

Positive nasal challenge responses to *Blomia tropicalis*

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Background: *Blomia tropicalis*, a dust mite commonly found in subtropical and tropical environments, is the fourth most common mite in the United States. Thirty-eight percent of 167 consecutive subjects evaluated for allergic respiratory symptoms in the Tampa Bay area had positive skin test responses to *B. tropicalis*.

Methods: Nasal challenges were performed in 19 subjects with allergic rhinitis; 12 had positive skin test responses to *B. tropicalis* (group I), and seven had negative skin test responses to *B. tropicalis* but positive skin test responses to *Dermatophagoides pteronyssinus* and *D. farinae* (group II). Subjects were challenged with a normal saline control solution and increasing concentrations of a 1 mg/ml in-house extract of *B. tropicalis* (1:125,000 vol/vol, 1:25,000 vol/vol, 1:5000 vol/vol, 1:1000 vol/vol, and 1:200 vol/vol). Inspiratory nasal airway resistance (was measured every 5 minutes for 15 minutes by posterior rhinomanometry at 50 Pa after each nasal challenge.

Results: Nasal challenge response was considered positive and stopped when the mean inspiratory nasal airway resistance was greater than twice the mean inspiratory nasal airway resistance after the normal saline challenge. Ten of 12 (83%) subjects in group I and none of seven (0%) subjects in group II had positive nasal challenge responses to *B. tropicalis* ($p = 0.0024$).

Conclusion: *B. tropicalis* is allergenic and should be considered as a cause of allergic rhinitis when evaluating a patient who lives in an area where it is endemic. (*J Allergy Clin Immunol* 1996;97:1045-9.)

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

Blomia tropicalis, a dust mite species commonly found in subtropical and tropical environments, is the fourth most common mite in the United States.¹ Several studies have suggested that *B. tropicalis* is a mite of allergenic importance. In 1990 Fernandez-Caldas et al.² detected *B. tropicalis* in 33% of Tampa house dust samples in concentrations greater than 150 mites per gram of dust. Other studies on the mite fauna in subtropical and tropical regions have demonstrated that *B. tropicalis* is very common and, in some instances, the predominant

Abbreviation used

iNAR: Inspiratory nasal airway resistance.

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Received for publication Dec. 6, 1994; revised June 12, 1995; accepted for publication June 23, 1995.

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0091-6749/96 \$5.00 + 0 1/1/67347

house dust mite species.³⁻⁶ In 1991, Arruda et al.⁷ demonstrated that 64% of *B. tropicalis* allergens are species-specific and that *Der p* 1, *Der f* 1, and group II *Dermatophagoides* allergens are not present in *B. tropicalis*. The authors detected specific IgE to *B. tropicalis* in 18 of 20 asthmatic children with mite allergy in São Paulo, Brazil.⁷ In 1993, Arlian et al.⁸ demonstrated that *B. tropicalis* is the source of at least 21 allergens, most of which are species-specific. In 1994, Stanaland et al.⁹ observed that 38% of 167 consecutive subjects evaluated for allergic respiratory symptoms in Tampa had positive skin test responses to *B. tropicalis*. In the 1994 study, RAST inhibition demonstrated minimal cross-reactivity among

TABLE I. Patient demographic data

	Group I (n = 12)	Group II (n = 7)
Men	4	5
Women	8	2
Mean age (yr)	29.5	31
Age range (yr)	19-47	18-44
AA and AR	3	2
AR alone	9	5

AA, Allergic asthma; AR, allergic rhinitis.

D. pteronyssinus, *D. farinae*, and *B. tropicalis*. Other studies have also demonstrated minimal to moderate cross-reactivity between *Dermatophagoides* spp. and *B. tropicalis* by using sera from patients with asthma who had positive skin test responses to *Dermatophagoides* spp. and *B. tropicalis*.¹⁰ As part of their study, Llerena et al.¹⁰ demonstrated a greater degree of cross-reactivity between *Lepidoglyphus destructor* and *B. tropicalis* than between *B. tropicalis* and *Dermatophagoides* spp. This is not surprising because these two mite species belong to the same Glycyphagidae family.

Although the allergenicity of *B. tropicalis* has been previously demonstrated by skin testing, RAST, and RAST inhibition, symptoms after inhalation of *B. tropicalis* allergens had not been demonstrated. The purpose of this study was to evaluate the allergenicity of *B. tropicalis* by performing nasal challenges and measuring inspiratory nasal airway resistance (iNAR) by rhinomanometry.

METHODS

Study population

Nineteen volunteer subjects with allergic rhinitis, with or without asthma, were recruited from the outpatient allergy and immunology clinics at the University of South Florida College of Medicine and private offices of Tampa area physicians (Table I). Two groups of subjects with allergic rhinitis were studied. These two groups were matched by age and disease severity (medication use and history of symptoms). Group I consisted 12 subjects, eight women and four men (age range, 19 to 47 years; mean age, 29.5 years), who had a positive skin prick test response to *B. tropicalis* extracts (wheals ≥ 3 mm). All except one of these subjects had positive skin prick test responses to both *D. pteronyssinus* and *D. farinae*; this subject was exclusively sensitized to *B. tropicalis*. Group II consisted of seven subjects, two women and five men (age range, 18 to 44 years; mean age, 31 years), who had negative skin prick test responses to *B. tropicalis* and positive responses to *D. pteronyssinus* and *D. farinae*. Subjects with a history of neurologic disease, cardiac disease,

irreversible lung disease, recent history of upper respiratory tract infection, or previous immunotherapy and pregnant women were excluded from this study. This study was approved by the Institutional Review Board of the University of South Florida, and informed consent was obtained from each patient before enrollment.

Allergen sources

Standardized extracts (10,000 Allergy Units/ml) of *D. pteronyssinus* and *D. farinae* were obtained from Greer Laboratories (Lenoir, N.C.). The *B. tropicalis* extract was prepared at the Division of Allergy and Immunology, University of South Florida College of Medicine by methods previously described.^{3,9,10} *B. tropicalis* mites were cultured for 2 months and then separated from the medium with a modified Tullgren apparatus and sieving. Whole mite bodies (purity estimated at $>95\%$ by light microscopy) were defatted for 4 hours with anhydrous ether in a Soxhlet extractor and dried at room temperature. Defatted mites were extracted 1/20 wt/vol in 0.2 mol/L ammonium bicarbonate/glycerin 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 molecular weight cutoff of 3500 d (Spectrum Medical Industries, Inc., Los Angeles, Calif.). The dialyzed extract was centrifuged, filtered through a 0.22 μ m filter and stored at -20° C in 1 ml aliquots. The protein concentration of the *B. tropicalis* extract was 1 mg/ml. Endotoxin content was determined by the QCL-1000 Limulus Amebocyte Lysate test (Bio Whittaker, Walkersville, Md.). The deionized water used for preparing the extract contained 0.2 endotoxin units/ml, and the extract used for the challenges contained 0.7 endotoxin units/ml.

Nasal challenges

Topical glucocorticosteroids and cromolyn sodium and systemic glucocorticosteroids were withheld for 2 and 4 weeks, respectively, before the nasal challenges. Short- and long-acting antihistamines and terfenadine and loratidine were withheld for 3 and 7 days, respectively; and astemizole was withheld for 30 days. Nasal challenges and rhinomanometry were performed with the patient in the upright position. Before the challenges, a nasal examination for obstruction, pulmonary function tests, and a physical examination for wheezing were performed. Patients were not challenged if their FEV₁ was less than 80% of predicted value or if they had complete obstruction of either nostril. Two actuations (0.12 $\pm$ 0.01 ml) from nasal spray bottles containing challenge material were nebulized into each nostril at end inspiration. The challenge included a normal saline control, followed by increasing concentrations of a 1 mg/ml in-house *B. tropicalis* extract given at 20-minute intervals. The initial dilution was 1:125,000 vol/vol, followed by 1:25,000 vol/vol, 1:5000 vol/vol, 1:1000 vol/vol, and 1:200 vol/vol dilutions.

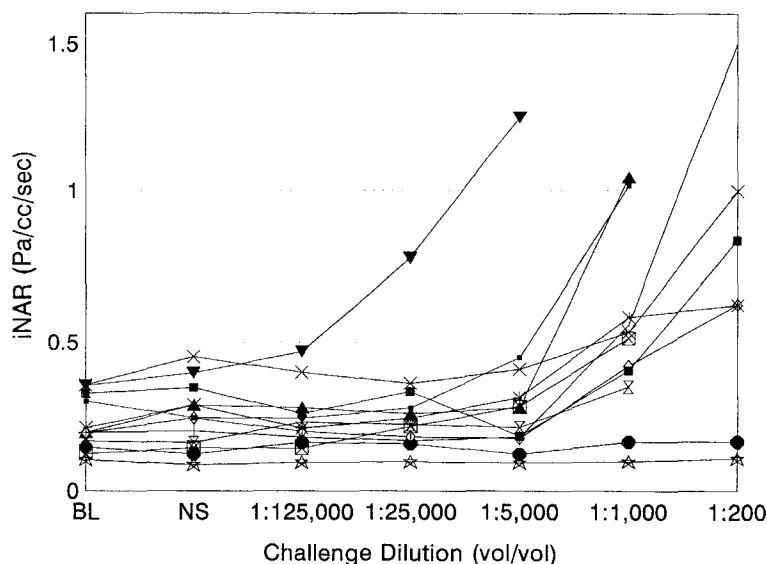


FIG. 1. Results of *B. tropicalis* nasal challenges in group I.

Rhinomanometry

Posterior rhinomanometry was performed with a Mercury Rhinomanometer (model NR8; Life-Tech, Inc., Austin, Texas) at baseline and after each nasal challenge, measuring iNAR at a fixed reference pressure of 50 Pa. The mean of three iNAR measurements, which were obtained every 5 minutes for 15 minutes after each nasal challenge, was recorded. The challenges were stopped when the iNAR was greater than twice the mean iNAR after the normal saline control challenge. A positive challenge was defined as a 100% change from the normal saline control value.

Statistical analysis

Statistical analysis was done with a Macintosh SE 30 computer (Apple Computer, Inc., Cupertino, Calif.) and the StatView 4.02 program (Abacus Concepts, Inc., Berkeley, Calif.). The results were compared by means of Student's *t* test, contingency table analysis, and chi square statistics with continuity correction. A *p* value of less than 0.05 was considered significant.

RESULTS

At baseline, the difference in mean iNAR was not significantly different between both groups (0.223 in group I and 0.201 in group II; *p* = 0.56). Group I results are shown in Fig. 1. This figure compares each of the mean iNAR measurements for each patient at each challenge dilution. Two patients had negative challenge results and did not have a significant increase in iNAR. Ten patients (83.3%) did have a significant increase in iNAR with increasing concentrations of *B. tropicalis* chal-

lenge material. One patient had the challenge stopped at a 1:5000 vol/vol dilution, four patients at a 1:1000 vol/vol dilution, and five at a 1:200 vol/vol dilution.

Fig. 2 shows the results obtained from the group with negative skin test responses to *B. tropicalis* and positive responses to *Dermatophagoides* spp. (group II). All seven of the patients with negative skin test responses to *B. tropicalis* had negative nasal challenge results.

A statistically significant difference between the two groups of subjects was seen at the 1:1000 vol/vol dilution with a *p* value of 0.007 and at the 1:200 vol/vol dilution with a *p* value of 0.0412. Comparison of the two sets of results by means of chi square statistics with continuity correction results in a significant *p* value of 0.0024.

DISCUSSION

This study demonstrates a high rate (83%) of positive nasal challenge results in subjects with allergic rhinitis who have positive skin test responses to *B. tropicalis*. The results are in agreement with other studies, which have demonstrated that about 95% of individuals who have symptoms of seasonal allergic rhinitis and a positive skin test response to the relevant allergen will experience an acute allergic reaction after nasal provocation with that allergen.¹¹ It has been argued that in subtropical and tropical regions, where house dust mites are the main perennial allergens, subclinical in-

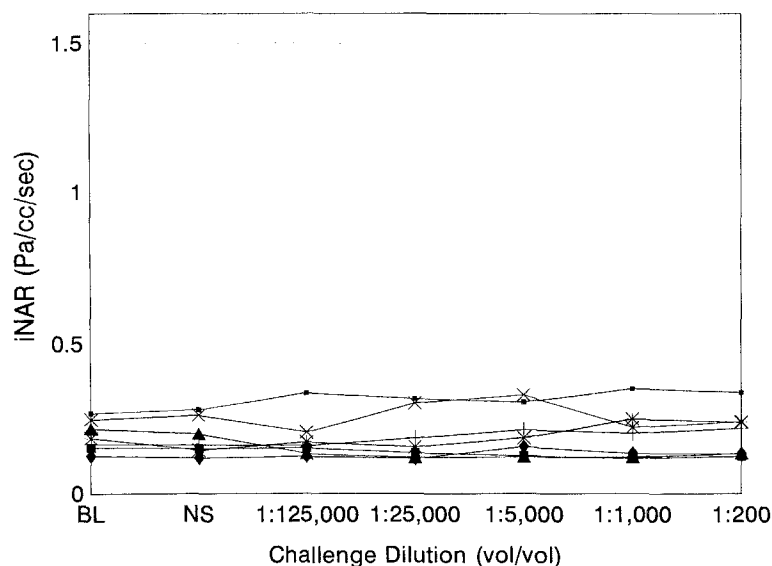


FIG. 2. Results of *B. tropicalis* nasal challenges in group II.

flammation present in the nasal mucosa probably compromises conclusions derived from nasal challenges.¹² However, because *B. tropicalis*, *D. pteronyssinus*, and *D. farinae* are common in house dust in Tampa throughout the year, there is constant exposure to mite allergens. Therefore nasal priming should not be a factor in the outcome of this study. In fact, there was no significant difference in the mean iNAR value at baseline in both groups.

Sensitization to *B. tropicalis* is species-specific because none of the subjects with rhinitis and positive skin test responses to *Dermatophagoides* spp. who had a negative skin test response to *B. tropicalis* had a positive nasal challenge result. *B. tropicalis* shows a high degree of in vivo and in vitro species specificity. In a previous study four of 167 (2%) atopic individuals had positive skin test responses to *B. tropicalis* and negative skin test responses to *Dermatophagoides* spp; five (3%) had positive responses to *D. pteronyssinus*, and four (2%) had positive responses to *D. farinae* alone.⁹ In these cases skin test reactivity seems to be species-specific and not caused by allergenic cross-reactivity.

Arlian et al.⁸ demonstrated that *B. tropicalis* shares three allergens with *D. pteronyssinus* and two with *D. farinae*. Our study suggests that these allergens may not be clinically relevant; that they account for a small proportion of the total allergenic load in these extracts; or, that when diluted, they do not produce a local inflammatory reaction in individuals who are not sensitized to *B. tropicalis*.

B. tropicalis has been detected in 33% of the dust samples in the Tampa area, and 38% of individuals evaluated for allergic respiratory complaints in this area have positive skin test responses to this mite. These two numbers are very similar and suggest that exposure to *B. tropicalis* is necessary for development of sensitization and symptoms. *B. tropicalis* is also common in other cities in the United States. In San Diego, California, it was found in 44% of the homes studied; in Delray Beach, Florida in 25%; in Galveston, Texas in 19%; in New Orleans, Louisiana in 5%; and in Memphis, Tennessee in 3%.¹

Seasonal changes in *B. tropicalis* mite counts and/or allergen levels remain to be established, but it is suspected that they will increase in the hot and humid months.

The clinical significance of sensitization to *B. tropicalis* may be even greater in other countries where *B. tropicalis* is the predominant mite species. In Venezuela, the Caribbean coast of Colombia, Costa Rica, and Brazil, *B. tropicalis* is the most important house dust mite species and is responsible for significant allergic respiratory disease.^{7, 10, 13, 14} Sensitization to *B. tropicalis* and other mite species is very common in patients with asthma living in five Latin American countries.¹³ This study included extracts of *D. pteronyssinus*, *D. farinae*, *B. tropicalis*, *Lepidoglyphus destructor*, *Aleuroglyphus ovatus*, and *Chortoglyphus arcuatus*. The highest rate of sensitization to *B. tropicalis* has been described in Sao Paulo, Brazil.⁷

Sensitization to multiple mite species has not

been studied extensively but seems to be a common phenomenon and may be a major risk factor for asthma.¹⁵ Further studies are needed to establish the clinical significance of sensitization to multiple mite species and to determine whether there is a synergistic effect on the overall symptomatology of allergic asthma.

B. tropicalis now fulfills Thommen's criteria, as modified for a nonpollen allergen. First, the allergen must be found in significant quantities. *B. tropicalis* is found in 33% of Tampa house dust samples and in other regions of the United States and throughout the world. Second, specific IgE must be detected in sensitized individuals. Thirty-eight percent of subjects evaluated for allergic disease in Tampa had positive skin test responses to *B. tropicalis*. Third, allergic signs and symptoms must result from challenge with the potential allergen. Eighty-three percent of subjects with positive skin test responses had positive nasal challenge responses to *B. tropicalis*.

In summary, *B. tropicalis* has species-specific allergens, and therefore subjects who have negative skin test responses to *Dermatophagoides* spp. and unexplained allergic symptoms may be sensitive to *B. tropicalis* in their environment. The fact that *B. tropicalis* induces nasal symptoms on inhalation may indicate that use of these extracts is warranted for a more accurate diagnosis and more effective treatment of allergic diseases.

REFERENCES

1. Arlian LG, Bernstein D, Bernstein IL, et al. Prevalence of dust mites in the homes of people with asthma living in eight different geographic areas of the United States. *J Allergy Clin Immunol* 1992;90:292-300.
2. Fernández-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;11:263-7.
3. Fernández-Caldas E, Puerta L, Mercado D, Lockey RF, Caraballo LR. Mite fauna, *Der p* I, *Der f* I and *Blomia tropicalis* allergen levels in a tropical environment. *Clin Exp Allergy* 1993;23:292-7.
4. Hurtado I, Parini M. House dust mite in Caracas, Venezuela. *Ann Allergy* 1987;59:128-30.
5. Mario Vargas V, Hazel Mairena A. House dust mites from the metropolitan area of San Jose, Costa Rica. *Int J Acarol* 1991;17:141-4.
6. Neto J, Croce J, Baggio D. Acaros da poeira domiciliar da cidade de Sao Paulo. Nota previa. *Rev Bras Alerg Imunopatol* 1980;2:140-5.
7. Arruda LK, Rizzo MC, Chapman MD, et al. Exposure and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil. *Clin Exp Allergy* 1991;21:433-9.
8. Arlian LG, Vyszynski-Moher DL, Fernández-Caldas E. Allergenicity of the mite, *Blomia tropicalis*. *J Allergy Clin Immunol* 1993;91:1042-50.
9. Stanaland BE, Fernández-Caldas E, Jacinto CM, Trudeau WL, Lockey RF. Sensitization to *Blomia tropicalis*: skin test and cross-reactivity studies. *J Allergy Clin Immunol* 1994;94:452-7.
10. Llerena LP, Fernández-Caldas E, Gracia LRC, Lockey RF. Sensitization to *Blomia tropicalis* and *Lepidoglyphus destructor* in *Dermatophagoides* spp.-allergic individuals. *J Allergy Clin Immunol* 1991;88:943-50.
11. Ilioupolos O, Proud D, Adkinson Jr NF, et al. Relationship between the early, late and rechallenge reaction to nasal challenge with an antigen. Observation on the role of inflammatory mediators and cells. *J Allergy Clin Immunol* 1990;86:851-61.
12. Togias AG. The role of environmental allergens in rhinitis. In: Busse WW, Holgate ST, eds. *Asthma and rhinitis*. Boston: Blackwell Scientific Publications, Inc., 1995:1009-29.
13. Puerta L, Fernández-Caldas E, Lockey RF, Caraballo LR. Mite allergy in the tropics: sensitization to six domestic mite species in Cartagena, Colombia. *J Investig Allergol Clin Immunol* 1993;3:198-204.
14. Fernández-Caldas E, Baena-Cagnani CE, López M, et al. Cutaneous sensitivity to 6 mite species in asthmatic patients from 5 Latin American countries. *J Investig Allergol Clin Immunol* 1993;3:245-9.
15. Fernández-Caldas E, Puerta L. Sensitization to various mite species. In: Johansson SGO, ed. *Progress in allergy and immunology*. Stockholm: Hogrefe & Huber, 1995;3:317-22.