

Specific profiles of house dust mite sensitization in children with asthma and in children with eczema

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Sensitization to house dust mites (HDM) is highly prevalent among the young atopic population in Singapore. Previously published data suggest that individuals with skin allergies show preferred sensitization to *Dermatophagoides pteronyssinus* while individuals with pure respiratory allergies show preferred sensitization to *Blomia tropicalis*. The aim of our study was to compare the sensitization profiles between children with asthma and those with eczema to *D. pteronyssinus* and *B. tropicalis* and their specific allergens. A total of 60 children, 30 with asthma and 30 with eczema were recruited. IgE levels specific for a panel of HDM allergens from the two mite species were measured using enzyme-linked immunosorbent assay. The asthma group showed highest sensitization to Blo t5 while the eczema group showed highest sensitization to Der p5. Comparison between the two disease groups showed that the eczema group had significantly higher IgE levels for Der p ($p = 0.042$) and its allergens Der p1 ($p = 0.019$) and Der p5 ($p = 0.001$). Generally, the eczema group was more sensitized to the panel of allergens compared to the asthma group. Individuals with asthma and those with eczema showed different sensitization profiles to HDM. These findings highlighted possible mechanisms for different manifestation of allergy.

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Allergic diseases such as asthma, rhinitis and eczema have become a worldwide epidemic, and their increasing prevalence for the last 20–30 yr makes it a major burden in westernized societies (1, 2). Allergic disorders are also common in Singapore, and prevalence is comparable to populations in the West (3). House dust mite (HDM) allergens constitute many of the clinically relevant allergens, with 90% of the atopic population in Singapore being sensitized to *Dermatophagoides pteronyssinus* (Der p) and *Blomia tropicalis* (Blo t) (4).

Allergic reactions can express themselves in any age group and in many different organ systems including the respiratory tract and the skin, leading to different clinical symptoms including asthma, rhinitis and eczema (5). The

exact reasons for the diversity of allergic clinical manifestations are still not known. Furthermore, a patient can develop various allergic symptoms at the same time or they may appear one after another (such as in the so-called Allergic March). One of the reasons for diversity of allergic manifestations could be that specific sensitization patterns correlate with specific symptoms. In one study, it was shown that allergic rhinitis (AR) patients with atopic dermatitis (AD) were more sensitized to *D. pteronyssinus* while patients with pure respiratory allergies (and no AD) were more sensitized to *B. tropicalis* (6).

In this study, we aimed to investigate differences in sensitization between patients with asthma and patients with eczema by comparing the sensitization profiles to *D. pteronyssinus* and

B. tropicalis. A unique sensitization profile to the mites by children with asthma and by children with eczema could suggest possible mechanisms for different manifestation of allergy. These findings could have future implications for the diagnosis (i.e. usage of extracts for skin prick testing) and treatment (i.e. specific immunotherapy) of allergic diseases.

Methods

Subjects

A total of 60 patients were included in this study, 30 patients with asthma (23 male) with a mean age of 12 ± 3.11 yr, and 30 patients with eczema (18 male) with a mean age of 10 ± 3.92 yr. Asthma was defined as the presence of recurrent wheezing in the past 12 months needing inhaled beta-agonists and inhaled corticosteroids. Eczema was defined as the presence of a chronic or relapsing rash in the limbs and/or neck (7). Subjects were only selected for the study if they had a positive skin prick test (SPT) to *D. pteronyssinus* and *B. tropicalis*. All of the 30 patients with eczema tested were sensitized to both mites while 2 of 32 patients with asthma (6.25%) tested were not sensitized to the dust mites and were excluded. Written consent from parents and child was required before blood sampling, and the study was approved by our institution's ethics review board. Serum samples were stored at -80°C before analysis.

House dust mite allergens

Subjects underwent SPT to a panel of *D. pteronyssinus* and *B. tropicalis* extracts. Histamine was used as a positive control and solvent (50% Cocas 50% Gly) as the negative control. Crude extract of *D. pteronyssinus* was obtained from commercial sources (Greer Laboratories, Lenoir, NC, USA), whereas the crude extract of *B. tropicalis* and the rest of the panel of allergens were prepared as previously described (8). Specific *D. pteronyssinus* and *B. tropicalis* allergens for enzyme-linked immunosorbent assay (ELISA) were obtained as follows. Der p1, Der p2, Der p5, Blo t4, Blo t5 Blo t6 and Blo t12 were expressed in transformed *Pichia pastoris* using the pPIC9 vector (9, 10). Native Blo t 11 (nBlo t 11) was immunoaffinity purified from the crude mite extracts using monoclonal antibodies to Blo t 11. Verification of antigenicity of these expressed proteins was examined by Western blot using relevant monoclonal and polyclonal antibodies.

Detection of HDM allergen-specific IgE by ELISA

ELISA was performed using a validated technique developed in our laboratory (11, 12). The volume of each step in the ELISA was 25 μl /well unless specified. The ELISA plates were coated with individual HDM allergens at 100 $\mu\text{g}/\text{ml}$ for crude extracts and 5 $\mu\text{g}/\text{ml}$ for recombinant allergens in 0.1 M sodium bicarbonate, pH 8.3 and left to incubate at 4°C overnight. Next, the wells were blocked with 50 μl blocking solution (TBS + 0.05% Tween20 + 1% BSA) at room temperature for 1 h. Sera diluted 5 \times (in blocking solution) were then added into the wells, and the plates were incubated at 4°C overnight. Specific IgE (sIgE) levels were detected by first adding 250 ng/ml biotinylated anti-human IgE (Southern Biotechnology Associates Inc., Birmingham, AL, USA) and incubated for 1 h. This was followed by the addition of 1:2000 diluted ExtrAvidin-alkaline phosphatase (Sigma, St Louis, MO, USA) in each well and left for another 1 h. Signal was developed by the addition of alkaline phosphatase substrate p-nitrophenylphosphate (Sigma), and the optical density was read at 405 nm with Infinite M200 microplate reader (Tecan, Tecan Group Ltd, Mannedorf, Switzerland). Extensive washing with washing buffer (TBS + 0.05% Tween 20) was performed following each incubation step. For standard curve, the wells were coated with purified mouse anti-human IgE (Southern Biotechnology Associates Inc.) as capture antibody, and semi-purified human IgE from human IgE myeloma (Chemicon International Inc., Temecula, CA, USA) was used as standard. Readings of absorbance were referred to these standards to obtain the relative IgE titre.

The value below which each sIgE was considered to be negative was determined by performing ELISA on a panel of 10 cord blood samples against each of the allergens. The mean IgE value in ng/ml of the 10 cord blood sera was determined, and the cut-off was finally calculated as mean + 3 s.d. From the calculation, the cut-off value for Der p crude extract was 21.5, Der p1 = 13.1, Der p2 = 13.5, Der p5 = 6.5, Blo t crude extract = 16.3, Blo t4 = 10.9, Blo t5 = 19.6, Blo t 6 = 14.7, Blo t 11 = 14.6 and Blo t 12 = 13.7 ng/ml, respectively.

Statistical analysis

Values obtained from ELISA were log transformed as the data were not normally distributed. To compare the sensitization profile, McNemar's test was performed for intra-group analysis, and

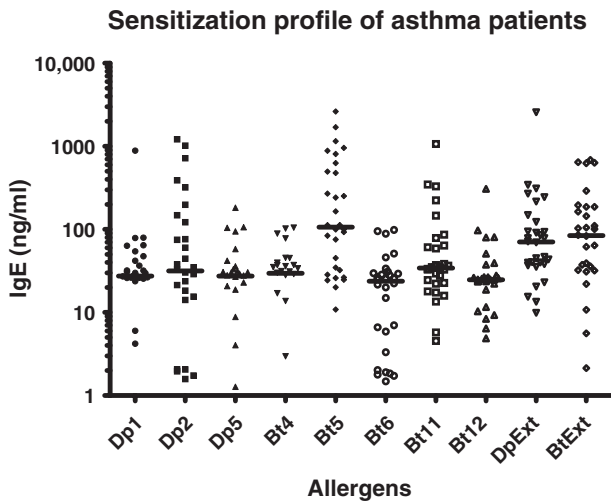


Fig. 1. IgE binding to Der p and Blo t allergens in the asthma group. Median IgE level is represented by the black bar. IgE levels < 1 ng/ml are not represented in this plot.

chi-square test was performed between the asthma and eczema group. Wilcoxon signed rank test was used to compare IgE levels within a disease group while Mann–Whitney test was used to compare IgE levels between the two groups of patients for each allergen. All statistical analyses were carried out using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). All graphs were drawn using GRAPHPAD Prism version 4.0 (GraphPad Software, San Diego, CA, USA). Significance was set at $p < 0.05$.

Results

Sensitization profile within each disease group

Within the asthma group, most patients were sensitized to Blo t5 (96.7%) when compared to the recombinant Der p and Blo t allergens ($p < 0.05$ for all) (Fig. 1, Table 1). The patients with asthma were also significantly more often sensitized to Blo t11 (86.7%) than Der p5, Blo t4 and Blo t6 ($p < 0.05$) (Fig. 1). Patients with eczema were most commonly sensitized to Der p5 (90.0%), Blo t5 (86.7%) and Der p1 (83.3%) compared to Blo t4 and Blo t12 ($p < 0.05$) (Fig. 2, Table 1). For both disease groups, Blo t5 was the allergen with the highest sIgE titre, and sensitization to Blo t5 was significantly higher than sensitization to its homologue Der p5 ($p < 0.001$) (Figs 1 and 2).

Comparing the sensitization profile between patients with asthma and patients with eczema

As a group, patients with eczema were more sensitized to the panel of allergens, displaying

Table 1. Median-specific IgE level (ng/ml) & prevalence of positive IgE response (%)

Allergen	Asthma n = 30	Eczema n = 30	Mann Whitney p-value
Der p 1	27.4 (13.2–886.7) 76.7%	45.3 (13.2–2883.8) 83.3%	0.019*
Der p 2	31.6 (13.5–1217.4) 73.3%	56.3 (13.5–5197.4) 76.7%	0.257
Der p 5	27.4 (6.5–182.2) 66.7%	42.6 (6.5–1633.8) 90%	0.001*
Blo t 4	29.5 (11.0–105.1) 63.3%	26.8 (11.0–86.2) 53.3%	0.480
Blo t 5	106.1 (19.6–2632.1) 96.7%	184.7 (19.6–2000.0) 86.7%	0.848
Blo t 6	23.8 (14.7–98.7) 63.3%	32.1 (14.7–156.4) 76.7%	0.039*
Blo t 11	34.3 (14.7–1066.8) 86.7%	46.3 (14.7–1264.9) 76.7%	0.279
Blo t 12	24.8 (13.8–306.4) 70.0%	28.6 (13.8–549.3) 63.3%	0.346
Der p Ext	70.7 (21.5–2571.9) 86.7%	252.0 (21.5–2571.9) 86.7%	0.042*
Blo t Ext	84.3 (16.3–685.4) 83.3%	120.3 (16.3–2734.7) 90.0%	0.111

Der p, *Dermatophagoides pteronyssinus*; Blo t, *Blomia tropicalis*.
*Asterisks denote statistical significance $p < 0.05$.

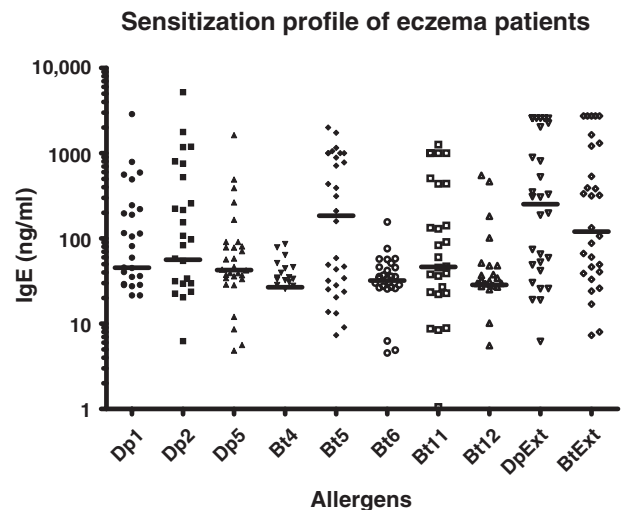


Fig. 2. IgE binding to *Dermatophagoides pteronyssinus* (Der p) and *Blomia tropicalis* (Blo t) allergens in the eczema group. Median IgE level is represented by the black bar. IgE levels < 1 ng/ml are not represented in this plot.

higher frequencies of sensitization and higher titres of sIgE when compared with the asthma group (Fig. 2, Table 1). In the eczema group, the rate of sensitization to Der p5 (90%) was higher compared to patients with asthma (66.7%), and this almost reached statistical significance ($p = 0.057$).

The patients with eczema had a significantly higher sIgE titres to Der p crude extract

($p = 0.042$), Der p1 ($p = 0.019$), Der p5 ($p = 0.001$) and Blo t6 ($p = 0.039$) when compared to patients with asthma. Although not statistically significant, the titres of sIgE for all the other allergens and Blo t crude extract were higher in the eczema group except for Blo t4, for which both groups had fairly similar degrees of sensitization.

Discussion

In this study, we looked at the sensitization profiles of patients with asthma and eczema to the ubiquitous mites, *D. pteronyssinus* and *B. tropicalis*. Both these mites are predominantly found concentrated in mattresses (Der p1 at geometric mean 1.2 $\mu\text{g/g}$ dust; Blo t at 2717 Allergen Units per gram dust [AU/g]) and carpets (Der p1 1.5 $\mu\text{g/g}$ dust; Blo t 1620 AU/g dust) (13). We showed that there was a difference in the sensitization profile between these two groups. The eczema group showed preferred sensitization to the crude extract of *D. pteronyssinus* and to the specific allergens Der p1 and Der p5 compared to patients with pure respiratory allergy. The association between *D. pteronyssinus* sensitization and eczema is not very well understood. The only study so far that supports the hypothesis of preferred sensitization is by Kidon et al. using SPT analysis, which showed that AR patients with eczema were more sensitized to the crude extract of *D. pteronyssinus* compared to patients with pure respiratory disease (6). Our results indicated that Der p5 could be the contributing allergen for the greater sensitization of patients with eczema to *D. pteronyssinus* mites.

It was also observed that patients with eczema were generally more sensitized to the panel of dust mite allergens. This suggests that patients with eczema could be more susceptible to dust mite allergy. There is good evidence that patients with eczema are more sensitized to allergens in general. It was shown that eczema in the first 3 months of life was a risk factor for sensitization to aeroallergens by 5 yr of age and subsequent manifestation of respiratory allergies (14). Another study showed that children with eczema were significantly more sensitized to a panel of allergens tested than those without skin diseases. The degree of sensitization was also observed to be directly associated with the severity of eczema, especially for HDM allergens (15).

In the asthma group, the highest frequency of sensitization was seen with Blo t5, a major allergen of the *B. tropicalis* mite. Our study suggests that asthmatics are more sensitized to

B. tropicalis or, more specifically, to its major allergen, Blo t5. A previous study of sensitization to these mites in patients with asthma was performed in Barbados, which also has a tropical climate. This study showed that *B. tropicalis* sensitization was more prevalent than *D. pteronyssinus* sensitization and that *D. pteronyssinus* sensitization without *B. tropicalis* sensitization was uncommon. In addition, the study found that dual sensitization to *D. pteronyssinus* and *B. tropicalis* resulted in higher risk of asthma (16).

Interestingly, in both the asthma and eczema groups, Blo t5 median sIgE levels were significantly higher than its homologue Der p5. Although Blo t5 and Der p5 are homologues, there is little cross-reactivity between them (17, 18). Blo t5 has been previously shown to result in higher IgE titres compared to Der p5 in tropical environments where there is dual sensitization to both mites (17). In addition, our study revealed the same phenomenon in patients with eczema where it has only been seen in patients with asthma so far (19).

Although HDM allergy is well documented, much about its pathogenesis and how it relates to skin or respiratory disease remains unclear. Our results raise new insights into this unsolved puzzle. In an environment rich in both mite species, individuals with skin allergies are found to be more sensitized to *D. pteronyssinus* while individuals with respiratory allergies are more sensitized to *B. tropicalis*. The manifestation of allergic diseases in different target organs could be because of the distinct aerodynamic characteristics of *D. pteronyssinus* and *B. tropicalis* allergens. The major allergen Der p1 of *D. pteronyssinus* is a 25 kDa cysteine protease and is carried by large particles of median size 25 μm (20). These large particles do not stay airborne and deposit onto surfaces rapidly (21), possibly aggravating eczema upon skin contact. On the other hand, the molecular weight of Blo t5 is only 14 kDa and thus can possibly remain airborne longer resulting in airway sensitization (22). Furthermore, sensitization through skin contact could be because of genetically defective skin barrier, which allows easier mite penetration. It could also be that lesions in the skin are caused by protease activity from mite allergens (23, 24). This suggests a possible role of a decreased barrier function in patients with eczema promoting increased sensitization, facilitated by protease activity of Der p1 and possible protease-independent activities of Der p5 on epithelial cells. Thus, it is possible that *D. pteronyssinus* could possess different enzymatic properties from

B. tropicalis mites that allow Der p to penetrate the skin barrier more easily. In fact, it could be the lack of production of the Der p1 protease inhibitor found in sweat, distinct to patients with eczema, which increases their susceptibility to *D. pteronyssinus* sensitization. The enzymatic property of Der p1 is well documented but the functional characterization of Blo t5 remains unknown. Thus, more studies need to be performed to characterize Blo t5 in order for this postulation to be supported, as well as investigate why individuals with respiratory allergies are more prone to *B. tropicalis* allergy.

There are two limitations to the current study. First, the actual mite exposure for each subject was not investigated, thus raising the possible interference of extrinsic factors. Secondly, recombinant yeast Der p1 was used instead of native Der p1 where the latter is proven to be more allergenic. Thus, the IgE levels for Der p1 were likely under-estimated but nonetheless it does not affect the purpose of our current study, which was to compare sensitization of asthma and eczema individuals to HDM. However, it is necessary to include native Der p1 allergen in future studies to elucidate the relative clinical relevance of Der p1 in allergic asthma and eczema in tropical countries.

In conclusion, asthma and eczema individuals showed different sensitization profiles to HDM. These findings highlighted possible mechanisms for different manifestation of allergy.

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