

Short communication

Determination of *Blomia tropicalis*-specific IgE and IgG subclasses in atopic dermatitis patients

Background: To verify the importance of *Blomia tropicalis* in atopic dermatitis (AD), we determined the cutaneous reactivity and the serum level of *B. tropicalis*-specific IgE and IgG subclasses in AD patients.

Methods: *B. tropicalis*-specific IgE and IgG subclasses were determined in AD patients and compared with bronchial asthma (BA) patients and a control group (CG) of nonatopic subjects. Specific IgE was obtained by skin prick test and RAST. *B. tropicalis*-specific IgG subclasses were determined by ELISA. The data were statistically analyzed by chi-square test (Mantel-Haenszel) and odds ratio (OR).

Results: We detected positive skin prick tests in 61.76% of AD and 83.33% of BA patients, and in 12.5% of the CG. RAST was positive in 44.12% of AD and in 61.90% of BA patients, but not in the CG. *B. tropicalis*-specific IgG1 and IgG2 subclasses showed no significant differences between the three groups. IgG3 subclass positivity was statistically significant in AD patients (41.17%) when compared to BA patients (14.29%) and the CG (16.67%). The determination of *B. tropicalis*-specific IgG4 was positive in 32.35% of AD patients, 21.43% of BA patients, and 8.33% of the CG.

Conclusions: These results confirm that the storage mite *B. tropicalis* is an important allergen in AD. It is possible that IgG3 activates the complement in AD patients, releasing vasoactive amines that further amplify the allergic reaction. The positive results of the *B. tropicalis*-specific IgG4 found in AD and BA were probably due to chronic exposure to this storage mite in the home environment.

**J. C. Mori¹, M. C. Pires¹,
C. E. S. Galvão¹,
J. Ferreira de Mello¹,
F. M. Golcher², F. Montealegre²**

¹Allergy and Immunology Division, Hospital Servidor Público Estadual de São Paulo (SP), Brazil;

²Immunochimistry Department, Medical School of Ponce University, Puerto Rico, USA

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José Carlos Mori, MD
Serviço de Alergia e Imunologia
Hospital do Servidor Público Estadual de São Paulo
CEP: 04039-901
Rua Pedro de Toledo, 1.800 – 4. andar
São Paulo – SP
Brazil

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Atopic dermatitis (AD) is associated with high serum levels of immunoglobulin E (IgE) and with an unbalance in cellular immunity. Although the pathogenic role of IgE in allergic diseases is well established, high levels of this immunoglobulin have not been explained, especially in AD, where 20% of the patients present normal IgE levels (1) and negative skin prick tests to environmental allergens (2).

It is known that IgG represents 75% of the total circulating immunoglobulins. Four subclasses of IgG have been identified with antiserum preparations against proteins from different human myelomas (3). They received the denominations of IgG1, IgG2, IgG3, and IgG4 according to serum concentrations in normal subjects. Several investigators have attributed a protective activity to IgG subclasses, mainly IgG4, which is produced as a result of a prolonged antigenic exposure. Some authors believe that the IgG4 subclass antibody could neutralize the antigen or block the antibody, and thereby aid immunotherapy (4).

Shakib et al. (5) observed high levels of IgE and IgG4

subclass in the serum of AD patients, suggesting the involvement of IgG4 in some immediate hypersensitivity diseases. Nakagawa et al. (6) and Merrett et al. (7) detected serum levels of specific IgG4 to environmental allergens and found high levels of this antibody in patients with AD and allergic respiratory diseases.

Walker (8) first reported an association between the exacerbation of eczema in patients with AD and the exposure to airborne allergens such as pollen and horse danders. There have been many studies of the correlation between worsening of symptoms and exposure to dust mites, as well as of the improvement after environmental control (9–12).

Blomia tropicalis is a storage mite that has adapted very well to the domestic environment and has been described as part of the house-dust fauna (13, 14). In Brazil, epidemiologic studies have demonstrated the prevalence of *Dermatophagoides pteronyssinus* and *B. tropicalis* (15). Mori et al. (16) observed that 85% of patients with allergic respiratory disease reported a worsening of symptoms after contact with house dust.

In the same study, they found a positive skin prick test in 80% of patients, and the principal allergens were *B. tropicalis*, *D. pteronyssinus*, and *D. farinae*. Other studies have demonstrated positivity to *B. tropicalis* at skin prick test (17–20). No study of *B. tropicalis*-specific IgG subclasses and AD has been published.

We aimed to determine the specific cutaneous reactivity and the serum level of *B. tropicalis*-specific IgE and IgG subclasses in AD patients in order to verify the importance of this allergen in AD.

Material and methods

One hundred patients were selected from the allergy department of Hospital do Servidor Estadual “Francisco Morato de Oliveira” in São Paulo (Brazil). Their ages varied from 1 to 65 years. Thirty-two were male and 68 were female. They were divided into three groups: group I, comprised 34 patients with a clinical diagnosis of AD according to the criteria of Hanifin & Lobitz (21); group II comprised 42 patients with a clinical diagnosis of asthma according to criteria of the First Brazilian Consensus on Management of Asthma (1994) (22); and group III comprised 24 nonallergic healthy subjects with no family history of atopy. They or their parents all gave informed consent to participate in this study.

All subjects were clinically evaluated to confirm diagnosis, and skin prick tests to house-dust mites were performed. A blood sample of 10 ml was collected for laboratory determinations.

The allergenic extracts of *B. tropicalis* were produced by the Immunochemical Laboratory, Medical School of Ponce, Puerto Rico (USA), and standardized by International Pharmaceutical Immunology (IPI-ASAC) in Spain. These antigens were standardized biologically (biological activity: 37.650 UBE/ml) according to the International Standard (IS) published by the World Health Organization.

The radioallergosorbent test (RAST) was used to detect *B. tropicalis*-specific IgE (Allercoat RAST isotope reagent, Sanofi Diagnostics Pasteur, Inc., Chaska, MN, USA). The specific IgG subclasses were determined by ELISA (Binding Site, San Francisco, CA, USA). The determination was calculated on the basis of a background equal to the median of 15 determinations. Results were considered positive when the determination was ≥ 0.1 nm. Data were statistically analyzed by the chi-square test (Mantel-Haenszel) and odds ratio (OR).

Results

Group I comprised 34 AD patients with ages ranging from 1 to 49 years. Twenty-eight (82%) were female and six (18%) were male. Based on the frequent association of AD and allergic respiratory diseases, this group was divided into a subgroup of 20 patients (58.82%) with only AD and another one of 14 patients (41.18%) with AD associated with respiratory allergy. Group II comprised 42 asthmatic patients, with ages ranging from 2 to 54 years; 29 (69%) were female and 13 (31%) were male. Group III comprised 24 nonallergic individuals with no family history of allergy. Their ages ranged from 1 to 45 years.

The results from skin prick tests to *B. tropicalis*, comparing AD patients, asthmatics, and the CG, are shown in Table 1. There was a positive association

Table 1. Skin prick test with *B. tropicalis* extract in AD patients, asthmatic patients, and CG

Skin prick test <i>B. tropicalis</i>	AD patients	Asthmatic patients	Control group
Positive	21 (61.76%)	35 (83.33%)	3 (12.50%)
Negative	13 (38.24%)	7 (16.67%)	21 (87.50%)
Total	34	42	24

between cutaneous reactivity to *B. tropicalis* and AD. The chi-square analysis (corrected by Mantel-Haenszel) was 13.84 ($P=0.0001$), and the OR was 11.31; therefore, the test was 11.31 times more positive in AD patients than in the CG. There was no difference between AD patients with or without associated respiratory disease. Although the skin prick test with *B. tropicalis* extracts detected a significant difference between AD and BA patients (chi-square = 4.45, with $P=0.035$), the odds ratio analysis (OR = 0.32) showed no significant difference between them.

Results from the *in vitro* determination of *B. tropicalis*-specific IgE, by RAST, comparing patients with AD, asthmatics, and the CG, are shown in Table 2. There was a positive association between the presence of *B. tropicalis*-specific IgE and respiratory allergy in AD patients. There was an OR of 7.5, a finding which means that for each patient with only AD, there were 7.5 patients with AD and respiratory allergy that presented *B. tropicalis*-specific IgE. These results showed a significant difference between groups with AD and CG. The chi-square analysis was 4.45 ($P=0.0002$), but it was not possible to define the OR. There was no significant difference between AD and BA patients. The chi-square test finding was 2.36 ($P=0.1244$), and the OR was 0.49.

The determination of *B. tropicalis*-specific IgG subclasses in AD patients, asthmatics, and the CG is shown in Table 3. Statistical analysis did not show any significant difference between the three groups. The chi-square test, corrected by the Mantel-Haenszel method, showed a positive association of *B. tropicalis*-specific IgG3 subclass and AD, compared to asthmatics and to the CG. For each asthmatic, there were 3.5 AD patients positive for *B. tropicalis*-specific IgG3, and for each CG subject, there were 4.2 AD patients with a positive IgG3 determination.

Data on the determination of the *B. tropicalis*-specific

Table 2. *B. tropicalis*-specific IgE in AD patients, asthmatic patients, and CG

<i>B. tropicalis</i> -specific IgE	AD patients	Asthmatic patients	Control group
Positive*	15 (44.12%)	26 (61.90%)	0 (0.00%)
Negative	19 (55.88%)	16 (38.10%)	24 (100%)
Total	34	42	24

* Results of RAST are expressed in classes 0–VI according to % total IgE standard. We considered results of class II to class VI to be positive.

IgG4 subclass have demonstrated a positive association with AD patients when compared to the CG. On the other hand, there was no significant difference compared with the asthmatic group. The determination of *B. tropicalis*-specific IgG4 was 5.6 times higher in AD patients than in the CG when we took into consideration the OR statistical analysis.

Our conclusions were as follows:

- 1) AD patients, with and without associated allergic diseases, were positive in the skin prick test to *B. tropicalis* when compared to the CG; and this reactivity was higher in the BA group.
- 2) *B. tropicalis*-specific IgE was more frequent in AD patients than in CG, and there was no statistical difference from the asthmatic group.
- 3) No correlation was found concerning the presence of *B. tropicalis*-specific IgG1 and IgG2 subclasses in AD and BA patients or the CG.
- 4) AD patients had *B. tropicalis*-specific IgG3 positivity more frequently than BA patients and the CG, suggesting the involvement of other modulating factors of the immune response in AD, such as the activation of the complement cascade and the release of vasoactive substances that could amplify the immunoallergic response.
- 5) AD and BA patients presented high levels of *B. tropicalis*-specific IgG4 more frequently than the CG. There was no significant difference between AD and BA patients. This finding could be explained by the prolonged antigenic exposure of the atopic patients.

Discussion

In AD, there is an exacerbation of humoral immunity, characterized by an increase in serum levels of IgE. There is also an intense cellular response in the tissues that recalls the clinical onset of allergic contact dermatitis (23). Serum levels of IgE are elevated in 80% of AD patients, and some authors associate these levels with the severity of the disease (2). Jones & Sampson (24) reported that the levels of IgE were higher in cases where AD was associated with asthma and/or rhinitis.

In the present study, *B. tropicalis*-specific IgE and IgG subclasses were determined in patients with AD, compared to a group of BA patients and a CG comprising nonallergic subjects. Despite the high prevalence of *B. tropicalis* in Brazil, its role in the pathogenesis of AD has been studied by only a few authors (25, 26). Therefore, we chose *B. tropicalis* as the subject of the experiments in this study.

The results from the skin prick test with *B. tropicalis* revealed a positivity of 61.8% in 34 patients with AD. Fourteen patients (41.2%) had associated respiratory allergy, and 20 patients (58.8%) had only AD. These

Table 3. *B. tropicalis*-specific IgG subclasses in AD patients, asthmatic patients, and CG

IgG subclasses anti- <i>B. tropicalis</i>	AD patients	Asthmatic patients	Control group
IgG1			
Positive	18 (52.94%)	30 (71.43%)	13 (54.17%)
Negative	16 (47.06%)	12 (28.57%)	11 (45.83%)
IgG2			
Positive	22 (64.71%)	35 (83.33%)	14 (58.33%)
Negative	12 (35.29%)	7 (16.67%)	10 (41.67%)
IgG3			
Positive	14 (41.17%)	6 (14.29%)	4 (16.67%)
Negative	20 (58.83%)	36 (85.71%)	20 (83.33%)
IgG4			
Positive	11 (32.35%)	9 (21.43%)	2 (8.33%)
Negative	23 (67.65%)	33 (78.57%)	22 (91.67%)

Results were considered positive when determination was ≥ 0.1 .

data are in accordance with several previous studies of *B. tropicalis* and AD. A positivity ranging from 61.5% to 92% has been reported (19, 20, 25). The differences in these results can be explained by the lack of a standardized *B. tropicalis* extract, the variable individual sensitization, the presence or not of this mite at home, and variations in the temperature and humidity (27, 28).

When we analyzed AD patients with associated respiratory allergy, we found 11 patients (78.57%) with a positive reactivity to *B. tropicalis*. In the group with AD without allergic respiratory disease, there were 10 (50%) positive skin prick tests to *B. tropicalis*. These data make it harder to understand the role of this mite. There are only a few studies on this problem in the medical literature, and no study has determined whether or not AD is associated with respiratory allergy. In Puerto Rico, Montealegre et al. (26) found that 47.7% of tests in AD patients were positive, a finding which coincides with our findings.

When we compared AD patients with and without associated respiratory allergy, we found, first, that there was no significant difference between the groups relating to the skin prick test to *B. tropicalis*, but that those who had associated respiratory allergy showed a reactivity 3.67 times higher than those with only AD. Secondly, we found that the presence of specific IgE in the serum of AD patients with associated respiratory allergy was statistically higher than in the group with only AD. The skin prick test and RAST positivity probably differed because the first one evaluates the presence of specific IgE bound to the Fc receptors on the mast-cell surface, and the second one determines the presence of circulating specific IgE (29).

In the comparison of total AD patients with the CG, both the skin prick test and RAST showed a statistically significant difference, demonstrating the importance of *B. tropicalis* in AD. When we compared AD patients with the asthmatic group, we observed a higher positivity in patients with respiratory allergy, both in

the skin prick test and the RAST. That was expected and confirmed what has been reported before in the medical literature.

There are only a few studies of specific IgG subclasses to house-dust mites, and they refer to *D. pteronyssinus* and *D. farinae* and to patients receiving specific immunotherapy. The determination of *B. tropicalis*-specific IgG1 showed no significant difference between AD and BA patients and/or the CG. This can be explained by the fact that these subjects have received repeated antigenic stimulus by this mite species. The same was found when we determined the positivity of *B. tropicalis*-specific IgG2. There was no significant difference between AD patients and asthmatic patients and the CG. We know that IgG2 is produced in response to polysaccharide antigens. It comprises 19–31% of the total IgG subclasses, with a low capacity of complement activation (30) and a low affinity to bind cell-surface receptors through the Fc portion (31). Studies of IgG2 production in man have shown that there is no elevation of serum levels in the secondary immune response (32). This must be because the polysaccharide antigens are T-cell independent and do not produce memory cells. Our findings seem to correspond to those of other authors reporting on other mites species, although we have not found any report on the *B. tropicalis*-specific IgG2 subclass.

For positivity to the *B. tropicalis*-specific IgG3 subclass, there was a statistically significant difference when AD and BA patients were compared to the CG. These results differ from other studies, which usually did not find elevated levels of IgG3 in atopic patients. The IgG3 subclass is the immunoglobulin with the highest capacity to activate the complement cascade, which is of great importance in the lysis of bacteria and viruses. Frequent infections by virus, bacteria, and

fungi, as well as other mechanisms, are involved in the onset of AD symptoms. We may consider the possibility that IgG3 activating the complement cascade could promote the release of vasoactive amines, amplifying the immune response.

The determination of *B. tropicalis*-specific IgG4 was positive in 32.35% of AD patients, a result which was statistically significant when compared to the CG, but not when compared to asthmatics. These findings are similar to those of other authors who analyzed IgG4 and other species of house-dust mites, such as Merrett et al. (7), who observed a strong correlation between anti-*D. pteronyssinus* IgE and IgG4 in atopic patients.

The observation that IgG4 rises substantially during immunotherapy has led some authors to consider that this IgG subclass blocks allergic reactions. Other investigators have demonstrated that IgG4 acts as an anaphylactic antibody in some cases of food allergy (33). Our findings could be explained by the fact that IgG4 is produced in the immune response as the result of prolonged antigenic exposure. IgG4 does not activate the complement cascade; it acts as a monovalent molecule, having a reduced capacity to bind cell-surface receptors, and producing less inflammation than the other IgG subclasses (8).

Our findings correspond to various studies of specific IgE in AD and show that *B. tropicalis* is an important allergen in our environment. Although we have evaluated a low number of subjects, AD patients had a very significant positivity to *B. tropicalis*-specific IgG3, which has a high capacity to activate the complement cascade. This suggests the participation of complement proteins in the pathogenesis of this disease. The significant detection of *B. tropicalis*-specific IgG4 in AD patients may be explained by the chronic exposure to this mite species in the home environment.

References

1. JUHLIN L, JOHANSSON SGO, BENNICHI H, HOGMAN C, THYRESON N. Immunoglobulin E in dermatitis. *Arch Dermatol* 1969;**100**:12–16.
2. OGAWA M, BERGER PA, MCINTYRE OR, CLENDENNING WE, ISHIZAKA K. IgE in atopic dermatitis. *Arch Dermatol* 1971;**103**:575–580.
3. TERRY WD, FAHEY JL. Subclasses of human gamma globulin based on differences in the heavy polypeptide chains. *Science* 1964;**146**:400–401.
4. AALBERSE RC, VAN MILLIGEN F, TAN KY, STAPEL SO. Allergen-specific IgG4 in atopic disease. *Allergy* 1993;**48**:559–569.
5. SHAKIB F, MCLAUGHLAN P, STANWORTH DR, SMITH E, FAIRBURN E. Elevated serum IgE and IgG4 in patients with atopic dermatitis. *Br J Dermatol* 1977;**97**:59–63.
6. NAKAGAWA T, TAKAISHI T, SAKAMOTO Y, ITO K, MIYAMOTO T, SKVARIL F. IgG4 antibodies in patients with house-dust-mite-sensitive bronchial asthma: relationship with antigen-specific immunotherapy. *Int Arch Allergy Appl Immunol* 1983;**71**:122–125.
7. MERRETT J, BARNETSON RStC, BURR ML, MERRETT TG. Total and specific IgG4 antibody levels in atopic eczema. *Clin Exp Immunol* 1984;**56**:645–652.
8. WALKER IC. Causation of eczema, urticaria and angioneurotic edema by proteins other than those derived from foods. *JAMA* 1918;**70**:897–900.
9. BECK HI, KORSGAARD J. Atopic dermatitis and house dust mites. *Br J Dermatol* 1989;**120**:245–251.
10. HARVING H, KORSGAARD J, DAHL R, BECK H-I, BJERRING P. House dust mites and atopic dermatitis. A case-control study on the significance of house dust mites as etiologic allergens in atopic dermatitis. *Ann Allergy* 1990;**65**:25–31.
11. COLLOFF MJ. Exposure to house dust mites in homes of people with atopic dermatitis. *Br J Dermatol* 1992;**127**:322–327.
12. SANDA T, YASUE T, OOHASHI M, YASUE A. Effectiveness of house dust-mite allergen avoidance through clean room therapy in patients with atopic dermatitis. *J Allergy Clin Immunol* 1992;**89**:653–657.
13. BAGGIO D, AMBRÓZIO LC. Household mites from Brazil: summary and annual seasonal variation. *Rev Bras Alerg Immunopat* 1992;**15**:Abstract 91.

14. FERNÁNDEZ-CALDAS E, PUERTA L, MERCADO D, LOCKEY RF. Mite fauna, Der p I, Der f I and *Blomia tropicalis* allergen levels in a tropical environment. Clin Exp Allergy 1993;23:292–297.
15. JORGE NETO J, CROCE J, BAGGIO D. Ácaros da poeira domiciliar na cidade de São Paulo [Abstract]. Rev Bras Alerg Imunopat 1980;2(3):140.
16. MORI JC, MELLO LM, JARDIM RF, AUN WT, MELLO JF. Alérgenos e análise crítica do controle ambiental. Rev Bras Alerg Imunopat 1993;16:174–180.
17. AMBRÓZIO LC, BAGGIO D, MORI JC, FERNANDES MF, KASE MT, MELLO JF. Avaliação de antígenos de *Blomia tropicalis* e comparação com outros ácaros do pó domiciliar. Cad Imunoalergol Pediatr 1989;4:24–30.
18. MALHEIROS MTSR, TOLEDO BARROS MAM, MACHADO L, AKAGAWA YT, LEÃO RC. Ácaros de estocagem: importância na sensibilização de doentes com sintomas de alergia respiratória. Rev Bras Alerg Imunopat 1990;13:233–247.
19. GUELLER M, FERNÁNDEZ-CALDAS E. Respiratory atopy and domestic mite sensitization in Rio de Janeiro. Rev Bras Alerg Imunopat 1995;18:1318.
20. MORI JC, KASE MT, FERREIRA MD, et al. *Blomia tropicalis*: reavaliação da reatividade das provas cutâneas de leitura imediata. Rev Bras Alerg Imunopat 1992;15:(Abstract 86).
21. HANIFIN JM, LOBITZ WC Jr. Newer concepts of atopic dermatitis. Arch Dermatol 1977;113:663–670.
22. Consenso Brasileiro no Manejo Da Asma. 1. Fortaleza CE, 1994:7–10.
23. LEUNG DYM. Atopic dermatitis: the skin as a window into the pathogenesis of chronic allergic diseases. J Allergy Clin Immunol 1995;96:302–318.
24. JONES SM, SAMPSON HA. The role of allergens in atopic dermatitis. Clin Rev Allergy 1993;11:471–490.
25. DUTRA CS, ALMEIDA LN. Dermatite atópica: estudo da reatividade cutânea imediata e tardia. Rev Bras Alerg Imunopat 1990;13:283–300.
26. MONTEALEGRE F, QUIÑONES C, VILLA J, et al. Ácaros domésticos son los agentes sensitizantes mas importantes en dermatitis atópica en el sur de Puerto Rico. In: XIII Congreso Ibero-Latinoamericano de Dermatología, 26 a 31 de agosto de 1995, San Juan. Anais, Puerto Rico, 1995 (Abstract).
27. ARLIAN LG, VYSZENSKI-MOHER DL, FERNANDEZ-CALDA E. Allergenicity of the mite, *Blomia tropicalis*. J Allergy Clin Immunol 1993;91:1042–1050.
28. SALLES VLFF. Estudo comparativo entre a antigenicidade da *Blomia tropicalis* com o *Dermatophagoides pteronyssinus* em pacientes com alergia respiratória na cidade de São Paulo. São Paulo, 1994. Dissertação de mestrado, Hospital do Servidor Público Estadual de São Paulo.
29. DAHL M. Atopic dermatitis. In: Clinical immunodermatology. 3rd ed. St Louis, MO: Mosby-Year Book, 1996:345–352.
30. FEINSTEIN A, RICHARDSON N, TAUSSING MJ. Immunoglobulin flexibility in complement activation. Immunol Today 1986;7:169–174.
31. ANDERSEN CL, LOONEY RJ. Human leukocyte IgG Fc receptors. Immunol Today 1986;7:264–267.
32. AMLOT PL, HAYES AE, GRAY D, GORDON-SMITH EC, HUMPHREY JH. Human immune responses *in vivo* to protein (KHL) and polysaccharide (DNP-Ficoll) neoantigens: normal subjects compared with bone marrow transplant patients on cyclosporine. Clin Exp Immunol 1986;64:125–131.
33. SHAKIB F, BROWN HM, PHELPS A, REDHEAD R. Study of IgG subclass antibodies in patients with milk intolerance. Clin Allergy 1986;16:451–458.