

Jaw-Ji Tsai^a
Huai Hui Wu^b
Horng-Der Shen^c
Err-Lieh Hsu^b
Soo-Ray Wang^d

^a Department of Allergy and Clinical
Immunology, Cathay General Hospital,

^b Graduate Institute of Plant Pathology and
Etymology, National Taiwan University,

^c Department of Medical Research,
Veterans' General Hospital and

^d Department of Allergy, Immunology and
Rheumatology, Veterans' General Hospital,
National Yang-Ming University,
Taipei, Taiwan, ROC

Sensitization to *Blomia tropicalis* among Asthmatic Patients in Taiwan

Abstract

House dust mites have been reported as one of the most important allergens in Taiwan especially in asthmatic patients. This study was conducted to determine the allergenicity of *Blomia tropicalis* and sensitization of asthmatic patients in Taiwan. Serial dust samples were collected every month between July 1993 and June 1994 from 13 houses of mite-allergic patients. About 1 m² surface area of a quilt was vacuumed. The floating method was used to collect mites, then identification and counting were performed. Results showed that *Dermatophagoides pteronyssinus* and *B. tropicalis* were the two most common species of mites found in allergenic patients' houses in Taipei. *D. pteronyssinus* accounted for 52.1% of the total number of mites and was found in every house. *B. tropicalis*, although not present in every sample, accounted for 44.3% of the total number of mites. The skin test positive reaction to *B. tropicalis*, *D. pteronyssinus* and *Dermatophagoides farinae* were 73.3, 88.3 and 85.0% as determined from 60 allergic patients who attended our allergy clinics. The extract prepared from *B. tropicalis* was used to determine the allergenicity and contained at least 30 protein components when silver stained. The most frequently detected allergens were proteins with molecular weights of 14.3, 106.5, 94.0, 72.0, 91.9, 63.7, 100.3, 43.6, 27.3, 62.0, 34.7, 18.3, 41.1 and 21.9 kD. The frequency of IgE binding of patient sera to those proteins were 87.0, 65.2, 56.5, 43.4, 39.1, 39.1, 34.8, 30.4, 30.4, 17.4, 17.4, 17.4, 13.0 and 8.7%. The results from immunoblot inhibition showed that there was IgE cross-reactivity among the *B. tropicalis* and *D. pteronyssinus*. However, there were two major allergenic components of *B. tropicalis* not inhibited by *D. pteronyssinus* with molecular weights of about 14.3 and 27.3 kD. The use of *B. tropicalis* extract for diagnostic purposes to identify patients with specific sensitivity should be considered in Taiwan.

Key Words

Bronchial asthma

Blomia tropicalis

Dermatophagoides pteronyssinus

Introduction

Sensitization to house dust mites is an important risk factor for the development of bronchial asthma [1, 2]. A previous study demonstrates that house dust mite is highly prevalent in Taiwan, where the climate is subtropical [3]. More than 80% of asthmatic patients are allergic to *Dermatophagoides pteronyssinus* as determined by skin test and *D. pteronyssinus*-specific IgE [4]. Several species of mites have been identified from house dust samples, including *D. pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis* in Taiwan [3]. Recently, *B. tropicalis* has been reported to be a clinically important allergic component of house dust [5]. The positive skin tests and/or presence of circulat-

goides *pteronyssinus* as determined by skin test and *D. pteronyssinus*-specific IgE [4]. Several species of mites have been identified from house dust samples, including *D. pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis* in Taiwan [3]. Recently, *B. tropicalis* has been reported to be a clinically important allergic component of house dust [5]. The positive skin tests and/or presence of circulat-

KARGER

E-Mail karger@karger.ch
Fax +41 61 306 12 34
www.karger.com

© 1998 S. Karger AG, Basel
1018–2438/98/1152–0144 \$15.00/0

This article is also accessible online at:
<http://BioMedNet.com/karger>

Correspondence to: Dr. Jaw-Ji Tsai
Department of Allergy and Clinical Immunology
Cathay General Hospital
No. 280, section 4, Jen-Ai Road
Taipei, Taiwan (ROC)

ing IgE antibodies to *B. tropicalis* in sera from individuals have been demonstrated after exposure to *B. tropicalis* [6, 7]. Several investigators, using radioallergosorbent test inhibition and crossed radioimmuno-electrophoresis assays on patients who were allergic to *D. farinae* and *B. tropicalis*, found only cross-reactivity between *D. farinae* and *B. tropicalis* [8, 9], and the specificity of the in vivo allergic reaction to *B. tropicalis* has been demonstrated. Recently, *B. tropicalis* allergen Blo t 5 has been identified by the molecular cloning technique, and showed 43% sequence homology to the *D. pteronyssinus* allergen Der p 5 [10]. Therefore, the purpose of this study was to survey dust mite prevalence in the homes of people with allergic asthma living in Taipei, Taiwan and to clarify the clinical significance of *B. tropicalis* by means of skin tests and immunoblot analysis. The cross-reactivity of *B. tropicalis* with *D. pteronyssinus* was also investigated by immunoblot inhibition analysis.

Materials and Methods

Subjects

Sixty allergic asthmatic patients who attended allergy clinics at the Cathay General Hospital, Taipei 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 allergens. All patients were sensitive to mites in terms of positive skin test and a positive mite-specific IgE as determined by the Pharmacia CAP system (Pharmacia, Sweden). Blood samples were drawn and sera stored at -70°C before use.

Collection of Dust and Counting of Mites from Houses of Asthmatic Patients

Random samples were collected every month between July 1993 and June 1994 from 13 households with asthmatic patients located in Taipei, Taiwan. Samples were collected from quilts, mattresses, pillows, carpets and sofas. About 1 m^2 of surface area of each site was vacuumed for 1 min, following which each dust sample in the container of the vacuum cleaner was brushed completely into a plastic bag and analyzed. 0.1 g of dust from each sample was added to 25 ml of normal saline. The suspension was shaken and left to settle for 5 min. The suspension was then poured through a $45\text{-}\mu\text{m}$ -pore filter and the material remaining on the filter was rinsed in an integrid petri dish. Mites were counted under an inverted phase contrast microscope and the mite concentration was expressed as the number of mites per gram of dust. For mite identification, mites were picked up by hand using a 25-gauge needle under an incident light microscope, then immersed in polyvinyl alcohol solution on a microscope slide and covered with a cover glass.

Preparation of Mite Extracts

B. tropicalis collected from house dust samples from the houses of allergic patients were cultured in our laboratory with autoclaved wheat and yeast powder. Crude extracts of *B. tropicalis* were prepared by the

method of Nakada et al. [11]. Extracts were made from isolated *B. tropicalis*. The lyophilized mite powder was defatted with ether (10 ml/g of mite) for 48 h, then homogenized and stirred continuously in 2 ml phosphate buffer saline for 36 h. After centrifugation (10,000 g, 5 min), the supernatant was filtered through a $0.22\text{ }\mu\text{m}$ membrane. The protein content was determined by Lowry's method using bovine serum albumin as standard [12]. The protein concentration of *B. tropicalis* was adjusted to 0.2 mg/ml for the skin prick test. Standardized extracts of *D. pteronyssinus* and *D. farinae* (10,000 BU/ml, 0.28 mg/ml) were purchased from Greer Laboratory (Lenoir, Calif., USA) for both skin test and immunoblot analysis.

Skin Prick Test and Measurement of Specific IgE to *D. pteronyssinus*

The skin was prick-tested with a disposable needle (Hollister-stier). Each allergen extract was applied 20 mm apart on the volar surface of the forearm. 15 min later, the reactions were read and the sizes (in millimeters) of wheals and flares were recorded. Reactions of $3 \times 3\text{ mm}$ or greater were considered positive. Specific IgE to *D. pteronyssinus* was measured using the Pharmacia CAP system. The assay procedure was automatically performed and the results were expressed as kU/l (with calibration against the WHO standard for IgE). The concentration range was from 0.35 to 100 kU/l.

Immunoblot Analysis

The immunoblot analysis of component of *B. tropicalis* recognized by human IgE antibodies was carried out according to the standard method [13]. The total protein concentration used for SDS-PAGE was 200 μg . *B. tropicalis* extract in SDS-PAGE sample buffer (containing 2% SDS and 5% 2-mercaptoethanol), which were boiled for 5 min, were then separated on a 12.5% SDS-polyacrylamide gel and transferred electrophoretically onto polyvinylidene difluoride (PVDF) membrane ($0.45\text{ }\mu\text{m}$, Millipore, Bedford, Mass., USA). After being blocked with 1% skim milk in Tris-buffered saline (TBS, 20 mmol/l of Tris, 500 mmol/l of NaCl, pH 7.5), the blots were incubated with diluted serum samples (1.5 dilutions in TBS containing 0.05% Tween 20 (TBST) and 1% skim milk) for 16 h at 4°C . After washing with TBST, the blots were incubated with alkaline phosphatase-conjugated monoclonal antihuman IgE antibodies (Pharmingen, San Diego, Calif., USA) for 1.5 h at room temperature. Following washing procedures, the blots were developed in a substrate solution of carbonate buffer (0.1 M NaHCO_3 , 1.0 M MgCl_2 , pH 9.8) containing 0.35 M 5-bromo-4-chloro-3-indoyl phosphate *p*-toluidine salt (BCIP) and 0.37 M *p*-nitro blue tetrazolium chloride (NBT) for about 30 min. After washing with water, the deposition of the purple stain which demonstrated antigen-antibody binding was recorded photographically. For control, serum samples obtained from cord blood were used.

Immunoblot Inhibition

To test the cross-reactivity between *B. tropicalis* and *D. pteronyssinus*, immunoblot inhibition was performed. An IgE-positive serum sample was mixed individually with an equal volume of *B. tropicalis* (200 μg /ml) and *D. pteronyssinus* (10,000 BU/ml) by end-over-end rotation at 4°C for 1 h. The PVDF blots were then incubated individually with the IgE-positive serum or with a different serum extract mixture at 4°C for 16 h. For control, the blots were incubated with an equal volume of *Penicillium notatum* (200 μg /ml) obtained from Greer Laboratories (Lenoir, Calif., USA). The blots were washed and incubated with alkaline phosphatase-conjugated monoclonal antihuman IgE antibodies and developed with the enzyme substrate as described above.

Table 1. Number of mites (/g dust) isolated from patients' houses in Taipei, Taiwan (mean $\pm$ SE, n = 13)

Species	Mites											
	July 1993	Aug.	Sep.	Oct.	Nov.	Dec.	Jan. 1994	Feb.	March	April	May	June
<i>D. pteronyssinus</i>												
Pillow	45 $\pm$ 6	105 $\pm$ 16	78 $\pm$ 07	99 $\pm$ 11	33 $\pm$ 4	13 $\pm$ 1	7 $\pm$ 1	10 $\pm$ 2	70 $\pm$ 9	28 $\pm$ 3	27 $\pm$ 4	68 $\pm$ 6
Quilt	191 $\pm$ 50	217 $\pm$ 28	165 $\pm$ 13	69 $\pm$ 5	26 $\pm$ 4	54 $\pm$ 21	55 $\pm$ 11	17 $\pm$ 3	79 $\pm$ 12	265 $\pm$ 64	57 $\pm$ 13	106 $\pm$ 18
Mattress	73 $\pm$ 14	357 $\pm$ 30	137 $\pm$ 23	150 $\pm$ 16	22 $\pm$ 5	6 $\pm$ 1	21 $\pm$ 5	28 $\pm$ 3	13 $\pm$ 1	74 $\pm$ 8	45 $\pm$ 5	69 $\pm$ 13
Carpet	14 $\pm$ 6	115 $\pm$ 32	9 ^a	50 ^a	33 ^a	64 ^a	53 ^a	70 ^a	0	31 ^a	4 ^a	1 ^a
Sofa	66 $\pm$ 6	327 $\pm$ 67	92 $\pm$ 34	71 $\pm$ 18	13 $\pm$ 29	64 ^a	20 ^a	25 $\pm$ 9	23 $\pm$ 4	89 $\pm$ 47	100 $\pm$ 45	35 $\pm$ 10
<i>D. farinae</i>												
Pillow	6 $\pm$ 3	183 $\pm$ 16	5 ^a	0	0	0	0	8 $\pm$ 2	0	0	0	50 ^a
Quilt	3 ^a	18 $\pm$ 14	57 $\pm$ 37	23 $\pm$ 9	9 $\pm$ 1	0	6 $\pm$ 3	7 ^a	20 ^a	80 $\pm$ 50	22 $\pm$ 12	235 $\pm$ 128
Mattress	28 $\pm$ 7	113 $\pm$ 41	6 $\pm$ 3	27 $\pm$ 10	6 $\pm$ 3	20 ^a	0	8 $\pm$ 3	0	0	26 $\pm$ 9	40 $\pm$ 14
Carpet	0	0	0	0	0	0	0	45 ^a	0	0	1 ^a	0
Sofa	10 ^a	0	0	0	70 ^a	0	0	20 ^a	118 $\pm$ 72	24 ^a	0	21 $\pm$ 4
<i>B. tropicalis</i>												
Pillow	95 $\pm$ 16	179 $\pm$ 25	75 $\pm$ 9	17 $\pm$ 1	10 ^a	0	0	0	10 $\pm$ 0.1	10 ^a	21 $\pm$ 0.4	89 $\pm$ 40
Quilt	55 $\pm$ 7	264 $\pm$ 31	63 $\pm$ 11	11 $\pm$ 2	2 $\pm$ 0.1	0	0	5 ^a	0	63 $\pm$ 25	3 ^a	10 $\pm$ 1
Mattress	115 $\pm$ 11	327 $\pm$ 32	207 $\pm$ 42	77 $\pm$ 7	10 $\pm$ 0.1	15 $\pm$ 4	10 ^a	5 $\pm$ 1	2 ^a	10 ^a	0	26 $\pm$ 6
Carpet	21 $\pm$ 8	108 $\pm$ 32	69 ^a	138 ^a	0	10 ^a	0	0	0	0	0	0
Sofa	116 $\pm$ 24	374 $\pm$ 63	127 $\pm$ 40	93 $\pm$ 24	26 $\pm$ 17	50 ^a	3 ^a	2 ^a	20 ^a	10 ^a	30 ^a	64 $\pm$ 31
<i>Miscellaneous</i>												
Pillow	2 $\pm$ 0.3	45 ^a	125 $\pm$ 71	0	0	0	0	0	0	0	0	0
Quilt	24 $\pm$ 7	21 $\pm$ 4	9 $\pm$ 4	4 ^a	5 $\pm$ 1	1 ^a	5 $\pm$ 2	0	0	0	0	0
Mattress	2 $\pm$ 0.4	58 $\pm$ 7	13 $\pm$ 1	45 $\pm$ 19	0	0	0	0	0	0	0	0
Carpet	45 $\pm$ 2	11 $\pm$ 2	69 ^a	0	0	20 ^a	0	0	0	0	0	0
Sofa	74 $\pm$ 10	13 $\pm$ 3	44 $\pm$ 11	0	0	0	0	0	0	0	0	0

^a n = 1.

Results

Seasonal Variations of Mite Density and Species Differences

The species and frequency of mites are shown in table 1. The two most common species of mites found in Taipei were *D. pteronyssinus* and *B. tropicalis*. *D. pteronyssinus* accounted for 52.1% of the total number of mites and was found in every house. *B. tropicalis*, although not present in every sample, accounted for 44.3% of the total number of mites. The mean mite density was highest from August to November and lowest in January (table 1).

Skin Test Reactivity to Various Groups of Mites

A total of 70 subjects (10 normal subjects and 60 asthmatics) were recruited for the skin prick test. None of the normal subjects showed a positive reaction to the mite extracts. The other 60 asthmatic patients were skin-tested to

evaluate the prevalence of allergic reactions to *B. tropicalis*, *D. pteronyssinus* and *D. farinae*. The results were 73.3, 88.3 and 85.0%, respectively. Positive skin test results to a single mite individually occurred in 4, 3 and 1 subjects for *B. tropicalis*, *D. pteronyssinus* and *D. farinae*, respectively. Positive skin test results to two mite species concomitantly occurred in 9 subjects to *D. pteronyssinus* and *D. farinae*, in 1 to *B. tropicalis* and *D. pteronyssinus* and in 1 to *B. tropicalis* and *D. farinae*. Positive skin tests to both *D. farinae* and *D. pteronyssinus* occurred in a total of 49 subjects, 38 of whom had positive skin test results to *B. tropicalis* (table 2).

Allergenic Components of *B. tropicalis* Extract

For the analysis of the components of *B. tropicalis* recognized by IgE antibodies, protein components of the mite extracts were separated by SDS-PAGE. The silver-stained protein profile of the crude extract is illustrated in figure 1. At least 30 different components were observed, with mo-

Table 2. Summary of the skin test results

Results	Number
Positive tests to at least one species of mite	60 (100)
Positive tests to Bt	44 (73.3)
Positive tests to Dp	53 (88.3)
Positive tests to Df	51 (85.0)
Positive tests to Bt alone	4 (6.7)
Positive tests to Dp alone	3 (5.0)
Positive tests to Df alone	1 (1.7)
Positive tests to Bt, Dp and Df	38 (63.3)
Positive tests to Dp and Df only	9 (15.0)
Positive tests to Bt and Dp only	1 (1.7)
Positive tests to Bt and Df only	1 (1.7)

Figures in parentheses represent percentage. Bt = *B. tropicalis*; Dp = *D. pteronyssinus*; Df = *D. farinae*.

lecular weights ranging from 10 to 100 kD in the crude extract. Sera of 23 allergic asthmatic patients were selected for immunoblotting to identify IgE-binding components in extracts of *B. tropicalis*. All patients showed a skin test positive to at least one type of house dust mite.

The results showed that there were no signals detected from the strips when control samples of cord blood sera were used. When using patients' sera, there were at least 14 IgE binding proteins in the immunoblot performed (fig. 2). Most sera detected more than three allergens. The maximum number of allergens detected by a single serum was 13 in lane 19 (fig. 2). The most frequently detected allergens were proteins with molecular weights of 14.3, 106.5, 94.0, 72.0, 91.9, 63.7, 100.3, 43.6, 27.3, 62.0, 34.7, 18.3, 41.1 and 21.9 kD and the frequencies of IgE binding were 87.0, 65.2, 56.5, 43.4, 39.1, 39.1, 34.8, 30.4, 30.4, 17.4, 17.4, 17.4, 13.0 and 8.7% (fig. 3).

Inhibition of the IgE Immunoblot Reactivity

To test for allergenic cross-reactivity between *B. tropicalis* and *D. pteronyssinus*, immunoblot inhibition was performed. Four positive serum samples (No. 2, 4, 10 and 19) were preincubated with either *B. tropicalis* (B strips) or *D. pteronyssinus* (D strips). *P. notatum* (P strips) was used as a negative control. Results showed that inhibition of IgE binding was observed in all B strips, and the inhibition of the IgE-immunoblot reactivity was not observed in all P strips; two allergenic components cannot be inhibited by *D. pteronyssinus* with molecular weights of about 14.3 and 27.3 kD (fig. 4).

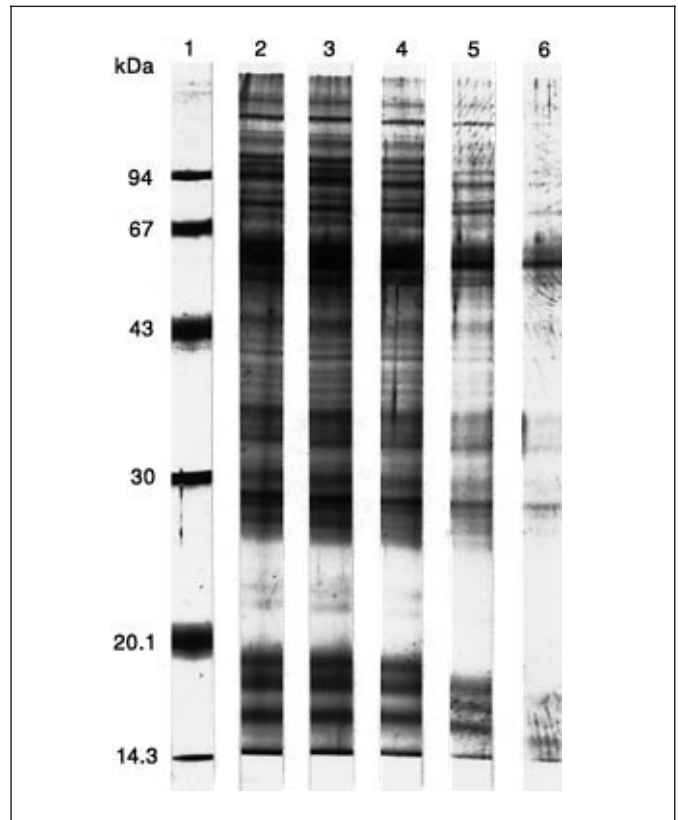


Fig. 1. Protein component of *B. tropicalis*. Series dilutions of crude *B. tropicalis* extract were separated by gel electrophoresis and stained with silver stain. Panel 1 represents protein markers, panels 2–6 series dilutions of *B. tropicalis* extract.

Discussion

B. tropicalis is prevalent in tropical and in subtropical regions world wide and has been detected in house dust samples in Taiwan. In this study, the means of the total counts of *B. tropicalis* per gram of dust in thirteen dust samples collected in August in homes of asthmatic patients from mattress, quilt, pillow and carpet were 327 ± 32 , 264 ± 31 , 179 ± 25 and 108 ± 32 , respectively. The large number of *B. tropicalis* in the house dust samples indicated that Taiwan is an endemic area. The low mite counts in carpets might be due to the fact that most carpets were small and machine-washable in this study. In addition, not all of the houses had carpets. Since the weather is warm and humid in the wintertime, the use of carpets in Taiwan is not as common as in western countries. It has been proposed that approximately 150 *B. tropicalis* mites/g dust are associated with sensitization or symptoms in persons sensitized to *B. tropicalis* [5]. A concentration greater than 150 mites/g

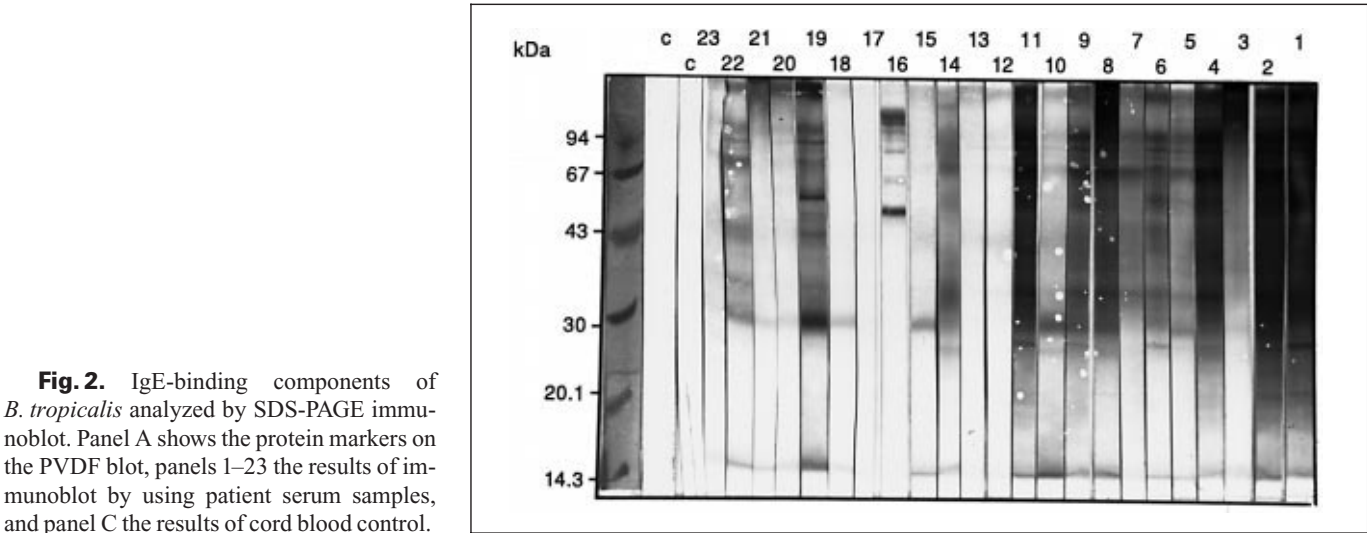


Fig. 2. IgE-binding components of *B. tropicalis* analyzed by SDS-PAGE immunoblot. Panel A shows the protein markers on the PVDF blot, panels 1–23 the results of immunoblot by using patient serum samples, and panel C the results of cord blood control.

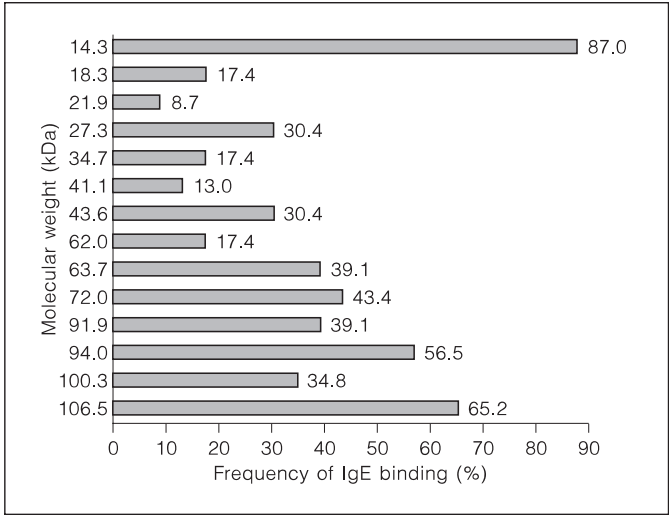


Fig. 3. Histogram showing the frequency of IgE binding of patients' sera to *B. tropicalis* body extract.

of dust in the Taipei area indicated that these asthmatic patients might be sensitized to *B. tropicalis*. Similar amounts of *B. tropicalis* have also been reported in the Tampa Bay Florida area of the United States [14] and in Cartagena, Colombia [5]. The evaluation of the role of *B. tropicalis* in asthma has been complicated by the fact that concurrent sensitization occurs in houses where both *B. tropicalis* and *D. pteronyssinus* are present. However, in 1996, Stanaland et al. [15] observed that only a skin test-positive reaction to *B. tropicalis* showed a positive nasal challenge response despite the fact that all allergic rhinitis subjects were sensitive to *D. pteronyssinus*. In this study, the high percentage (73.3%) of asthmatic patients showing a positive skin test

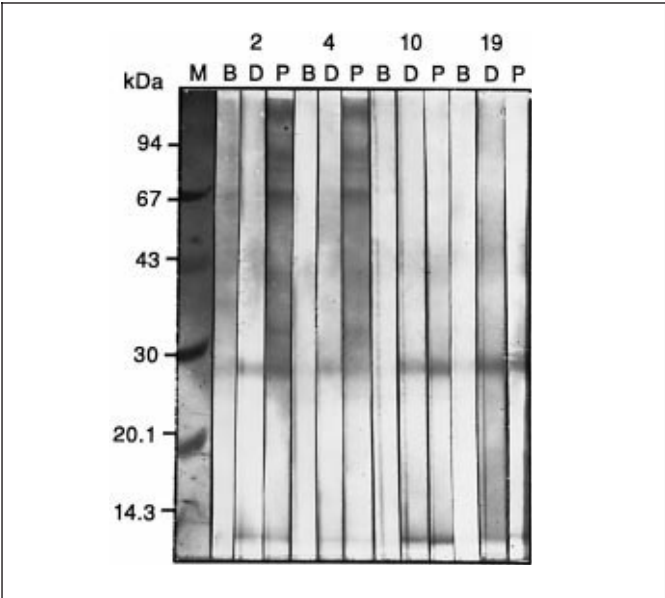


Fig. 4. The cross-reactions of *B. tropicalis* and *D. pteronyssinus*. Patients' sera (2, 4, 10, 19) were selected from figure 2 and mixed with *B. tropicalis* and *D. pteronyssinus* or *P. notatum* and then analyzed by immunoblotting with their IgE binding to the PVDF blot of *B. tropicalis*. Panel B was the result of the absorption by *B. tropicalis*. Panel D represents results after the absorption of *D. pteronyssinus*, panel P after the absorption of *P. notatum*.

reaction to *B. tropicalis* might suggest that *B. tropicalis* plays a role in the pathogenesis of asthma in Taiwan. In this study, there were more than 30 antigens in the *B. tropicalis* extract as determined by silver staining. The allergenic profiles analyzed by SDS-PAGE immunoblot using sera from skin test-positive patients demonstrated that there are more

than 14 different IgE-binding proteins with different molecular weights ranging from 10 to 100 kD. It has been reported that *B. tropicalis* has 21 different IgE-binding antigens detected by crossed radioimmuno-electrophoresis [7], and 25 antigens detected by immunoblotting and autoradiography analysis [8]. This discrepancy might be due to the less sensitive nature of the nonisotope immunoblotting methods that we used. More than 80% of the patients' sera reacted with the 14.3-kD allergens, which might be considered as the major allergen. Similar results have recently reported that *B. tropicalis* has the major allergen component with a molecular weight of approximately 11–13 kD [7]. This major allergen should be analyzed further. The high number of mite-specific IgE antibodies in most patients' sera could further indicate that there were high levels of exposure of our asthmatic patients to *B. tropicalis*.

The results from immunoblot inhibition showed that there was IgE cross-reactivity between *B. tropicalis* and *D. pteronyssinus*. However, two major allergenic components of *B. tropicalis* could not be inhibited by *D. pteronyssinus* with molecular weights of about 14.3 and 27.3 kD. Arruda et al. [10] have demonstrated that 33% inhibition was achieved by *D. pteronyssinus* when *B. tropicalis* was used in the solid phase. Recently, Blo t 5 and Der p 5 have been analyzed and sequenced to show that there was 43% amino

acid homology. The 33% cross-reactivity of *B. tropicalis* with *D. pteronyssinus* and 43% sequence homology of Blo t 5 and Der p 5 suggested that several IgE-binding proteins of *B. tropicalis* could be partially inhibited by *D. pteronyssinus*. However, the non-cross-reactive component of *B. tropicalis* and *D. pteronyssinus* indicated that *B. tropicalis* has species-specific allergens which need to be described in more detail.

In conclusion, *B. tropicalis* is highly prevalent and can be commonly detected in house dust samples in Taiwan. A high frequency of sensitization to *B. tropicalis* can also be demonstrated. Immunoblotting indicated that *B. tropicalis* possesses many IgE binding allergens. Some IgE-binding proteins are species-specific allergens based on the immunoblotting inhibition assay. The use of *B. tropicalis* extract for diagnostic purposes to identify patients with specific sensitivity to *B. tropicalis* with or without a concurrent sensitivity to *Dermatophagoides* spp. should be taken into consideration in Taiwan.

Acknowledgement

This study was supported by a grant (NSC 84-2331-B281-001) from the National Science Council and partially by a grant (DOH 85-HR-508) from the Department of Health, Taiwan.

References

- 1 Platts-Mills TAE, De Weck AL: Dust mite allergens and asthma – A worldwide problem. *J Allergy Clin Immunol* 1989;83:416–472.
- 2 Platts-Mills TAE, Chapman MD: Dust mites: Immunology, allergy disease and environmental control. *J Allergy Clin Immunol* 1987;80:755–775.
- 3 Chang YC, Hsieh KH: The study of house dust mites in Taiwan. *Ann Allergy* 1989;62:101–106.
- 4 Kam KL, Hsieh KH: Comparison of three in vitro assays for serum IgE with skin testing in asthmatic children. *Ann Allergy* 1994;73:329–336.
- 5 Fernandez-Caldas E, Puerta L, Mercado D, Lockey R, Caraballa L: Mite fauna, *Der p* I, *Der f* I and *Blomia tropicalis* allergen levels in tropical environment. *Clin Exp Allergy* 1993;23:292–297.
- 6 Puerta L, Fernandez-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.
- 7 Arlian LG, Vyszynski-Mober D, Fernandez-Caldas E: Allergenicity of the mite *Blomia tropicalis*. *J Allergy Clin Immunol* 1993;91:1042–1050.
- 8 Caraballo L, Puerta L, Martinez B, Moreno L: Identification of allergens from mite *Blomia tropicalis*. *Clin Exp Allergy* 1993;24:1056–1060.
- 9 Stanaland BE, Fernandez-Caldas E, Jacinto CM, Trudeau WL, Lockey R: Sensitization to *Blomia tropicalis*: Skin test and cross-reactivity studies. *J Allergy Clin Immunol* 1994;94:452–457.
- 10 Arruda LK, Vailes LD, Platts-Mills TAE, Chapman MD, et al: Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blo t 5. *Am J Respir Crit Care Med* 1997;155:343–350.
- 11 Nakada S, Itok K, Urata C: Allergenicity and immunogenicity of house-dust mite (*Dermatophagoides farinae*) antigens treated with glutaraldehyde. *Ann Allergy* 1995;54:437–441.
- 12 Lowry OH, Rosebrough NJ, Farr AL, Randal RJ: Protein measurement with Folin phenol reagent. *J Biol Chem* 1951;193:265–275.
- 13 Towbin H, Stachelin T, Gordon J: Electrophoretic transfer of proteins from acrylamide gels to nitrocellulose sheets. Procedure and some applications. *Proc Natl Acad Sci* 1979;76:4350–4354.
- 14 Fernandez-Caldas E, Fox RW, Bucholtz GA, Trudeau WL, Lockey RF: House dust mite allergy in Florida. Mite survey in households of mite-sensitive individuals in Tampa, Florida. *Allergy Proc* 1990;11:263–267.
- 15 Stanaland BE, Fernandez-Caldas E, Jacinto CM, Trudeau WL, Lockey RF: Positive nasal challenges with *Blomia tropicalis*. *J Allergy Clin Immunol* 1996;97:1045–1049.

Copyright: S. Karger AG, Basel 1998. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.