

Sensitization to *Blomia tropicalis*: Skin test and cross-reactivity studies

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This study evaluated the prevalence of positive percutaneous skin test results to Blomia tropicalis, Dermatophagoides pteronyssinus, and D. farinae in 167 consecutive persons (61 males, 106 females; mean age, 38.7 years; range, 14 to 75 years) with asthma or rhinitis, or both, in Tampa, Fla. Forty-nine had asthma with or without rhinitis, and 118 had rhinitis alone. RAST and RAST inhibition analysis of all three mite species were performed by using pooled patient sera. Prepared extracts of B. tropicalis and standardized D. pteronyssinus and D. farinae (1:50 wt/vol) were used for percutaneous skin tests. One hundred twelve subjects (67%) had a positive result to at least one mite species; 38%, 62%, and 60% reacted to B. tropicalis, D. pteronyssinus, and D. farinae, respectively. Positive skin test results to both D. pteronyssinus and D. farinae occurred in 96 subjects (57%); 56 of these subjects (58%) were also positive to B. tropicalis. Positive skin test results to B. tropicalis, D. pteronyssinus, or D. farinae alone occurred in four (2%), five (3%), and four (2%), respectively. RAST and RAST inhibition data indicate that B. tropicalis contains unique allergens. The high rate of skin test reactivity to B. tropicalis extracts, and its unique allergenic composition, warrants its use in the diagnosis and treatment of allergic disorders in Tampa. (J ALLERGY CLIN IMMUNOL 1994;94:452-7)

Key words: *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, *D. farinae*, allergens, mites, house dust, allergic rhinitis, asthma, cross-reactivity

There is increasing interest in the study of nonpyroglyphid storage mites as a source of important environmental allergens. The glycyphagid storage mite *Blomia tropicalis* has been detected in 16% to 96% of subtropical and tropical house dust samples.¹⁻⁵ *B. tropicalis* was found in 30% of Tampa, Fla., house dust samples, occurring more commonly in mattresses than in carpets.⁶ *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* were the predominant mite species in these dwellings, but other storage and predator mites were also found.

Skin test sensitivity to the pyroglyphid mites,

Dermatophagoides pteronyssinus and *Dermatophagoides farinae*, and the nonpyroglyphid mite *Aleuroglyphus ovatus* is prevalent in the Tampa Bay area.^{6, 7} Several studies suggest that *B. tropicalis* is a mite of allergenic importance, since specific IgE to *B. tropicalis* can be detected by skin tests and by RAST in mite-sensitive persons.^{1, 8-11} Presently, there are no *B. tropicalis* extracts commercially available for skin testing or immunotherapy. Studies have demonstrated little or no cross-reactivity between *Dermatophagoides* spp. and *B. tropicalis* extracts, and that *Der p* I, *Der f* I and group II *Dermatophagoides* allergens could not be identified in *B. tropicalis* extracts.¹⁰ Immunoabsorption experiments by Arruda et al.¹⁰ demonstrated that 64% of *B. tropicalis* allergens are species specific.

In the United States *B. tropicalis* is one of the four most important house dust mites, along with *D. pteronyssinus*, *D. farinae* and *Euroglyphus maynei*.¹² *B. tropicalis* has been identified in homes in New Orleans, La., Memphis, Tenn., Galveston, Texas, Delray Beach, and San Diego, Calif., accounting for five of the eight geographic areas surveyed.

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FIG. 1. Scanning electron micrograph of *B. tropicalis*. (Original magnification = $\times 200$).

The purposes of this study were (1) to evaluate the prevalence of positive percutaneous skin test results in subjects from Tampa, Fla., to *B. tropicalis*, *D. pteronyssinus*, and *D. farinae* in consecutive individuals evaluated for an allergic cause of their upper and lower respiratory complaints, and (2) to determine allergenic cross-reactivity among the mite species tested by RAST inhibition assays by using pooled patient serum.

METHODS

Patient population

The patient population consisted of 167 consecutive individuals from Tampa evaluated for an allergic cause of their upper and lower respiratory complaints. There were 61 males and 106 females, with a mean age of 38.7 years (range, 14 to 75 years). Forty-nine subjects had allergic asthma, with or without allergic rhinitis, defined as a history of reversible obstructive airway disease associated with exacerbation resulting from allergen exposure and concomitant skin reactivity to exacerbat-

ing allergens. One hundred eighteen subjects had allergic rhinitis alone, defined as a history of rhinitis caused by seasonal or perennial allergens with an historical correlation between skin reactivity and exacerbating allergens and no history of asthma. Informed consent was obtained from each patient before enrollment in the study. This study was approved by the Institutional Review Board of the University of South Florida and the James A. Haley Veterans Hospital.

Allergen sources

Standardized extracts (10,000 Allergy Units) of *D. pteronyssinus* and *D. farinae* were obtained from Greer Laboratories (Lenoir, N.C.), and the *B. tropicalis* extract was prepared by the Division of Allergy and Immunology, University of South Florida College of Medicine. *B. tropicalis* mites (Fig. 1) were cultured for 2 months with autoclaved rodent chow and then separated from the food medium with a modified Tullgren apparatus. Whole mite bodies were defatted for 4 hours with anhydrous ether in a Soxhlet extractor and dried at room temperature. Defatted mites were extracted in 0.1

TABLE I. Skin test and RAST data on seven patients used for RAST inhibition study

Patient No.	Skin test			RAST*		
	<i>Dp</i>	<i>Df</i>	<i>Bt</i>	<i>Dp</i>	<i>Df</i>	<i>Bt</i>
1	4+	4+	4+	7	8	6
2	4+	4+	4+	30	20	10
3	4+	4+	4+	27	15	9
4	4+	4+	4+	35	28	9
5	4+	4+	4+	25	17	11
6	4+	4+	4+	3	5	17
7	4+	4+	4+	34	26	31

Dp, *D. pteronyssinus*; *Df*, *D. farinae*; *Bt*, *B. tropicalis*.

*RAST results expressed as a percent of the total counts added.

mol/L of ammonium bicarbonate for 24 hours at 4° C. The extract was clarified by centrifugation at 18,000 rpm and dialyzed overnight at 4° C against deionized water with Spectra/Por 3 membranes with a 3500 MW cutoff (Spectrum Medical Industries, Inc., Los Angeles, Calif.). The dialyzed extract was centrifuged, filtered through a 0.22 µm membranes, diluted in glycerin to a final concentration of 1:50 wt/vol, and stored at -20° C. The protein concentrations of the *B. tropicalis*, *D. pteronyssinus*, and *D. farinae* were 1.3, 1.9, and 1.9 mg/ml, respectively.

Skin tests

Percutaneous skin testing was performed with bifurcated needles (Allergy Laboratories of Ohio, Columbus). The results were recorded using a scale from 1+ to 4+, according to the size of the induced wheal and erythema: erythema less than 20 mm without a wheal, 1+; wheal less than 3 mm with erythema greater than 20 mm, 2+; wheal greater than 3 mm with surrounding erythema, +3; greater than 3 mm wheal with pseudopods and surrounding erythema, 4+. A 3+ or greater reaction was considered positive.

RAST inhibition

Specific IgE to each mite species and RAST inhibition assays were performed with plastic microtiter plates (Immulon 4; Dynatech Laboratories, Alexandria, Va.), as previously described.¹ Fifty µl of serial dilutions (1:8000, 1:6000, 1:4000, 1:2000, 1:1000, 1:500, 1:200, and 1:100) of the mite extracts were added in duplicate along with 50 µl aliquots of a serum pool comprising the sera of seven individuals with a positive RAST result to the three mite species (Table I). The protein concentrations added ranged from 0.1625 to 13.0 µg/ml for *B. tropicalis* and from 0.2375 to 19.0 µg/ml for *D. pteronyssinus* and *D. farinae*. The plates were incubated overnight at room temperature, washed, and 100 µl of iodine 125-radiolabeled anti-human IgE were added to the wells. After overnight incubation and wash, individual wells were counted in a gamma counter. In Fig. 2 the amount of inhibitor is

expressed as the log of the protein concentration per milliliter of each mite extract. Serial dilutions of a short ragweed extract failed to produce any inhibition in the three allergen systems.

Statistical analysis

Statistical analysis was done using a Macintosh SE 30 computer (Apple Computer, Inc., Cupertino, Calif.) and the StatView II program (Brainpowers Inc., Calabasas, Calif.). Results were compared by contingency table analysis and chi square statistics. A *p* value less than 0.05 was considered significant.

RESULTS

Patient population and skin tests

One hundred sixty-seven consecutive subjects from Tampa, Fla., were skin tested to evaluate their upper and lower respiratory symptoms. Fifty five (33%) subjects had negative skin test results, whereas 112 (76%) subjects had a positive skin test to at least one of the three mite species. The prevalence of positive skin test reactions to *B. tropicalis*, *D. pteronyssinus*, and *D. farinae* was 38%, 62%, and 60%, respectively. Positive skin test results to a single mite individually occurred in four (2%), five (3%), and four (2%) subjects for *B. tropicalis*, *D. pteronyssinus*, and *D. farinae*, respectively. Positive skin test results to two mites, concomitantly, occurred in 40 (24%) subjects to *D. pteronyssinus* and *D. farinae*, in three (2%) to *B. tropicalis* and *D. pteronyssinus*, and in none to *B. tropicalis* and *D. farinae*. Fifty-six (34%) subjects had positive skin test results to all three mites. Positive skin tests to both *D. farinae* and *D. pteronyssinus* occurred in a total of 96 (57%) subjects, 56 (58%) of whom had positive skin test results to *B. tropicalis* (Table II).

The prevalence of positive skin test reactions to dust mites was higher in the group of asthmatic persons with or without rhinitis than in the group

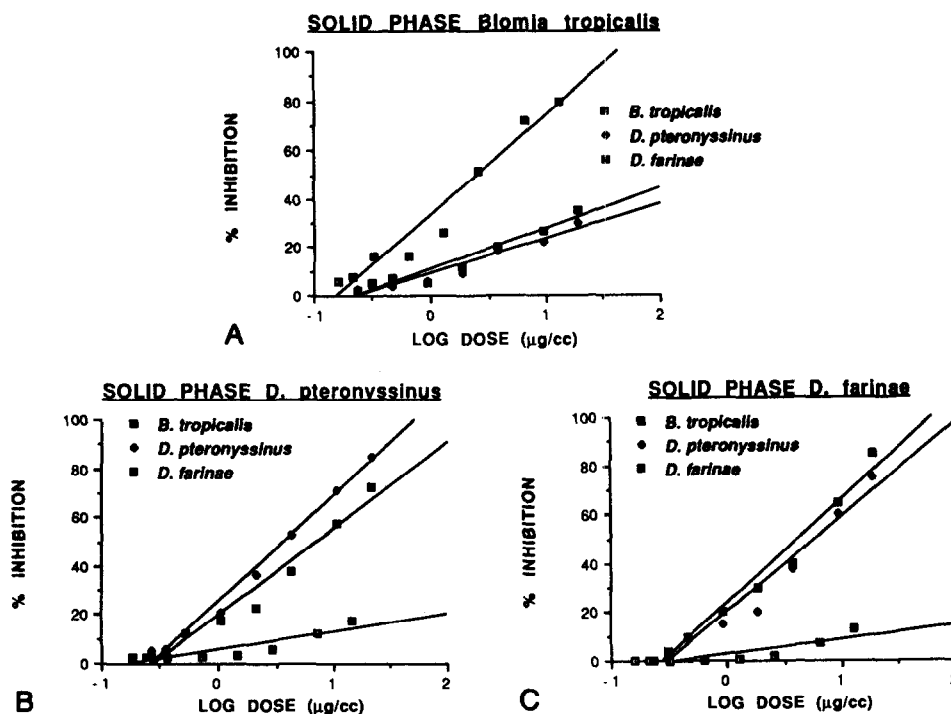


FIG. 2. RAST inhibition assays: A, *B. tropicalis* solid phase; B, *D. pteronyssinus* solid phase; C, *D. farinae* solid phase.

of subjects with rhinitis alone: 51% versus 32% ($p = 0.02$) for *B. tropicalis*, 73% versus 58% ($p = 0.05$) for *D. pteronyssinus*, and 73% versus 54% ($p = 0.02$) for *D. farinae*. A summary of the results is given in Table III.

Cross-reactivity between *D. pteronyssinus*, *D. farinae*, and *B. tropicalis*

The inhibitory capacities of different concentrations of *D. pteronyssinus*, *D. farinae*, and *B. tropicalis* extracts on the serum pool are illustrated in Fig. 2. When *B. tropicalis* was used in the solid phase, *B. tropicalis* inhibited 80% at 13 $\mu\text{g/ml}$; *D. pteronyssinus* inhibited 33% and *D. farinae* 35% at 19 $\mu\text{g/ml}$. *D. pteronyssinus* and *D. farinae* revealed similar inhibitory capacities when *D. pteronyssinus* and *D. farinae* were used in solid phase, with little inhibition by *B. tropicalis*. When *D. pteronyssinus* was used on the solid phase, *D. pteronyssinus* reached 82% inhibition at the highest concentration used (19 $\mu\text{g/ml}$). At the same concentration, *D. farinae* produced 73% inhibition. *B. tropicalis*, produced 19% inhibition at 13 $\mu\text{g/ml}$. When *D. farinae* was used on the solid phase, *D. farinae* produced 84% inhibition at the highest concentration used (19 $\mu\text{g/ml}$). The same dilution of *D. pteronyssinus* inhibited 78%; *B. tropicalis* produced 13% inhibition at 13 $\mu\text{g/ml}$.

TABLE II. Summary of the skin test results

Result	No. (%)
Negative skin tests	55 (33)
Positive to at least one mite	112 (76)
Positive tests to <i>Bt</i>	63 (38)
Positive tests to <i>Dp</i>	104 (62)
Positive tests to <i>Df</i>	100 (60)
Positive to <i>Bt</i> alone	4 (2)
Positive to <i>Dp</i> alone	5 (3)
Positive to <i>Df</i> alone	4 (2)
Positive to <i>Bt</i> , <i>Dp</i> , and <i>Df</i>	56 (34)
Positive to <i>Dp</i> and <i>Df</i> only	40 (24)
Positive to <i>Bt</i> and <i>Dp</i> only	3 (2)
Positive to <i>Bt</i> and <i>Df</i> only	0 (0)

Bt, *B. tropicalis*; *Dp*, *D. pteronyssinus*; *Df*, *D. farinae*.

DISCUSSION

The prevalence of skin test reactivity to *B. tropicalis* in the United States has not been previously studied. This study demonstrates a high prevalence (38%) of skin test reactivity to *B. tropicalis* in subjects from Tampa, Fla., who were evaluated for allergic respiratory disease. A large number of these subjects (34%) demonstrated skin sensitivity to the three mite species tested, and 7% were sensitive to only one species. The rate of positive skin

TABLE III. Comparison of skin test results in patients with allergic asthma, allergic rhinitis, or both, compared with patients with allergic rhinitis alone

	Allergic asthma (n = 49)		Allergic rhinitis (n = 118)		p Value
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
<i>Bt</i>	25 (51)	24 (49)	33 (32)	80 (68)	0.02
<i>Dp</i>	36 (73)	13 (27)	68 (58)	50 (42)	0.02
<i>Df</i>	36 (73)	13 (27)	64 (54)	54 (46)	0.05

Bt, *B. tropicalis*; *Dp*, *D. pteronyssinus*; *Df*, *D. farinae*.

test reactivity to *B. tropicalis* alone (2%) is comparable to that of *D. pteronyssinus* (4%) or *D. farinae* (2%) alone. Evaluation of allergic subjects with *D. pteronyssinus* and *D. farinae* extracts identified 96% of the mite-sensitive subjects. However, 62% of these mite-sensitive subjects were also sensitive to *B. tropicalis*.

Studies in other countries have obtained comparable results. In Cartagena, Columbia, Puerta Llerena et al.¹ found that 85.5% of patients skin test positive to both *D. pteronyssinus* and *D. farinae* had a positive RAST to *B. tropicalis*. Similarly, in São Paulo, Brazil, Arruda et al.¹⁰ found that 65% of asthmatic children who were skin test positive to *D. pteronyssinus* and *D. farinae* had a positive RAST to *B. tropicalis*.

Although a high rate of simultaneous sensitivity to *D. pteronyssinus*, *D. farinae*, and *B. tropicalis* occurs, *B. tropicalis* appears to induce specific sensitivity. In our study four subjects were skin test positive to *B. tropicalis* and negative to both *D. pteronyssinus* and *D. farinae*. Therefore patients who have unexplained allergic symptoms and are skin test negative to *D. pteronyssinus* and *D. farinae* may be sensitive to *B. tropicalis* and not be identified by skin testing or RAST, because *B. tropicalis* extracts are not yet commercially available. The clinical significance of a possible synergistic effect of multiple mite sensitivity and the clinical benefits of immunotherapy with *B. tropicalis* extracts remain to be established when more mite extracts are available for diagnosis and treatment. The incorporation of *B. tropicalis* extracts in immunotherapy regimens may prove to be effective in treating allergic respiratory symptoms in sensitized persons.

RAST inhibition assays confirmed a high degree of cross-reactivity between *D. pteronyssinus* and *D. farinae*. Little cross-reactivity was demonstrated between *B. tropicalis* and the other two mite species, suggesting that *B. tropicalis* has species-specific allergens. The 33% inhibition achieved by

D. pteronyssinus when *B. tropicalis* was used in the solid phase, confirms the findings by Arruda et al. suggesting that approximately 65% of *B. tropicalis* allergens are species specific. Arlian et al.¹³ demonstrated by crossed immunoelectrophoresis and crossed radioimmuno-electrophoresis that *B. tropicalis* is a source of at least 21 allergens, most species specific, and that *B. tropicalis* shared three cross-reacting allergens with the dust mites *D. pteronyssinus* and *D. farinae*. Low IgE binding to the cross-reacting allergens suggested that there is limited structural homology and few cross-reacting epitopes in these three allergens. The same investigators also found that *B. tropicalis* is the source of several "major" allergens and that patient sensitivity to the various *B. tropicalis* allergens varied.

In Tampa the rate of sensitization to *B. tropicalis*, *D. pteronyssinus*, and *D. farinae* was significantly higher in asthmatic persons with or without allergic rhinitis (51%, 73%, and 73%, respectively) than in subjects with allergic rhinitis alone (32%, 58%, and 54%, respectively). Similar results were reported by Siltan et al.,⁷ who found that a positive RAST to the storage mite *A. ovatus* was more common in subjects with allergic asthma with or without allergic rhinitis than in subjects with allergic rhinitis alone. Farmers with allergic asthma alone, or in combination with allergic rhinitis, are also more frequently sensitized to storage mites and house dust mites than are farmers with allergic rhinitis alone.¹⁴ In general, asthmatic persons appear to be more susceptible to dust mite sensitization than do rhinitic patients, although the genetic basis or immunologic mechanisms have not been elucidated.

B. tropicalis is prevalent in tropical and in subtropical regions of the world and has been detected in house dust samples in the Tampa Bay area in concentrations greater than 150 mites per gram of dust.⁶ In Cartagena, Colombia, the geometric mean of the total counts of *B. tropicalis* and *D. pteronyssinus* mites in 50 dust samples collected

in homes of persons sensitized to *B. tropicalis* and *D. pteronyssinus* was 147.5 and 101.7, respectively.¹⁵ Based on these findings, it has been proposed that approximately 150 *B. tropicalis* mites per gram of dust are associated with sensitization or symptoms in persons sensitized to *B. tropicalis*. This figure is similar to the 100 *D. pteronyssinus* per gram of dust suggested by Korsgaard¹⁶ and Peat et al.¹⁷

Geographic patterns of dust mite sensitivity may be due to differences in mean dust mite allergen levels and in mite fauna. Asthmatic persons in Tampa were less frequently sensitized to *B. tropicalis* than to *Dermatophagoides* spp. However, in São Paulo, Brazil, *B. tropicalis* was the most frequent sensitizer of asthmatic children.¹⁰ *B. tropicalis* seems to be more abundant in Cartagena, Colombia, and in São Paulo than in the United States. This fact could explain the higher rate of sensitization to this mite in these areas.^{1, 18}

Dust mite levels in Tampa Bay vary with the seasons and are highest during the summer months.¹⁹ Seasonal variations in dust mite allergens and lower mean temperatures and humidity in the winter months could account for a less frequent sensitivity to *B. tropicalis*, as compared with Cartagena, Colombia, and São Paulo, Brazil, which are situated in tropical environments.¹⁰ Cultural as well as genetic factors may also account for these differences.

Several issues concerning the allergenicity of *B. tropicalis* are presented in this article. First, a high frequency of sensitization to *B. tropicalis* has been demonstrated in the Tampa Bay area. Second, RAST inhibition data indicate that there is minimal cross-reactivity between *B. tropicalis* and *D. pteronyssinus* and *D. farinae*, using a serum pool derived from persons from this area who are allergic to mites. Third, the use of *B. tropicalis* extracts for diagnostic purposes identifies patients with specific sensitivity to *B. tropicalis* with or without a concurrent sensitivity to *Dermatophagoides* spp. Finally, this study suggests that the utilization of *B. tropicalis* extracts for diagnostic purposes may be indicated in areas where this mite species is common. The role of *B. tropicalis* extracts in immunotherapy remains to be established.

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