid N₂ and

kept at –80°C before RNA isolation for constructing the cDNA library [11]. *B. tropicalis* extract was prepared as described previously [13]. Briefly, whole mite bodies were defatted with anhydrous ether and dried at room temperature. Defatted mites were extracted 1:20 w/v in 0.1 mol/l ammonium bicarbonate. The extract was clarified by centrifugation and dialyzed overnight against deionized water, filtered using 0.22 µm filters and lyophilized. The extract was reconstituted in water to obtain a concentration of 2.8 mg/ml as assessed by Bradford assay (Bio-Rad, Hercules, Calif., USA). *D. pteronyssinus* extract was obtained from Greer Laboratories (Lenoir, N.C., USA) and used at a concentration of 2.8 mg/ml. For skin testing, the *B. tropicalis* extract was used 50% glycerinated at 0.23 mg/ml; the *D. pteronyssinus* extract, 10,000 AU/ml, was obtained glycerinated from Greer Laboratories.

Subcloning and Expression of BtM Insert into pGEX Vector

The insert from an *Eco*RI and *Not*I digestion of lambda gt11 DNA from the *B. tropicalis* cDNA library [11] was purified with the Magic PCR Preps kit, ligated to pGEX-4T-3 vector (Pharmacia Biotech, Piscataway, N.J., USA) and transformed into *Escherichia coli* BL21. Recombinant clones were confirmed by restriction analysis of a miniprep from a transformed BL21. The insert subcloned in pGEX-4T-3 was sequenced in both directions by the polymerase chain reaction-dideoxy sequencing method using the appropriate primers for this vector. Expression of BtM as a fusion protein with glutathione S-transferase (GST-BtM) was induced with 0.1 mM IPTG and purification from bacterial lysate was done over glutathione-Sepharose (Pharmacia Biotech) followed by elution with 10 mM reduced glutathione according to the protocol of the GST Purification Module (Pharmacia Biotech). The IgE binding to the fusion protein (GST-BtM) was established by immunoblotting using a patient's serum and ¹²⁵I-labeled horse anti-human IgE (Kallestad Diagnostic, Inc. Chaska, Minn., USA). IgE binding was also detected by RAST and dot blot. GST was produced and purified from pGEX-4T-3-transformed *E. coli* BL21.

Expression of Der p 5 cDNA

Der p 5, was subcloned into pGEX-2T at the Graduate Institute of Immunology, National Taiwan University College of Medicine, as described previously [9]. The expression and purification of Der p 5 as a fusion protein (GST-Der p 5) was done as described for BtM (fig. 1).

Serum Samples

Forty-eight sera from non-immunotherapy-treated asthmatic patients allergic to *B. tropicalis* and *D. pteronyssinus* were used to establish the IgE-binding frequencies to Der p 5 and BtM and to make the serum pools. These sera were previously characterized in regard to RAST and were positive for both standardized *B. tropicalis* and *D. pteronyssinus* body extracts. Patients were skin test positive for both extracts and were informed about the objectives of the investigation. These individuals belonged to the prevalent ethnic group in Cartagena, an admixture of African Blacks and European Caucasians named Mulattos [14]. Allergic asthma was diagnosed following the criteria proposed by the expert panel of the Allergy Foundation of America [15] namely, recurrent episodes of wheezing or dyspnea; objective evidence of abnormality in pulmonary function during episodes, as well as positive skin tests. All patients had a clear history of atopic symptoms related to house dust exposure and were negative when skin-tested with a *B. tropicalis* and *D. pteronyssinus* culture medium extract. Negative control sera were obtained from healthy subjects, with no history of atopy and negative skin tests and RAST to mite allergens.

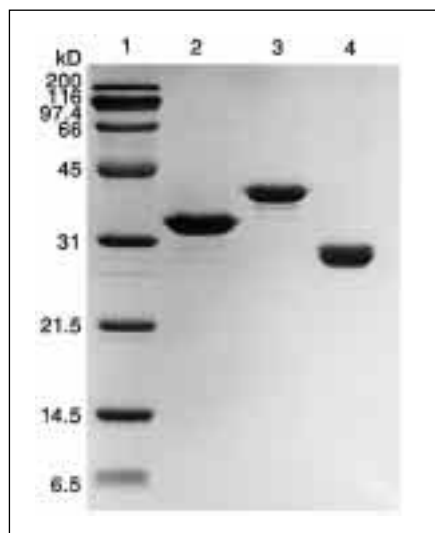


Fig. 1. SDS-PAGE (15%, reducing conditions) analysis of the recombinant proteins used in RAST, RAST inhibition and immunoblot inhibition experiments. The gel was stained with Coomassie Brilliant Blue R-250. Lane 1, molecular mass markers (Bio-Rad); lanes 2, 3, and 4, purified GST-BtM, GST-Der p 5 and GST, respectively.

Detection of the Serum Reactivity against *B. tropicalis* and *D. pteronyssinus* Extracts

Specific IgE antibodies to *B. tropicalis* and *D. pteronyssinus* extracts were determined in 20 asthmatic patients by RAST using microtiter plates (Immulon-4, Dynatech Laboratories, Alexandria, Va., USA). This additional group of patients also filled the diagnostic criteria for asthma described above, but were not skin-tested when the blood samples were obtained. Mite extracts were diluted 1:50 in 0.2 M sodium carbonate/bicarbonate buffer (pH 9.2) and 100 µl were added to each well, incubated overnight at room temperature and washed three times (PBS pH 7.4, 0.1% Tween 20). One hundred microliters of each serum sample were added to the wells, incubated overnight in a humid chamber, washed three times and incubated with 100 µl of ¹²⁵I-labeled horse anti-human IgE, 25,000 cpm. After overnight incubation, the plates were washed three times and each well was counted in a gamma counter (Gamma 5500 Beckman). The results were expressed as the mean percentage of total count bound. The RAST was considered positive when a serum bound > 1% total count bound. The results were also expressed as count index which was calculated by dividing the average reading (cpm) of an individual test serum by the average reading of the four negative control serum samples included in the same assay. Nonspecific binding was subtracted for all the cpm readings.

Serum Reactivity against Der p 5 and BtM

The presence of specific IgE antibodies to GST-Der p 5 and GST-BtM in the sera of 48 mite-allergic patients and 4 nonallergic controls was investigated by RAST as described above. All the sera were known to react to *B. tropicalis* and *D. pteronyssinus* mite extracts. Microtiter plates were coated with 0.5 µg/well of GST-Der p 5 (1.19 µM) and GST-BtM (1.34 µM). After overnight incubation with sera at

room temperature, specific IgE antibody was detected as mentioned above.

RAST Inhibition Experiments

To evaluate the ability of GST-BtM and GST-Der p 5 to inhibit IgE antibody binding to the mite extracts, two serum pools were prepared from ten sera with the highest IgE antibody levels to *B. tropicalis* [A35, CB19, CB29, A118, CB25 (pool A)] and *D. pteronyssinus* [A21, A121, A82, A72, A62 (pool B)]. The RAST values (expressed as percent total count bound) to *B. tropicalis* and *D. pteronyssinus* for pool A were 44 and 11, respectively; for pool B, 45 and 37, respectively. The serum pools were adsorbed overnight with a lysate of *E. coli* BL21 transformed with pGEX-4T-3. Each microtiter well was coated with 5 µg of the mite extracts. A serum pool dilution of 1:20 was used to avoid antibody excess in the fluid phase and enhance the sensitivity of the assay. One hundred microliters of pool A were adsorbed with increasing micromolar concentrations of GST-BtM (4.2×10^{-4} , 2.1×10^{-3} , 1.1×10^{-2} , 1.34, 6.68, 13.33, 18.72), GST-Der p 5 (3.8×10^{-4} , 1.9×10^{-3} , 0.95×10^{-2} , 1.19, 5.95, 11.90, 16.67) and GST (5.5×10^{-4} , 2.8×10^{-3} , 1.38×10^{-2} , 1.72, 8.62, 17.24, 24.14). The same procedure was performed with pool B, prior to incubation with the extracts of *B. tropicalis* and *D. pteronyssinus*, respectively. After an overnight adsorption, 100 µl of serum pool were loaded into the wells and the plates incubated overnight at room temperature. The reaction was developed as described above. Each experimental determination was done in duplicate using the mean cpm value. The percentage of inhibition was calculated as follows: percent inhibition = $\frac{\text{cpm without inhibitor} - \text{cpm with inhibitor}}{\text{cpm without inhibitor}} \times 100$. To determine the overall cross-reactivity between the two mite extracts, we used the pools of serum (1:20) and protein concentration of extracts ranging from 0.063 to 40 µg/ml.

RAST inhibition assays between GST-BtM and GST-Der p 5 were done with adsorbed serum pools A (1:40) and B (1:20). The recombinants were used at 0.5 µg/well. Pool A was adsorbed with increasing concentrations of GST-BtM, GST-Der p 5, GST (as above) and GST-Bt6 (3.5×10^{-4} , 1.7×10^{-3} , 0.87×10^{-2} , 1.09, 5.43, 10.87, and 15.22 µM). The same procedure was done with pool B, before incubation with GST-BtM and GST-Der p 5, respectively. GST-Bt6 is a BtM-unrelated recombinant allergen from *B. tropicalis* [16]. After adsorptions, the experiment was continued as described above. Each experimental determination was carried out in triplicate and the mean cpm was used for calculations.

In an attempt to identify the epitopes involved in the cross-reactivity between BtM and Der p 5, we used the following six five-residue overlapping peptides designed on the basis of the deduced amino acid sequence of BtM p1 (residues 1–18), p2 (14–30), p3 (26–39), p4 (35–50), p5 (46–61), p6 (57–72). Peptides were synthesized and purified at the Institut de Biochimie, University of Lausanne, Switzerland. Using these peptides, we have previously shown that most of the IgE reactivity to BtM is directed to a linear epitope corresponding to peptide 4 [17]. RAST inhibitions with peptides as inhibitors and 0.5 µg/well of BtM and Der p 5 on solid phase, were done with serum pools A and B, respectively. The adsorption with the peptides was made by mixing equal volumes of serum pool (diluted 1:25) and the corresponding peptide at 2, 1, 0.5, 0.1, and 0.01 mM dissolved in DMSO. Then, the experiment was continued as described above. RAST inhibition using homologous BtM or Der p 5 as inhibitors was included as controls. In addition, an irrelevant peptide from a nonrelated allergen Amb a V [18] was used as inhibitor, as negative control.

Immunoblot Inhibition

To provide a qualitative confirmation of RAST inhibition studies, IgE inhibition experiments with immunoblot were performed. SDS-PAGE (15%, reducing conditions) and immunoblotting were performed according to standard methods [19, 20]. GST-BtM and GST-Der p 5 were loaded at 1 µg/lane, electrophoresed in a Mini-Protean II cell (Bio-Rad, Hercules, Calif., USA) and transferred to a nitrocellulose membrane (Trans-blot NC, Bio-Rad). Nitrocellulose strips were incubated overnight with 2 ml of 1:60 pool A (GST-BtM) and 1:40 pool B (GST-Der p 5). Before incubation, serum pools were adsorbed with 15, 30, 60 and 120 µg/ml of the homologous, heterologous and control inhibitors. IgE was detected by using ¹²⁵I-labeled horse anti-human IgE, followed by autoradiography with Kodak X-OMAT films.

Statistical Analysis

Graphs and statistical analysis, including Spearman rank correlation coefficient of the RAST results, were performed with the SYSTAT program (Version 5 Edition, Evanston, Ill., 1994).

Results

Specific IgE Levels to B. tropicalis and D. pteronyssinus Extracts in Sera from Mite-Allergic Patients Show a Positive Correlation

IgE antibodies to *B. tropicalis* and *D. pteronyssinus* in sera of 20 allergic patients were compared by RAST and the results expressed as count index. The average reading of negative-control sera was 75.2 cmp for both mite extracts. Serum samples with a count index equal to or greater than 2.6 (which is equivalent to the mean +3 SD of the negative control sera) were considered positive. Eighteen sera were positive for *B. tropicalis* (90%) and 13 (75%) for *D. pteronyssinus*. Eleven sera (55%) were positive for both extracts. The results were compared by the Spearman rank correlation coefficient ($r = 0.62$, $p = 0.001$).

There Is a Strong Correlation between IgE Antibody Reactivity to GST-BtM and GST-Der p 5 in Sera from Mite-Allergic Patients

Specific IgE antibodies in sera from 48 patients were detected by RAST. The count indices for recombinant GST-BtM and GST-Der p 5 were 2.5 and 1.4, respectively. Thirty-eight (79%) of the 48 sera were positive for both GST-BtM and GST-Der p 5. Forty (83%) were positive for GST-Der p 5 ($r = 0.79$, $p < 0.0001$). No serum was positive for GST alone.

There Is Moderate Cross-Reactivity between B. tropicalis and D. pteronyssinus Extracts

Since both *B. tropicalis* and *D. pteronyssinus* coexist in Cartagena, a positive correlation between RAST to these mite extracts does not mean that cross-reactivity exists. Therefore, RAST inhibition experiments were done with the two extracts. The inhibitory capacities of *B. tropicalis* and *D. pteronyssinus* extracts on the serum pools from allergic patients were almost the same. When *B. tropicalis* was on the solid phase, homologous inhibition was 96% at 40 µg/ml and *D. pteronyssinus* inhibited 28% at the same concentration. With *D. pteronyssinus* on the solid phase, *B. tropicalis* produced 25% inhibition at 40 mg/ml. 95% inhibition was obtained with the homologous system at the same concentration.

GST-Der p 5 and GST-BtM Induce Important Inhibition of IgE Antibody Binding to D. pteronyssinus and B. tropicalis Extracts

RAST inhibition was done to investigate the significance of BtM and Der p 5 IgE antibody binding relative to *B. tropicalis* and *D. pteronyssinus* extracts. Using *B. tropicalis* on the solid phase, 7.4% and 6% of inhibition was obtained with 4.2×10^{-4} µM GST-BtM and 3.8×10^{-4} µM GST-Der p 5, respectively. Maximum inhibition of IgE antibody binding to *B. tropicalis* extract was 25% (6.68 µM GST-BtM) and 24% (5.95 µM GST-Der p 5) (fig. 2a). With *D. pteronyssinus* on the solid phase, 8.2 and 12.1% of inhibition were obtained with 4.2×10^{-4} µM GST-BtM and 3.8×10^{-4} µM GST-Der p 5, respectively. Maximum inhibition of IgE antibody binding to *D. pteronyssinus* was 21.1% (6.68 µM GST-BtM) and 22% (16.67 µM GST-Der p 5). Preadsorptions with control GST yield no important inhibition (fig. 2b).

GST-BtM and GST-Der p 5 Exhibit Cross-Reactivity

RAST inhibition was carried out to evaluate the potential cross-reactivity between GST-BtM and GST-Der p 5, using serum pools A and B. In homologous systems, 4.2×10^{-4} µM GST-BtM and 3.8×10^{-4} µM GST-Der p 5 achieved 37.5% inhibition. Maximum inhibition (90%) was reached by preadsorption with 13.33 µM GST-BtM or 11.9 µM GST-Der p 5. In heterologous systems, when GST-BtM was on the solid phase, 3.8×10^{-4} µM GST-Der p 5 inhibited 31.6%. Maximum inhibition (72.4%) was obtained with 5.95 µM GST-Der p 5 (fig. 3a). The same procedure was done using GST-Der p 5 on the solid phase. In this case, 4.2×10^{-4} µM GST-BtM inhibited 20.2%. The maximum inhibition (74.5%) was obtained with 6.68 µM GST-BtM (fig. 3b). Using pool A, maximum inhibitions obtained with GST-BtM

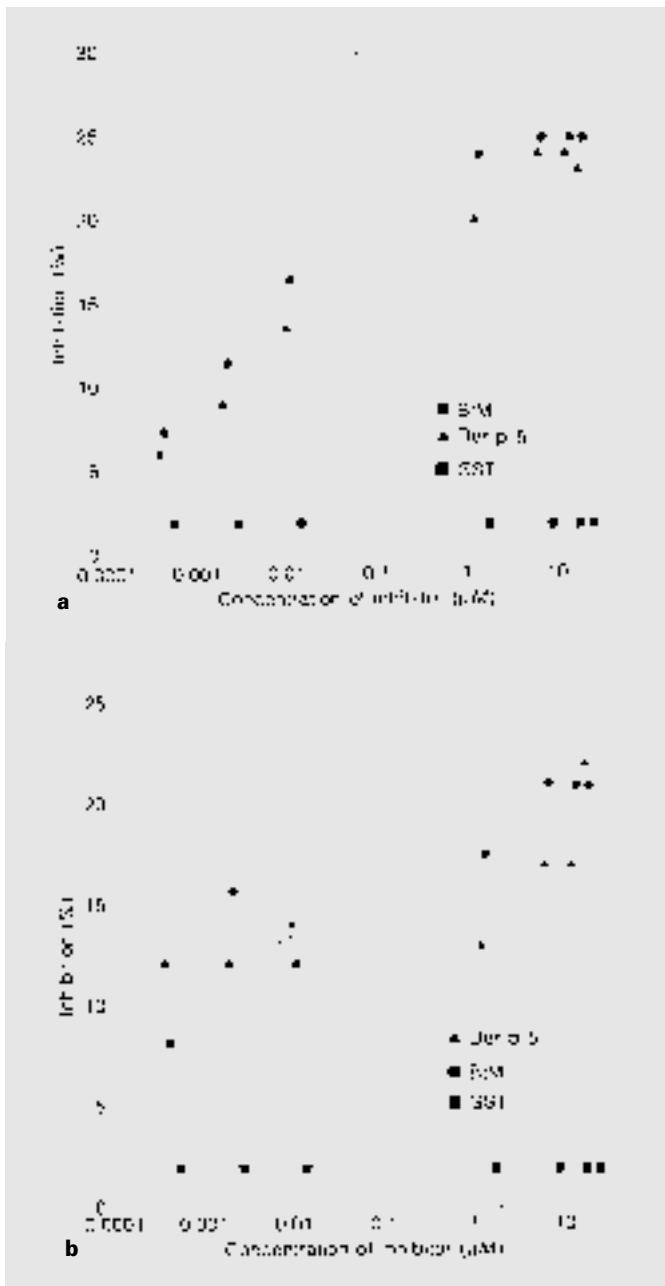


Fig. 2. Dose-dependent inhibition of specific IgE antibody binding to *B. tropicalis* and *D. pteronyssinus* extracts by the recombinant allergens. **a** *B. tropicalis* extract on a solid phase. **b** *D. pteronyssinus* extract on a solid phase.

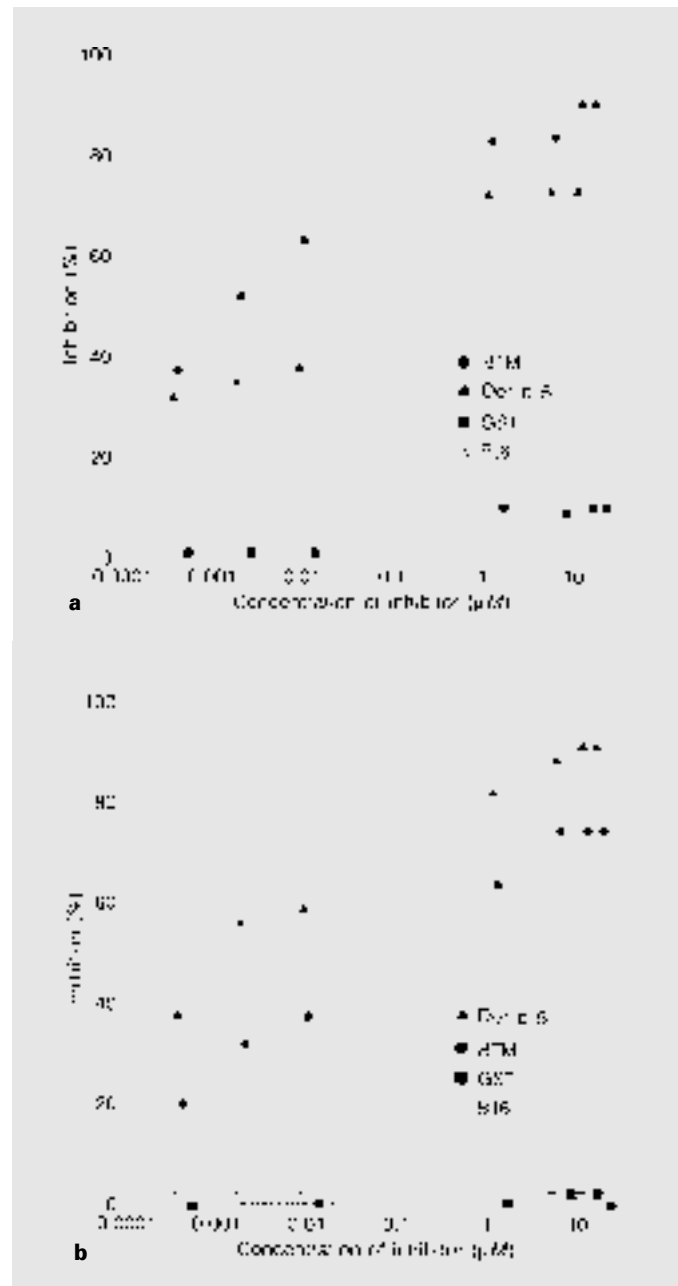


Fig. 3. a Dose-dependent inhibition of IgE-binding to GST-BtM (solid phase) by GST-BtM, GST-Der p 5, GST and GST-Bt6. **b** Dose-dependent inhibition of IgE-binding to GST-Der p 5 (solid phase) by GST-Der p 5, GST-BtM, GST and GST-Bt6.

and GST were 5.2 and 10%, respectively; with pool B, maximum inhibition with these recombinants was 2.4%.

These results were qualitatively confirmed by immunoblot inhibition experiments. In the homologous system, complete inhibition of IgE binding was obtained with 15 µg of GST-BtM and GST-Der p 5 (fig. 4, lanes 2 and 7).

In the heterologous system, a significant degree of inhibition between the two recombinants was observed, starting at 30 µg of inhibitors. Maximum inhibitions were obtained with 60 µg of GST-BtM (lane 3) and GST-Der p 5 (lane 8). No inhibition was observed with any amount of GST-Bt6 or GST.

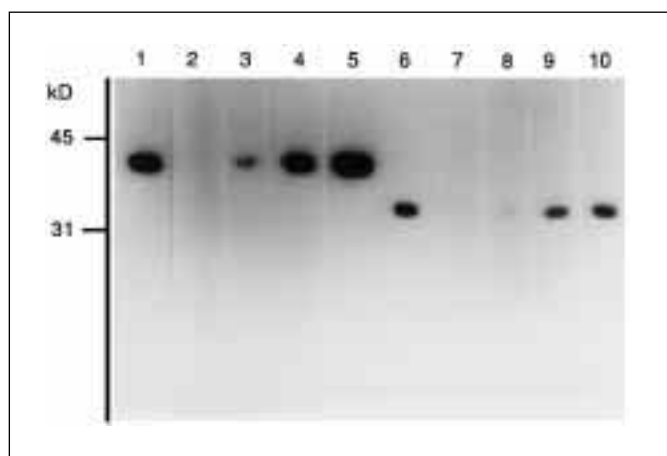


Fig. 4. Immunoblot inhibition of IgE-binding to Der p 5 (lanes 1–5) and BtM (lanes 6–10). Serum pool A (1:60) and B (1:40) were adsorbed with the following inhibitors: lanes 1 and 6, no inhibitor; lanes 2 and 7, GST-Der p 5 and GST-BtM, respectively; lanes 3 and 8, GST-BtM and GST-Der p 5, respectively; lanes 4 and 9, GST-Bt6 (60 µg/ml); lanes 5 and 10, GST (60 µg/ml).

Cross-Reactivity between BtM and Der p 5 Relies on C-Terminal epitopes of Both Molecules

Peptide 4 produced the maximum inhibition (36.7%, at 2 mM concentration) of the IgE antibody binding to BtM on the solid phase and a dose-response curve was obtained. A lower degree of inhibition was observed when peptide 2 (25.7%) and peptide 5 (16%) were used as inhibitors at the same concentration. With the remaining peptides, no dose-response curve was observed and their highest values of inhibition were no more than 18.5%. When Der p 5 was on solid phase, only peptide 5 inhibited IgE antibody binding (31.6% at 2 mM) and a dose-response curve was obtained. The inhibition with the remaining peptides was no more than 2.7%. The maximum RAST inhibition value in the homologous system with GST-BtM and GST-Der p 5 was 90%. No inhibition was observed with the irrelevant peptide. The same experiments were done using serum A21, which has high RAST values to GST-BtM and GST-Der p 5. With Der p 5 on the solid phase, 31.6 and 27.4% of inhibition and a dose-response curve was obtained with peptide 5 and peptide 2 (2 mM), respectively. When BtM was on the solid phase, only peptide 2 produced a dose-response curve with 56.2% of inhibition at 2 mM. No important inhibition was obtained with the irrelevant peptide.

Discussion

In this study, we analyzed several aspects of the IgE antibody response to the mite extracts and to two recombinant allergens of the house dust mites *B. tropicalis* and *D. pteronyssinus*. As demonstrated for several plants and mite allergens, cross-reactivity is likely to occur between allergens showing amino acid sequence homology, such as BtM and Der p 5. The RAST and RAST inhibition experiments allowed us to show the existence of an important degree of cross-reactivity between these two major recombinant allergens, and this was further confirmed with immunoblot inhibition assay. Since the percentage of inhibition of the IgE antibody binding between the two molecules is almost the same and reaches more than 70%, it seems that they share one immunodominant epitope. The amino acid sequence homology of these allergens is mainly at the C-terminal segment of each molecule, and our results of RAST inhibition experiments, using sera from mite-allergic individuals and synthetic peptides suggest that epitopes in this region can define this cross-reactivity. In fact, most of the IgE antibodies to both recombinant allergens can be inhibited by two overlapping peptides spanning residues 35–61 of the C-terminal portion of BtM. Although we did not use Der p 5-derived peptides, the high degree of homology at this segment of the two molecules suggests that the IgE-binding inhibition obtained is due to the existence of a common epitope.

Cross-reactivity is a frequent feature of mite allergens, especially those from taxonomically related species. Cross-reactivity between extracts and purified allergens from *Dermatophagoides* spp. is well known. A lower degree of cross-reactivity has also been described between extracts of *B. tropicalis* and *D. pteronyssinus* [4–7], which belong to the Echinomphididae and Pyroglyphidae, respectively [21]. Based on the results of several immunochemical experiments, we confirmed the cross-reactivity between these extracts and report, for the first time, cross-reactivity between purified allergens from these two mites. Since these allergens also show high amino acid sequence homology, they are probably conserved proteins with an important physiological role. As mentioned, in spite of being a partial clone, BtM has most of the allergenic epitopes of its counterpart natural allergen [11], and this study provides additional evidence about this fact and its clinical relevance. However, based on our data, the existence of shared epitopes in the N-terminal portion of this molecule and that of Der p 5 cannot be excluded.

Our study also shows that in a Mulatto population living in a tropical Caribbean city, the prevalence of IgE antibodies to *B. tropicalis* and *D. pteronyssinus* extracts in asthmatic

ic patients (as determined by RAST) is 90 and 75%, respectively, and that there is a good correlation between the RAST values. Both the cross-reactivity between BtM and Der p 5 and the coexistence of the two mite species in the region can explain the significant correlation observed between RAST values of sera reacting with the two mite extracts and the recombinants. It has been shown that BtM and Der p 5 have equivalent native allergens in whole mite extracts [11, 22]. We now describe that these allergens account for almost 25% of the IgE antibody binding capacity of their mite extracts. Since the extent of RAST inhibition between the extracts of *B. tropicalis* and *D. pteronyssinus* found in this study is approximately 25%, which is very similar to that obtained by others [4], our results suggest that group 5 allergens account for the majority of the cross-reactivity between the two mites, and that these native allergens are well represented in the extracts.

The frequencies of IgE reactivity to BtM and Der p 5 among asthmatic patients were 79 and 83%, respectively, which is higher than that reported by us [11] and Lin et al. [9] using plaque immunoassay and dot-blot. In addition, count indices for most of these sera were high, showing that the IgE reactivity is strong. The differences among IgE-binding frequencies could be explained by the higher sensitivity of RAST, but racial factors and levels of mite allergen exposure [23, 24] have to be considered. Cross-reactivity reflects the quality of the IgE responses to allergens among specific populations and level of exposure. The clinical importance of BtM and Der p 5 in Mulattos living in a tropical climate is suggested by the high level of sensitization to these molecules among mite-allergic patients. This also indicates that, even being a partial clone, BtM exhibits major allergen properties and could be useful, together with other recombinants, for diagnosing mite allergy in the tropics. In

fact, preliminary studies in our laboratory have shown that this small peptide is able to induce positive skin tests in *B. tropicalis*-allergic patients [unpubl. obs.]. Since positive skin tests with both purified Der p 5 and Blo t 5 have been observed in other groups of patients allergic to *D. pteronyssinus* and *B. tropicalis* [9, 25], it would appear that the clinical relevance of these allergens is more extensive. Other tests, like allergen-induced histamine release, and nasal and bronchial challenge, will document more accurately their clinical significance.

According to this and previous reports, cross-reactivity between *B. tropicalis* and *D. pteronyssinus* is low to moderate. This study shows that most of it relies on group 5 allergens, and more specifically in immunodominant epitope (s) shared by the two major allergens Blo t 5 and Der p 5. Although a previous study implies that this cross-reactivity involves at least one additional allergen [5], our results suggest that the relative IgE-binding capacity of this allergen might be low. Therefore, the use of both mite extracts or a combination of several major recombinant allergens from *B. tropicalis* and *D. pteronyssinus* is mandatory for diagnosis and treatment in tropical regions. Considering the potential clinical use, of the major recombinant allergen BtM, it is advantageous to define its allergenic properties since small peptides can be genetically engineered more easily than large molecules.

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References

- 1 Arlian L, Bernstein D, Bernstein I, et al: Prevalence of dust mites in the homes of asthmatics living in eight different geographical areas of the United States. *J Allergy Clin Immunol* 1992;90:292–300.
- 2 Fernández-Caldas E, Puerta L, Mercado D, Lockey R, Caraballo L: Mite Fauna, Der p I, Der f I and *Blomia tropicalis* allergens levels in a tropical environment. *Clin Exp Allergy* 1993; 23:292–297.
- 3 Fernández-Caldas E, Baena-Cagnani C, López M, Patiño C, Neffen H, Sanchez-Medina M, Caraballo L, Huerta J, Malka S, Naspitz C, Lockey R: Cutaneous sensitivity to six mite species in asthmatic patients from five Latin American countries. *J Invest Allergol Clin Immunol* 1993;3:245–249.
- 4 Stanaland B, Fernández-Caldas E, Jacinto C, Trudeau W, Lockey R: Sensitization to *Blomia tropicalis*: Skin test and cross-reactivity studies. *J Allergy Clin Immunol* 1994;94:452–457.
- 5 Arlian L, Vyszynski-Moher D, Fernández-Caldas E: Allergenicity of the mite, *Blomia tropicalis*. *J Allergy Clin Immunol* 1993;91:1042–1050.
- 6 Stanaland B, Fernández-Caldas E, Jacinto C, Trudeau W, Lockey R: Positive nasal challenge responses to *Blomia tropicalis*. *J Allergy Clin Immunol* 1996;97:1045–1049.
- 7 Arruda K, Fernández-Caldas E, Naspitz C, Montealegre F, Chapman M: Identification of *Blomia tropicalis* allergen Blo t 5 by cDNA cloning. *Int Arch Allergy Immunol* 1995;107: 456–457.
- 8 Arruda K, Rizzo C, Chapman M, Fernández-Caldas E, Baggio D, Platt-Mills T: Exposure and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil. *Clin Exp Allergy* 1991;21:433–439.

- 9 Lin K, Hsieh K, Thomas W, Chiang B, Chua K: Characterization of Der p V allergen, cDNA analysis, and IgE-mediated reactivity to the recombinant protein. *J Allergy Clin Immunol* 1994;94:989–996.
- 10 O'Neil G, Donovan G, Baldo B: Sequence analysis of the native form of a major house dust mite allergen and its nascent polypeptide. *Mol Immunol* 1994;31:1447–1448.
- 11 Caraballo L, Avgioglou A, Marrugo J, Puerta L, Marsh D: Cloning and expression of cDNA coding for an allergen with common antibody binding specificities with three allergens of the house dust mite *Blomia tropicalis*. *J. Allergy Clin Immunol* 1996;98:573–579.
- 12 Vailes L, Rizzo M, Naspitz C, Montealegre F, Fernández-Caldas E, Platts-Mills T, Chapman M, Arruda K: Analysis of IgE antibody responses to *D. pteronyssinus* and *Blomia tropicalis* using recombinant group 5 allergens (abstract). *J Allergy Clin Immunol* 1996;97:213.
- 13 Puerta L, Fernández-Caldas E, Caraballo L, Lockey R: Sensitization to *Blomia tropicalis* and *Lepidoglyphus destructor* in *Dermatophagoides* spp. allergic individuals. *J Allergy Clin Immunol* 1991;88:943–950.
- 14 Caraballo L, Marrugo J, Erlich H, Pastorizo M: HLA alleles in the population of Cartagena, Colombia. *Tissue Antigens* 1992;39:128–133.
- 15 Busse W, Reed C: Asthma: Definition and pathogenesis; in Middleton E, Reed C, Ellis E, Adkinson N, Yunginger J (eds): *Allergy Principles and Practice*. St Louis, Mosby, 1988, p 969.
- 16 Caraballo L, Puerta L, Jiménez S, Martínez B, Mercado D, Avjioglou A, Marsh D: Cloning and IgE-binding of a recombinant allergen from the mite *Blomia tropicalis*, homologous with fatty acid-binding proteins. *Int Arch Allergy Immunol* 1997;112:341–347.
- 17 Moreno L, Hamilton R, Rafnar T, Jiménez S, Marsh D, Caraballo L: Identification of an IgE-binding epitope of a recombinant allergen from the mite *Blomia tropicalis* (Bt) (abstract). *J Allergy Clin Immunol* 1997;99:S351.
- 18 Rafnar T, Ghosh B, Metzler W, Huang S, Perry M, Mueller L, Marsh D: Expression and Analysis of Recombinant Amb a V and Amb t V. *J Biol Chem* 1992;267:21119–21123.
- 19 Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–685.
- 20 Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Nat Acad Sci USA* 1979;76:4350–4354.
- 21 O'Connor B: Astigmata; in Parker S (ed): *Synopsis and Classification of Living Organisms*. New York, Mc Graw Hill, 1982, vol 2, pp 146–169.
- 22 Tovey E, Johnson M, Roche A, Cobon G, Baldo B: Cloning and sequencing of a cDNA expressing a recombinant house dust mite protein that binds human IgE and correspond to an important low molecular weight allergen. *J Exp Med* 1989;170:1457–1462.
- 23 Puerta L, Fernández-Caldas E, Mercado D, Lockey R, Caraballo L: Sequential determinations of *Blomia tropicalis* allergens in mattress and floor dust samples in a tropical city. *J Allergy Clin Immunol* 1996;97:689–691.
- 24 Li C, Wan G, Hsieh K, Chua K, Lin R: Seasonal variation of house dust mite allergen (Der p 1) in a subtropical climate. *J Allergy Clin Immunol* 1994;94:131–134.
- 25 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 the recombinant group 5 allergens among mite allergic patients living in UK (abstract). *J Allergy Clin Immunol* 1996;97:421.

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