

Identification of allergens from the mite *Blomia tropicalis*

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Summary

In some tropical areas the mite *Blomia tropicalis* is a clinically important allergenic component of house dust, inducing specific IgE immune response in patients with allergic respiratory diseases such as asthma and rhinitis. The identification of allergens of this mite is necessary to obtain appropriate reagents for diagnostic and treatment procedures. We carried out this study using immunoblotting to detect the allergens of *B. tropicalis*. Our results demonstrate that this mite has one major allergen (11–13 kDa) and three other important allergens with about 50% binding (64, 36 and 33 kDa). Therefore, *B. tropicalis* should be regarded as an important source of allergens in the house dust in tropical areas, besides those derived from other mites.

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Introduction

House dust mites are an important source of allergens which can cause allergic asthma (AA) and allergic rhinitis (AR) [1–3]. *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are associated with allergic respiratory diseases in several areas of the world [2,4,5]. *Blomia tropicalis* is a domestic mite of the Glycyphagidae family commonly found in tropical and subtropical areas as a component of house dust, together with other mites with well known sensitizing properties [6–8]. The important allergenic role of this mite as an inducer of AA and AR has been described recently [9–11]. The identification of major, intermediate and minor allergens [12,13] is important to ensure standardized mite allergenic extracts for diagnosis and treatment can be created. In addition, this identification is helpful when characterizing recombinant allergens and performing basic studies as, for example, the genetic control of IgE immune response.

Little work has been done regarding the identification of the spectrum and relative clinical importance of *B. tropicalis* allergens. Recently, it has been reported that *B. tropicalis* has 21 different IgE binding antigens, as detected by crossed radioimmunoelectrophoresis (CRIE). Most of these allergens are species-specific [14]. The present study reports the allergenic properties of an extract of this mite. The prevalence of the IgE immune response to its allergens was investigated with

patients' sera using an immunoblotting technique. The results show that a whole body extract of *B. tropicalis* has, at least, 25 allergens ranging between 11 and 85 kDa.

Materials and methods

Allergen extracts

B. tropicalis collected from house dust samples from homes of allergic patients were cultured in our laboratory as previously described [10]. Lyophilized extract was reconstituted in water to obtain a concentration of 3.5 mg/ml. A standardized *D. pteronyssinus* extract was obtained commercially.

Skin-prick test (SPT)

A skin-prick test was carried out. Papules greater than 5 mm were considered positive. To skin-test with *B. tropicalis* we prepared an extract containing 0.23 mg/ml of protein in 50% glycerol, 0.125% sodium bicarbonate, 0.25% NaCl and 0.4% phenol. Positive (histamine, 1 mg/ml) and negative (50% glycerol) controls and an extract of *B. tropicalis* culture medium only were included in all skin tests.

Patients

Sera from 96 non-immunotherapy-treated AA patients with positive SPT and radioallergosorbent test (RAST)

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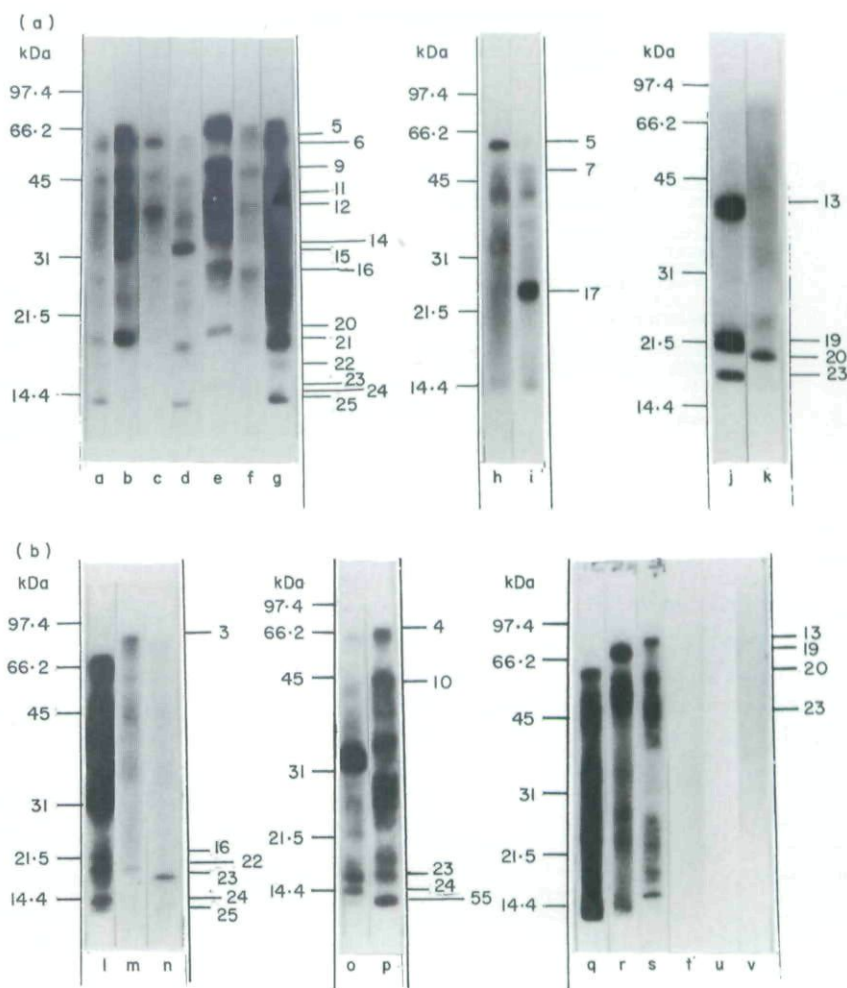


Fig. 1. Autoradiography of SDS-PAGE analysis of the allergens of a *B. tropicalis* body extract. Lanes a–k (A) and l–s (B) show patterns of reactivity of strips incubated with selected sera and discriminating the different allergens. Lanes t, u and v (B): strips incubated with control serum pool, PBS and individual serum from a subject ragweed positive/mite negative respectively. Overexposure of some gels was necessary to better show specific bands.

(total count bound > 1%) to *B. tropicalis* were used for the immunoblotting assays. Patients (41 men and 55 women aged 18–54 years) were mulattos, the prevalent ethnic group in Cartagena [15]. AA was diagnosed following the criteria proposed by the expert panel of the Allergy Foundation of America [16]. The average total IgE level in patients was 620 UI/ml (Phadezym PRIST, Pharmacia, Sweden). Seventy per cent of patients were allergic to mites only. The other 30% were only allergic to a few additional allergens, mainly cockroach, cat and *Aspergillus*. All patients were negative when skin-prick tested with a *B. tropicalis* culture medium extract.

A pool of 10 sera from mulatto subjects (aged 18–35 years) without any allergy history, negative SPT and RAST to *B. tropicalis* and *Dermatophagoides* spp. were employed as negative control. In addition, a serum from one patient allergic to ragweed but non-allergic to mites was used as negative control. A pool of 21 sera from subjects with AA (positive SPT and RAST to *B. tropicalis*) and previously tested with immunoblotting was used as

positive control. An additional serum (SPT and RAST positive to both *D. pteronyssinus* and *B. tropicalis*) was used to perform immunoblotting of a *D. pteronyssinus* extract parallel with the extract of *B. tropicalis*. This serum was able to identify *D. pteronyssinus* group II allergens and several allergens of *B. tropicalis*. Each subject signed a written informed consent before entry into the study.

Radioallergosorbent test (RAST)

Specific IgE to *B. tropicalis* extract was determined in plastic microtitre plates (Immulon 4, Dynatech Laboratories, Alexandria, VA, USA) using methods previously described [10].

Immunoblotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (12%) and immunoblotting

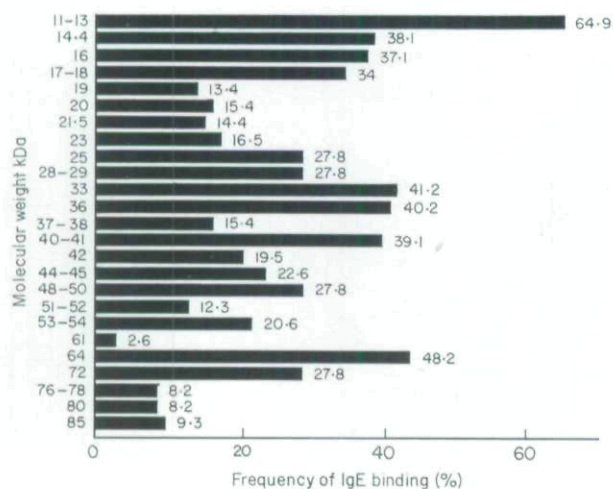


Fig. 2. Histogram showing the frequency of IgE binding of patients' sera to each allergen of *B. tropicalis* body extract.

were carried out according to standard methods [17,18]. To ensure electrophoresis could take place under reduced conditions, 2, beta-mercaptoethanol was added and the mixture heated at 95°C for 10 min. The electro-

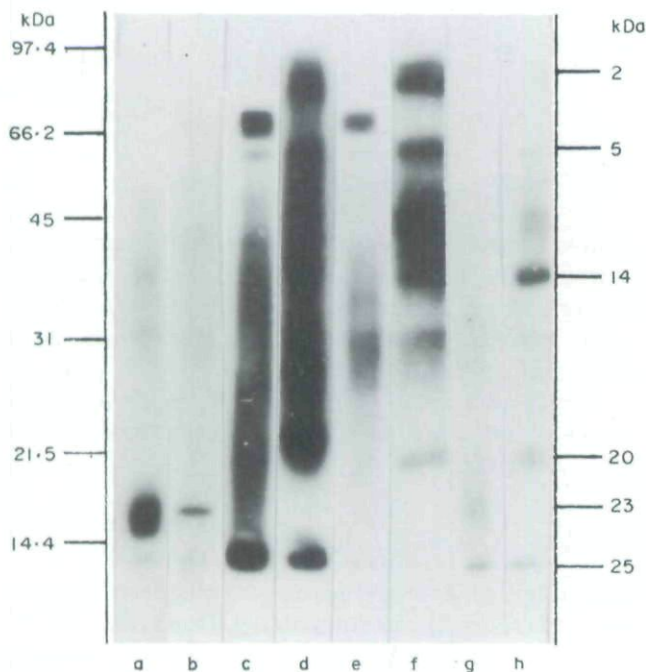


Fig. 3. Autoradiography of SDS-PAGE analysis of the allergens of a *B. tropicalis* body extract, showing differences between reducing and non-reducing conditions using the same serum for each pair of lanes (see text). Lanes a, c, e and g: non-reducing conditions. Lanes b, d, f and h: reducing conditions.

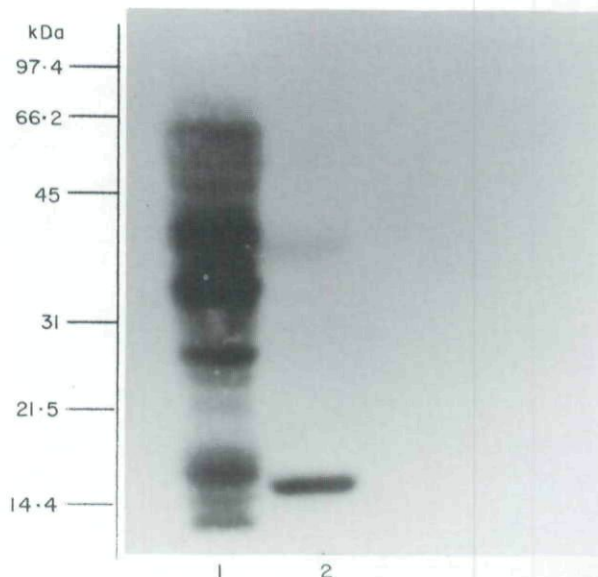


Fig. 4. Autoradiography of SDS-PAGE analysis of *B. tropicalis* (Lane 1) and *D. pteronyssinus* (Lane 2) extracts under reducing conditions. This experiment shows that the 11–13 kDa allergen of *B. tropicalis* (the lowest band, below 14.4) is clearly different from the 15 kDa group II band of *D. pteronyssinus* (this 15 kDa band was identified by 90% of a panel of allergic sera, with the same intensity under reducing and non-reducing conditions).

phoresis was run at 200 V on a Mini-Protean II cell (Bio-Rad) and transferred in cold conditions for 2 h at 80 V. The NC strips were incubated for 10 h with 2 ml of patients' sera (diluted 1:4 in phosphate buffered saline [PBS]) and the controls described. After three washes with PBS/Tween 20, the strips were incubated for 16 h with 2 ml of ^{125} I-labeled rabbit anti-human IgE (Pharmacia), washed with PBS and exposed to a X-OMAT AR (Kodak) film at –70°C, for 10–72 h.

Results

RAST

RAST of *B. tropicalis* showed that the % TCB in patients' sera ranged between 1.1 and 43% (mean 18%) and in negative control sera ranged between 0.16% and 0.30% (mean 0.24%).

Allergens of *B. tropicalis* extract

No signals were detected from the strips when negative serum pools, ragweed-positive/mite-negative controls and PBS were used. In addition, when proteins of culture medium blotted on NC strips were incubated with the positive and negative serum pools, no bands

were detected. When using patients' sera, there were bands in all immunoblots performed, even when using sera with very low levels of *B. tropicalis* IgE antibodies.

The extract prepared from *B. tropicalis* contains at least 25 IgE-binding components between 11 and 85 kDa (Fig. 1). Under reducing conditions, the most frequently detected allergens were the 11–13 kDa, 64 kDa, 33 kDa, and 36 kDa, with a reactivity of 64.9%, 43.2%, 41.2% and 40.2% respectively. The remaining allergens were detected with frequencies between 2.6% and 39.5% (Fig. 2). Under non-reducing conditions most of the allergens mentioned were also detected, although, in general, they were better defined under reducing conditions. The allergens with the strongest binding were: 11–13 kDa, 14.4 kDa, 21.5 kDa, 25 kDa, 33 kDa, 36 kDa, 37–38 kDa, and 64 kDa. Applicable to all of them, a strong signal in the autoradiography usually appeared before 48 h. Most sera (77%) detected between five and nine allergens. The maximum number of allergens detected by a single serum was 11. No unique pattern of sera reacting with several allergens could be established.

The following differences between reducing and non-reducing conditions were observed (Fig. 3):

- 1 The 16 kDa allergen shows a stronger IgE binding capacity under non-reducing conditions (lanes a and b).

- 2 Under non-reducing conditions, the electrophoretic mobility of most allergens with a mol.wt. over 64 kDa increases. Lanes c and d and e and f show this finding with two different sera (A154 and A104): the 80 kDa allergen (reducing conditions) appears as a 72 kDa allergen under non-reducing conditions.

- 3 Under non-reducing conditions, the 36 kDa allergen was not detected with 10 sera, which is shown in lanes g and h for serum A192. Another group of sera detected this band under both conditions, but it was always weaker under non-reducing conditions. This pattern of stronger IgE binding reactivity under reducing conditions was also observed for the 37–38 kDa allergen (not shown).

Since we used non-gradient gels, the possibility that the 11–13 kDa allergen was, in fact, a 14 kDa group II equivalent was ruled out by parallel immunoblotting of *D. pteronyssinus* and *B. tropicalis* extracts (Fig. 4).

Discussion

The present study shows that a whole body extract of *B. tropicalis* has at least 25 allergens, which supports the evidence of its allergenic role, already proven by RAST and skin-test studies. More than 50% of the patients' sera reacted with the 11–13 kDa allergen (Fig. 1, band 25); so that it can be considered a major allergen. Three other allergenic components: 64, 36 and 33 kDa (Fig. 1, bands 5, 14 and 15 respectively) were recognized by

patients' sera with frequencies ranging from 40 to 50%. These four allergens showed a very strong IgE binding capacity, according to their autoradiographical signal. Thus, it can be concluded that the crude whole body extract of *B. tropicalis* includes four important allergen components with molecular weights of approximately 11–13, 33, 36 and 64 kDa.

The number of allergens detected in this study is near the number of IgE binding components found in other mite extracts. For example, 32 IgE-binding components have been described for *D. pteronyssinus* [19]; 14 for *D. farinae* [20]; 25 for *Euroglyphus mainey* [21] and 11 for *Lepidoglyphus destructor* [22]. As most of these mites are present in tropical environments [7], the allergenic potential of house dust in those regions should be high, which explains the elevated prevalence of asthma found in previous studies [23,24]. In addition, although genetic factors may be involved, the expected high level of exposure of the population to *B. tropicalis* could explain our finding regarding the number of allergens (5–9) recognized by IgE antibody from most patients, which is higher than the number of allergens commonly recognized by sera (2–6) when immunoblotting *Dermatophagoides* spp. extracts [25, 26].

Although patients included in this study were also allergic to *D. pteronyssinus* and *D. farinae*, it is unlikely that allergens detected in the body extract of *B. tropicalis* were the result of crossreactivity because it has been demonstrated that this mite shows only weak crossreactivity with clinically important Pyroglyphid mites (10, 11, 14). In addition, although we have found a considerable degree of crossreactivity between *B. tropicalis* and *Lepidoglyphus destructor* [10] the latter has not been found in house dust in Cartagena [7]. Therefore, even though the present investigation cannot rule out that some bands are from related products as has been suggested for *Der f* III [27], the non-crossreacting allergens could increase the allergenic properties of house dust in tropical environments and, as a consequence, they should be considered for the diagnosis and treatment of allergic diseases in these regions.

This study is a first step in the standardization of reagents for the diagnosis and treatment of allergic diseases caused by *B. tropicalis*. Cloning allergens from this mite, to ensure better characterization of them is now underway in our laboratory.

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