

Sensitization to dust mites in children with allergic rhinitis in Singapore: does it matter if you scratch while you sneeze?

M. I. Kidon*, W. C. Chiang*, W. K. Liew*, S. H. Lim*, Y. See*, A. Goh*, J. P. L. Tan*, O. M. Chay* and A. Balakrishnan†

Departments of *Paediatric Medicine and †Otolaryngology, KK Children's Hospital, Singapore, Singapore

Summary

Background Previously published data established *Blomia tropicalis*, as the major source of allergic sensitization in asthmatic children in tropical Singapore.

Objective To define the prevalence, clinical characteristics and risk factors of species-specific mite sensitization in paediatric allergic rhinitis (AR) patients in this unique environment.

Methods We performed a prospective evaluation of newly diagnosed AR patients, from 1 May 2003 to 30 April 2004, from the otolaryngology and allergy outpatient clinics of the Kendang Kerbau Children's Hospital in Singapore. Patients included in the study showed evidence of sensitization to at least one respiratory allergen source and completed a detailed questionnaire. Relative risk of 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. Continuous values were compared using ANOVA, SPSS 9.0 for Windows (SPSS Inc., 1999).

Results One hundred and seventy-five patients were included, 119 (68%) males, 142 (81%) Chinese, age mean 7.9 years (range 2–16). Sixty-eight patients (39%) reported a concomitant diagnosis and/or clinical complaints of bronchial asthma and 84 (48%) of atopic dermatitis. Skin prick test results were positive for traditional house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae* mix) in 85% of patients and for *B. tropicalis* in 62%. Overall mite sensitization was 98%, household pets 10%, moulds 9% and food proteins 12%. By far the single most significant factor associated with *Dermatophagoides* sensitization in this group was the presence of allergic eczema (odds ratio (OR) 31.8%, 95% confidence interval (CI) 3.6–285, $P = 0.002$). Allergic eczema was negatively associated with *B. tropicalis* sensitization (OR 0.26%, 95% CI 0.14–0.5).

Conclusions Children with AR and concomitant atopic dermatitis show a preferential sensitization to the *Dermatophagoides* mites. In our population, *B. tropicalis* sensitization is more prominent in children with pure respiratory allergy.

Keywords allergic rhinitis, atopic dermatitis, *Blomia tropicalis*, mites

Submitted 20 July 2004; revised 15 January 2005; accepted 17 January 2005

Introduction

Studies in Singapore and other tropical environments, have identified the storage mite *Blomia tropicalis* as the major sensitizing agent, associated with symptomatic allergic asthma in children [1–5]. *B. tropicalis* has also been identified as the major source of allergens in Singaporean homes [6, 7]. Although molecular studies have shown significant differences between IgE epitopes of the pyroglyphid mites (*Dermatophagoides pteronyssinus*, *D. farinae*, *Euroglyphus maynei*) and *B. tropicalis* [8–11], dual sensitization or cross-sensitization is quite prevalent in environments rich in both.

This can be demonstrated using either skin prick tests (SPTs) or *in vitro* specific IgE determination [12–14]. In Singapore, about a third of young atopic children, before the age of 3 years, show sensitization to dust mites, with *D. pteronyssinus* being slightly more prominent than *B. tropicalis* [15]. Increasing rates of sensitization are seen with age, so that 97.5% of asthmatic children 6–14 years old, are sensitized to *B. tropicalis* and 94.1% to *D. pteronyssinus* [16].

The international study of allergy in childhood (ISAAC) has demonstrated a significant overlap between asthma, allergic rhinitis (AR) and allergic eczema in children [17], as have the published ISAAC studies in Singapore [18, 19]. Wang et al. [18] also showed a further increase in the prevalence and severity of allergic eczema in the paediatric age group in Singapore, in the past few years.

In different settings, specific patterns of sensitization have been associated with increased severity of disease, for example mould sensitization and asthma disease severity [20, 21].

Correspondence: Mona Iancovici Kidon, Department of Paediatric Medicine, KK Children's Hospital, 100 Bukit Timah Road, 229899 Singapore, Singapore.
E-mail: drkidon@yahoo.com

We have previously shown that patterns of sensitization to aeroallergens, specifically to moulds in our area, are modulated by environmental factors such as home air-conditioning and passive smoking [22]. Our initial observations, also showed a significantly lower over all sensitization to *B. tropicalis* in our patient population (unpublished data), when compared with the previously published data in Singapore [23].

Therefore we aimed to define the prevalence, clinical characteristics and risk factors, of species-specific mite sensitization in paediatric AR patients in this, unique environment.

Methods

Patients

From 1 June 2003 to 31 May 2004, all new patients with a clinical diagnosis of AR from the otolaryngology and allergy outpatient clinics of the Kendang Kerbau children's hospital in Singapore, were offered a screening SPT evaluation with a panel of aeroallergen as well as food allergen extracts. All completed a standardized questionnaire, including information 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 bedroom. All were asked to fill a detailed disease activity score.

Patients were included in the analysis, if they had clear evidence of environmental sensitization (ascertained by at least one positive SPT on our aeroallergen panel), and documentation of a symptomatic period of minimum 2 months, with at least two out of the following: watery rhinorrhea, blocked nose, itchy nose, sneezing or night cough. All patients were seen by a paediatric otolaryngologist or allergist.

Skin prick tests

All tests were performed in the respiratory laboratory by an experienced technician using commercial allergen extracts and the GreerPick SPT device (Greer Laboratories, Lenoir, NC, USA) and evaluated by an experienced paediatric allergist. A weal diameter of 3 mm or more in excess of the negative control was considered a positive test result. 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 includes: house dust mite mix (HDM; *D. farinae* 5000 AU/mL + *D. pteronyssinus* 5000 AU/mL, standardized), *B. tropicalis* 0.2 mg protein/mL, 50% v/v glycerol, cockroach mix (*Periplaneta americana*, *Blattella germanica*), mixed feathers (chicken, duck, goose), canary feathers (*Serinus canaria*), kapok seeds (*Alternaria alternata*, *Curvularia spicifera*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *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), acacia,

melaleuca, beefwood (Australian pine), oil palm, mango blossom, sage mix and weed mix. Food protein extracts include cow's milk, soybean, egg whole, egg white, peanut, sesame seed, rice, wheat, fish mix and shellfish mix.

Questionnaire

The questionnaire is a culturally adjusted, self-administered, multiple-choice tool, focusing on five areas on interaction: early childhood events, maternal socio-economic data, environmental exposures, familial risk factors and patient oriented, i.e. past medical history, hospitalizations, diagnoses, use of medication, etc.

The disease activity score used, is modified from the rhinoconjunctivitis and asthma symptom score, for use as an outcome measure in clinical trials, published by Wasserfallen et al. [24], in which the severity of disease was classified into three groups only: none = 0, mild = 2 (including trivial and mild-existent symptoms that do not interfere with a persons daily routine) and moderate-severe = 5 (including moderate and severe, disease significantly interfering with one's schedule, school work, etc.). This truncation was necessary in view of language and cultural barriers as well as use of observer information as opposed to direct questioning of patients. A linear symptom scales normalized to a maximum score of 100 was used in the subsequent data analysis. We have also added three items in the evaluation of associated skin manifestations (dryness, erythema and itching), with similarly graded severity.

Statistical analysis

Relative risk of sensitization and associated risk factors were calculated using binary logistic regression analysis with the forward stepwise model. Multivariate regression analysis was performed to adjust for confounding interactions. Continuous values were compared using one way ANOVA, SPSS 9.0 for Windows (SPSS Inc., 1999).

Results

Two hundred and seven patients fulfilling the clinical criteria, underwent SPT for environmental allergens during the study period. Thirty-two (15%) of patients did not show a positive SPT on our environmental panel and therefore were excluded from analysis. One hundred and seventy-five patients were included, 119 (68%) males, 142 (81%) Chinese, 13 (7%) Indian, 12 (7%) Malay, eight (5%) others. The mean age was 7.9 years (range 2–16). The most prevalent clinical symptoms were: nasal congestion 78%, sneezing 77%, watery rhinorrhea 65% and nasal itch 62%. The prevalence of asthma and atopic dermatitis was 39% and 48%, respectively, in this group of children and 22% had concomitant symptoms of all three related diseases. The average combined symptom score was 24 (range 2–78, on a scale of 0–100), asthma-specific score 12.5 (range 0–87), rhinoconjunctivitis score 26 (range 0–67), eczema score 29 (range 0–100). A history of allergy-related hospitalization was elicited in 11% of children, and was significantly associated with a concurrent diagnosis of

asthma (odds ratio (OR) 8.1, 95% confidence interval (CI) 2.5–25.9, $P < 0.001$).

SPT results were positive for HDMs (*D. pteronyssinus* and *D. farinae* mix) in 85% of patients and for *B. tropicalis* in 62%. Overall mite sensitization was 98%, household pets 10%, moulds 9% and food proteins 12%. Concomitant sensitization to both *B. tropicalis* and *Dermatophagoides* species was seen in 50% of patients. Twenty-two patients (12%) were sensitized to *B. tropicalis* but not to *Dermatophagoides* and 62 (35%) were positive for *Dermatophagoides* but not *B. tropicalis*. In the younger age group (patients less than 5 years old) concomitant sensitization was much less likely. Only 28% of patients in this age group showed positive SPT results to both *B. tropicalis* and *Dermatophagoides* spp., compared with the older age group where 57% had positive results to both (OR 0.3, 95% CI 0.1–0.6).

Atopic dermatitis was present in 69.4% of patients uniquely sensitized to *Dermatophagoides* but only in 4.5% of patients uniquely sensitized to *B. tropicalis*, $P < 0.01$.

In our whole studied population, significant risk factors for *B. tropicalis* sensitization were: older age (> 5 years) (OR 2.1, 95% CI 1.1–4.3, $P < 0.05$) and breastfeeding less than 3 months (OR 2.3, 95% CI 1.1–4.8, $P < 0.05$). Sensitization was significantly less likely in patients with concomitant atopic dermatitis (OR 0.26, 95% CI 0.14–0.5, $P < 0.001$), patients with food protein sensitization (OR 0.45, 95% CI 0.2–0.96, $P < 0.05$), in households with air conditioning (OR 0.33, 95% CI 0.12–0.92, $P < 0.05$) and where English was the primary household language (OR 0.4, 95% CI 0.2–0.8, $P < 0.01$), see Table 1. *Dermatophagoides* spp. sensitization was a major risk factor for the presence of atopic dermatitis (OR 31.8, 95% CI 3.6–285, $P < 0.01$), see Table 2.

We performed a multivariate regression analysis using a forward stepwise model chosen to maximize the predictive value for *B. tropicalis* sensitization. In this analysis only older age (> 5 years) remained a significant positive risk factor (OR 2.9, 95% CI 1.1–7.8, $P < 0.05$) while the presence of atopic dermatitis was negatively associated with *B. tropicalis* sensitization (OR 0.25, 95% CI 0.1–0.6, $P < 0.01$).

Asthmatic patients with *B. tropicalis* sensitization had a higher asthma-specific symptoms score (25 vs. 13 in asthmatic children without *B. tropicalis* sensitization, $P < 0.01$). Asthmatic patients with sensitization to both *B. tropicalis* and *Dermatophagoides*, had a higher mean total symptom score (33 vs. 21, $P < 0.05$) and rhinitis symptom score (35 vs. 24, $P < 0.05$) than patients without dual sensitization.

Overall, the skin response to our *B. tropicalis* extract, 0.2 mg protein/mL, 50% v/v glycerol, was smaller than the response to the standardized mite mix 10 000 AU/mL preparation. In patients with concomitant sensitization, the average *Dermatophagoides* weal size was 13.6 mm compared with 11.4 mm for *B. tropicalis*, $P < 0.05$. In atopic dermatitis patients this difference was marked, see Fig. 1, average weal size for the mite mix was 13.3 mm compared with 7.4 mm for *B. tropicalis*, $P < 0.001$. The average SPT weal size elicited by *B. tropicalis* in allergic eczema patients (7.4 mm) was also significantly smaller than that elicited in patients without skin allergy (9.7 mm), $P < 0.05$.

Discussion

A differential species-specific mite sensitization pattern is seen in this population of children with AR. Almost 70% of

Table 1. Comparison of patients with and without *Blomia tropicalis* sensitization

Demographics	All patients (175)	Blomia+ (109)	Blomia – (66)	P
Males (%)	68	72	63	NS
Chinese (%)	81	83	73	NS
Age mean (range) (years)	7.9 (2–16)	8.5	7.3	< 0.05
Age < 5 years (%)	25	19	34	< 0.05
Sibs	2.3	2.0	3.0	NS
Maternal education $>$ high school (%)	41	42	39	NS
English primary language (%)	44	35	56	< 0.01
Early childhood data				
Birth weight mean (kg)	3.1	3.15	3.07	NS
Delivery (% cesarian)	25	21	30.3	NS
Breastfeeding > 3 months (%)	23	17	32	< 0.05
Early introduction of solids (< 6 months) (%)	72	73.5	70.4	NS
Environmental				
Passive smoking (%)	25.6	25.0	26.6	NS
Air conditioning in house (%)	84.3	79.6	92.2	< 0.05
Carpets/rugs (%)	10.5	11.1	9.5	NS
Furred pets (%)	17.7	17.4	18.2	NS
Clinical data				
Atopic eczema (%)	48	35	68	< 0.001
Asthma (%)	39	42	35	NS
Total symptom score	24.2	24.8	21.2	NS
Asthma symptom score	12.5	15.2	8.0	< 0.01
Rhinoconjunctivitis symptom score	25.5	26.6	23.8	NS
Allergy-related hospitalizations	12.2	13.8	9.7	NS
Positive family history of atopy (%)	69	69	69	NS

Table 2. Comparison of patients with and without *Dermatophagoides* sensitization

Demographics	All patients (175)	Der+ (149)	Der – (26)	P
Males (%)	68	69	65	NS
Chinese (%)	81	83	73	NS
Age mean (range) (years)	7.9 (2–16)	8.2	7.5	NS
Age <5 years (%)	25	21	46	< 0.05
Sibs	2.3	2.3	2.5	NS
Maternal education > high school (%)	41	42	39	NS
English primary language (%)	44	42	56	NS
Early childhood data				
Birth weight mean (kg)	3.1	3.1	3.0	NS
Delivery (% cesarian)	25	25	23	NS
Breastfeeding >3 months (%)	23	24	17	NS
Early introduction of solids (<6 months) (%)	72	71	80	NS
Environmental				
Passive smoking (%)	25.6	26	23	NS
Air conditioning in house (%)	84.3	77	62	NS
Carpets/rugs (%)	10.5	10	12	NS
Furred pets (%)	17.7	17	19	NS
Clinical data				
Atopic eczema (%)	48	55	11	< 0.001
Asthma (%)	39	38	48	NS
Total symptom score	24.2	25.1	19.2	NS
Asthma symptom score	12.5	11.5	18.3	NS
Rhinoconjunctivitis symptom score	25.5	26.5	19.4	NS
Allergy-related hospitalizations	12.2	13%	10%	NS
Positive family history of atopy (%)	69	69	67	NS

patients with unique sensitization to the *Dermatophagoides* mites have associated allergic eczema while only 4.5% of patients sensitized solely to *B. tropicalis* have any evidence of skin allergy. In the entire population, responses to the *B. tropicalis* extract were smaller than those elicited by the *D. pteronyssinus*/*D. farinae* mix, which may be because of the relative allergenic potency of the preparations. However, this difference was exaggerated in the group of children with allergic eczema, where the mean SPT weal diameter caused by *B. tropicalis* was significantly smaller than that caused by the *Dermatophagoides* mites (7.5 vs. 13.3 mm, respectively, $P < 0.001$), suggesting that in some instances the skin response to the *B. tropicalis* extract in this population may be secondary to cross-reactivity and not concomitant sensitization.

In this study group, the presence of allergic eczema was the strongest positively associated factor with *Dermatophagoides* sensitization (OR 31.8, 95% CI 3.6–285, $P < 0.01$), while it was negatively associated with *B. tropicalis* sensitization (OR 0.2, 95% CI 0.1–0.6, $P < 0.01$). In the classical view of an ordered sequential process in the development of allergic disease, we would tend to postulate that a significant early exposure to *Dermatophagoides* mites, caused sensitization and this in turn is a causative risk factor for the development of atopic dermatitis on subsequent exposure to the offending allergen. However, both *B. tropicalis* and *Dermatophagoides* spp. are abundant in the indoor environment in which our children live. We have seen in other as yet unpublished work that significant levels of allergen from both the pyroglyphid mites and *B. tropicalis* can be found in cord blood serum of healthy term newborn in Singapore, testifying to the early

ubiquitous exposure of our children. All patients included in this study have proven, clinically relevant atopy according to the Pepys criteria [25] and significant presumed exposure. So why do young children with atopic dermatitis, living in an environment rich in both, get sensitized to the pyroglyphid mites preferentially? The new classification of allergic eczema dermatitis syndrome (AEDS) [26], takes into account the clinical and laboratory observations that not all features of AEDS can be explained or treated as a direct allergen-immune system interaction even in those patients where, similar to our group, an IgE response to environmental or food-related proteins can be found. Findings from basic research of structural deficiencies/differences in atopic skin [27, 28], raise the possibility that an inherent quality of the skin causes a specific vulnerability/permeability allowing early sensitization to specific allergens. The possibility of sensitization to aeroallergens through the skin and its systemic implications is well documented and summarized by Beck and Leung [29]. The possibility that this process may be unique to the *Dermatophagoides* mites and not extendable to other mite species has not been previously published.

The allergenic differences between the pyroglyphid mites (*D. pteronyssinus*, *D. farinea* and *E. maynei*) and *B. tropicalis* are significant and well described [30, 31]. In our context it seems of interest that the major sensitizing allergens of the pyroglyphid mites, the group 1 allergens, are gastrointestinal tract-derived cysteine proteases [32–34], whose enzymatic activity seems to be important in the pathogenicity of the skin irritation associated with mite exposure in patients with pyroglyphid mite sensitization [35–37]. While recently, a homologue of the group 1 allergens, has been isolated from a

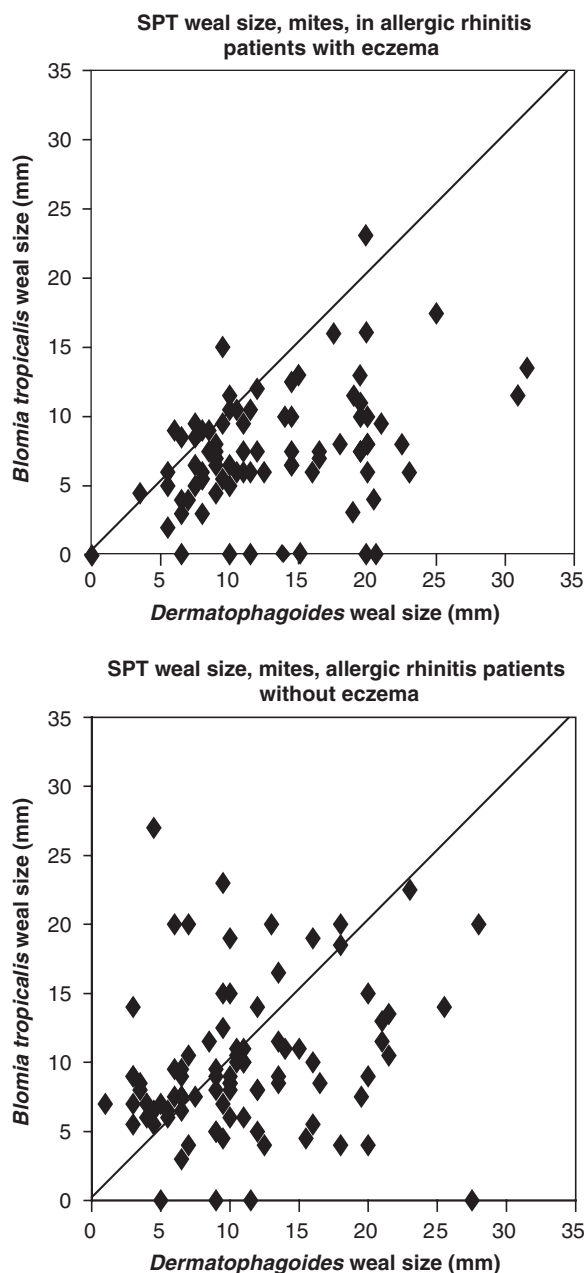


Fig. 1. Comparison of skin prick test weal diameter (mm), *Dermatophagoides* vs. *Blomia* in patients with and without allergic eczema. The diagonal line is equidistant to the x and y axes.

cDNA library of *B. tropicalis* [38], the major sensitizing allergen in this mite is the Blo t 5 [39–41], a protein without a functional characterization as yet but no enzymatic activity.

In view of the above, one may speculate, that the basis of this preferential sensitization to *Dermatophagoides* mites observed in atopic patients with allergic eczema, in an environment rich in both *Dermatophagoides* and *Blomia* mites [42], may be because of the combined direct enzymatic and allergenic effects of the major group 1 allergens on the skin. While the *B. tropicalis* major allergens, acting as pure respiratory sensitizing agents, similar to pollen allergens in temperate climates, will largely cause sensitization in older children, after a longer exposure period and be associated

with mainly respiratory symptoms. The positive association seen between *B. tropicalis* sensitization and the asthma symptom score, as well as that between dual sensitization and increased total and rhinitis symptoms score, seem to indicate that further elucidation the factors determining species-specific mite sensitization in a dual exposure environment may be of clinical importance.

In the studied population, air conditioning seemed, at least in univariate regression analysis, a significant 'protective' factor against *Blomia* sensitization. The known optimal growth requirements of the *B. tropicalis* mites are a mean annual temperature of 30 °C and a mean relative humidity (RH) of 80% [43], while *Der* spp. has been shown to survive environments with an RH of 50% for more than 10 weeks [44]. This may be the basis of decrease exposure and therefore decreased sensitization in households using air-conditioning in tropical climates.

The seemingly '*Blomia* protective' effect exerted by 'English speaking' again in univariate analysis can probably be explained as a surrogate marker for a more westernized life style, for example less storage of rice which has been shown to be associated with increased quantities of this storage mite [45].

All the patients in this group had a clinical diagnosis of AR, augmented by a positive aeroallergen SPT and so unsurprisingly the most common clinical symptoms reported were nasal congestion, sneezing, watery rhinorrhea and nasal itch. The relatively high incidence of associated allergic asthma (39%) and allergic eczema (48%) is probably somewhat influenced by a referral bias to the allergy clinic, preferentially seeing patients with 'systemic allergy', whereas patients with 'monosystem' allergic disease may be referred to other individual specialist clinics. This bias towards patients with systemic allergy, may explain the slight variation in aeroallergen sensitization prevalence, compared with our previously published data [46]. As a group these patients were less sensitized to the HDMs (85% vs. 97% *Dermatophagoides* sensitization) when compared with the previously studied group of patients with AR referred mainly from the otolaryngology clinic of our hospital, where the incidence of other associated atopic diseases was markedly lower. Nevertheless the data from the ISAAC studies [18, 19, 47] supports a significant overlap of the atopic phenotypes in all allergic children. The increased relative prevalence of allergic eczema in our study may be influenced by the relatively young age of the population studied (mean 7.9 years compared with 14 years in the ISAAC phase 1 studies).

There are some weaknesses associated with our study. As serological testing was not performed we do not have corroborative data from *Dermatophagoides* or *B. tropicalis* specific IgE levels, nor can we perform inhibition studies to determine the incidence of cross-reactivity as opposed to dual sensitization. This issue is particularly relevant in the group of patients with allergic eczema, where the mean SPT weal diameter induced by the *B. tropicalis* extract was significantly smaller than that induced by *Dermatophagoides* extracts in the same group, implying that in this group of patients, sensitization to the pyroglyphid mites may be the primary response with some of the smaller responses induced by *B. tropicalis* because of cross-sensitivity alone.

We also did not collect dust samples and so lack data on actual indoor aeroallergen exposure in our patients and

therefore base our exposure assumptions on previously published studies in our environment [48, 49].

Conclusions

Children with AR and concomitant atopic dermatitis in Singapore's tropical climate, show a preferential sensitization to the *Dermatophagoides* species. In our population, *B. tropicalis* sensitization is more prominent in children with pure respiratory allergy. This pattern of species-specific sensitization may be because of intrinsic differences in the structure and activity of the major allergenic group proteins in the *Dermatophagoides* and *Blomia* mites and seems to have a clinical as well as a statistical impact. Further elucidation of the temporal sequence as well as the clinical correlates of this differential sensitization is needed and may only be achieved in a long term prospective cohort study.

References

- Puerta L, Fernandez-Caldas E, Lockey RF, Caraballo LR. Mite allergy in the tropics: sensitization to six domestic mite species in Cartagena, Colombia. *J Invest Allergol Clin Immunol* 1993; 3: 198–204.
- Arruda LK, Vailes LD, Platts-Mills TA et al. Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blo t 5. *Am J Respir Crit Care Med* 1997; 155:343–50.
- Caraballo L, Puerta L, Fernandez-Caldas E, Lockey RF, Martinez B. Sensitization to mite allergens and acute asthma in a tropical environment. *J Invest Allergol Clin Immunol* 1998; 8:281–4.
- Chew FT, Lim SH, Goh DY, Lee BW. Sensitization to local dust-mite fauna in Singapore. *Allergy* 1999; 54:1150–9.
- Yeoh SM, Kuo IC, Wang DY et al. Sensitization profiles of Malaysian and Singaporean subjects to allergens from *Dermatophagoides pteronyssinus* and *Blomia tropicalis*. *Int Arch Allergy Immunol* 2003; 132:215–20.
- Chew FT, Zhang L, Ho TM, Lee BW. House dust mite fauna of tropical Singapore. *Clin Exp Allergy* 1999; 29:201–6.
- Yi FC, Chew FT, Jimenez S, Chua KY, Lee BW. Culture of *Blomia tropicalis* and IgE immunoblot characterization of its allergenicity. *Asian Pac J Allergy Immunol* 1999; 17:189–94.
- Cheong N, Soon SC, Ramos JD et al. Lack of human IgE cross-reactivity between mite allergens Blo t 1 and Der p 1. *Allergy* 2003; 58:912–20.
- Kuo IC, Cheong N, Trakultivakorn M, Lee BW, Chua KY. An extensive study of human IgE cross-reactivity of Blo t 5 and Der p 5. *J Allergy Clin Immunol* 2003; 111:603–9.
- Yi FC, Cheong N, Shek PC, Wang DY, Chua KY, Lee BW. Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2002; 32: 1203–10.
- Chew FT, Yi FC, Chua KY et al. Allergenic differences between the domestic mites *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1999; 29:982–8.
- Simpson A, Green R, Custovic A, Woodcock A, Arruda LK, Chapman MD. Skin test reactivity to natural and recombinant *Blomia* and *Dermatophagoides* spp. allergens among mite allergic patients in the UK. *Allergy* 2003; 58:53–6.
- Yeoh SM, Kuo IC, Wang DY et al. Sensitization profiles of Malaysian and Singaporean subjects to allergens from *Dermatophagoides pteronyssinus* and *Blomia tropicalis*. *Int Arch Allergy Immunol* 2003; 132:215–20.
- Chew FT, Lim SH, Goh DY, Lee BW. Sensitization to local dust-mite fauna in Singapore. *Allergy* 1999; 54:1150–9.
- Khoo J, Shek L, Khor ES, Wang DY, Lee BW. Pattern of sensitization to common environmental allergens amongst atopic Singapore children in the first 3 years of life. *Asian Pac J Allergy Immunol* 2001; 19:225–9.
- Chew FT, Lim SH, Goh DY, Lee BW. Sensitization to local dust-mite fauna in Singapore. *Allergy* 1999; 54:1150–9.
- ISAAC: The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema. *Lancet* 1998; 351:1225–32.
- Wang XS, Tan TN, Shek LP et al. The prevalence of asthma and allergies in Singapore; data from two ISAAC surveys seven years apart. *Arch Dis Child* 2004; 89:423–6.
- Goh DY, Chew FT, Quek SC, Lee BW. Prevalence and severity of asthma, rhinitis, and eczema in Singapore schoolchildren. *Arch Dis Child* 1996; 74:131–5.
- Zureik M, Neukirch C, Leynaert B, Liard R, Bousquet J, Neukirch F. Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. *BMJ* 2002; 325:411–4.
- Newson R, Strachan D, Corden J, Millington W. Fungal and other spore counts as predictors of admissions for asthma in the Trent region. *Occup Environ Med* 2000; 57:786–92.
- Kidon MI, See Y, Goh A, Chay OM, Balakrishnan A. Aeroallergen sensitization in pediatric allergic rhinitis in Singapore: is air-conditioning a factor in the tropics? *Pediatr Allergy Immunol* 2004; 15:340–3.
- Chew FT, Lim SH, Goh DY, Lee BW. Sensitization to local dust-mite fauna in Singapore. *Allergy* 1999; 54:1150–9.
- Wasserfallen JB, Gold K, Schulman KA, Baraniuk JN. Development and validation of a rhinoconjunctivitis and asthma symptom score for use as an outcome measure in clinical trials. *J Allergy Clin Immunol* 1997; 100:16–22.
- Pepys J. Atopy. In: Gill PGH, Coombs RRA, Lachmann PJ, eds. *Clinical aspects of immunology*. Oxford: Blackwell, 1975; 877–902.
- Johansson SGO, Bieber T, Dahl R et al. Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004; 113:832–6.
- Arikawa J, Ishibashi M, Kawashima M, Takagi Y, Ichikawa Y, Imokawa G. Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum corneum from patients with atopic dermatitis to colonization by *Staphylococcus aureus*. *J Invest Dermatol* 2002; 119:433–9.
- Imokawa G. Lipid abnormalities in atopic dermatitis. *J Am Acad Dermatol* 2001; 45:S29–32.
- Beck L, Leung D. Allergen sensitization through the skin induces systemic allergic responses. *J Allergy Clin Immunol* 2000; 106: 258–63.
- Chew FT, Yi FC, Chua KY et al. Allergenic differences between the domestic mites *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1999; 29:982–8.
- Yi FC, Cheong N, Shek PC, Wang DY, Chua KY, Lee BW. Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2002; 32: 1203–10.
- Dilworth RJ, Chua KY, Thomas WR. Sequence analysis of cDNA coding for a major house dust mite allergen, Der f I. *Clin Exp Allergy* 1991; 21:25–32.
- Chua KY, Stewart GA, Thomas WR et al. Sequence analysis of cDNA coding for a major house dust mite allergen, Der p 1. Homology with cysteine proteases. *J Exp Med* 1988; 167: 175–82.

- 34 Smith W, Mills K, Hazell L, Hart B, Thomas W. Molecular analysis of the group 1 and 2 allergens from the house dust mite, *Euroglyphus maynei*. *Int Arch Allergy Immunol* 1999; 118:15–22.
- 35 Baker SF, Yin Y, Runswick SK et al. Peptidase allergen Der p 1 initiates apoptosis of epithelial cells independently of tight junction proteolysis. *Mol Membr Biol* 2003; 20:71–81.
- 36 Wan H, Winton HL, Soeller C et al. Quantitative structural and biochemical analyses of tight junction dynamics following exposure of epithelial cells to house dust mite allergen Der p 1. *Clin Exp Allergy* 2000; 30:685–98.
- 37 Wan H, Winton HL, Soeller C et al. The transmembrane protein occludin of epithelial tight junctions is a functional target for serine peptidases from faecal pellets of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2001; 31:279–94.
- 38 Mora C, Flores I, Montealegre F, Diaz A. Cloning and expression of Blo t 1, a novel allergen from the dust mite *Blomia tropicalis*, homologous to cysteine proteases. *Clin Exp Allergy* 2003; 33: 28–34.
- 39 Simpson A, Green R, Custovic A, Woodcock A, Arruda LK, Chapman MD. Skin test reactivity to natural and recombinant *Blomia* and *Dermatophagoides* spp. allergens among mite allergic patients in the UK. *Allergy* 2003; 58:53–6.
- 40 Arruda LK, Vailes LD, Platts-Mills TA et al. Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blo t 5. *Am J Respir Crit Care Med* 1997; 155:343–50.
- 41 Tsai JJ, Yi FC, Chua KY, Liu YH, Lee BW, Cheong N. Identification of the major allergenic components in *Blomia tropicalis* and the relevance of the specific IgE in asthmatic patients. *Ann Allergy Asthma Immunol* 2003; 91:485–9.
- 42 Zhang L, Chew FT, Soh SY et al. Prevalence and distribution of indoor allergens in Singapore. *Clin Exp Allergy* 1997; 27:876–85.
- 43 Yi FC, Chew FT, Jimenez S, Chua KY, Lee BW. Culture of *Blomia tropicalis* and IgE immunoblot characterization of its allergenicity. *Asian Pac J Allergy Immunol* 1999; 17:189–94.
- 44 Arlian LG, Confer PD, Rapp CM, Vyszenski-Moher DL, Chang JC. Population dynamics of the house dust mites *Dermatophagoides farinae*, *D. pteronyssinus*, and *Euroglyphus maynei* (Acari:Pyroglyphidae) at specific relative humidities. *J Med Entomol* 1998; 35:46–53.
- 45 Franzolin MR, Baggio D. Mite contamination in polished rice and beans sold at markets. *Rev Saude Publica* 2000; 34:77–83.
- 46 Kidon MI, See Y, Goh A, Chay OM, Balakrishnan A. Aeroallergen sensitization in pediatric allergic rhinitis in Singapore: is air-conditioning a factor in the tropics? *Pediatr Allergy Immunol* 2004; 15:340–3.
- 47 ISAAC: The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema. *Lancet* 1998; 351:1225–32.
- 48 Chew FT, Zhang L, Ho TM, Lee BW. House dust mite fauna of tropical Singapore. *Clin Exp Allergy* 1999; 29:201–6.
- 49 Zhang L, Chew FT, Soh SY et al. Prevalence and distribution of indoor allergens in Singapore. *Clin Exp Allergy* 1997; 27: 876–85.