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Exposure and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil

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Summary

A group of 20 mite allergic asthmatic children aged 6–12 years old, living in São Paulo, Brazil, was studied regarding their degree of sensitization to house dust mites and exposure to mite allergens in their homes. In 18 out of 20 houses at least one dust sample was obtained which contained >10 μg *Der p* I/g of dust. The highest levels of *Dermatophagoides pteronyssinus* allergens, *Der p* I and Group II, were measured in bedding samples (geometric mean 38·4 and 36·6 μg/g, respectively), followed by bedroom floor, TV room and kitchen. Mite allergen levels in Brazilian houses were as high as those reported to be associated with sensitization and acute attacks of asthma in other parts of the world. In keeping with previous reports that *D. farinae* is rarely found in Brazil, *Der f* I was undetectable or found in very low levels (<0·5 μg/g). Levels of cat allergen *Fel d* I of >8 μg/g of dust were obtained only in 2 houses only. Cockroach allergen *Bla g* I was detected in five out of 20 houses. Levels of IgE antibodies to *D. pteronyssinus* were >200 RAST U/ml in 19 out of 20 children (geometric mean 1588 RAST U/ml). IgE antibodies to cat, cockroach, *A. fumigatus*, ragweed and rye grass pollens were undetectable or <80 RAST U/ml. IgE antibodies to the mite *Blomia tropicalis* were also measured, and levels >200 RAST U/ml were observed in 13 out of 20 sera. Immunoabsorption studies demonstrated that the bulk of the IgE antibody to *B. tropicalis* (64%) was to species-specific allergens and that 36% were cross-reactive with *D. pteronyssinus*. The implication of our results is that management of children with asthma in São Paulo should include skin testing for allergy to both *Dermatophagoides* and *B. tropicalis* as well as recommendations about environmental control of house dust mite exposure.

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Introduction

The importance of house dust mites as a source of allergens and their contribution to bronchial asthma, rhinitis and atopic dermatitis has been recognized for many years [1,2]. Epidemiologic studies have demonstrated that the presence of IgE antibodies to dust mites and other indoor allergens are major risk factors for asthma [3–5]. Some of the studies have come from tropical countries such as Zambia, Papua New Guinea and Brazil [6,7]. The development of sensitive monoclonal antibody-based immunoassays for measuring *Dermatophagoides* sp. Group I and group II allergens have both improved the standardization of extracts and greatly simplified the assessment of exposure to dust mite allergens [3].

Although pyroglyphid mites have been shown to dominate the mite fauna in house dust, IgE-mediated reactions to storage mites have been documented by skin tests, RAST and bronchial challenge tests [8–13]. These nonpyroglyphid mites include *Glycyphagus domesticus* and *Lepidoglyphus destructor* (family Glycyphagidae), *Tyrophagus putrescentiae* and *Acarus siro* (family Acaridae). *Blomia tropicalis* is regarded as a storage mite, however high densities of this species have been reported

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in house dust samples from several countries, including the U.S.A [14–17].

Studies carried out in São Paulo, Brazil, have identified *D. pteronyssinus* and *B. tropicalis* as the most frequent mite species in house dust [7]. At Paulista School of Medicine, two-thirds of the patients who are referred to the Paediatric Allergy Clinic give positive immediate skin tests to house dust mite extracts (*Dermatophagoides* species). A recent report from São Paulo on 123 adult patients with asthma and/or rhinitis showed that 77% were skin test positive to *D. pteronyssinus* and 78% were positive to *B. tropicalis* [18]. In populations exposed to both mites, it is not clear how much of the skin response is specific for *B. tropicalis* or is shared with antigens from *D. pteronyssinus*. The present study was designed to quantify exposure to house dust mite allergens and IgE antibodies in a group of mite-allergic asthmatic children living in São Paulo. We also investigated the antigenic relationships between *B. tropicalis* and *D. pteronyssinus*.

Subjects and methods

Subjects

Twenty children (11 girls, nine boys) with moderate to severe asthma, aged 6–12 years old, were enrolled in the present study, from patients who attended the Paediatric Allergy Clinic of Paulista School of Medicine, São Paulo, Brazil. Children were selected based on positive immediate skin test to *D. pteronyssinus* (Dpt) and/or *D. farinae* (Df) extracts (Alergomed, Merck, Munich, Germany). A positive test was defined by a weal size of $> 5 \times 5$ mm in diameter on prick test. Diagnosis and classification of asthma was done by history, physical exam and spirometry [19]. Serum samples were obtained in August to October, 1988, and patients had their houses visited for collection of house dust samples over the same period of time.

B. tropicalis *and* D. pteronyssinus *extracts*

Two *B. tropicalis* extracts were used in the present study. A whole culture extract was produced in São Paulo, Brazil, as follows: *B. tropicalis* was grown in a medium containing horse skin scales, fish flour, beer yeast, manioc toasted flour and soybean flour. Mites, faecal particles and culture medium were defatted with ether, extracted for 1 hr at 4°C in saline, sonicated and centrifuged at 48 000 *g* for 30 min.

The supernatant was dialysed against saline for 24 hr. A mite body extract was obtained in Tampa, FL, according to the following method: *B. tropicalis* was cultured on a medium of autoclaved nude mice food and Brine shrimp 1:1 at 25°C and 75% relative humidity. Cultures were transferred to a modified Tullgren apparatus. Live mites were forced to pass through six layers of cheese cloth to escape the heat produced by a 60 W light bulb. Mite bodies were frozen and subsequently defatted with ether, extracted overnight in 100 mM ammonium bicarbonate and centrifuged at 12 000 *g*. The supernatant was dialysed against distilled water and frozen at −20°C until use. Extract made from isolated *D. pteronyssinus* bodies was kindly provided by Shirley Williams, Hollister Stier, Spokane, WA.

Total IgE and specific IgE antibodies

Total serum IgE was measured by two-site radioimmunoassay (RIA) [4,20]. Briefly, removawell plates were coated overnight with monoclonal antibodies (MoAbs) to heavy chain determinants on IgE (CIA/E/7.12 and CIA E 4.15, kindly provided by Dr Andrew Saxon, University of California, Los Angeles, CA). Dilutions of a control serum or patients' sera were added, followed by ^{125}I-labelled goat anti-human IgE. Anti-human IgE was prepared by immunization of a goat with purified Fc fragment of the IgE PS myeloma protein in Freund's complete adjuvant and subsequent affinity purification of the antibodies over a PS Fc epsilon fragment immunosorbent [21]. IgE results were expressed as international units ml (1 IU/ml, about 2·4 ng/ml of IgE). Specific IgE antibodies to *D. pteronyssinus*, *D. farinae*, cat epithelium, German cockroach (*Blattella germanica*), *Aspergillus fumigatus*, short ragweed pollen and rye grass pollen were measured by RAST [4]. In order to decrease background binding on RAST, horse serum was used as diluent in all steps of the assay. Extracts of *B. tropicalis* mite bodies and whole *B. tropicalis* culture were used to coat CNBr-activated RAST discs (10 μg protein/disc) for measuring IgE antibody to *B. tropicalis*. Preliminary experiments with four sera showed that optimal binding was seen with 10 μg *B. tropicalis* protein/disc. All assays for IgE antibodies were quantitated on the basis of a control curve, using *D. farinae* discs and serial two-fold dilutions of a mite allergic serum pool (UVA 87/01) that had been substandardized against an International Reference Serum Pool (NIBSC Code no. 82/528). The UVA 87/01 control serum pool contains 1000 RAST U/ml of IgE antibody to *D. farinae*. One unit of IgE antibody in the RAST is approximately equivalent to 0·1 ng of IgE [4].

Allergen levels in house dust

Samples of house dust were obtained from four sites of each patient's house: bedding, bedroom floor, TV room and kitchen, using a hand-held vacuum cleaner (Electrolux, São Paulo, Brazil). Floor dust was collected by vacuuming an area of 1 m^2 for 2 min [22]. Kitchen samples

included cabinet and floor dust. Fine dust was obtained by sieving through a 0·3-mm mesh screen. Samples of 100 mg were extracted overnight in 4°C in 2 ml borate-buffered saline (BBS), pH 8·0. After centrifugation, supernatants were stored at −20°C prior to immunoassay. Group I *Dermatophagoides* allergens (*Der p* I and *Der f* I), cat allergen *Fel d* I, and cockroach (*B. germanica*) allergen *Bla g* I were measured by two-site MoAb-based ELISA [23–25]. Group II *Dermatophagoides* sp. allergens were quantified by MoAb-based RIA [26]. Reference standards containing known levels of each allergen were used to make control curves and the results were expressed as μg/g of dust or ng/ml of extract. Cockroach assays were quantitated based on a reference German cockroach extract (UVA 89/01), and results were expressed as arbitrary units of *Bla g* I/g of dust [25].

Immunoabsorption studies

Absorption experiments were carried out as described by Heymann *et al.* [27]. CNBr-activated Sepharose was coupled with 10 mg *D. pteronyssinus* whole body extract/g of gel, or with 10 mg *B. tropicalis* body extract/g of gel, or with 10 mg human serum albumin (HSA)/g of gel. It was estimated that 1·2 mg *Der p* I/g of gel bound to the Sepharose (99% of *Der p* I added). Sera from six Brazilian children (1·2 ml diluted 1/2 or 1/8) who had >200 U of IgE antibody to both *D. pteronyssinus* and *B. tropicalis* were absorbed with 0·25 ml 40% Sepharose beads for 6 hr and then separated by centrifugation. Serum from an American patient allergic to *D. pteronyssinus* but without measurable IgE antibody to *B. tropicalis* was also absorbed. Measurements of IgE antibody to *D. pteronyssinus* and *B. tropicalis* were carried out by RAST on all samples post-absorption. Absorption results were calculated as the mean of the percentage of reduction in IgE antibody observed with each dilution. Serum IgG antibody levels to *Der p* I were measured by antigen-binding RIA [28]. Absorption by mite immunosorbent was compared with absorption by HSA immunosorbent for each sample.

Results

IgE response to inhalant allergens

Total serum IgE levels were >400 IU/ml in 18 out of 20 children (geometric mean (gm) 1092 IU/ml, range 96–7031). In each serum the highest levels of IgE antibodies were to mite antigens; IgE antibodies to *D. pteronyssinus* were >200 RAST U/ml in 19 out of 20 children (Fig. 1). The levels of IgE antibodies to *D. farinae* were on average 2·3-fold lower; however, there was a close correlation

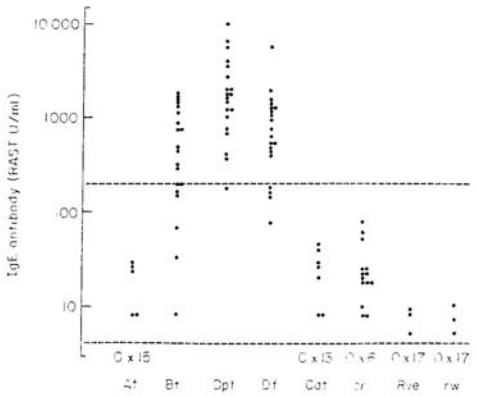

Fig. 1. Levels of serum IgE antibodies to *A. fumigatus* (Af), *B. tropicalis* (Bt), *D. pteronyssinus* (Dpt), *D. farinae* (Df), cat, cockroach (cr), rye grass and ragweed (rw) pollens in 20 mite-allergic asthmatic children from São Paulo. Values shown below the lower dashed line (for example, 0 × 15 for *A. fumigatus*) represent undetectable IgE antibodies (<4 U/ml). The upper dashed line represents levels of 200 U/ml of specific IgE antibody (equivalent to about 20 ng of IgE) which we consider as a moderate positive RAST [4].

between the results for the two *Dermatophagoides* species ($r = 0{\cdot}90$, $P < 0{\cdot}001$). Levels of IgE antibodies to *B. tropicalis* of >200 RAST U/ml were observed in 11 out of 20 sera or 13 out of 20 sera using the whole mite culture or the mite body *B. tropicalis* extracts, respectively. A good quantitative correlation was seen between the results for the two *B. tropicalis* extracts ($r = 0{\cdot}80$, $P < 0{\cdot}05$). Levels of IgE antibodies to *D. pteronyssinus* (gm 1585 RAST U/ml) were significantly higher than the levels of *B. tropicalis* (gm 358 RAST U/ml) ($t = 3{\cdot}78$, $P < 0{\cdot}001$, Student's *t*-test). RAST analysis using *B. tropicalis* food medium (10 μg protein/disc) showed <1% binding, as compared to up to 31% binding on *B. tropicalis* discs. IgE antibodies to *B. tropicalis* in a group of nine patients from Virginia, U.S.A., allergic to *D. pteronyssinus* (345–10 000 RAST U/ml) were very low and in no case greater than 5% the results obtained in the *D. pteronyssinus* RAST (data not shown). IgE antibodies to cat, cockroach, *A. fumigatus*, ragweed and rye grass were <80 RAST U/ml (Fig. 1).

Exposure to indoor allergens

Mite, cat and cockroach allergens were measured in 79 dust samples from the 20 houses visited (one kitchen dust sample was not available). The highest levels of *Der p* I and group II allergens were found in bedding samples

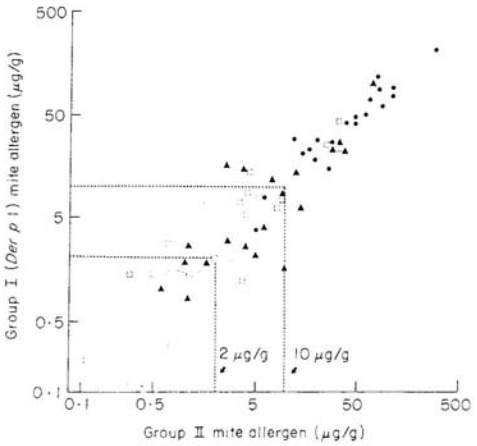

Fig. 2. Measurements of *Der p* I and Group II allergens, expressed in μg/g of fine dust, in 79 samples of house dust, collected from four sites of each patient's home: bedding (●), bedroom floor (▲), TV room (□) and kitchen (○). Levels of 2 μg/g and 10 μg/g of dust, which have been proposed as risk factors for sensitization and development of acute asthma in mite-allergic individuals, respectively, are indicated as dashed lines.

(gm 38·4 μg/g and 36·6 μg/g, respectively), followed by bedroom floor (gm 5·7 μg/g and 5·6 μg/g), TV room (gm 4·1 μg/g and 5·6 μg/g) and kitchen (gm 0·5 μg/g and 0·9 μg/g) (Fig. 2). There was an excellent correlation between levels of *Der p* I and group II allergens ($r = 0{\cdot}94$, $P < 0{\cdot}001$). *Der f* I was undetectable or found in very low levels ($< 0{\cdot}5$ μg/g). Levels of cat allergen *Fel d* I > 8 μg/g of dust (a level which has been consistently associated with presence of cat(s) in the house) were found in two houses. Cockroach allergen was detected in five houses; however, the levels were < 10 U/g in each case.

Analysis of cross-reactivity between D. pteronyssinus *and* B. tropicalis

Extracts of *D. pteronyssinus* and *D. farinae* prepared in Brazil had Group I and Group II allergen levels ranging from 4·7 to 9·3 μg/ml. Extracts of *B. tropicalis*, prepared from two different cultures, had undetectable or < 50 ng/ml *Dermatophagoides* sp. allergens. The absence of Group I allergens in *B. tropicalis* was confirmed by both monoclonal and polyclonal antibody-based immunoassays (Table 1) [29]. These results suggested that *B. tropicalis* produced allergens that were distinct from the major *D. pteronyssinus* allergens.

The specificity of IgE antibodies to *D. pteronyssinus* and *B. tropicalis* was compared by immunoabsorption using six selected sera from Brazilian patients with asthma and RAST > 200 U/ml to both mite species. Most IgE antibodies to *D. pteronyssinus* were removed by absorption of the sera with *D. pteronyssinus* extract ($95{\cdot}8 \pm 3\%$), but only 32% of the IgE antibodies to *D. pteronyssinus* were absorbed with *B. tropicalis* extract (Fig. 3a). Similarly, most IgE antibodies to *B. tropicalis* were absorbed with *B. tropicalis* extract ($95{\cdot}1 \pm 3{\cdot}9\%$), but only 36% of the IgE antibodies to *B. tropicalis* were removed following absorption with *D. pteronyssinus* extract (Fig. 3b). Absorption of sera with *B. tropicalis* had no effect on levels of IgG antibodies to *Der p* I, while absorption with *D. pteronyssinus* removed $> 95\%$ of IgG-anti *Der p* I from all sera (Fig. 3c).

Discussion

The children in our study were all exposed to levels of *Der p* I > 2 μg/g of dust, and levels > 10 μg/g of *Der p* I were detected in 18 out of 20 of the houses. Those levels have been proposed as risk factors for sensitization and development of acute attacks of asthma, respectively [6]. The mean levels of mite allergens in Brazilian houses were higher than levels reported in similar studies performed in the U.S.A [22] and in England [5]. These high levels probably reflect the fact that the relative humidity in São Paulo is 70–80% year round, with temperatures of 20–25°C, i.e. favorable conditions for mite growth. The *Der p* I:Group II ratio in our dust samples was 0·96:1. Sakagushi *et al.* [30] have reported a similar ratio (0·8:1) in Japanese floor dust samples. Levels of cat allergen were undetectable in most houses; only one out of 20 patients reported the presence of cats in the house. Our results suggest that cockroach may not be as significant a cause of sensitization in São Paulo as house dust mites. However, the population we have studied was primarily selected for mite sensitivity, and it is possible that cockroach allergy has a role in asthma among a different group of patients. Although *B. germanica* has a cosmopolitan distribution, the cockroach fauna in São Paulo may include other species, which do not produce antigen detected by the assay for *Bla g* I.

The Brazilian children with asthma had high levels of specific IgE antibodies to mites in their sera. We have presented the results for *D. pteronyssinus* IgE antibodies and *B. tropicalis* IgE antibodies as if the RAST units for each were equivalent. The comparison of RAST units for different allergens assumes that the preparations of the discs is optimal for each allergen. We tested different extracts and concentrations of *B. tropicalis* in order to optimize the RAST, and believe that the RAST values

Table 1. Group I and Group II allergen levels in mite extracts

Mite extracts	Source	*Der p* I (ng/ml)	*Der f* I (ng/ml)	Group I* (ng/ml)	Group II (ng/ml)
D. pteronyssinus	São Paulo, Brazil	6580	< 10	8875	3800
D. farinae	São Paulo, Brazil	158†	4730	5125	9300
Blomia tropicalis	São Paulo, Brazil	< 10	< 10	< 50	20
Food medium	São Paulo, Brazil	< 10	< 10	< 50	< 10
Blomia tropicalis	Tampa, FL	< 10	< 10	< 50	< 10

* Assayed by inhibition RIA using affinity purified rabbit antibodies to cross-reacting determinants on *Der p* I and *Der f* I [29].
† Positive results for the *D. farinae* extract in the *Der p* I assay reflect weak cross-reactivity in the MoAb assay for *Der p* I, which has previously been estimated at 1–2% [23].

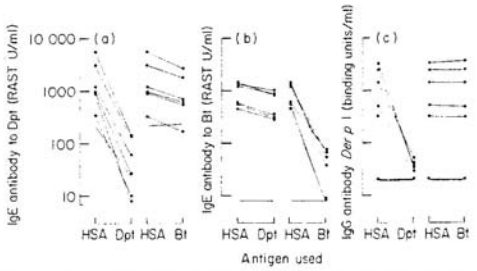

Fig. 3. Sera from six selected Brazilian asthmatic children allergic to both *D. pteronyssinus* (Dpt) and *B. tropicalis* (Bt) (●), and from one American asthmatic patient with IgE antibodies to *D. pteronyssinus* but not to *B. tropicalis* (○) were absorbed with either *D. pteronyssinus*, *B. tropicalis* or human serum albumin (HSA) Sepharose. Comparisons of IgE antibody levels to either *D. pteronyssinus* (a) or *B. tropicalis* (b) following absorption with these antigens are shown. (c), Effect of immunoabsorption on IgG antibodies to *Der p* I, as determined by antigen-binding RIA [28].

obtained are broadly comparable with those of *D. pteronyssinus*. Positive results observed in the *D. farinae* RAST were presumably due to the fact that IgE antibody to antigens of either *Dermatophagoides* species are highly cross-reactive in humans. Rye-grass- and ragweed-specific IgE antibodies were not found in these sera, probably because of lack of exposure. With the exception of the Southern provinces, no grass pollens of allergenic importance have been reported in Brazil [31].

Children from Third World countries are exposed to intestinal parasites (helminths) which can be potent stimuli for inducing marked elevation of IgE and can potentiate the production of IgE against non-parasitic antigens [32]. Although the specificity of the potentiated IgE has not been established, there is good evidence that it is not directed against inhalant allergens [32]. Thus, it seems unlikely that the high levels of IgE to mites we see in our patients are a consequence of parasitic infection. High levels of total IgE may increase background binding on RAST; however, the low results obtained on rye grass and ragweed RAST do not support this hypothesis. The total IgE levels found in sera of our children were at least five-fold higher than mean levels reported in two separate studies on epidemiology of acute asthma in adults, carried out in Charlottesville, Virginia [4] and in Wilmington, Delaware [33]. These results suggest that some of the children may have had parasitic infection. In future studies, it would be prudent to examine for parasitic infections and to investigate whether parasites influence IgE antibody responses in urban populations of Brazilian children.

Previous studies performed in São Paulo, based on mite counts and microscopic identification of mites, have shown *D. pteronyssinus* and *B. tropicalis* to be the most prevalent mite species in house dust. In addition, seasonal variation of both mite species has been reported, with peaks of frequency in March–April and in August–November in house dust [7]. The present study did not focus on counting mites in dust; however, analysis of house dust samples has shown that *D. pteronyssinus* represented > 50% of the mite species in all but one house; *B. tropicalis* was identified in dust from four of the houses, representing 5–26% of the mites present. These numbers appeared to be lower than has been reported previously. Other mites identified included *Euroglyphus maynei* and *Cheyletus* sp., and no species of *D. farinae* were found.

Absorption experiments revealed that most of the IgE antibodies to *B. tropicalis* were directed against species-specific antigens, however a smaller proportion recognized antigens shared with *D. pteronyssinus*. Since we found undetectable or very low levels of *Der p* I, *Der f* I or Group II *Dermatophagoides* allergens in two different *B. tropicalis* extracts, it seems likely that cross reactivity is associated with other allergens [34]. Van Hage-Hamsten *et al.* [35] have recently reported that *B. kulagini* is another important *Blomia* species in Brazil; however, no comparative studies on prevalence or on the frequency of sensitization to both species have been performed. Their RAST inhibition results using sera from Swedish farmers or allergic subjects from Brazil also suggested that *D. pteronyssinus* and *B. kulagini* have predominantly species-specific allergens [35].

The implication of our results is that environmental control measures to reduce exposure to mite allergens in houses of children in São Paulo may help control symptoms or alternatively may reduce the prevalence of sensitization and the development of symptomatic asthma. In São Paulo, *B. tropicalis* should be considered in the evaluation of immediate hypersensitivity in patients with asthma, given that the greater proportion of its allergens appear to be species specific.

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