
Identification of the major allergenic components in *Blomia tropicalis* and the relevance of the specific IgE in asthmatic patients

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Background: *Blomia tropicalis* has been reported to be a clinically important allergen in house dust. High prevalence of sensitization to *B tropicalis* has been noted in asthmatic patients in Taiwan; however, the allergenic components and its impact on asthmatic patients remain to be clarified.

Objective: To analyze the prevalence of IgE against *B tropicalis* and each allergenic component in asthmatic patients.

Methods: A series of recombinant allergenic components were used for skin tests. The *B tropicalis* specific IgE in the serum were measured using the Pharmacia CAP System and immunoblot analysis.

Results: A total of 131 patients were included in this study: 44% of these 131 patients were allergic to *B tropicalis*, 43% of the 80 *B tropicalis*-sensitive patients were allergic to Blo t 5, and 75% of the 65 Blo t 5-sensitive patients were allergic to Blo t 5 fragment 3 (Blo t 5 70–117). The sera IgE binding activity to *B tropicalis* was repeatedly tested after *Dermatophagoides pteronyssinus* absorption, and results showed that most patients were concurrently sensitized to *D pteronyssinus* and *B tropicalis*. In addition, in 2 (18%) of 11 patients, the *B tropicalis* sensitization was caused by the cross-reactivity of *D pteronyssinus*.

Conclusion: A high prevalence of *B tropicalis* sensitization was detected in our asthmatic patients, and most of them were concurrently sensitized to *D pteronyssinus* and *B tropicalis*. The major allergenic component and its IgE binding fragments in Blo t 5 have been identified. These allergenic components can be used for the allergenic determination in *B tropicalis* and for further immunotherapy.

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INTRODUCTION

There is increasing evidence that sensitization to indoor allergens is a causative factor for the development of asthma among people who are genetically predisposed to making IgE antibody responses.^{1–7} *Blomia tropicalis* and *Dermatophagoides pteronyssinus* occur with high frequency and at high levels of infestation in houses from tropical and subtropical areas of the world. These regions include the southern states of the United States, Central and South America, Hong Kong, Taiwan, India, and Egypt.^{8–12} Epidemiologic studies in Taipei, Taiwan, have shown that sensitization to *Blomia* is strongly associated with asthma.¹³

The results of our previous report demonstrated that *B tropicalis* accounted for 44.3% of the total number of mites obtained from the household of asthmatic patients and that 73.3% of asthmatic patients were sensitized to *B tropicalis*.¹³ However, concurrent sensitization to *B tropicalis* and *D pteronyssinus* occurred in 63.3% of the asthmatic patients sensitized to both species of mites.¹³ Inhibition studies sug-

gested that *B tropicalis* allergens are species specific, and the extent of cross-reactivity between *B tropicalis* and *D pteronyssinus* was estimated at 30% to 43%.^{14–17} Recently, most of the allergenic components of *B tropicalis* have been cloned and sequenced, and the patterns of sensitization to *B tropicalis* and its recombinant allergens in tropical populations (Indonesia, Singapore, Taiwan, and India) have been described.¹⁸ Despite the high prevalence and high variability of *B tropicalis* sensitization (40% in India and 82% in Indonesia) in atopic populations, there are no data for the asthmatic population. The objective of this study is, therefore, to clarify the prevalence of allergenicity of each allergenic component, particularly *B tropicalis*, using both the skin prick test and specific IgE measurement.

MATERIALS AND METHODS

Patients

Allergic, asthmatic patients who attended the Allergy Clinic at Cathay General Hospital, Taipei, Taiwan, were recruited for this study. Allergic asthma was defined as a history of reversible obstructive airway disease associated with exacerbation resulting from allergen exposure and concomitant skin reactivity to exacerbating allergens. All patients were sensitive to mites and had positive skin test results and mite-specific IgE as determined using the Pharmacia CAP System (Uppsala, Sweden). Serum samples were drawn and stored at –70°C before testing. The study was approved by the Human

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Research Ethics Committee of Cathay General Hospital and informed, written consent was obtained from all participants.

Preparation of *B tropicalis* Allergens

All recombinant *B tropicalis* allergens were prepared at the University of Singapore at a concentration of 25 µg/mL. Blo t A2, Blo t 2, Blo t 3, Blo t 5, Blo t 10, Blo t 11, Blo t 12, Blo t 13, and fragments of Blo t 5 expressed in *Escherichia coli* and Blo t 4, Blo t 6p, and Blo t 6m expressed in *Pichia pastoris* were used for percutaneous skin prick test and immunoblot analysis. Blo t 5 has 5 IgE binding fragments, which contain 1 to 117 peptide fragments: fragment 1, 1 to 45; fragment 2, 41 to 80; fragment 3, 70 to 117; fragment 4, 1 to 80; and fragment 5, 41 to 117. Crude *B tropicalis* were extracted by homogenizing after resuspending in phosphate-buffered saline containing aprotinin (0.1 IU/mL; Sigma Chemicals, St. Louis, MO) and phenylmethylsulfonyl fluoride (1 mmol/L; Sigma). After centrifugation at 10,000g for 30 minutes, the supernatant was dialyzed against 0.05 mol/L ammonium hydrogen carbonate (pH 8.0). It was then freeze-dried in aliquots and stored at 4°C until used. The protein content was determined using the Lowry method, with bovine serum albumin as the standard.

Skin Prick Test and Measurement of Specific IgE

All recombinant *B tropicalis* allergens and crude *B tropicalis* extract were used for skin prick testing at the concentration of 0.06 µg/µL after the end point titration using 10-fold dilutions. Both purified Blo t 5 and commercially available *D pteronyssinus* were used in this study. Histamine, 1 mg/mL, and 50% glycerin solution buffer were used as positive and negative controls, respectively. The skin was prick tested with a disposable needle (Greer Derma Pit system; Greer Laboratories, Lenoir, NC). Each allergen extract was applied 20 mm apart on the polar surface of the forearm. Fifteen minutes after application, the reactions were read and the sizes (in millimeters) of wheals and flares were recorded. Reactions of 3 × 3 mm or greater were considered positive.

Measurements of specific IgE to *B tropicalis* were performed using the Pharmacia CAP System. The assay procedure was automatically performed, and the results were calibrated against the World Health Organization standard for IgE. The concentration ranged from 0.35 to 100 kU/L. The titers of antigen-specific IgE were further classified as follows: class 1, 0.35 to 0.7 kU/L; class 2, 0.7 to 3.5 kU/L; class 3, 3.5 to 17.5 kU/L; class 4, 17.5 to 50.0 kU/L; class 5, 50 to 100 kU/L; and class 6, more than 100 kU/L.

Immunoblot Analysis

Immunoblot analysis of Blo t 5 recognized by *B tropicalis* using human IgE antibodies was performed according to the standard method.¹³ The total protein concentration used for the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was 20 µg in 20 µL. The Blo t 5 in the SDS-PAGE sample buffer (containing 2% SDS and 5% 2-mercaptoethanol) was boiled for 5 minutes, separated on a 12.5% SDS-polyacrylamide gel, and then transferred electro-

phoretically onto a polyvinylidene difluoride membrane (0.45 µm; Millipore, Bedford, MA). All of the serum samples were used after absorption (16 hours at 4°C) with or without *D pteronyssinus*. After being blocked with 1% skim milk in Tris-buffered saline (TBS; 20 mmol/L Tris, 500 mmol/L sodium chloride, pH 7.5), the blots were incubated with diluted serum samples (1 in 5 dilution in TBS containing 0.05% Tween 20 [TBST] and 1% skim milk) for 16 hours at 4°C. After washing with TBST, the blots were incubated with alkaline phosphate–conjugated monoclonal antihuman IgE antibodies (Pharmingen, San Diego, CA) for 1.5 hours at room temperature. After washing, the blots were developed in a substrate solution of carbonate buffer (0.1 mol/L sodium bicarbonate, 1.0 mol/L magnesium chloride, pH 9.8) containing 0.35 mol/L 5-bromo-4-chloro-3-indolyl phosphate β-toluidine salt and 0.37 mol/L nitroblue tetrazolium chloride for approximately 30 minutes. After washing with water, the deposition of the purple stain demonstrating antigen-antibody

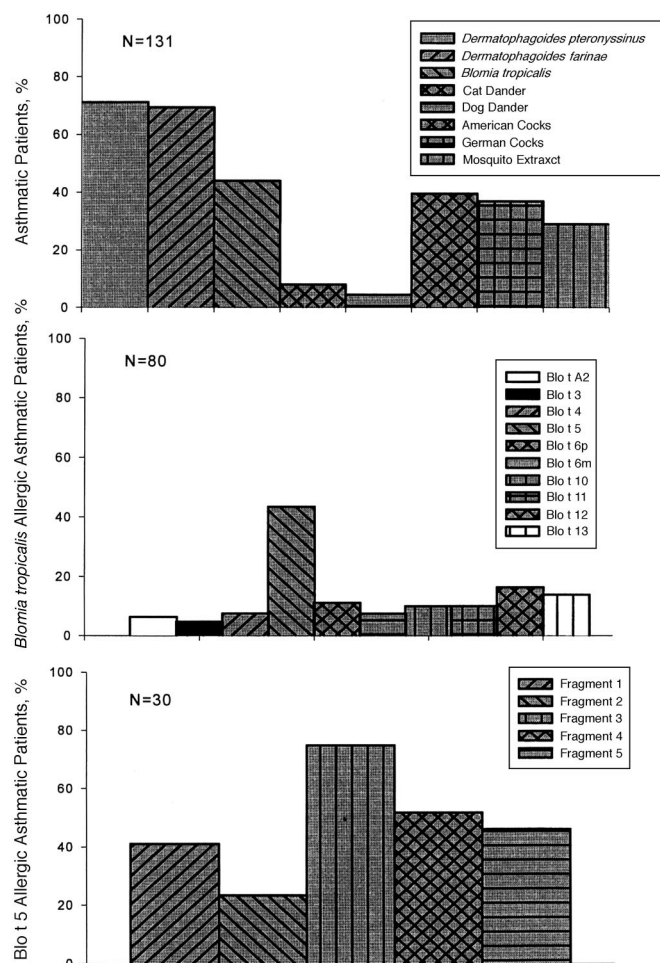


Figure 1. The positive rate of skin test reaction to *Blomia tropicalis* (top), *B tropicalis* allergen component (middle), and Blo t 5 fragments (bottom) in asthmatic patients.

Table 1. Percentage of Fragment Allergy in the Different Age Groups of *Blomia tropicalis*-Allergic Individuals

Age group, y	Blo t 2	Blo t 4	Blo t 5	Blo t 10	Blo t 11	Blo t 12	Blo t 13
≤20 (n = 32)	9*	6	28	9	9	22	16
21-30 (n = 12)	8	0	42	17	17	0	17
≥31 (n = 21)	0	10	48	5	5	19	14

* Percentage of patients who were allergic to *B tropicalis* in the same age groups.

binding was recorded photographically. For the negative controls, serum samples obtained from cord blood were used.

RESULTS

Prevalence of Positive Skin Reaction to *B tropicalis* and Its Allergenic Components

A total of 131 asthmatic patients were included in the skin test study. The results showed that 71% had positive skin reactions to *D pteronyssinus*, 69% to *Dermatophagoides farinae*, 44% to *B tropicalis*, 37% to American cockroach, 36% to German cockroach, 4% to dog dander, 8% to cat dander, and 29% to mosquito (Fig 1, top).

When the *B tropicalis*-allergic, asthmatic patients were tested with the allergenic components of *B tropicalis*, the results of the study of 80 patients showed that 43% had positive reactions to Blo t 5, 16% to Blo t 12, 14% to Blo t 13, and 11% to Blo t 6p. The prevalence of the other allergenic components were all less than 10% (Fig 1, middle). Of the patients, 10% had negative skin test results to all allergenic components except to the crude extract of *B tropicalis*.

Different age groups of patients who were allergic to *B tropicalis* were analyzed for their skin test reactivity to *B tropicalis* fragments. A total of 65 patients were recruited for this study. The results showed that patients who were 20 years or younger had a high percentage of allergy to Blo t 5 (28%) and Blo t 12 (22%), whereas the patients older than 20 years had a high percentage of allergy to Blo t 5 (42% for those between 21 and 30 years old and 48% for those older than 30 years) (Table 1). When the Blo t 5 allergenic, asthmatic patients were tested with the fragments of Blo t 5, the results showed that 75% had positive reactions to fragment 3, 52% to fragment 4, and less than 50% to fragments 1, 2, and 5 (Fig 1, bottom).

Cross-reactivity of Mite Specific IgE to *B tropicalis* and *D pteronyssinus*

A total of 21 asthmatic patients with positive skin test results to *D pteronyssinus* and *B tropicalis* were recluded for the mite specific IgE measurements in the serum. The results showed that most of the patients were moderately to severely allergic to both species of dust mites (Table 2).

Eleven asthmatic patients were selected for the *D pteronyssinus* absorption study. The results showed that 2 individuals (cases 3 and 8), whose *B tropicalis* specific IgE antibodies were lower than that of *D pteronyssinus*, were totally

absorbed by *D pteronyssinus* and 3 individuals (cases 2, 5, and 11), whose *B tropicalis* specific IgE antibodies were equal or higher than that of *D pteronyssinus*, were not absorbed by *D pteronyssinus*. Despite the significant decrease of *D pteronyssinus* specific IgE, the titer of *B tropicalis* specific IgE was only slightly decreased after *D pteronyssinus* absorption (Table 3). The same results were shown in the Western blotting. There was only one Blo t 5 specific IgE (case 2b) that could be absorbed by *D pteronyssinus* (Fig 2).

DISCUSSION

The pattern of sensitization to *B tropicalis* and its recombinant allergens has been identified. The prevalence of sensitization to *B tropicalis* was 44% in asthmatic patients. Among the *B tropicalis*-sensitized patients, 43% tested positive to Blo t 5 and 16% tested positive to Blo t 12. For the group younger than 20 years, the percentage of patients sensitive to Blo t 5 was lower and the percentage sensitive to Blo t 12 was higher than that of patients older than 20 years. The major allergenic component in Blo t 5 appeared to be fragment 3 (70 to 117 peptide fragments), and 75% of the Blo t 5-sensitized asthmatic patients had positive reactions.

It has been reported that *B tropicalis* is an important allergen in tropical and subtropical areas.⁹⁻¹² Our results

Table 2. Comparison of Specific IgE to *Blomia tropicalis* and *Dermatophagoides pteronyssinus*

Patient	Age, y	Specific IgE (class)*	
		<i>B tropicalis</i>	<i>D pteronyssinus</i>
A	38	3	4
B	8	5	6
C	12	5	6
D	16	4	6
E	32	4	4
F	12	4	4
G	8	4	6
H	8	4	6
I	12	5	4
J	14	4	6

* The titers of mite specific IgE were classified as follows: class 1, 0.35 to 0.7 kU/L; class 2, 0.7 to 3.5 kU/L; class 3, 3.5 to 17.5 kU/L; class 4, 17.5 to 50 kU/L; class 5, 50 to 100 kU/L; and class 6, more than 100 kU/L.

Table 3. Comparison of Specific IgE to *Blomia tropicalis* and *Dermatophagoides pteronyssinus* before and after *D pteronyssinus* Absorption

Patients	Age, y	Specific IgE, kU/L			
		Before <i>D pteronyssinus</i> absorption*		After <i>D pteronyssinus</i> absorption	
		<i>B tropicalis</i>	<i>D pteronyssinus</i>	<i>B tropicalis</i>	<i>D pteronyssinus</i>
1	22	16.4	88.1	1.43	1.03
2	14	67.5	37.0	25.6	0.39
3	36	2.2	16.9	<0.35	0.36
4	14	11.5	59.9	1.55	1.45
5	32	9.06	4.22	2.13	<0.35
6	11	5.55	37.3	0.56	0.73
7	12	39.4	>100	5.5	7.79
8	31	0.81	4.51	>0.35	<0.35
9	12	5.33	76.2	0.93	1.43
10	12	26.9	>100	1.88	3.21
11	12	13.5	48.5	4.73	<0.35

* Patients' serum samples were incubated with *D pteronyssinus* extract at room temperature for 30 minutes then measured by the CAP system as described in the "Materials and Methods" section.

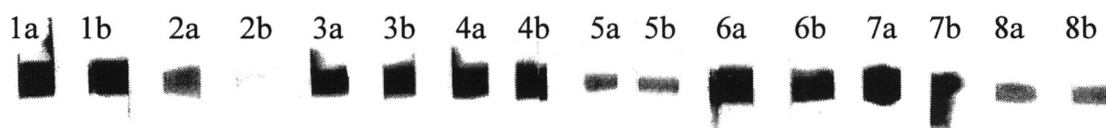


Figure 2. Comparison of specific IgE to Blo t 5 after buffer (a) and *Dermatophagoides pteronyssinus* (b) absorption. Lanes 1 to 8 show the patients' serum samples as described in the "Material and Methods" section.

indicated that *B tropicalis*, as well as *D pteronyssinus* and *D farinae*, was the major source of allergen that can cause skin test positive reactions in patients with bronchial asthma. Since most *B tropicalis* allergens are species specific, and it has been reported that Blo t 1, Blo t 2, and Blo t 5 share 30% to 50% amino acid sequence homology with Der p 1, Der p 2, and Der p 5,^{18,19} it could be suggested that the sensitization of *B tropicalis* was the result of cross-reactivity with *D pteronyssinus*. Currently, *B tropicalis* specific IgE can be measured using commercial kits with the Pharmacia CAP system. Although serum samples from skin test results positive to *B tropicalis* can further be confirmed by the presence of *B tropicalis* specific IgE, it cannot exclude the possibility of concurrent sensitization of *B tropicalis* with *D pteronyssinus*. The absorption studies showed that most asthmatic patients were concurrently sensitized by *B tropicalis* and *D pteronyssinus*. In the study, only 3 of 11 of the *B tropicalis* specific IgE samples were completely absorbed by *D pteronyssinus*. Most of the *B tropicalis* specific IgE samples were only partially absorbed by *D pteronyssinus*. In the recent study, by using a large panel of asthmatic serum samples and a combination of in vitro and in vivo assays, the major allergen of *B tropicalis* in tropical and subtropical regions, Blo t 5, exhibits low levels of IgE cross-reactivity with homologous Der p 5.²⁰⁻²³ Additionally, our previous surveys demonstrated that homes with *B tropicalis* are usually cohabited by *D pteronyssinus* and *D farinae*.¹³ These findings suggest that most patients were concurrently sensitized by *B tropicalis* and *D pteronyssinus*.

In the allergenic component and fragment studies, there were 8 patients (10%) who had negative skin test results to all allergenic components of *B tropicalis*, despite the fact that all asthmatic patients had positive skin test reactions to *B tropicalis*. These results might be due to either the low allergenicity of these allergenic components or the cross-reactivity between the crude mite extracts of *B tropicalis* and *D pteronyssinus*.

The results showed that Blo t 5 was the major allergen in *B tropicalis*, and 43% of asthmatic patients had skin test reactions to Blo t 5. Similarly, researchers reported that large proportions of *B tropicalis*-sensitized, atopic individuals had positive skin test reactions to Blo t 5 in other Asian countries (55.7% in Singapore and 57.5% in Indonesia).²⁴ Despite the fact that different age groups might be sensitized by different major allergens of *B tropicalis*, Blo t 5 remained the most important allergen in *B tropicalis*. These findings indicated that Blo t 5 hypersensitivity may at least play a part in the pathogenesis of atopic diseases in the *B tropicalis*-sensitive asthmatic patients. The high percentage of skin test reactions to fragment 3 suggested that the C-terminal of Blo t 5 may be the major IgE binding site. However, the detail residuals of the IgE binding site need further clarification.

In conclusion, the prevalence of *B tropicalis* hypersensitivity in asthmatic patients is very high. The pattern of its allergenic components and its allergenicity were clarified. The major allergenic determinant of Blo t 5 can be used as diagnostic tools for *B tropicalis*-allergic, asthmatic patients.

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