

Sensitization Profiles of Malaysian and Singaporean Subjects to Allergens from *Dermatophagoides pteronyssinus* and *Blomia tropicalis*

S.M. Yeoh^a I.C. Kuo^a D.Y. Wang^b C.K. Liam^d C.K. Sam^e J.A. De Bruyne^d
B.W. Lee^a N. Cheong^c K.Y. Chua^a

Departments of ^aPaediatrics and ^bOtolaryngology, National University of Singapore, ^cBioprocessing Technology Centre, BMSI, A*STAR, Singapore; ^dDepartment of Medicine, ^eInstitute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

Key Words

Asthmatics · Rhinitis · *Blomia tropicalis* ·
Dermatophagoides pteronyssinus · ELISA · Sensitization
to allergens

Abstract

Background: The house dust mites *Dermatophagoides pteronyssinus* (Der p) and *Blomia tropicalis* (Blo t) are the most common house dust mite species in Southeast Asia. To date, there have only been a few studies on the sensitization profile of the general populations in Southeast Asia to house dust mites. The aim of this study was to determine the profiles of Der p and Blo t sensitization among Singaporean and Malaysian subjects. **Methods:** Enzyme-linked immunosorbent assay was used to detect specific IgE to Der p and Blo t mite crude extracts as well as purified Der p 1, Der p 2 and Blo t 5 allergens. Sera used were from 229 Singaporean subjects (124 with rhinitis, 105 without rhinitis) and 143 Malaysian subjects (94 adults and 49 children with asthma). **Results:** The sensitization profile of rhinitis subjects to the dust mite allergens used in this study was as follows: Blo t extract positive: 91/124 (73%); Blo t 5 positive: 62/124 (50%); Der p extract positive: 61/124 (49%); Der p 1 positive: 53/124

(43%); Der p 2 positive: 45/124 (36%). The nonrhinitis subjects' sensitization profile was as follows: Blo t extract positive: 60/105 (57%); Blo t 5 positive: 24/105 (23%); Der p extract positive: 38/105 (36%); Der p 1 positive: 14/105 (13%); Der p 2 positive: 17/105 (16%). The study of Malaysian asthmatic adults showed that 39% of them were sensitized to Der p 1, 32% to Der p 2 and 37% to Blo t 5. Among the asthmatic children, sensitization to Blo t 5, Der p 1 and Der p 2 was 90, 57 and 39%, respectively. **Conclusion:** This study clearly revealed that dual sensitization to *B. tropicalis* and *D. pteronyssinus* is common in the general populations of Singapore and Malaysia. Sensitization to Blo t 5 is more prevalent than to Der p 1 and Der p 2.

Copyright © 2003 S. Karger AG, Basel

Introduction

The incidence of allergic asthma and rhinitis increased over the last few decades of the 20th century [1–3], although the prevalence of asthma symptoms was highly variable from country to country [4]. It is well known that sensitization to dust mite allergens is strongly associated with these allergic diseases [5]. The major dust mite aller-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2003 S. Karger AG, Basel
1018–2438/03/1323–0215\$19.50/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Nge Cheong, PhD
Bioprocessing Technology Centre, National University of Singapore
MD11, Level 5, 10 Medical Drive
Singapore 117597 (Singapore)
Tel. +65 68744500, Fax +65 67754933, E-Mail btccn@btc.a-star.edu.sg

gens in the tropical region come primarily from two species of house dust mite: *Dermatophagoides pteronyssinus* (Der p) and *Blomia tropicalis* (Blo t) [6–8].

In an earlier study in Singapore, Kuo et al. [9] found that subjects from Singapore and Taiwan showed dual sensitization to Der p and Blo t. They also showed that although Der p 1, Der p 2 and Blo t 5 allergens were important in both Singapore and Taiwan, Blo t 5 appeared to be more important among Singaporean subjects compared to Taiwanese subjects [9]. Nevertheless, the number of Singaporean subjects who were tested for their specific IgE against the three allergens in that study was small. Therefore, one of the aims of this study was to further extend the previous study to determine the sensitization profile in the Singaporean population with a larger panel of 229 sera. In addition, 143 sera from Malaysia, a neighboring country that shares identical climatic conditions with Singapore, were also included in this study.

Subjects and Methods

Subjects

A total of 229 sera were obtained from randomly selected adult volunteers recruited for a national rhinitis survey study in the ENT outpatient clinic of the National University Hospital, Singapore. Out of 229 subjects, 124 (62 males and 62 females; age range 7–73 years, mean age 34 years) were diagnosed with persistent rhinitis [10]. The definition of rhinitis was based on the International Consensus Report algorithm recommendation for treatment of rhinitis [11]. Subjects who had two or more symptoms (nasal obstruction, rhinorrhea, sneezing and itchy nose) on most days during the past year were defined as having perennial rhinitis. The remaining 105 subjects (59 males and 46 females; age range 7–73 years, mean age 38 years), who had no history of rhinitis, were recruited for comparison. Approval to conduct this study was granted by the National Medical Research Council of Singapore and the institutional review board of the Medical Faculty, National University of Singapore.

For the Malaysian study, a total of 94 sera from adult asthmatic patients visiting the Asthma Clinic in the University Hospital, University of Malaya, Malaysia, were used. The mean age of these patients was 46.4 years. None of the patients had chronic obstructive pulmonary disease. In addition, 49 sera from asthmatic children attending the Pediatric Allergy Clinic in the same hospital were also included in this study.

Preparation of Blo t and Der p Allergens

Native Der p 1 was purified from spent mite medium by affinity chromatography, using the monoclonal antibody 4C1 [12]. Recombinant Der p 2 [13, 14] and Blo t 5 [15] were produced in the *Pichia pastoris* yeast expression system and purified using chromatographic methods [9, 16].

Preparation of Mite Crude Extracts

Blo t and Der p mite crude extracts were prepared as described previously [9]. Briefly, 1 g of frozen dust mites (Blo t grown in-house

as described previously [17] or lyophilized *Der p* from Commonwealth Serum Laboratories, Parkville, Australia) was homogenized in the presence of liquid nitrogen. Protein extraction was performed using PBS containing 2 mM phenylmethyl-sulfonyl fluoride and 1 mM EDTA. The mixture was centrifuged at 15,000 *g* for 15 min and the supernatant was dialyzed overnight at 4 °C against PBS. The protein concentration was determined by using Coomassie® Plus Protein Assay Reagent Kit (Bio-Rad Laboratories, Calif., USA). The protein extracts were stored in aliquots at –80 °C.

Enzyme-Linked Immunosorbent Assay

Specific IgE against Blo t 5, Der p 1 and Der p 2 in the sera were determined using enzyme-linked immunosorbent assay (ELISA) as previously described [9]. Briefly, 250 ng of allergen was coated onto each well of a 96-well microtiter plate (Corning) overnight in 0.1 M NaHCO₃ (Sigma). For detection of specific IgE to mite crude extract, the plate was saturated with 10,000 ng of Blo t or Der p mite crude extracts. The plate was then washed 3 times with PBS-Tween 20 (0.05%) (washing solution) using Columbus washer (Tecan). All subsequent washing steps were performed in a similar manner. The plate was then blocked with PBS-Tween 20 (0.05%) containing 1% BSA (Sigma) (blocking and dilution solution) for 2 h. Then, the plate was washed. Sera diluted 1:5 in dilution solution were dispensed at 50 µl/well and incubated overnight. The next day, the plate was washed and incubated with mouse anti-human biotin-conjugated IgE (Southern Biotechnology; 1:2,000 dilution) for 1 h at room temperature. Again, the plate was washed and incubated with alkaline phosphatase-conjugated ExtrAvidin® (Sigma; 1:2,000 dilution) at room temperature for 1 h. The plate was washed and the substrate, β-nitrophenyl phosphate (Sigma), was then added. Optical density (OD_{405 nm}) readings were obtained using a Shell Rainbow Reader (Tecan) at 30 min and 1 h after the addition of substrate.

Control sera for Singapore subjects were from a set of sera that showed negative results in ELISA against the allergens tested, whereas control sera for Malaysian asthmatic subjects were from patients who had no observable wheals to all the allergens in skin prick tests (data not shown). The cutoff value for individual allergen ELISA readouts was based on the mean of all the cutoffs for individual allergens, which were determined using the mean plus two standard deviations of these control sera against the respective allergen. However, cutoffs for mite crude extracts (Der p extract and Blo t extract) were determined independently from individual allergens. Their cutoffs were determined using the mean plus two standard deviations of the ELISA readings of control sera to the respective extract.

Statistical Analysis

The OD_{405 nm} readings from Singaporean subjects were analyzed using a Mann-Whitney statistical test (SPSS for Windows, version 10.0.5). Correlation coefficients were calculated using Excel [Microsoft® Excel 2002 (10.3506.3501)SP-1].

Results

In this study, we determined the dust mite allergen sensitization profile for four subgroups of subjects: subjects from Singapore with and without rhinitis, and adults and children with asthma from Malaysia.

Fig. 1. Allergen-specific IgE titers from rhinitis (R) (n = 124) and nonrhinitis (NR) (n = 105) subjects from Singapore. Significant differences were observed between the two groups for all allergens when analyzed using Mann-Whitney statistical analyses. Ext = Extract; Bt = Blo t; Dp = Der p. Cutoff = 0.093 for Der p 1, Der p 2, Blo t 5; 0.101 for Blo t Ext; 0.090 for Der p Ext. *A significant difference was observed.

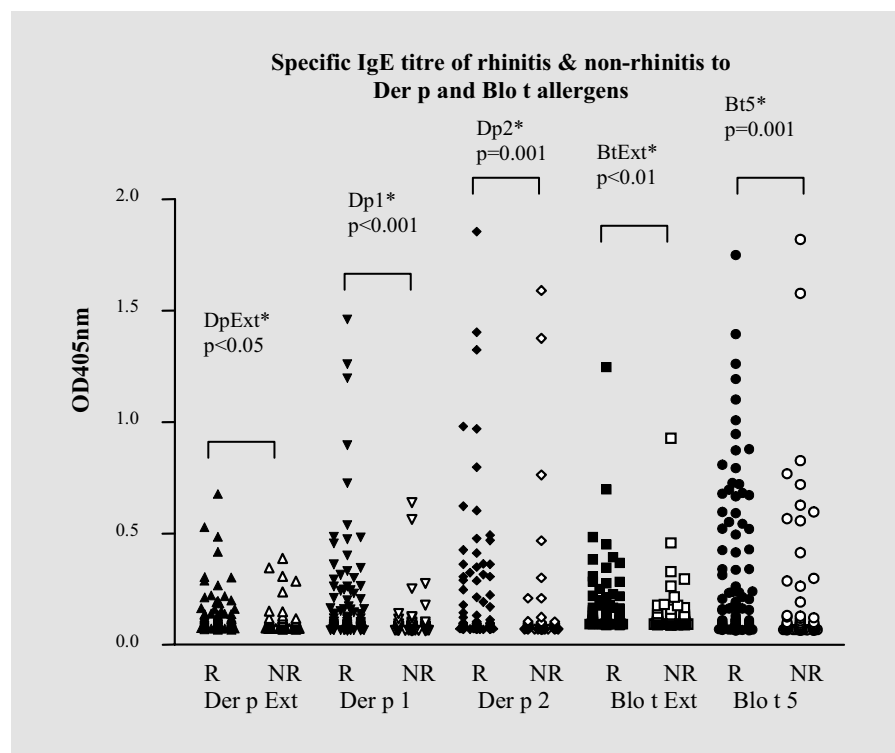
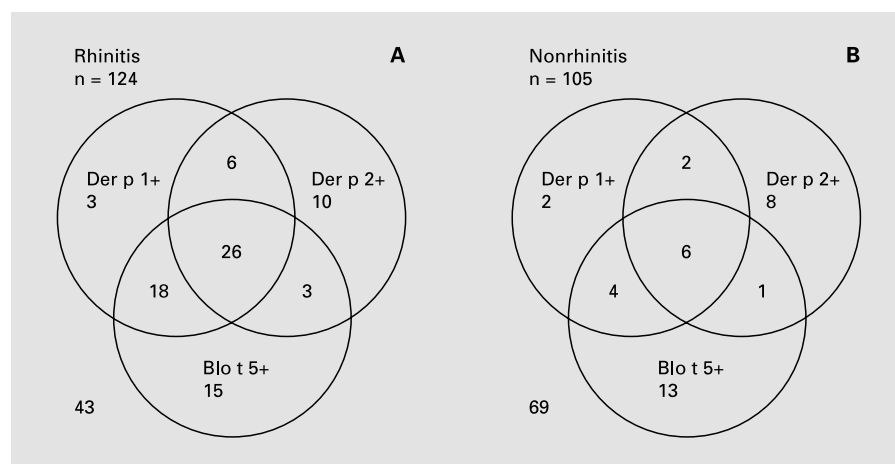


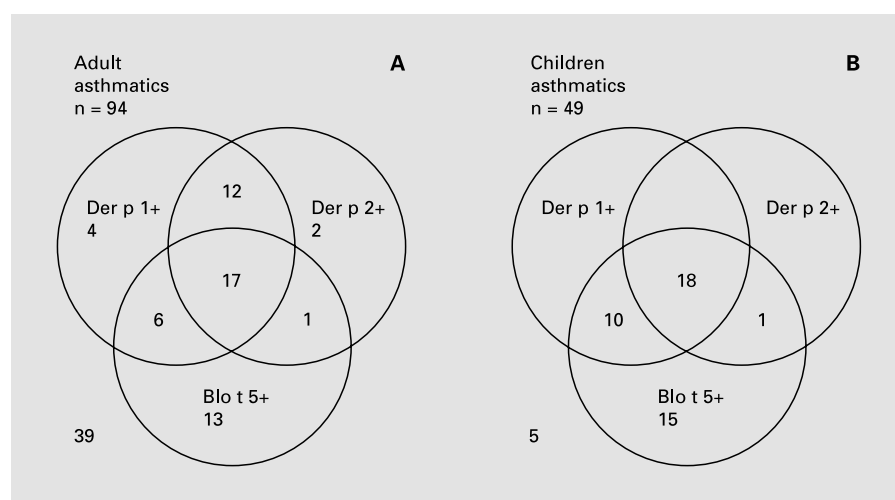
Fig. 2. Sensitization profile of Singaporean rhinitis (A) and nonrhinitis (B) subjects based on ELISA results. Cutoff = 0.093 for all allergens.



The sensitization profile of rhinitis subjects to the dust mite allergens used in this study was as follows: Blo t extract positive: 91/124 (73%); Blo t 5 positive: 62/124 (50%); Der p extract positive: 61/124 (49%); Der p 1 positive: 53/124 (43%); Der p 2 positive: 45/124 (36%). The nonrhinitis subjects' sensitization profile was as follows: Blo t extract positive: 60/105 (57%); Blo t 5 positive: 24/105 (23%); Der p extract positive: 38/105 (36%); Der p 1 positive: 14/105 (13%); Der p 2 positive: 17/105 (16%). There was a significant difference in the levels of allergen-

specific IgE in rhinitis versus nonrhinitis subjects. In fact, the rhinitis subjects generally had a higher titer of IgE (inferred from the higher OD reading) than nonrhinitis subjects (fig. 1). In addition, a greater number of rhinitis subjects were sensitized to each of the allergens compared to the nonrhinitis group. In the rhinitis cohort, 81 of 124 (65%) reacted to at least one allergen from Blo t and/or Der p (Blo t 5/Der p 1/Der p 2), while in the nonrhinitis subjects, the corresponding figure was 36 of 105 (34%) (fig. 2).

Fig. 3. Sensitization profile of adult (**A**) and children asthmatics (**B**) from Malaysia based on ELISA results. Cutoff = 0.15 for all allergens.



Correlation between the single allergens among rhinitis subjects was as follows: Der p 1 and Der p 2: $r = 0.60$; Blo t 5 and Der p 1: $r = 0.64$; Blo t 5 and Der p 2: $r = 0.78$. Correlation for the same parameters for nonrhinitis subjects was as follows: Der p 1 and Der p 2: $r = 0.71$; Blo t 5 and Der p 1: $r = 0.51$; Blo t 5 and Der p 2: $r = 0.45$.

The results for Malaysian adult asthmatics showed that 39% of these subjects were sensitized to Der p 1, 32% to Der p 2 and 37% to Blo t 5. The rates of allergen sensitization in the asthmatic children from Malaysia were 57% to Der p 1, 39% to Der p 2 and 90% to Blo t 5 (fig. 3).

Moreover, the correlation between Der p 1 and Der p 2 sensitization was 0.77 for adult asthmatics and 0.94 for children. There was less correlation between Blo t 5 sensitization and either Der p 1 or Der p 2 (Blo t 5 and Der p 1: 0.47 for adults, 0.22 for children; Blo t 5 and Der p 2: 0.41 for adults, 0.27 for children).

Discussion

Our study on Singaporean subjects showed that a high percentage of the rhinitis patients were sensitized to both Der p and Blo t mites; these subjects showed sensitivity to at least one major allergen from Blo t and Der p mites. Our results also revealed that some nonrhinitis subjects were also sensitized to house dust mite allergens (fig. 2). The data suggest that mite sensitization in our general population is highly prevalent and that allergen sensitization does not always lead to clinical symptoms, implying that the relevance of serum IgE in disease prediction remains controversial [18–20]. For instance, Burrows et

al. [20] reported that serum IgE could be predictive of rhinitis and asthma; in contrast, others [19] have shown that the experimental asthma phenotype could be independent of IL-4 and allergen-specific IgE. Although our study showed that in general, rhinitis subjects had higher allergen-specific IgE levels than did nonrhinitis subjects (fig. 1), allergen-specific IgE in serum is not the only nor the best indicator for allergic diseases, as suggested by other investigators [21, 22]. Therefore, diagnosis by a trained clinician is still highly desirable. However, a patient's sensitization profile can serve as helpful information in the diagnosis of allergic rhinitis because the odds of a rhinitis subject being sensitized to any of the three allergens used is generally greater than that in a nonrhinitis subject.

The sensitization profile of Malaysian asthmatic adults, as determined by ELISA, was quite different to that of children asthmatics, especially with respect to Blo t 5 sensitization (fig. 3). This observation can probably be attributed to the age factor. The influence of age on IgE responsiveness to dust mite allergens has been reported by Shibasaki et al. [23] and Boulet et al. [24]. Though the frequency decreased, the prevalence of sensitization to the three house dust mite allergens remained quite high in adults. Although this observation is contrary to a report that sensitization actually increases with age [25], it is in accordance with the observation by Burrows et al. [20] that serum IgE levels decline significantly with age among asthmatics ($p < 0.0001$). Furthermore, Boulet et al. [24] also showed that allergen sensitization among older adults (>46 years old) was much lower than that in the age group 6–15 years. This could probably help to explain our observation, because the age of the adult asthmatics in our

study was generally skewed towards middle age rather than the early twenties.

The sensitization profiles of Malaysian asthmatic children observed in this study revealed that sensitization to Blo t 5 allergen was as high as 90%, and sensitization to Der p 1 was 57%. The data are in good agreement with results previously reported by Leung et al. [26]. They showed that the frequency of positive skin prick test reactivity to Der p extract in older children (age range 12–18 years) in the general population from Kota Kinabalu, Sabah, Malaysia, was about 60%. The study also indicated that mite allergy was a risk factor for asthma and rhinitis [26]. Another observation from the present study worth noting is that the sensitization to Der p 2 was below 50% in both Singaporean and Malaysian subjects. This is rather surprising, as a previous epidemiological study indicated that an extremely high number of both Der p and Blo t mites were found in Singaporean homes [6], and the levels of Blo t and Der p allergens found in these homes were very high [27]. This result also shows a discrepancy with other published studies in which it was reported that about 80% of mite-allergic asthmatic patients were sensitized to Der p 2 [28–30]. Since those studies were conducted in temperate and subtropical regions, the relatively lower prevalence of sensitization to Der p allergens than Blo t allergens seen in our study is probably unique for the populations living in the tropics. Further studies are required to fully address this discrepancy.

Although the individual allergens used in the present study (Blo t 5, Der p 1, Der p 2) were able to detect a majority of the house dust mite extract-positive subjects, there was a subset of subjects who went undetected by

these three major allergens, indicating that other additional allergens are needed for a complete-spectrum diagnosis of mite allergy. There were also some subjects who reacted to the individual allergens used but to neither of the extracts used. This could also be due to an insufficient amount of allergenic components in the extract. Therefore, it would be feasible and useful to use a panel of individual allergens to replace the use of mite crude extracts for more precise component-resolved diagnosis of mite allergy [31]. Such diagnostic reagents are particularly useful for patient-tailored allergen-specific immunotherapy.

In summary, our study confirms the findings of a previous study [9] that Der p 1, Der p 2 and Blo t 5 are important sensitizing allergens among Singaporean and Malaysian subjects. In addition, we also showed that the prevalence of sensitization to Blo t 5, Der p 1 and Der p 2 and the levels of specific IgE to these allergens were significantly higher in subjects with rhinitis than in the sensitized subjects without rhinitis. For the Malaysian asthmatic subjects, sensitization to these three mite allergens was much more prevalent in children than in their adult counterparts. Further studies with a larger panel of sera from well-defined populations should be performed to confirm these observations.

Acknowledgements

This research was funded by grants from the Academic Research Fund, National University of Singapore (R178-000-010-112), and the Agency for Science, Technology and Research (A*STAR), Singapore. We would like to express our thanks to Ms. Madeline Wong for her technical assistance.

References

- 1 Platts-Mills TAE, Wheatley LM, Aalberse RC: Indoor versus outdoor allergens in allergic respiratory disease. *Curr Opin Immunol* 1998; 10:634–639.
- 2 Åberg N, Hesselmar B, Åberg B, Eriksson B: Increase of asthma, allergic rhinitis and eczema in Swedish school children between 1979 and 1991. *Clin Exp Allergy* 1995;25:815–819.
- 3 Linneberg A, Nielsen NH, Madsen F, Frølund L, Dirksen A, Jørgensen T: Increasing prevalence of allergic rhinitis symptoms in an adult Danish population. *Allergy* 1999;54:1194–1198.
- 4 Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* 1998; 351:1225–1232.
- 5 Arlian LG, Platts-Mills TAE: The biology of dust mites and the remediation of mite allergens in allergic disease. *J Allergy Clin Immunol* 2001;107:S406–S413.
- 6 Chew FT, Zhang L, Ho TM, Lee BW: House dust mite fauna of tropical Singapore. *Clin Exp Allergy* 1999;29:201–206.
- 7 Puerta L, Fernandez-Caldas E, Mercado D, Lockey RF, Caraballo LR: Sequential determination of *Blomia tropicalis* allergens in mattress and floor dust samples in a tropical city. *J Allergy Clin Immunol* 1996;97:689–691.
- 8 Chew FT, Lim SH, Goh DYT, Lee BW: Sensitization to local dust-mite fauna in Singapore. *Allergy* 1999;54:1150–1159.
- 9 Kuo IC, Yi FC, Cheong N, Shek LPC, Chew FT, Lee BW, Chua KY: Sensitization to *Blomia tropicalis* and *Dermatophagoides pteronyssinus* – a comparative study between Singapore and Taiwan. *Asian Pac J Allergy Immunol* 1999;17: 179–188.
- 10 Bousquet J, Van Cauwenberge P, Khaltaev N: Allergic Rhinitis and its Impact on Asthma (ARIA). ARIA Workshop Report. *J Allergy Clin Immunol* 2001;108(5 suppl):S147–S334.
- 11 Lund VJ, et al: International Consensus Report on the Diagnosis and Management of Rhinitis. International Rhinitis Management Working Group. *Allergy* 1994;49(19 suppl):1–34.
- 12 Chapman MD, Heymann PW, Platts-Mills TA: Epitope mapping of two major inhalant allergens, Der p I and Der f I, from mites of the genus *Dermatophagoides*. *J Immunol* 1987; 139:1479–1484.

- 13 Chua KY, Doyle CR, Simpson RJ, Turner KJ, Stewart GA, Thomas WR: Isolation of cDNA coding for the major mite allergen Der p II by IgE plaque immunoassay. *Int Arch Allergy Immunol* 1990;91:118–123.
- 14 Chua KY, Huang CH, Shen HD, Thomas WR: Analysis of sequence polymorphism of a major mite allergen, Der p 2. *Clin Exp Allergy* 1996; 26:829–837.
- 15 Arruda LK, Vailes LD, Platts-Mills TA, Fernandez-Caldas E, Montealegre F, Lin KL, Chua KY, Rizzo MC, Naspitz CK, Chapman MD: Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blo t 5. *Am J Respir Crit Care Med* 1997; 155:343–350.
- 16 Goh LT, Kuo IC, Luo S, Chua KY, White M: Production and purification of recombinant *Blomia tropicalis* group 5 allergen from *Pichia pastoris* culture. *Biotechnol Lett* 2001;23:661–665.
- 17 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;17:189–194.
- 18 Lynch NR, Puccio FS, Di Prisco MC, Escudero JE, Nozzolino M, Hazel LA, Smith WA, Thomas WR: Association between allergic disease and reactivity to recombinant Der p 2 allergen of house dust mites in a tropical situation. *J Allergy Clin Immunol* 1998;101:562–564.
- 19 Hogan SP, Mould A, Kikutani H, Ramsay AJ, Foster PS: Aeroallergen-induced eosinophilic inflammation, lung damage, and airways hyper-sensitivity in mice can occur independently of IL-4 and allergen-specific immunoglobulins. *J Clin Invest* 1997;99:1329–1339.
- 20 Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG: Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989;320:271–277.
- 21 Droste JHJ, Kerkhof M, de Monchy JGR, Schouten JP, Rijcken B: Association of skin test reactivity, specific IgE, total IgE, and eosinophils with nasal symptoms in a community-based population study. The Dutch ECRHS Group. *J Allergy Clin Immunol* 1996;97:922–932.
- 22 Burrows B, Sears MR, Flannery EM, Herbison P, Holdaway MD: Relations of bronchial responsiveness to allergy skin test reactivity, lung function, respiratory symptoms, and diagnoses in thirteen-year-old New Zealand children. *J Allergy Clin Immunol* 1995;95:548–556.
- 23 Shibasaki M, Itoyama S, Takita H: Influence of age on IgE responsiveness to *Dermatophagoides farinae*: An immunoblot study. *Int Arch Allergy Immunol* 1994;103:53–58.
- 24 Boulet L-P, Turcotte H, Laprise C, Lavertu C, Bédard P-M, Lavoie A, Hebert J: Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clin Exp Allergy* 1997;27:52–59.
- 25 Gerritsen J, Koëter GH, De Monchy JGR, Knol K: Allergy in subjects with asthma from childhood to adulthood. *J Allergy Clin Immunol* 1990;85:116–125.
- 26 Leung R, Ho P, Lam CWK, Lai CKW: Sensitization to inhaled allergens as a risk factor for asthma and allergic diseases in Chinese population. *J Allergy Clin Immunol* 1997;99:594–599.
- 27 Zhang L, Chew FT, Soh SY, Yi FC, Law SY, Goh DYT, Lee BW: Prevalence and distribution of indoor allergens in Singapore. *Clin Exp Allergy* 1997;27:876–885.
- 28 Tsai JJ, Shen HD, Chua KY: Purification of Group 2 *Dermatophagoides pteronyssinus* allergen and prevalence of its specific IgE in asthmatics. *Int Arch Allergy Immunol* 2000;121: 205–210.
- 29 Lynch NR, Thomas WR, Garcia NM, Di Prisco MC, Puccio FA, Lopez RI, Hazell LA, Shen HD, Lin KL, Chua KY: Biological activity of recombinant Der p 2, Der p 5 and Der p 7 allergens of the house-dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Immunol* 1997; 114:59–67.
- 30 Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE: Antigenic and structural analysis of group II allergens Der f II and Der p II from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989;83: 1055–1067.
- 31 Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H: The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;29:896–904.