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Allergenic differences between the domestic mites *Blomia tropicalis* and *Dermatophagoides pteronyssinus*

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Summary

Background House dust mite allergens are the most important indoor allergens associated with asthma and rhinitis in Singapore and the tropics. Recent data to suggest that besides the *Dermatophagoides* spp., the domestic mite *Blomia tropicalis* (Bt) is also an important source of allergens in these regions.

Objective To evaluate the degree of allergenic cross-reactivity between Bt and *D. pteronyssinus* (Dp).

Methods Cross-reactivity between extracts of Bt and Dp was evaluated by fluorescent allergosorbent (FAST) inhibition studies and cross enzyme immunoelectrophoresis. Additionally, the major Dp allergens — Der p 1, Der p 2 and Der p 5, were also compared with the Bt extract by dot blot inhibition. Skin prick and intradermal end-point titration were then carried out to compare the homologous allergens of the mite species, Blo t 5 and Der p 5.

Results FAST inhibition studies showed low to moderate cross-reactivity between the two dust mite extracts (maximum cross-inhibition, 60%). Native allergens studied by cross enzyme immunoelectrophoresis using mite allergic sera also showed similar results but with at least four cross-reactive IgE binding antigens. Dot blot inhibition studies using allergens of Dp, Der p 1, Der p 2, and Der p 5, showed little cross-reactivity between these allergens with components of the crude Bt extracts. Further, evaluation of a recombinant allergen of Bt, Blo t 5, showed low levels of cross-reactivity even with its homologous Dp counterpart, Der p 5.

Conclusion These results provide evidence that Bt allergens are distinct and have relatively low to moderate cross-reactivity with *Dermatophagoides* spp. allergens. Bt allergens should therefore be included in the diagnostic panel for the evaluation of allergic disorders in the tropics, and the development of new diagnostic and therapeutic strategies should include allergens of Bt.

Keywords: Blomia tropicalis, Dermatophagoides pteronyssinus, mite allergy, tropics

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Introduction

Immediate hypersensitivity to indoor allergens is strongly associated with bronchial asthma [1,2]. In Singapore, one in five school children have been identified with doctor-diagnosed asthma [3]. With most populations spending more time indoors [4], increased exposure to indoors allergen has been postulated to play a causal role in the world-wide increase in asthma prevalence [5]. The majority (>90%) of local asthmatic children are sensitized to the common house dust mites, *Dermatophagoides pteronyssinus* (Dp) and *D. farinae* (Df) [6]. Besides the *Dermatophagoides* spp.,

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we have recently shown that an equally high proportion of local atopics are sensitized to the domestic mite, *Blomia tropicalis* (Bt) [7]. In a survey on indoor allergens, we have also shown high prevalence of *Dermatophagoides* spp. and Bt allergens in dust samples of local homes [8].

With molecular cloning of the allergens of *Dermatophagoides* spp., it has been shown that allergens of Dp and Df are homologous and cross-reactive [9,10]. In addition, the cloning of one of the Bt allergens, designated Blo t 5, has been shown to have 43% homology with the Dp allergen, Der p 5 [11]. Apart from Blo t 5, however, little is known about the structure of the other allergens of Bt. It is therefore possible that Bt allergens are also highly cross-reactive with those of *Dermatophagoides* spp. In this study, we set out to further characterize the Bt allergens and determine their cross-reactivity with *Dermatophagoides* spp. allergens.

Materials and methods

Screening of serum for Bt and Dp-specific IgE antibodies

To screen for mite-specific serum IgE antibodies to Dp and Bt, the quantitative FAST (BioWhittaker, Walkersville, MD, USA) was carried out. The Dp coated wells were obtained from the manufacturer (BioWhittaker, USA) while the Bt wells were coated as previously described [8]. Sera from 20 subjects (Table 1) were pooled for studies on cross-reactivity.

Table 1. Specific IgE binding of the 20 sera used in the serum pool for inhibition studies (expressed in IU/mL equivalent)

1	44.12 (4+)	11.23 (3+)	15.47 (3+)
2	36.89 (4+)	11.95 (3+)	12.45 (3+)
3	35.01 (4+)	15.20 (3+)	25.17 (4+)
4	32.12 (4+)	7.02 (3+)	9.71 (3+)
5	26.47 (4+)	15.75 (3+)	21.35 (4+)
6	24.79 (4+)	5.59 (3+)	6.30 (3+)
7	21.63 (4+)	12.47 (3+)	18.56 (4+)
8	15.64 (3+)	8.14 (3+)	9.40 (3+)
9	15.60 (3+)	10.20 (3+)	13.36 (3+)
10	13.54 (3+)	11.47 (3+)	14.35 (3+)
11	12.57 (3+)	10.40 (3+)	14.08 (3+)
12	11.56 (3+)	7.46 (3+)	9.91 (3+)
13	9.89 (3+)	7.31 (3+)	8.24 (3+)
14	9.89 (3+)	8.87 (3+)	11.29 (3+)
15	5.76 (3+)	3.98 (3+)	4.12 (3+)
16	5.64 (3+)	15.24 (3+)	17.01 (3+)
17	5.46 (3+)	3.99 (3+)	4.51 (3+)
18	4.87 (3+)	6.98 (3+)	7.45 (3+)
19	4.75 (3+)	10.90 (3+)	13.49 (3+)
20	3.19 (3+)	3.02 (3+)	3.57 (3+)

*FAST Negative < 0.35 IU/mL; FAST Class 1+ = 0.35–0.75 IU/mL; FAST Class 2+ = 0.76–2.99 IU/mL; FAST Class 3+ = 3.00–17.5 IU/mL; FAST Class 4+ > 17.5 IU/mL. †Sorted in a descending order by specific IgE levels to *B. tropicalis*.

Mite allergen extracts

For these studies, Dp, Df and Bt mites were cultured on a mixture of rodent food pellets, tetramin fish food (25%) and yeast extract (25%), and separated from food medium using a modified Tullgren apparatus to ensure minimal contamination by medium [12]. The whole mite bodies were defatted for 4 h with anhydrous ether and lyophilized prior to storage at −80 °C. Extraction overnight at 4 °C in phosphate-buffered saline was carried out. The supernatant was filtered through a 0.22 μm membrane, and quantified according to the method described by Bradford [13].

Cross-reactivity studies

FAST inhibition The FAST inhibition assay [8] was used to evaluate the degree of cross-reactivity between crude extracts of Bt, Dp and Df. Serial concentrations ranging from 0.001 to 1000 μg/mL of protein were added to equal volume of serum pool of 20 Bt-, Dp- and Df-sensitized subjects and incubated for 1.5 h. BSA was used as the control inhibiting antigen. The results were then expressed as percentage inhibition. A level of greater than 30% inhibition was considered to be significant. FAST inhibition was also carried out to evaluate cross-reactivity between recombinant Blo t 5 and Der p 5, with serial concentrations of protein from 0.001 to 5 μg/mL.

Cross-immunoelectrophoresis (CIE) and cross enzyme-immunoelectrophoresis (CEIE) Cross-reactivity was also evaluated via CIE and CEIE. Rabbit antisera against Dp and Bt were prepared by immunizing New Zealand white rabbits subcutaneously with 3.5 mg of protein emulsified in complete Freund's adjuvant, followed by subsequent injections every 3 weeks with 3.5 mg protein in incomplete Freund's adjuvant until potent antisera were produced. CIE was performed as previously described [14]. A total of 25 μL of Bt mite extracts (1.5 mg/mL) were horizontally electrophoresed in the first dimension at 200 V, at 14 °C for 30 min. The second dimension agarose gels were electrophoresed at 50 V at 14 °C and for 16 h, with the Bt antiserum (50 μL/mL) absorbed to a filter paper and laid on the gel according to Kuusi [15]. To identify the number of native cross-reactive epitopes, heterologous CIE gels using antiserum against Dp were run in a similar fashion. Electrophoresed gels were washed, pressed and stained with Coomassie Brilliant Blue R-250 (Sigma, St Louis, MO, USA).

CEIE was then performed according to the method described by Savolainen and Viander [16]. Both homologous

and heterologous CIE gels were processed further by incubating in Bt- and Dp-allergic patients' sera (1 : 2 dilution in PBS) overnight. These were then washed and incubated for 2 h with 1 mL of a antihuman IgE β-galactosidase-labelled antisera (Pharmacia, Uppsala, Sweden) (diluted 1 : 2 in PBS). The gels were then washed and the substrate solution containing 2-napthyl-β-D-galactopyranoside monohydrate (Sigma) added to detect the bound IgE precipitates.

Cross-reactivity studies using recombinant allergens

Dot blot inhibition studies A dot blot inhibition study using the major Dp allergens: native Der p 1, rDer p 2 (expressed in yeast) and rDer p 5 (expressed in *E. coli*) was carried out. The group 1, 2 and 5 allergens were chosen as they were the major sensitization Dp allergens identified by Western blotting in our population (> 50% of sera have specific IgE binding to these allergens [unpublished]). Native Der p 1 was used as the expressed recombinant allergen showed poor allergenic reactivity. The allergens were blotted in duplicate at 2 μg/dot (recombinant allergens) and 5 μg/dot (crude extract), on a nitrocellulose membrane. Sera were pre-absorbed (1 : 2) with 2 mg/mL of Dp and Bt extract separately, in the presence of 0.1% of nonfat milk. BSA was used as a control-inhibiting antigen. The blots were incubated overnight in pre-absorbed sera, respectively, at 4 °C and followed by washing with PBS-Tween 20 (0.05%). For IgE detection, the blots were then incubated with biotinylated-antihuman IgE for 1 h at room temperature. The membranes were stringently washed as before and were subsequently incubated with ExtrAvidin conjugated to horseradish peroxidase for another hour. The blots were visualized using the ECL plus Western blotting detection system (Amersham Life Science, Amersham, England).

Skin prick and intradermal testing To test sensitization and cross-reactivity between the homologous recombinant allergens rBlo t 5 and rDer p 5 (both expressed as fusion protein with glutathione-S-transferase), skin prick tests were carried out on 75 subjects and intradermal tests on 31 subjects. Crude extracts of Dp (Miles Laboratories, Elkhart, IN, USA), and Bt extracts (prepared from Bt cultures as described above, to a concentration of 20 μg/mL) were used to document sensitization to both mite species. Glutathione-S-transferase in buffer and histamine (1 mg/mL) were included as negative and positive controls, respectively. A reaction of greater than 4×4 mm diameter 20 min after prick was regarded as a positive prick test. Quantitative intradermal skin tests were carried out sequentially with 0.03 mL of 10-fold dilutions of extracts, from 10^{-5}–10^{0} μg/mL or until a 6×6 mm diameter weal, which was considered the positive end point. The end-point titration values for rBlo t 5 and rDer p 5 of the 31 subjects sensitized to both Dp and Bt extracts were analysed. Informed consent was obtained from all volunteers.

Results

Evaluation of cross-reactivity between allergens of Bt and Dp

FAST inhibition using crude allergen extracts The dose–response curves for FAST inhibition between extracts of Bt and *Dermatophagoides* spp. are presented in Fig. 1. When homologous protein extracts were used, $\approx$ 100% inhibition was achieved at protein concentrations of > 10 μg/mL. With heterologous FAST inhibitions, the maximum percentage inhibition achieved was < 60% and only at very high concentration of the heterologous protein (> 10 μg/mL). At 10 μg/mL of heterologous protein, $\approx$ 40% inhibition was achieved. Control inhibition with BSA only achieved a maximum of 30% inhibition at the maximum concentration of 5 mg/mL. This result suggests that only a low to moderate degree of cross-reactivity exists between allergens of Bt and *Dermatophagoides* spp.

Cross-immunoelectrophoresis and Cross enzyme-immunoelectrophoresis The homologous Bt CEIE gel incubated with rabbit anti-Bt serum and then patients' sera revealed the presence of at least 13 native IgE binding proteins (Fig. 2). The heterologous Bt CEIE gel (Fig. 2) incubated with Dp antiserum, and then sera of mite-allergic patients, showed the presence of at least four cross-reactive IgE binding antigens between the two mites.

Cross-reactivity studies using recombinant allergens To determine whether the cross-reactive antigens in Bt were related to the major Dp allergens, cross-reactive studies using the major Dp recombinant allergens were performed. Figure 3 shows the dot blots of nDer p 1, rDer p 2, rDer p 5, crude Dp and Bt extracts and a control protein (BSA) incubated in pooled sera of Bt- and Dp-positive patients. The pooled sera, after absorption with 2 mg/mL BSA, and without any prior absorption, was found to react to all allergen spots except to the control BSA protein. After absorption with 2 mg/mL crude Bt extract, serum still bound intensely to nDer p 1, rDer p 2, rDer p 5 and the crude Dp extract, but not to crude Bt spots. In contrast, no binding with the exception of the crude Bt spot, was detected after absorption with 2 mg/mL crude Dp extract. The results indicate that the cross-reactive allergens were not closely related to the major Dp allergens, namely Der p 1 and Der p 2 and Der p 5, despite 43% homology of the latter allergen with Blo t 5.

Cross-reactivity between Der p 5 and Blo t 5 Of the 75 subjects tested, skin prick tests showed that 45% of 46

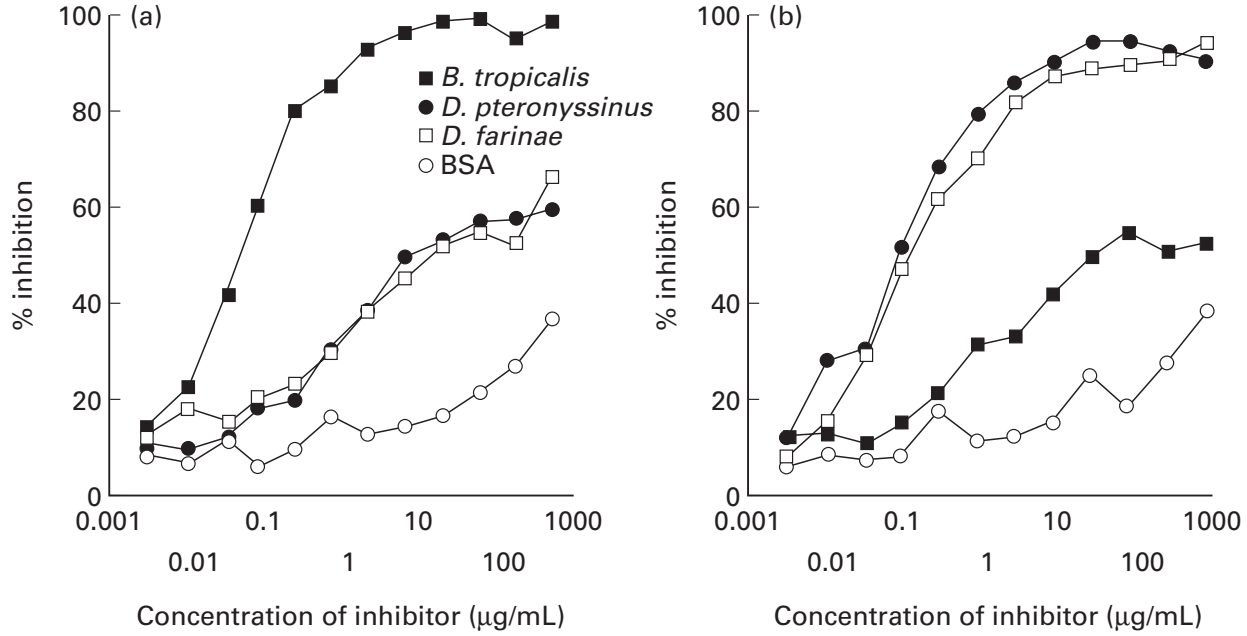

Fig. 1. FAST inhibition curve with (a) *Blomia tropicalis* and (b) *Dermatophagoides pteronyssinus* on the solid phase (bound on the wells). Extracts of *B. tropicalis*, *D. pteronyssinus*, *D. farinae* and bovine serum albumin were used as the inhibiting allergens.

individuals positive for crude Bt extracts reacted to rBlo t 5, but only 19% of these 46 reacted to rDer p 5. In addition, only 32% of the rBlo t 5-positive individuals reacted to rDer p 5. Intradermal skin test end-point titration using rDer p 5 and rBlo t 5 in 31 mite sensitized individuals also showed lower titration end-points (lower concentration end-point) with rBlo t 5 as compared to rDer p 5 ($P < 0.05$) (Fig. 4). In contrast, there was no difference in the distribution of the end points for crude Bt and Dp extracts (data not shown).

FAST inhibition tests further demonstrated only moderate cross-reactivity between rBlo t 5 and rDer p 5 (Fig. 5). With homologous cross inhibition, more than 80% inhibition was achieved at protein concentration of > 0.1 μg/mL but heterologous inhibition could only achieve a maximum

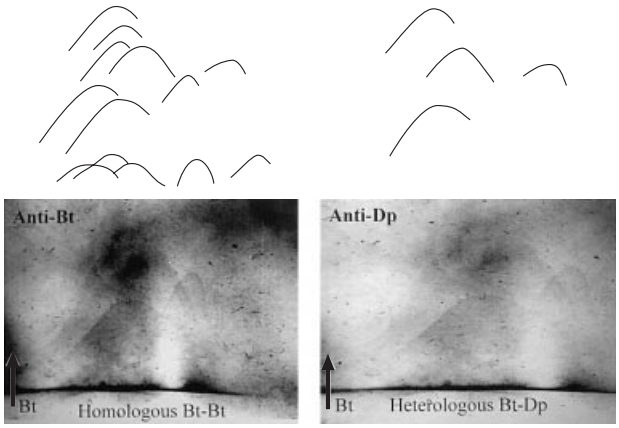

Fig. 2. CEIE gels. The heterologous CEIE gel (right panel) showing the cross-reactive components obtained by running Bt extract into a rabbit anti-Dp serum-soaked gel and further incubated in a pooled Dp positive sera. The homologous CEIE gel (left panel) was obtained running Bt extract into a rabbit anti-Bt serum-soaked gel and further incubated with pooled Bt positive sera.

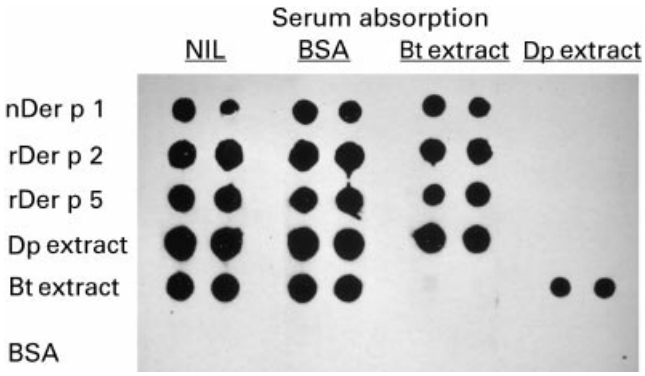

Fig. 3. Dot blots of nDer p 1, rDer p 2, rDer p 5, crude *Dermatophagoides* spp. and *Blomia* spp. extracts and a control protein (BSA) incubated in a pooled sera of *D. pteronyssinus*-positive patients (FAST 4 +) (a) without any absorption prior to incubation (NIL), (b) after absorbed with 2 mg/mL BSA, (c) after absorbed with a 2 mg/mL crude *Blomia tropicalis* extract, and (d) after absorbed with a 2 mg/mL crude *D. pteronyssinus* extract.

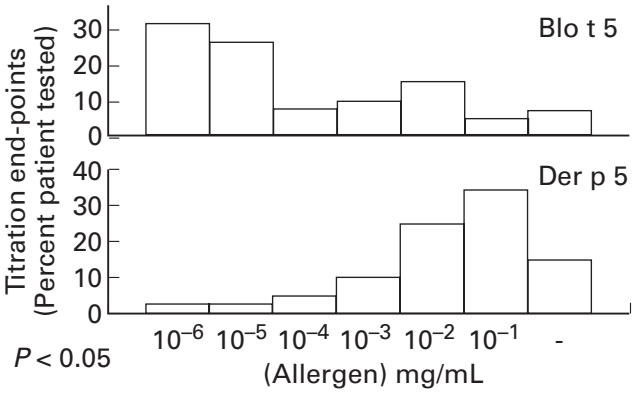

Fig. 4. Titration end-points of the intradermal skin test using rDer p 5 and rBlo t 5 allergen extracts in 31 atopic individuals in Singapore. Comparisons between the reactions to *Dermatophagoides pteronyssinus* and *Blomia tropicalis*, and rBlo t 5 and rDer p 5 were performed pair-wise**.

of ≈80% inhibition at concentration of >1 μg/mL. At 0.1 μg/mL heterologous inhibition, only less than 30% inhibition was achieved. Control inhibition with BSA could only achieve a maximum of 30% inhibition at the maximum concentration of 5 μg/mL.

Discussion

Unlike mites of *Dermatophagoides* spp., which are found world-wide, the domestic mite Bt have only been found in the tropics and subtropical areas. This mite has been found in house dust from homes in Hong Kong, Brazil, Venezuela, Columbia, Taiwan, Malaysia, Spain, Egypt and Florida in USA [17], but not in Thailand [18]. Our earlier studies have shown that Bt allergens are highly prevalent in Singapore [8]. Mite counting and identification, confirmed that it is the most prevalent mite in house dust, with Dp a close second (unpublished data). Testing with crude allergen extracts showed that the majority (>90%) of our local atopics are sensitized to both mites [7]. It was therefore important to define the allergens of Bt and determine their cross-reactivity with allergens from Dp. These findings would have important practical implications in determining the allergen panel to be used for diagnosis and immunotherapy of allergic diseases in Singapore.

Similar to studies from South America [19], IgE immunoblotting studies using local positive sera showed at least 28 allergenic components of Bt, with up to eight major allergens, indicating a substantial number of allergenic components (unpublished). Except for four possible allergenic components as shown in the CEIE studies, the results in this study revealed little or moderate cross-reactivity between allergens of Bt and Dp. In this paper, the cross-reactivity studies between crude allergens of Bt and Dp using FAST and immunoblot inhibition tests indicated low or moderate allergenic cross-reactivity between these mites. Similarly our CEIE studies, which provide information on the native protein also provided similar information. Similar results were also described by Arlian *et al*. using heterologous Bt CIE and crossed radioimmunoelectrophoresis (CRIE) with anti-Df and anti-Dp sera, where cross-reactivity was limited to only three and two antigenic peaks [20]. These data suggest that although these mites share some degree of allergenic cross-reactivity, it was likely that Bt also contains unique allergens with relatively low cross-reactivity with allergens of Dp. In support,

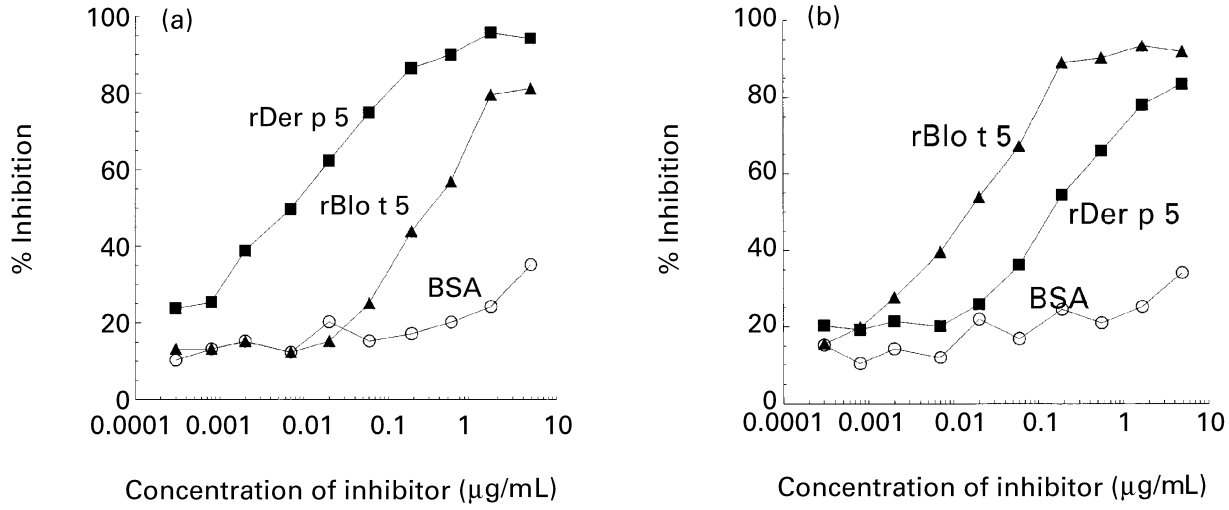

Fig. 5. FAST inhibition curve with (a) rBlo t 5 and (b) rDer p 5 and on solid phase (bound on the wells). Recombinant Blo t 5, Der p 5, and bovine serum albumin were used as the inhibiting allergens.

previous studies have also shown only moderate cross-reactivity between the crude extracts of Bt and other *Dermatophagoides* mite species, Df, and *D. siboney* [21,22].

The molecular cloning of *Dermatophagoides* spp. allergens has provided us with an understanding of the nature of mite allergens [23]. However, to date, only three Bt allergens have been cloned [11,24–26]. Both the Blo t 5 allergen [11] and the 310 bp partial clone [25] have homology with Der p 5, a Dp allergen. The remaining clones [25,26] did not have sequence homology with allergens of *Dermatophagoides* spp., supporting our notion that Bt contained allergenic components unrelated to Dp. The availability of these recombinant allergens provided us with the opportunity to study cross-reactivity between recombinant allergens. Of significance were the results of *in vitro* (FAST inhibition) and *in vivo* skin tests which showed that rDer p 5 and rBlo t 5 had only moderate cross-reactivity despite sharing 43% sequence homology. The lower potency and percentage of skin test reactivity of rDer p 5 is unlikely to be due to the conformational change in the recombinant Der p 5, as both were expressed as fusion protein with glutathione-S-transferase. Moreover, similar data were obtained in our laboratory when recombinant Der p 5 and Blot 5 produced in yeast were used in our recent skin test studies. In addition, Lin *et al*. conducted skin test studies on 45 Taiwanese asthmatic children using rDer p 5 from the same source, 51 percentage of the patients tested showed strong reactivity [27]. Our findings are also supported by studies of atopic populations in temperate regions, where subjects have been exposed to Dp and not Bt (Bt is not found in temperate climates) and reactivity to rBlo t 5 was much lower than reactivity to rDer p 5 [28–30]. Taken together, these results suggest conformational differences between these homologous allergens, and therefore dissimilar allergenic epitopes. Dot blot inhibition studies using nDer p 1, rDer p 2 and rDer p 5 also indicate that the Bt allergens have little cross-reactivity with these major Dp allergens.

In conclusion, Bt is one of the most important sources of mite allergens in Singapore. It is the most prevalent mite identified in house dust and our atopic population is highly sensitized to Bt allergens. Immunochemical and cross-reactivity studies show that Bt contains allergens that are either unrelated or have little cross-reactivity with the allergens of the other common local mite, Dp. These findings indicate that diagnostic and therapeutic strategies for respiratory allergic diseases in our population should include the allergens of Bt.

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