

# Reactivity of anti-*Blomia tropicalis* IgG and IgE in patients with atopic dermatitis

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## Summary

Patients with atopic dermatitis (AD) commonly develop antibodies of subclasses IgG and IgE to house dust mites, such as *Dermatophagoides pteronyssinus* and *D. farinae*. The domestic mite *Blomia tropicalis* is prevalent in Brazil and can cause the exacerbation of AD. The objectives of this study were to assess skin reactivity to *B. tropicalis* extracts of AD patients and a control group of nonallergic subjects, and to determine the *in vitro* reactivity of anti-*B. tropicalis* IgG and IgE in the serum of AD patients and a control group of nonallergic individuals. Subjects were 36 patients with confirmed AD and the controls were 25 nonallergic individuals. Skin sensitivity to *B. tropicalis* extracts was tested by the prick-test, and anti-*B. tropicalis* IgG reactivity in the serum was detected by the Western blot. Anti-*B. tropicalis* IgE reactivity in the serum was measured by RAST. Patients with AD reacted 7.12 times more often to extracts from *B. tropicalis* than the control group. A positive association was observed between the presence of anti-*B. tropicalis* IgG and IgE and AD. AD patients showed a high degree of sensitization to *B. tropicalis*; it can thus be considered a risk factor for the development of AD exacerbations in patients exposed to this mite species.

## Introduction

In spite of increasing evidence that house dust mites are triggers of atopic dermatitis (AD) and its lesions in susceptible individuals, this association remains controversial. Huang *et al.*<sup>1</sup> conducted a prospective study with healthy newborns until they reached 3 years of age. During this period, *Der p1* concentrations in house dust and total and specific immunoglobulin E against mite allergens were determined when each baby was 18 and 36 months old. It was found that in those individuals exposed to mite allergen concentrations > 1 µg/g dust, 21.4% had developed AD and that exposure in general could be associated with a higher incidence of AD. In other studies conducted by Ricci *et al.*<sup>2</sup> it was demonstrated that there is a dose-response relationship between IgE-mediated response and exposure to *Der p1*

mite allergen levels > 2 µg/g dust (a concentration presumed to be a risk factor for sensitization). Also 90% of these patients presented positive allergometric [prick test, patch test and radioallergosorbent test (RAST)] response to *Dermatophagoides pteronyssinus*. The authors concluded that exposures to high concentrations of mite allergens may elicit *D. pteronyssinus* IgE sensitization and delayed hypersensitivity in children with AD. Similar results have been reported by Harving *et al.*<sup>3</sup> Reversibility of the exacerbation and clinical improvement has been documented when environmental exposures to dust mites have been reduced.<sup>4,5</sup> From the evidence presented, it is reasonable to propose that allergens from *D. pteronyssinus* and *Blomia tropicalis*, a domestic mite species found in Brazil,<sup>6</sup> share biological importance in the induction of allergic responses and their clinical manifestations.

The domestic mite *B. tropicalis*, a member of the family *Echymyopodidae*<sup>7</sup> is an important source of aeroallergens in tropical and subtropical parts of the world.<sup>8</sup> Evidence supporting the allergenicity and

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clinical importance of *B. tropicalis* in atopic diseases, notably asthma and allergic rhinitis, has been provided by several authors. Arruda *et al.* demonstrated the presence of *B. tropicalis*-specific IgE in asthmatic children, and showed that the RAST score in this population was moderate to high, indicating sensitization to this mite.<sup>9</sup> Since this initial study, several authors have reported IgE-mediated reactivity to *B. tropicalis* in allergic patients.<sup>10–15</sup> Evidence that exposure triggers a response in allergic patients has been provided by *in vivo* challenge studies. Stanaland *et al.* challenged subjects with allergic rhinitis with *B. tropicalis* extracts.<sup>16</sup> In their study 10 out of 12 (83%) subjects had positive nasal challenge responses to *B. tropicalis* as measured by rhinometry. The authors concluded that *B. tropicalis* should be considered as a risk factor for allergic rhinitis when evaluating a patient who lives in an area where it is endemic.

Little is known about the association between *B. tropicalis* and AD, therefore the present study was undertaken in an effort to elucidate the possible role of *B. tropicalis* in this disease. In a case-control study the presence of anti-*B. tropicalis* serum IgG and IgE was analysed, together with skin reactivity by using the prick-test.

## Patients and methods

### Sample size

To study the association between *B. tropicalis* and AD, we conducted a case-control study. The sample size used in the present study was calculated using published data on prevalence studies in Brazil by Rizzo *et al.*<sup>14</sup> The authors found that allergic children had 82% skin reactivity to dust mites. With an expected prevalence of 80% positive skin reactions to *B. tropicalis* in AD patients, the proposed sample size of 54 individuals was calculated to be able to reach at least an odds ratio (OR) of 6.0 when comparing 27 cases to 27 controls, reaching 80% power and a type I error. Sample size determinations were conducted using EPI-INFO 6.0 (Centers for Disease Control, Atlanta, Georgia, USA).

### Patient population

A total of 36 AD patients who presented a dermatologist-based diagnosis of AD according to established criteria were included in the study.<sup>17</sup> Patients were excluded from the study if they were using systemic corticosteroids or immunosuppressive drugs, had signs

or symptoms of systemic immunodeficiency or any other severe disease, were pregnant or using antihistamines or other drugs for allergies that could interfere with the skin test. Those patients with skin lesions on the forearm were also excluded from skin testing because of the possibility that the lesions could interfere with the measurement of the reaction. For the control population, 25 nonallergic individuals were included in the study. None of the controls had signs or symptoms of allergic diseases. Patients were recruited from the outpatient clinic of the Hospital do Servidor Público Estadual de São Paulo (Dermatology and Allergy) and Complexo Hospitalar Padre Bento de Guarulhos. Controls were recruited from the same institutions including routine or follow-up visits for reasons other than allergic disease. All patients signed an approved consent form prior to participating in the study.

### Skin testing and blood sampling

A series of prick tests were conducted on the volar surface of the arm by a trained nurse or a physician following standard procedures. Participants were tested using standardized glycerinated *B. tropicalis* extracts (IPI-ASAC, Sao Paulo, Brazil). Skin reactivity was recorded at 15 min. The mean of the major diameter and its perpendicular for the wheal-and-flare were measured. The smallest reaction considered positive was a mean of diameters  $\geq 3$  mm<sup>2</sup>.<sup>18</sup> Histamine diphosphate with base equivalent of 1 mg/mL was used as a positive control. The vehicle alone was used as a negative control. For the standardized prick tests, disposable polymethacrylate needles were used (Satalergens, France). Prick tests performed with standardized extracts of *D. pteronyssinus*, *B. tropicalis*, *D. farinae*, *Suidasa pontificeae* and *Tyrophagus putrescentiae* in all of the participants. Also, from each participant a total of 10 mL of venous blood was collected for the detection of specific IgE and IgG using RAST and Western blotting respectively.

### Data analysis

Data entry and analysis were carried out by using EPINFO. Frequency distributions were performed for all of the variables. Arithmetic means for the major diameters of the prick test were calculated. The comparison of skin reactivity, RAST and IgG reactivity rates between cases and controls was carried out by using  $2 \times 2$  tables. The crude ORs were used as a measure of rate difference, Cornfield's 95% confidence intervals (CI) for the ORs and Fisher's exact test were

calculated according to Pagano.<sup>19</sup> Chi-square tests were used to assess the significance of the OR.  $P < 0.050$  was considered significant.

## Results

### Study population

The age range of patients with AD was 1–49 years (mean, 17.72 years) and that of the controls was 1–45 years (mean, 17.03 years). Of the AD patients 83% were female, whereas only 44% of the controls were female.

### Skin reactivity

The results of the prick tests with *B. tropicalis* extracts are presented in Table 1. There was a positive association between the presence of skin reactivity to *B. tropicalis* by the prick test and AD ( $P < 0.05$ ). The presence of anti-*B. tropicalis* IgE by RAST in the serum of AD patients and the control group is illustrated in Table 1. These results show that there was a positive association between the presence of anti-*B. tropicalis* IgE in the serum and AD ( $P < 0.05$ ).

The presence of anti-*B. tropicalis* IgG in the serum of patients with AD and individuals in the control group, as detected by Western blotting, is shown in Fig. 1 and Table 1. Statistical analysis showed a positive association between the presence of anti-*B. tropicalis* IgG reactivity and AD.

## Discussion

The role of *B. tropicalis*-derived allergens in the pathogenesis of AD has not been studied fully, and the available information indicates that it induces a strong skin reactivity in allergic patients.<sup>20</sup> In our study, specific IgE to *B. tropicalis* in AD patients was detected using both *in vivo* and *in vitro* tests. The results show that AD patients were seven times more likely to have a positive skin test to *B. tropicalis* than the nonallergic control group. Similarly, AD patients had 12.8 times more

positive *in vitro* IgE activity than the nonallergic control group. These results strongly suggest that *B. tropicalis* is an important sensitizing agent in AD and are in parallel with the findings of several authors with other mite species.<sup>21</sup> In this regard, a strong IgE response in the AD patients indicates environmental exposures to *B. tropicalis*-derived allergens in sufficient amounts to induce allergic sensitization. Although our study did not quantify these mite allergens environmentally, it is reasonable to conclude that *B. tropicalis* is associated with patient sensitization that could lead to development of AD under chronic environmental exposures.

The skin lesions in AD may be precipitated by numerous factors, including aero-allergens that would trigger type I hypersensitivity (house dust mites, pollen, fungi and others), food (milk, egg-white, wheat), microbial organisms (*Staphylococcus aureus*, *Pityrosporum ovale*), emotional disturbance, sweating and weather changes.<sup>22</sup> Also, it has been demonstrated that the complement system actively participates in the inflammatory process in AD.<sup>23,24</sup> One of the most important activators of the classical complement cascade is the IgG complexed to antigens, in this case dust mite-derived allergens from environmental exposures. In our study,

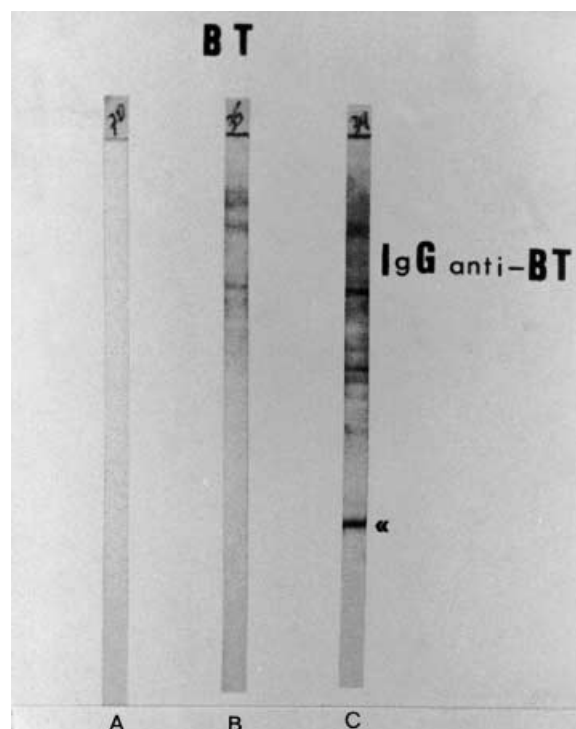


**Figure 1** Lane A represents the negative control. Lane B, where visible dark bands are observed, illustrates the anti-*Blomia tropicalis* IgG reactivity.

**Table 1** Characteristics of the reactivity of the AD patients and controls to *B. tropicalis* extracts ( $n = 61$ ).

| Variable                 | Crude OR | 95% CI     | P     |
|--------------------------|----------|------------|-------|
| Positive skin prick test | 7.07     | 2.19–22.57 | <0.05 |
| Positive RAST            | 12.85    | 2.87       | <0.05 |
| Positive Western Blot    | 3.25     | 1.06–9.97  | 0.030 |

AD patients had 3.25 times more positive IgG reactions to *B. tropicalis* extracts than the nonallergic control group. Although the mechanism for the phenomenon is presently not known, it is possible that the IgG regulatory mechanisms in AD patients favour a high reactivity state that it is reflected in the *in vitro* laboratory analysis. Local and chronic activation of complement by anti-*B. tropicalis* IgG could produce complement-derived components leading to cell infiltrates and skin lesions. Our data also show that the nonallergic controls had a 50% prevalence in IgG reactivity. This could be explained by the environmental exposure of nonallergic individuals to *B. tropicalis* antigens, but without the clinical manifestations of AD. The development of an allergic disease in the individuals with mite-specific IgG reactivity would depend on genetic predisposition, quantity of exposure, cytokine production pattern and the presence of specific IgE in sufficient amounts to induce cell degranulation.

## Conclusions

- (a) Patients with AD showed greater positivity in skin tests with *B. tropicalis* antigens than did normal controls.
- (b) Patients with AD had anti-*B. tropicalis* IgE antibodies more frequently than normal controls.
- (c) The presence of anti-*B. tropicalis* IgG reactivity was greater in patients with AD than in the control group.
- (d) This mite species should be considered in clinical evaluations, and the promotion of patient-based measures of environmental hygiene is highly recommended to reduce the mite population overall, particularly that of *B. tropicalis*.

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