

Mite component-specific IgE repertoire and phenotypes of allergic disease in childhood: The tropical perspective

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Sensitization to perennial aeroallergens correlates with the risk of persistent asthma (AS) in children. In tropical Singapore, multiple codominant species of mites abound in the indoor environment, and preferential species-specific sensitization has been associated with different phenotypes of allergic disease. We investigated the pattern of mite component-specific IgE (mcsIgE) in children with different phenotypes of clinical allergic disease in an environment with multiple mite species exposure. A prospective evaluation of newly diagnosed patients with clinical diagnosis of allergic rhinitis (AR), atopic dermatitis (AD), or AS and sensitization to one or more aeroallergens were performed. Sera were tested for specific IgE against an extensive panel of *Dermatophagoides pteronyssinus* and *Blomia tropicalis* allergens. A total of 253 children were included, mean age 7.3 yr, 79% fulfilled criteria for AR, 46% AS, 71% AD, and 31% for all three. Sensitization to one or both mites was observed in 91% of children, 89% were sensitized to *D. pteronyssinus*, and 70% to *B. tropicalis*. The most common mite allergens recognized by these atopic children were Der p 1 (64%), Der p 2 (71%), Blo t 5 (45%), Blo t 7 (44%), and Blo t 21 (56%). Specific IgE responses to an increased number of distinct mite allergens correlated with the complexity of the allergic phenotype. In multivariate analysis, an increased risk for the multi-systemic phenotype (AR + AS + AD) was associated with sensitization to an increased repertoire of mite components (three or more) (OR 4.3, 95% CI 2.1–8.8, $p = 0.001$) and a positive parental history of AS (OR 2.4, 95% CI 1.2–2.9, $p = 0.013$). A highly pleiomorphic IgE response to the prevalent indoor mites is associated with the presence of a multi-systemic allergic phenotype in childhood in a tropical environment.

Abbreviations:

AD, atopic dermatitis; AR, allergic rhinitis; AS, asthma; anova, analysis of variance; ELISA, enzyme-linked immunosorbent assay; nDer p 1, natural or native Der p 1 allergen; rBlo t 1, recombinant Blo t 1 allergen; NBT, nitro-blue tetrazolium; Ni-NTA, nickel-nitrilotriacetic acid; PBS, phosphate buffered saline; SD, standard deviation; SOS, sum of the specificities; SPT, skin prick test; v/v, volume to volume; 95% CI, 95% confidence interval; 3Dx, multi-systemic allergic phenotype (i.e., the presence of atopic dermatitis, allergic rhinitis and asthma).

Early sensitization to perennial allergens has been implicated as a major risk factor for the persistent type of respiratory allergic disease in childhood (1–3). The best-studied perennial allergen sources are the domestic mites. More than 10 different species have been found in house dust, three of which are very common worldwide (4) – *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, and in tropical climates, *Blomia tropicalis*, which mostly cohabit with the *Dermatophagoides* mites.

Previous studies from Singapore established *B. tropicalis* and *Dermatophagoides* mites as codominant indoor allergen sources involved in allergic sensitization and disease in atopic

individuals (5, 6). A relatively recent study reported high prevalence of these mites not only in homes but also in child-care centers in Singapore (7). Allergens, Blo t 5 and Der p 1 used as surrogate markers for the presence of *B. tropicalis* and *D. pteronyssinus* were detected in 99.2% and 73.2%, respectively, in the samples collected from these centers. The relative prevalence of *B. tropicalis* and the pyroglyphid mites in areas of cohabitation is highly dynamic, depending on both geographic and micro-environmental factors (6, 8, 9). Although locally more prevalent in the indoor environment, *B. tropicalis* seems to be preferentially involved as a cause of respiratory allergy while sensitization to *Dermatophagoides* mites is a major factor in children with atopic dermatitis (AD) (10, 11). In a different geographic region dual sensitization was shown to increase the risk of lower (asthma [AS]) and upper (allergic rhinitis [AR]) respiratory allergic manifestations (12).

Component- and epitope-resolved diagnosis of specific food sensitizations has revolutionized our diagnostic capabilities in the field of food allergy. These new modalities seem to predict with significantly better accuracy the risk of clinical persistence vs. tolerance as well as the risk for severe clinical reactions to foods in sensitized children (13). This evolving field has sparked the idea that component resolved diagnosis in the field of respiratory allergy may as well reveal additional information on the clinical patterns of disease.

In achieving these goals, the use of microarray immunoassays has been an irreplaceable tool, enabling the concomitant measurement of multiple allergenic epitopes in a relatively minute amount of serum (14).

Concomitantly, significant advances have been made in the characterization of new mite allergens (15, 16), their structure (17, 18), function, and localization (19), as well as their relative importance for species-specific sensitization and cross-reactivity with both other mites (9) and non-acarid allergens. The major sensitizing components of the pyroglyphid mites in atopic adults (20, 21) and children (21) have been characterized and cloned with a view toward building new and improved vaccines. Tools enabling speedy cloning, identification, production, and measurement of IgE reactivity of recombinant allergens, such as expressed sequence tag catalogs (22), proteomics (22, 23), and allergen microarrays (14, 24) have become available in our laboratory and many others. These tools have now enabled laboratories to reliably produce a large repertoire of the known mite allergens and present them on a microarray-type test, enabling simultaneous establishment of the presence of mite component-specific IgE in very small sera samples. These can then be verified using techniques such as streptavidin-linked allergen-specific Immuno-CAP, enzyme allergeo-sorbent test, IgE dissociation-enhanced lanthanide fluoroimmuno-assay or via conventional solid phase allergen-specific enzyme-linked immunosorbent assay (ELISA) and ELISA inhibition assays.

The aim of our study is defining the prevalence, clinical characteristics, and risk factors associated with component-specific mite sensitization in allergic children in an environment where multiple species of mites coexist.

Methods

We performed a prospective evaluation of newly diagnosed allergic patients presenting to the outpatient allergy clinic of the Kendang Kerbau Children's Hospital in Singapore between January 2004 and December 2007. The study was approved by the KK Hospital's Institutional Review Board committee.

Patients

During the study period, patients were offered a screening medical examination, a skin prick test (SPT), *in vitro* specific IgE measurements against *D. pteronyssinus* and *B. tropicalis* (ImmunoCAP; Pharmacia Diagnostics AB, Uppsala, Sweden) and an immuno-dot blot test evaluating specific IgE to a panel of recombinant and natural mite allergens (16–18, 23, 25). Patient guardians expressed written informed consent and fulfilled a detailed questionnaire. Data were collected on early birth and exposure data, social, economical, and maternal factors, additional diagnoses and medication usage, family history of allergic disease, exposure to house pets, passive smoking and use of air conditioning in households and children's bedrooms. They also filled a detailed disease activity score and were followed up at the clinic periodically. Patients were included if they had evidence of sensitization to at least one allergen source, either by skin prick or by serum IgE test and documentation of clinical criteria sufficient for the diagnosis of AR, AD, or AS.

Clinical diagnostic criteria

For the diagnosis of AR: a symptomatic period of minimum 2 months, with at least two of the following: watery rhinorrhea, blocked nose, itchy nose, sneezing, or night cough, as detailed in our previous publication (10). For the diagnosis of AD: we used the age appropriate clinical criteria for allergic eczema in childhood, summarized by Muraro et al. (26). For the diagnosis of AS: a previous diagnosis of AS with proof of reversibility on pulmonary function tests and response to bronchodilators. With younger children or where such evidence was not readily available: a history of three or more episodes of at least two of the following – persistent day time or night time cough, doctor-diagnosed non-febrile wheezing, recurrent episodes of shortness of breath or exercise-induced shortness of breath or cough. These data were obtained from the questionnaire, clinical records, their clinic interview, and medical examination (26).

Skin prick tests (SPTs)

SPTs were performed in the respiratory laboratory by an experienced technician using commercial extracts and the GreerPick prick test device (Greer Laboratories, Lenoir, NC, USA). Wheal diameter of 3 mm or more in excess of the negative control was considered positive. SPTs Wheal diameter of 3 mm or more in excess of the negative control was considered positive. The allergen extracts included in our panel

are commercially produced by Greer (Greer Laboratories), except for the *B. tropicalis* extract produced by the allergy and molecular immunology laboratory, National University of Singapore, Singapore. Our SPT panel included: house dust mite mix (HDM; *D. farinae* 5000 AU/ml and *D. pteronyssinus* 5000 AU/ml, standardized), *B. tropicalis* 0.2 mg protein/ml, 50% v/v glycerol, cockroach mix (*Periplaneta americana*, *Blattella germanica*), mixed molds (*Alternaria alternata*, *Curvularia spicifera*, *Cladosporium herbarum*, *Aspergillus fumigatus* and *Candida albicans*), cat hair (standardized cat hair, *Felis catus domesticus* 10,000 BAU/ml) and dog epithelia (*Canis familiaris*), grass mix (nine grass mix, standardized) and weed mix.

Questionnaires

The questionnaire is a culturally adjusted, self-administered, multiple-choice tool, focusing on five areas: early childhood events, maternal socioeconomics, environmental exposure, familial risks, and patient-oriented factors, i.e., past medical history, hospitalizations, diagnoses, and medication use, previously standardized and used for the evaluation of allergic children in Singapore (10, 27). The disease activity score is a modified form of the rhino-conjunctivitis and AS symptom score used as an outcome measure in clinical trials, as detailed previously (10).

Mite component-specific IgE in serum

Specific IgE responses to an array of recombinant and natural mite antigens were conducted on serum samples collected from each patient. The allergens selected and used in this study were natural or recombinant allergens considered to contain the majority of the IgE epitope specificities of naturally occurring allergen sources. Natural Der p 1 was purchased from Indoor Biotech (Charlottesville, VA, USA) while rBlo t 1 (GenBank accession number, AY291322) was produced as a recombinant protein with a N-terminal hexahistidine tag. The IgE binding profile of rBlo t 1 and its natural counterpart, nBlo t 1 (isolated by affinity pull-down via Seize™ Primary Immunoprecipitation Kit [Pierce, Rockford, Illinois, USA]), was highly correlated, $r = 0.7971$, $p < 0.001$ (data not shown). Therefore, in our study, rBlo t 1 was used for all comparisons. Natural Der p 3 and Blo t 3 were individually purified via ion-exchange chromatography and affinity chromatography. Recombinant Der p 2 (AF276239), Blo t 2 (DQ677253), Der p 4 (AF144060), Blo t 4 (AY291324), Der p 5 (S76340), Blo t 5 (U59102), Der p 7 (U37044), Blo t 7 (AY291326), Der p 8 (S75286), Blo t 8 (AY283284), Der p 10 (AF016278), Blo t 10 (EU106615), Der p 13 (EX162433), Blo t 13 (AY283286), Der p 14 (Mag 1) (AF373221), Der p 14 (Mag 3) (AF373221), Blo t 14 (Mag 1) (EU106616), Blo t 14 (Mag 3) (EU106616), Der p 15 (DQ078741), Blo t 15 (AY291330), Der p 20 (EU684970), Blo t 20 (CB283067), Der p 21 (DQ354124), and Blo t 21 (AY800348) were produced as N-terminal hexahistidine tagged recombinant proteins and further purified by Nickel-Nitrilotriacetic Acid (Qiagen, Hilden, Germany) and high-resolution size exclusion chromatography.

IgE responses to these allergens were screened using an immuno-dot blot. A total of 1 µg/ml of each allergen was loaded in duplicate on nitrocellulose membranes, together with IgE standards (National Institute for Biological Standards; 250 IU/ml serially diluted twofold to 0.24 IU/ml), 1 µg of bovine serum albumin and 1 µl of buffer as negative controls. Membranes were air-dried and blocked with PBS-0.1% Tween-20 and incubated with sera (1/1 v/v in PBS) at 4°C overnight, followed by 2 h in anti-human IgE antibodies conjugated with alkaline phosphatase (1/1000 v/v in PBS; Sigma-Aldrich, St. Louis, Missouri, USA). The membranes were then developed with nitro-blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (Promega, Madison, Wisconsin, USA) for 30 min, and spot intensities were measured with an imaging software (Microimage v.3.01, Hamburg, Germany). Spot intensities (range 0–255) were normalized after subtracting the local background, and intensities > 10 (equivalent to 2 s.d. above the mean negative sera responses) were considered positive. This was equivalent to approximately 1 IU/ml when compared to the IgE standard curve. The inter-assay and intra-assay concordances were $> 85\%$ and 95% , respectively, demonstrating good reproducibility of the assay. The specific IgE levels were then confirmed via allergen-specific ELISA and specificity of IgE binding to the allergens was further validated via ELISA inhibition assays. Assays performed in this study have previously been used and validated in multiple other publications (16–18, 23, 25).

Statistical analysis

Relative risk for sensitization and associated risk factors were calculated using logistic regression analysis with the forward stepwise model. Multivariate regression analysis was performed to adjust for confounding interactions. Correlations between the antibody responses were analyzed by Spearman correlation. Continuous values were compared using ANOVA and independent Student's *t*-test and performed on the SPSS 11.5 for Windows statistical software.

Results

A total of 253 children, mean age 7.3 yr (range 0.4–18.4), were included, 79% fulfilled diagnostic criteria for AR, 47% AS, 72% AD and 31% for all three (classified here as the multi-systemic phenotype). There were 62.9% males, and 75.9% of Chinese descent. Preschoolers (< 6 yr) comprised 43.5% of the children. As a group, patients with AD were slightly younger (mean age 6.9 yr, $p < 0.05$). Clinical severity score was generally mild to moderate in all patients. For additional demographic and clinical characteristics, refer to Table 1.

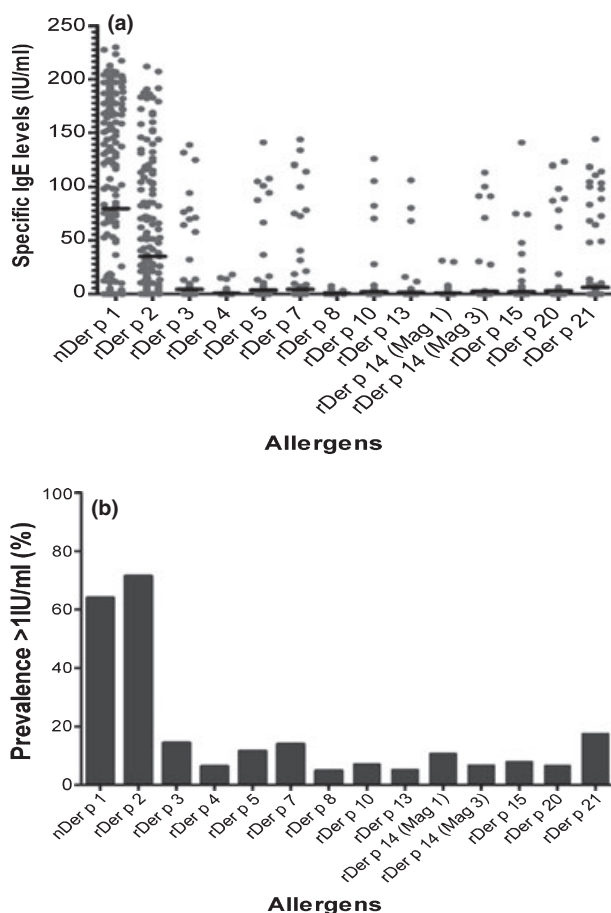
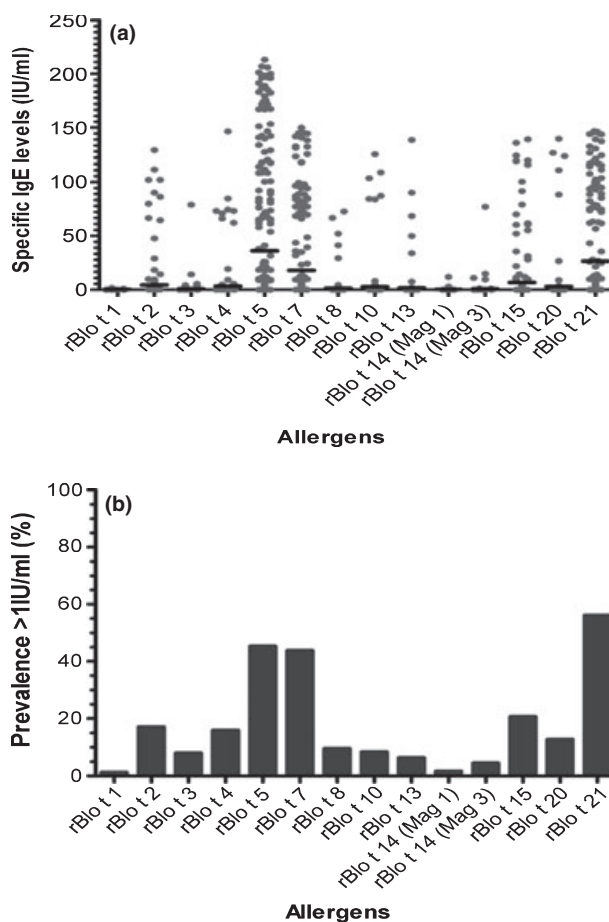
Overall mite sensitization (positive skin prick or serum IgE test with mite extracts) was 91%, with 89% sensitized to *D. pteronyssinus* and 70% to *B. tropicalis*. A small minority (3.8%) did not respond to either the skin prick or serum IgE tests for mites but had a positive response to one or more mite component allergens. Patients lacking evidence of mite sensitization were not included in the analysis of component specific data.

Table 1 Patients' characteristics and percentage of specific IgE responses to house dust mites tabulated according to clinical diagnosis

	All patients	Atopic dermatitis	Allergic rhinitis	Asthma	p-Value
n (%)	253	182 (72%)	200 (79%)	119 (47%)	–
Age (yr)	7.3	6.9	7.9	8.0	p < 0.05
Male gender	62.9%	62.4%	62.7%	63.9%	NS
Chinese (ethnic group)	75.9%	76.8%	76.1%	71.2%	NS
<i>Dermatophagoides</i> +	89%	88%	91%	91%	NS
<i>Blomia tropicalis</i> +	70%	68%	70%	75%	NS

Additional aeroallergen sensitizations identified via SPT in this population: Cockroach 8%, cats 5%, and molds 4%.

The most prevalent component-specific IgE responses were to Der p 1 (64%), Der p 2 (71%), Blo t 5 (45%), Blo t 7 (44%), and Blo t 21 (56%); however, 14 different allergens showed at least a 10% response rate, see Figs 1a, b and 2a, b. There were no significant differences between the preva-

**Figure 1** (a) sIgE response to *Der p* allergenic components. (b) Prevalence of sensitization to *Der p* allergenic components.**Figure 2** (a) sIgE response to *Blo t* allergenic components. (b) Prevalence of sensitization to *Blo t* allergenic components.

lence of sensitization to specific components, nor mean specific IgE levels to these allergens when patients were stratified by individual allergic diagnosis.

The average number of mite component allergens eliciting an IgE response per individual was 2.8 (95% CI: 2.4–3.2; range 0–14) for *D. pteronyssinus*, and 2.7 (95% CI: 2.3–3.1; range 0–14) for *B. tropicalis*. Fig. 3a, b shows the distribution in the number of *D. pteronyssinus* and *B. tropicalis* allergen responses per individual, respectively. Of *D. pteronyssinus* responders, <25% react to only one allergen (mostly Der p 1 or 2), while the majority responded to two or more. With *B. tropicalis*, <14% responded to only one, while the majority responded to between 2 and 4 allergens (mostly Blo t 5, 7, 15, and/or 21).

Der p 1 and 2 alone identified close to 64% and 71% of the *D. pteronyssinus* extract responders, respectively, while a combination of the two identified approximately 78% of them. An increased number allergenic components used in combination would correspondingly identify more *D. pteronyssinus* responders. Nevertheless, inclusion of allergens beyond the top 7 did not further increase the number (maximum at 82.5%) suggesting that there were other isoforms or

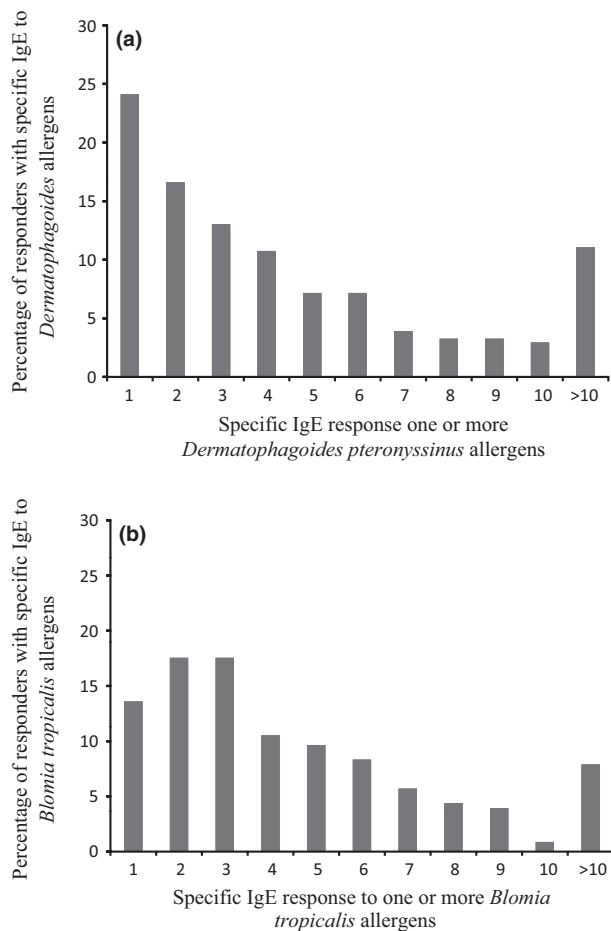


Figure 3 Percentage of individuals with specific IgE responses to distinct number of allergens from *Dermatophagoides pteronyssinus* (a) and *Blomia tropicalis* (b).

groups of allergens not listed here that would define these responders. With *B. tropicalis*, Blo t 21 alone identified ~56% of the responders while a Blo t 21, 5, and 7 combination identified approximately 65% of the responders. The full panel of *B. tropicalis* allergens identified only 70% of the responders, again suggesting the presence of other isoforms or additional *B. tropicalis* allergens as yet not identified.

Quantitatively, Der p 1 and 2 represented approximately 46% and 29% of the total specific IgE (sum of the specificities, SOS) against *D. pteronyssinus* allergens, respectively, and 77.3% SOS when combined, 95% CI: 75.3–79.4%. The other allergens represented on average between 4.3% and 7.0% of the SOS. With *B. tropicalis*, Blo t 21, 5, and 7 represented on average 36.2%, 21.0% and 18.1% of the SOS and as a combination, on average 77.4% of the *B. tropicalis* SOS, 95% CI: 75.3–79.4%.

IgE binding to Der p 21, 7, and 5 (representative of mid-range allergens) generally occurs only in individuals with high titer specific IgE to *D. pteronyssinus* and are not strongly correlated to the ImmunoCAP measurements ($r = 0.3098$ – 0.3953 ; slope, $\beta = 0.06$ – 0.07). Similar observations were seen

with the other minor *D. pteronyssinus* allergens. This suggests that the majority of individuals with lower specific IgE to *D. pteronyssinus* tend to respond only to Der p 1 and 2, while those with higher levels respond to a larger repertoire of allergens.

With the mid-range *B. tropicalis* allergens (e.g., Blo t 15, 4 and 2), specific IgE levels were more correlated with ImmunoCAP measurements to *B. tropicalis* extracts ($r = 0.4751$ – 0.6174 , $\beta = 0.08$ – 0.15). The percentage of IgE binding to these allergens is generally similar in sera with high or low specific IgE titers to *B. tropicalis*. The slopes of the relationships (β values) show that the contribution to the total specific IgE binding to *B. tropicalis* for these mid-range allergens was typically 8–15%.

Sensitization to mite allergens generally increased across age quartiles, in terms of both new sensitization and increased specific IgE levels. This effect was more pronounced for *B. tropicalis* Table 2(a), while most age-dependent changes in the sensitization to *Dermatophagoides* occurred between the first and second quartile, Table 2(b). By 2 yr of age, more than 50% of atopic patients were sensitized to either Der p 1 or Der p 2, while allergens from groups 5, 7, and 21 (including both of the major allergenic groups of *B. tropicalis*) were positive in 50% of children only at 6–7 yr of age. The repertoire of responses to *B. tropicalis* allergens also increased across age quartiles from approximately 30% responding to two or more in the first quartile to 70% in the fourth.

There was no particular pattern of component-specific IgE sensitization associated with any individual diagnosis.

A wider repertoire of component-specific IgE responses correlated with an increased complexity of the allergic phenotype (see Table 3). On average, patients with a single allergic diagnosis responded to 1.75 *B. tropicalis* components (95% CI 1.2–2.3), patients with two systems involvement responded to 2.80 components (95% CI 2.2–3.5) and patients with multi-systemic phenotype responded to 3.66 *B. tropicalis* allergens (95% CI 2.9–4.4), $p < 0.001$. A similar progression was seen with the *Dermatophagoides* allergens and the combined mite components.

In univariate analysis, the following variables were associated with an increased risk for a multi-systemic (3Dx) phenotype: sensitization to *B. tropicalis* extract (77% vs. 61% in patients without 3Dx, $p = 0.017$), sensitization to all major *Blomia* allergens–Blo t 21 (68% vs. 51%, $p = 0.014$); Blo t 7 (60% vs. 37%, $p = 0.001$); Blo t 5 (57% vs. 40%, $p = 0.012$); and Blo t 15 (29% vs. 17%, $p = 0.039$), sensitization to *Dermatophagoides* extract (97% vs. 83%, $p = 0.001$) and specific *Dermatophagoides* allergens–Der p 1 (83% vs. 55%, $p = 0.001$), Der p 2 (82% vs. 67%, $p = 0.015$), Der p 5 (18% vs. 9%, $p = 0.028$) and Der p 7 (23% vs. 10%, $p = 0.004$), an increased number of sensitizations to discrete mite allergens, with a mean number of *B. tropicalis* responses of 3.7 vs. 2.3 in non-3Dx patients $p = 0.001$, a mean number of *Dermatophagoides* responses of 3.9 vs. 2.3, $p = 0.001$, and a combined mite response index of 7.5 vs. 4.6, $p = 0.001$. The 3Dx phenotype was also associated with increased allergen-specific IgE titers to both

Table 2 Serum IgE responses to (a) *Blomia tropicalis* and (b) *Dermatophagoides pteronyssinus* extract and component-specific allergens by age quartile, all children

n = 253	1st quartile <1–3 yr	2nd quartile 4–6 yr	3rd quartile 7–10 yr	4th quartile >10 yr	p-Value
<i>(a)</i>					
<i>Blomia tropicalis</i> extract	42.2%	58.8%	83.6%	80.9%	<0.001
Blo t 21	20.3%	57.4%	76.7%	70.2%	<0.001
Specific IgE, IU/ml (95% CI)	28.92 (12.61–45.24)	71.18 (51.48–90.87)	69.96 (52.84–87.09)	57.37 (37.15–77.59)	<0.01
Blo t 7	25.0%	48.5%	50.7%	51.1%	<0.01
Specific IgE, IU/ml (95% CI)	7.93 (1.06–14.81)	31.20 (17.86–44.54)	24.16 (14.01–34.30)	33.63 (17.08–50.18)	<0.05
Blo t 5	14.1%	45.6%	60.3%	63.8%	<0.001
Specific IgE, IU/ml (95% CI)	14.48 (2.12–26.83)	45.27 (28.39–62.15)	42.83 (28.94–56.73)	41.82 (24.14–59.51)	<0.05
Blo t 15	9.4%	27.9%	23.3%	21.3%	NS
Specific IgE, IU/ml (95% CI)	2.07 (0.42–4.56)	11.11 (2.82–19.41)	8.24 (1.99–14.48)	12.54 (2.20–22.87)	NS
Blo t 4	3.1%	16.2%	17.8%	29.8%	<0.01
Specific IgE, IU/ml (95% CI)	0.45 (0.33–0.56)	5.05 (0.12–10.22)	1.03 (0.48–1.58)	8.44 (1.92–14.96)	<0.05
Blo t 2	3.1%	16.2%	27.4%	21.3%	<0.01
Specific IgE, IU/ml (95% CI)	0.42 (0.36–0.48)	4.20 (0.24–8.16)	8.01 (2.53–13.49)	4.92 (1.20–11.05)	NS
No. of Blo t responses	1.16	2.96	3.34	3.51	<0.001
At least one Blo t response	32.8%	67.7%	80.8%	80.9%	<0.001
Two or more Blo t responses	29.7%	60.3%	71.2%	70.2%	<0.001
Three or more Blo t responses	18.8%	39.7%	50.7%	51.1%	<0.001
<i>(b)</i>					
<i>Dermatophagoides</i> extract	78.1%	82.4%	94.5%	95.7%	<0.01
Der p 1	39.1%	66.2%	74.0%	78.7%	<0.001
Specific IgE, IU/ml (95% CI)	36.07 (19.11–53.03)	85.48 (64.69–106.26)	98.29 (78.91–117.66)	101.58 (78.70–124.45)	<0.001
Der p 2	56.3%	73.5%	80.8%	74.5%	<0.05
Specific IgE, IU/ml (95% CI)	13.17 (5.64–20.69)	38.75 (24.82–52.69)	47.01 (33.70–60.31)	40.60 (22.66–58.54)	<0.01
Der p 21	3.1%	20.6%	21.9%	25.5%	<0.01
Specific IgE, IU/ml (95% CI)	3.20 (2.61–9.01)	16.51 (5.07–27.96)	15.12 (5.84–24.40)	11.35 (1.18–21.53)	NS
Der p 7	3.1%	14.7%	16.4%	23.4%	<0.05
Specific IgE, IU/ml (95% CI)	1.53 (0.80–3.87)	9.88 (0.85–18.91)	16.72 (5.73–27.70)	9.40 (0.77–18.03)	NS
Der p 5	0.0%	8.8%	19.2%	19.1%	<0.01
Specific IgE, IU/ml (95% CI)	0.28 (0.24–0.32)	11.42 (1.49–21.35)	11.85 (2.92–20.79)	17.69 (3.11–32.26)	NS
Der p 3	10.9%	19.1%	12.3%	14.9%	NS
Specific IgE, IU/ml (95% CI)	2.51 (0.29–5.31)	7.21 (1.77–12.65)	3.81 (0.72–8.34)	7.35 (1.32–16.02)	NS
No. of Der p responses	1.55	2.98	3.26	3.51	<0.001
At least one Der p response	68.8%	85.3%	90.4%	83.0%	<0.01
Two or more Der p responses	39.1%	67.7%	74.0%	72.3%	<0.001
Three or more Der p responses	20.3%	44.1%	49.3%	55.3%	<0.001
No. of Der p or Blo t responses	2.70	5.94	6.60	7.02	<0.001

B. tropicalis and *Dermatophagoides* extracts and many of their respective allergen components, as well as with a positive family history of parental AS.

In stepwise multivariate logistic analysis, however, only measures of the increased sensitization to a larger repertoire of allergens, such as a combined mite response index of three or more (OR 4.3, 95% CI 2.1–8.8, $p = 0.001$), and a positive parental history of AS (OR 2.4, 95% CI 1.2–2.9, $p = 0.013$). Controlling for age, these two variables (mite response index of three or more, and parental history of AS), remained significantly associated with the 3Dx phenotype.

Discussion

Sensitization to perennial allergens is a significant risk factor for the development of respiratory disease in children with

AD and for the development of persistent AS in wheezy infants (2, 28). No explanation has been available as to why some sensitized individuals display no symptom of allergy, others develop only one system involvement (isolated food allergy, AD, AS, or AR alone), while others run through the gamut of the 'allergic march' to develop upper and lower respiratory disease with or without skin involvement (29). Our data suggest that one possible explanation may be the individual's qualitative immunologic/allergic responses to perennial allergens in his/her's environment.

Among the 14 allergenic components of *D. pteronyssinus* tested, Der p 1 and 2 accounted for >75% of the *Dermatophagoides* IgE response in atopic children in Singapore. More than 50% of these individuals also responded to additional allergenic specificities; however, 14 components could only identify up to 83% of the *D. pteronyssinus* responders. With

Table 3 Serum IgE responses to mites and their component-specific allergens tabulated according to the clinical diagnostic complexity of allergic disease, all children

n = 253	One diagnosis, AD or AR or AS	Two diagnoses, (AD + AR) or (AD + AS) or (AR + AS)	Three diagnoses, (AD + AR + AS)	p-Value
No. of Blo t responses	1.75	2.80	3.66	<0.001
At least one Blo t response	48.8%	67.0%	80.5%	<0.001
Three or more Blo t responses	26.2%	41.8%	52.0%	<0.01
No. of Der p responses	2.00	2.64	3.86	<0.001
At least one Der p response	75.0%	80.2%	92.2%	<0.05
Two or more Der p responses	48.8%	61.5%	80.5%	<0.001
Three or more Der p responses	29.8%	40.7%	55.8%	<0.01
Tot No of components	3.75	5.44	7.52	<0.001
Parental AS	19.2%	21.3%	35.3	<0.001

AD, atopic dermatitis; AR, allergic rhinitis; AS, asthma.

B. tropicalis, 3–4 allergenic groups (Blo t 5, 7, 15 and 21) accounted for >75% of the IgE response. But all 14 *B. tropicalis* allergens could only identify 70% of *Blomia* extract responders suggesting further specificities yet to be defined for both mite species. These may include the other dust mite allergens not studied here, e.g., the groups 6, 9, 11, and 12 allergens, and possibly many other cross-reactive isoforms or unique specificities. Conversely, in some individuals, the calculated SOS (sum of IgE responses to the individual components) was higher than the IgE response to the whole mite extracts. One likely explanation is that mite allergenic extracts, although standardized and containing good amounts of the major allergenic mite proteins, may relatively or completely lack various minor epitopes and so will under-estimate the true overall magnitude of response. In addition, although the 14 individual components, chosen for testing in this study, represent distinct families of allergenic proteins, there is a certain amount of structural homology between groups, for example between groups 2 and 22, as well as groups 5 and 21. This structural homology may translate to shared IgE epitopes, with IgE directed against one, reacting with the second molecular entity as well. This type of problem would cause an over-estimation of the true response by the SOS.

On the average, individual patients sensitized to mites respond to 2.8 and 2.7 *D. pteronyssinus* and *B. tropicalis* allergens, respectively, and to 5–6 allergens in combination. Fourteen allergenic specificities evaluated here have a response rate of at least 10%. Additionally, many of these allergens, if found to be positive, make up 5% or more of the total sum of IgE specificities to the dust mite. All these suggest that the combination of responses among atopic children in Singapore is diverse and more wide-ranging than those reported elsewhere (20, 21). This may be because of the presence of more than one predominant dust mite in the environment, the influence of other related allergens, e.g., minor mites, insects, and sea foods, or other genetic and environmental variables. This wider repertoire and diverse profile may have important implications on the choice and selection of immunotherapeutic extracts and reagents as well as the overall clinical expression of allergic disease.

A distinct age-related pattern of sensitization is seen, with groups 1 and 2 allergens (mainly from *Dermatophagoides*) being early sensitizing agents, with more than 50% of atopic children <2 yr of age, already showing positive sensitizations to these allergenic components. Sensitization to *Blomia tropicalis* t major allergenic components, Blo t 5, blo t 21, blo t 7 but also to Der p 21, seems to occur later with 50% prevalence of sensitization reached only around school age. This pattern is unlikely to be caused by differences in exposure, because both mite species have been documented in large quantities in the indoor environment in Singapore, with *Blomia* t, usually appearing in higher abundance. A plausible explanation could be found in the now known structural identity of the molecules. The group 1 allergens of *Dermatophagoides* are cysteine proteases, capable of both skin/mucosal penetration and facilitation of allergen sensitization through this route (30). Der p 2, in addition to its intrinsic protease activity, has been classified as a ‘complete immunogen’, displaying both T-cell-specific and B-cell-specific epitopes (31), favoring a rapid sensitization profile. Group 5 allergens and the group 5 homologs group 21 are not enzymes capable of disrupting skin and membrane barriers and behave in a manner similar to other aeroallergens in requiring prolonged repeated exposure periods before sensitization occurs.

Although children seen in the allergy clinic, preferentially tend to display complex phenotypes (referral bias), as opposed to mono-symptomatic patients who may attend other specialist clinics (otolaryngologists, respirologists, etc.), data from the International Study of Allergy and AS in Childhood in Singapore, show a very significant overlap of the different phenotypes of allergic disease in childhood (32). The higher incidence of allergic eczema/AD in our study is probably influenced by the relatively young age of our study population.

Our data suggest that an increasingly pleiomorphic response to mite allergens in this dual exposure environment is a marker of the systematization of the allergic inflammatory response in atopic children. Even though the prevalence of sensitization to *Dermatophagoides* allergens is higher in all age groups and across all diagnoses, than that for *B. tropical-*

is, concomitant sensitization to both seems to contribute to the risk of developing both AS and a multi-systemic allergic phenotype. While responses to *Dermatophagoides* allergens beyond Der p 1 and 2 are seen mainly in patients with higher IgE titer, many of the minor *B. tropicalis* allergens are positive even in patients without high titers but increasingly so in patients displaying multi-systemic disease. This finding expands and corroborates work by Lynch et al.(33), who in a different tropical environment, using a much smaller panel of allergens, showed that patients with combined AR and AS, when compared to those with either one alone, were predisposed to react to the minor *D. Pteronyssinus* allergens, Der p 5 and 7.

One of the major limitations of this study, apart from its cross-sectional design, is the use of the dot immunoblot technique for the determination of multiple component IgE specificities in children's sera. This was necessary to minimize the amount of sera required for triplicate measurements of multiple allergenic groups from two concomitant allergen sources. Notoriously, allergen dot immunoblot arrays are generally prone to non-specific IgE binding as well as non-specific inhibition of IgE binding by the presence of large quantities of antigen specific IgG in patients sera (34). We did not ascertain the dust mite IgG; however, we did rule out non-specific IgE binding using ELISA inhibition tests.

Cross-sectional studies such as this can act as a 'hypothesis generator', in this case supporting a possible correlation between a pleiomorphic specific IgE response to mites and increased risk for the development of complex phenotypic allergic disease.

However, the question remains open as to the timing for the appearance of such response in the course of the allergic

march. Whether this is a causal relationship, and the immunologic response precedes and may be at least partially responsible for the phenotypic expression of such a condition, is a question that can be answered only by a prospective longitudinal birth cohort study. If confirmed in such a setting, this could be used as an effective early marker for the systemic allergic phenotype, and in effect brings us closer to the possibility of early intervention and prevention. Very recently, specific patterns of multiple/early IgE responses to perennial environmental allergens have been shown to correlate with the increased risk of AS in a prospective birth cohort, albeit in a different geographic area (35). This very robust finding supporting that early sensitization to multiple allergenic components is a significant factor in the phenotypic expression of allergic disease seems to corroborate our conclusions.

Conclusion

We have shown that sensitization to a wider repertoire of the prevalent indoor mite component allergens is associated to a certain extent with age, but more significantly with an increased complexity and systematization of the allergic phenotype in Singaporean atopic children.

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