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Sensitization to *Blomia tropicalis* and *Dermatophagoides pteronyssinus*-A Comparative Study between Singapore and Taiwan

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SUMMARY ***Blomia tropicalis*** **(Bt) and** ***Dermatophagoides pteronyssinus*** **(Dp) are the predominant domestic mites species in Singapore and Taiwan. This study aims to characterize and compare the mite sensitization profiles in both countries. Skin prick tests were performed on 203 Singaporeans with Dp and Bt crude extracts.** ***In vitro*** **IgE and IgG4 reactivity to extracts and specific allergens (Der p 1, Der p 2 Der p 5 and Blo t 5) were determined by immunoassays. Approximately 91% of the tested Singaporeans were skin test positive for both Bt and Dp. Both populations share similar frequencies of** ***in vitro*** **IgE reactivity to all the allergens tested, but they differ in the pattern and magnitude of allergen sensitization. Although Der p 1, Der p 2 and Blo t 5 are major sensitizing allergens in both countries, Blo t 5 is a more potent one in Singapore, probably reflecting the high level of exposure to Bt. The unique major Bt and Dp allergens should be included for precise diagnosis and effective immuno-therapeutic treatment of mite allergy in both countries.**

It is well established that house dust mite allergy is associated with the increasing prevalence of allergic diseases.[1] The domestic mites of the families Pyroglyphidae and Glycyphagidae are the main sources of house dust allergens worldwide.[1-4] Epidemiological studies clearly indicated that *Dermatophagoides pteronyssinus* (Dp) and *Blomia tropicalis* (Bt) mites from the Pyroglyphidae and Glycyphagidae, respectively, are the most common mite species in the tropical and sub-tropical regions of the world.[2-4]

In Singapore, the general population has been sensitized by a number of domestic mite species. These include Pyoglyphidae species such as Dp and *Sturnophagoides brasiliensis*. Species from the family Glycyphagidae such as Bt and *Austroglycyphagus malaysiensis* are also present in high number in domestic dust samples collected from Singaporean homes.[5] Bt mite is the most prevalent mite in domestic dust along with Dp and sensitization to both mite species has been demonstrated.[6,7] Other species such as *Euroglyphus maynei* and *Lepidoglyphus destructor*, which are commonly found in some tropical and subtropical regions,[4,8] are not found in the domestic environment in Singapore.

Studies from Taiwan also revealed that both Dp and Bt species could be found in dust samples from the houses of allergic patients. IgE from allergic sera reacted with numerous proteins in the crude Dp and Bt extracts[9] indicating that these patients have been sensitized to both species. Further, IgE inhibition data indicate the presence of both cross reactive and unique allergens in both mite species.

Dp and Bt species are endemic in both Singapore and Taiwan, but Bt is the predominant species in Singapore and Dp pre-

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dominates in Taiwan. To date, sensitization to these mites has only been examined using crude mite extracts, and little is known about how the pattern of responsiveness to major allergens of these mites compare. Therefore, the main objective of this study is to compare and further characterize the reactivity profiles of mite sensitive subjects from these two countries to highly purified major mite allergens. For this purpose, sera obtained from allergic subjects from Taiwan and Singapore has been tested for IgE binding to purified and recombinant Dp and Bt allergens. Skin prick tests were also conducted for subjects from Singapore.

MATERIALS AND METHODS

Sera

Allergic sera were obtained from thirty asthmatic patients attending the Allergy Clinic of the National Taiwan University Hospital, Taiwan. All the patients were skin test positive for Dp extract, but sensitization to Bt extract was not assessed by skin tests. However, subsequent screening by IgE immunodot assay revealed that all the Taiwanese sera showed positive IgE reactivity to both Dp and Bt extracts. Some of these patients (aged between 10-14 years) underwent immunotherapy using Dp extract. Another thirty sera were obtained from Singaporean subjects that were skin prick test positive for Bt and Dp crude extracts. Fourteen and eleven allergic sera from Singapore and Taiwan, respectively, were selected for further analysis for serum IgE and IgG4 against a panel of purified Der p 1, Der p 2, Der p 5 and Blo t 5 by ELISA. All the sera donors, except those underwent immunotherapy, were adults with approximate age ranging from 20-40 years old.

Mites source

Lyophilized Dp mites were purchased from Commonwealth Serum Laboratory (CSL, Melbourne, Australia). Bt mites were grown in our laboratory and the starter cultures were prepared by collecting mites from house dust samples in Singapore.[10]

Preparation of mite crude extracts

One gram of frozen or lyophilized mites was homogenized using pestle and mortar in the presence of liquid nitrogen. Twenty milliliters of phosphate-buffered saline (PBS) containing 2 mM phenylmethyl-sulfonyl fluoride and 1 mM EDTA were used for protein extraction at 4°C overnight. After centrifugation at 15,000 x *g* for 15 minutes, the supernatant of the extract was dialyzed overnight at 4°C against PBS. The protein concentration of the mite extracts was determined by Bio-Rad protein assay (Bio-Rad Laboratories, CA). The extracts were stored at -80°C.

Preparation of Dp and Bt allergens

Native Der p 1 was purified from spent mite medium by using the monoclonal antibody 4C1 affinity chromatography.[11] Recombinant Der p 2, Der p 5, and Blo t 5 were produced in the *Pichia pastoris* yeast expression system and purified using chromatographic methods (manuscripts in preparation). For *in vitro* assays, recombinant Der p 5 and Blo t 5 were also prepared in the pGEX *E. coli* expression system[12,13] following thrombin cleavage and removal of the GST by glutathione agarose or Superdex-75 gel filtration.

Human IgE immunodot analysis

The dot-blot immunoassay for IgE was performed by a protocol previously described.[14] Two micrograms of each purified protein were applied onto nitrocellulose membrane in duplicate. The dot-blots were blocked with 5% skim milk in PBS containing 0.05% Tween-20 (PBS/T). Allergic sera were diluted 1:1 in PBS and incubated with the dot-blots at 4°C for 12-16 hours. The incubation with the secondary antibodies was performed with 1:1,000 dilution of biotinylated mouse anti-human IgE (PharMingen) for 1 hour, followed by another hour incubation with ExtrAvidin peroxidase (Sigma). The signal of the reaction was developed using enhanced chemiluminescent detection reagent (Amersham) and autoradiography. Extensive washing of the dot-blots was performed with PBS/T following each treatment step throughout the immunoassay protocol.

Detection of house dust mite allergens-specific IgE and IgG4 by ELISA

The sera of selected subjects were further analysed for Der p 1, Der p 2, Der p 5, and Blo t 5-specific IgE and IgG4. The volume of each step in the ELISA assay was 50 µl/well unless specified. The ELISA plates were coated with individual HDM allergens (250 ng/well in 0.1 M sodium bicarbonate, pH 8.2) and blocked with 100 µl 10% fetal calf serum in PBS/T. The sera were diluted 1:5 and 1:30 in blocking solution for allergen-specific IgE and 1:5 for allergen-specific IgG4 detection. The plates were incu-

bated with diluted sera at 4°C for 16 hours. The specific IgE was detected by 1 μg/ml biotinylated mouse anti-human IgE (Phar-Mingen) followed by 1:2,000 diluted ExtrAvidin-alkaline phosphatase (Sigma) each for 1 hour. Signal was developed by addition of p-nitrophenylphosphate (Sigma). The specific IgG4 was detected by 1:1,000 diluted monoclonal anti-human IgG4 peroxidase conjugate (Janssen Biochimica) and developed by 100 μl 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid). Extensive washing with PBS/T was performed following each incubation step. One positive control serum was included in each plate to normalize the OD_{405} nm readings.

Skin prick tests

Skin prick test was performed according to the protocol previously described.[7] A drop of allergen was applied on the volar of the forearm and the skin was pricked with a disposable lancet. A reaction of greater than 3 x 3 mm wheal diameter after subtracting the negative control 30 minutes after the prick was regarded as a positive prick test. Glycerol-buffer (50% glycerol) and histamine (1 mg/ml) were included as negative and positive controls, respectively. All the purified mite allergens were used at 25 μg/ml and the recombinant mite allergens used in this study were produced in *Pichia pastoris* yeast.

RESULTS

Prevalence and sensitization profiles of Dp and Bt mites

The prevalence of mite species in domestic environment in tropical Singapore and Colombia and subtropical Taiwan has been reported.[5,9,15] Table 1a summarizes the number of Bt and Dp mites isolated from different sampling sites in Singaporean homes and the

Table 1 (a) Comparison of Bt and Dp mite counts* among Singapore, Colombia and Taiwan

Countries	Singapore[a] (median), n = 50		Colombia (Cartagena)[b] (geometric mean), n = 25		Taiwan (Taipei)[c] (mean ± SE), n = 13	
Species niches	Bt	Dp	Bt	Dp	Bt	Dp
Mattress	7,250	1,150	146.8	137	327 ± 32	357 ± 30
Carpet	8,250	2,150	N.D.	N.D.	108 ± 32	115 ± 32
Floor	1,100	900	148.3	72.3	N.D.	N.D.

*Mite counts were expressed as number of mites per gram of dust
a: data obtained from reference 5
b: data obtained from reference 15
c: data obtained from reference 9, data for mid-summer (August) were used in the table
N.D. = not done

(b) Summary of skin test reactivity to Dp and Bt crude mite extracts

Skin test reactivity to mite species	Subjects	
	Singaporean[a] n = 203	Taiwanese[b] n = 60
Positive to either Dp, Bt or both	203 (100)	59 (98.3)
Positive to Dp	198 (97.5)	53 (88.3)
Positive to Bt	189 (93.1)	44 (73.3)
Positive to Bt alone	5 (2.5)	4 (6.7)
Positive to Dp alone	14 (6.9)	3 (5)
Positive to both Dp and Bt	184 (90.6)	52 (86.7)
Negative to Dp and Bt but positive to Df	N.D.	1 (1.7)

Figures in parentheses represent percentage
a: data from this study
b: data interpreted from reference 9
N.D. = not done

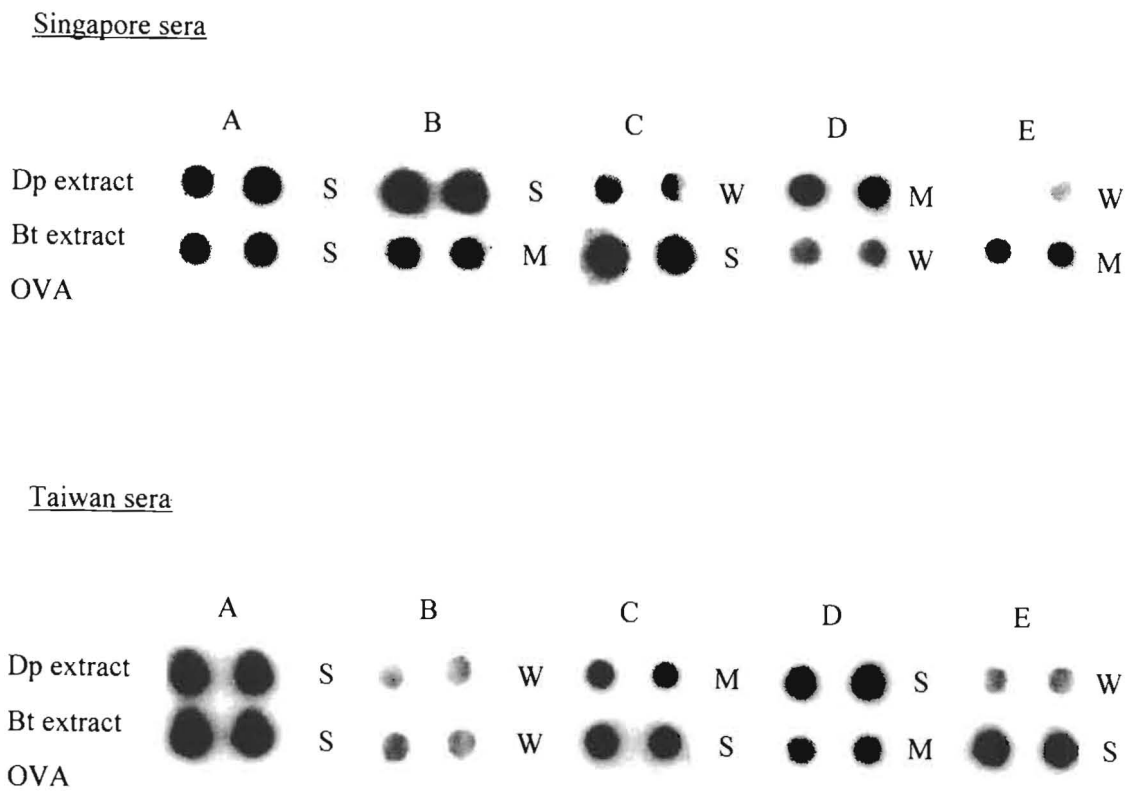

Fig. 1 Serum IgE reactivity of Singaporean and Taiwanese subjects to Dp and Bt crude extracts determined by immunodot assay. Five μg each of the Dp and Bt extracts and 2 μg of ovalbumin (OVA) control antigen were dotted on nitrocellulose membranes. The negative control serum was from a non-atopic individual. S, M, W represents strong, medium, weak reactivity, respectively.

results reported from Taiwan and Colombia were included for comparison. The number of mites found in the domestic environment was extremely high in Singapore, ranging from 7.4 to 76 times the numbers found in Colombia or Taiwan. The sensitization profiles to these two mite species in Singapore were studied by skin prick tests and the results were compared to previously published data from similar studies performed in Taiwan (Table 1b). The data show that 90.6% of the Singapore subjects and 86.7% of the Taiwanese subjects exhibit dual sensitization to Dp and Bt mite allergens. Exclusive sensitization to either Bt or Dp mites was rare in both populations.

Table 2 Summary of IgE reactivity pattern for the mites sensitized population in Singapore and Taiwan

(a) IgE reactivity pattern for either Bt or Dp species

	Singaporean	Taiwanese
Bt strong	13	12
Bt moderate	17	9
Bt weak	1	10
Dp strong	8	11
Dp moderate	11	7
Dp weak	12	13

(b) IgE reactivity pattern for both Bt and Dp species

	Singaporean n = 31(%)	Taiwanese n = 31 (%)
Strong to both	6 (19.4)	7 (22.6)
Strong to Bt and weak to Dp	2 (6.5)	2 (6.5)
Strong to Bt and moderate to Dp	5 (16.0)	3 (9.7)
Moderate to both	6 (19.4)	3 (9.7)
Moderate to Bt and weak to Dp	9 (29.0)	2 (6.5)
Moderate to Bt and strong to Dp	2 (6.5)	4 (12.9)
Weak to both	1 (3.2)	9 (29.0)
Weak to Bt and moderate to Dp	0 (0)	1 (3.2)
Weak to Bt and strong to Dp	0 (0)	0 (0)

In vitro IgE reactivity to Dp and Bt mite extracts

The sensitization profiles to Bt and Dp mites were further analyzed by IgE immunodot assay with 31 allergic sera each from Singapore and Taiwan. Fig. 1 shows the IgE reactivity profiles of a selected panel of allergic sera. The pattern of IgE reactivity to both mite extracts are shown in Table 2. Ninety-seven percent of the Singapore sera (30/31) show moderate to strong IgE reactivity to Bt extract, whereas 61% (19/31) of these sera show moderate to strong IgE reactivity to Dp extract. Unlike the Singapore sera, a significant number (9/31) of Taiwanese sera reacted weakly to Bt extract, 21/31 and 17/31 of them show moderate to strong IgE reactivity to Bt and Dp extracts, respectively.

In vitro reactivity of IgE and IgG4 to specific mite allegens

Purified Der p 1, Der p 2, Der p 5 and Blo t 5 were used to perform the ELISA tests with 11 and 14 mite allergic sera from Taiwan and Singapore, respectively. Two non-atopic sera were included as controls. The IgE reactivity of the selected sera to Dp and Bt extracts ranged from weak to strong. All the tested allergic sera showed IgE reactivity to at least one of four tested allergens. The sera were diluted 1/5 and 1/30 for IgE assay, in most cases IgE was still detectable when the sera were diluted 1/30 indicating that the allergen-specific IgE titers were high in these sera. Fig. 2 shows the IgE reactivity profiles for sera diluted 1/5. Intriguingly, a number of Taiwanese sera have significant levels of Dp allergen-specific IgG4. This group of sera was from asthmatic patients, who underwent immunotherapy with Dp crude extracts (Fig. 3).

DISCUSSION

The domestic mite fauna of Singapore consists predominantly of the Pyroglyphid mite *Dermatophagoides pteronyssinus* and the Glycyphagidae mite *Blomia tropicalis*. Both mite species exist in very high number as compared to that found in Cartagena, Colombia and in Taipei, Taiwan (Table 1a). The highest number of Bt and Dp mites in Taipei was found during the summer months (July-August). The number dropped significantly during the winter months.[9] Like Singapore, Cartagena is warm and humid throughout the year, and there is little seasonal variation in

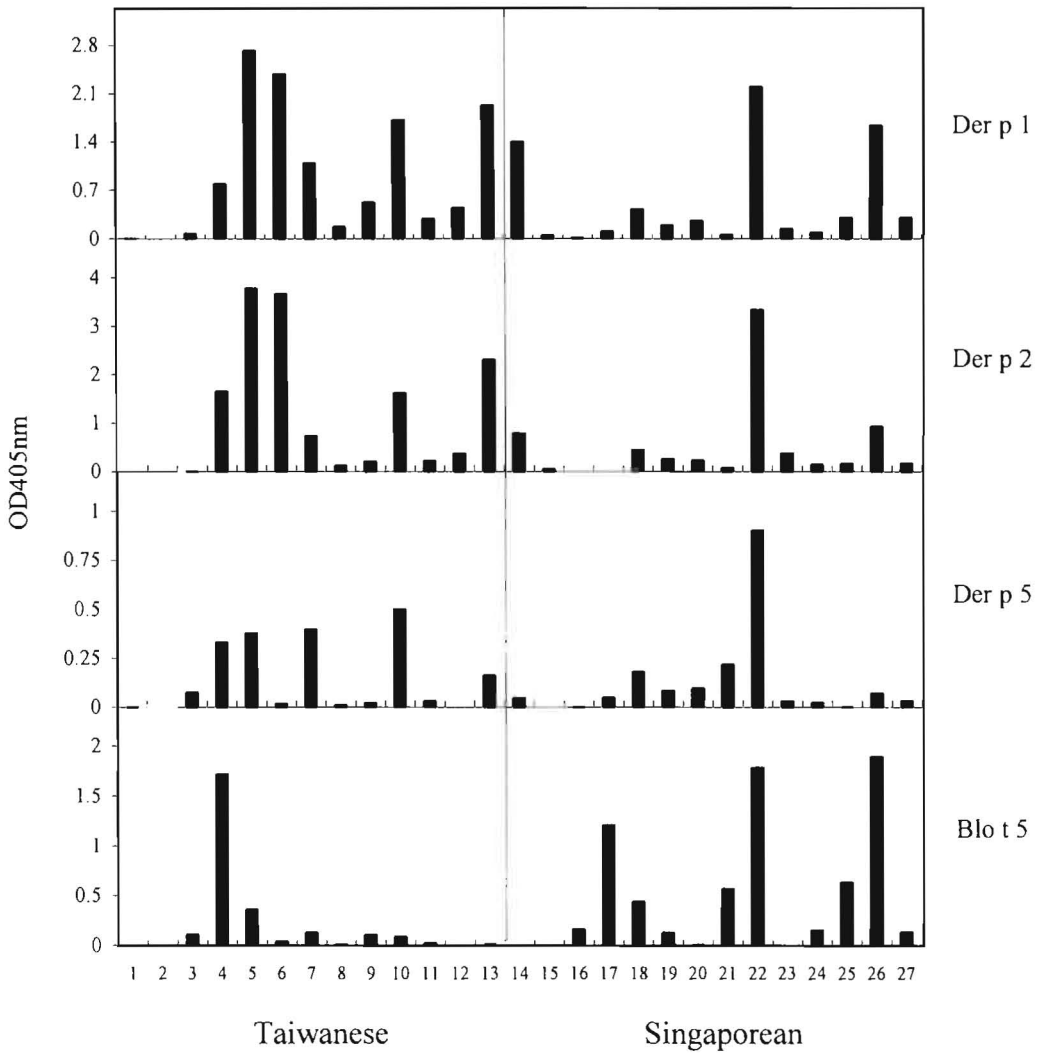

Fig. 2 Serum IgE reactivity to major mite allergens determined by ELISA. Purified native Der p 1 and recombinant Der p 2, Der p 5, and t 5 were used as the coating allergens. Sera 1 and 2 were from non-atopic controls, the rest were sera from mite allergic subjects in Singapore and Taiwan.

mite numbers.[15] However, the number of Bt and Dp mites found in the domestic dust samples was much lower in Cartagena than in Singapore, the number of both mites reported was very similar to that found in Taipei during the summer months (Table 1a). Despite the climatic similarity between Singapore and Cartagena, there is a significant difference in mite number between these two cities. Incidentally, very high Pyroglyphid mite counts in the domestic dust samples have also been reported in Bangalore City of India.[16] The number of Pyroglyphids per gram of dust ranged from 19-7,103, with an average number of 768. The authors claimed that there was no direct correlation between mite number and the changes in relative humidity. Extremely high Pyroglyphid mite numbers also reported in Malaysia.[17] Taken together, these data suggest that humidity may not be the only critical determining factor for excess mite growth in the tropics.

Despite the significant difference in Dp mite counts between Singapore and Cartagena, the

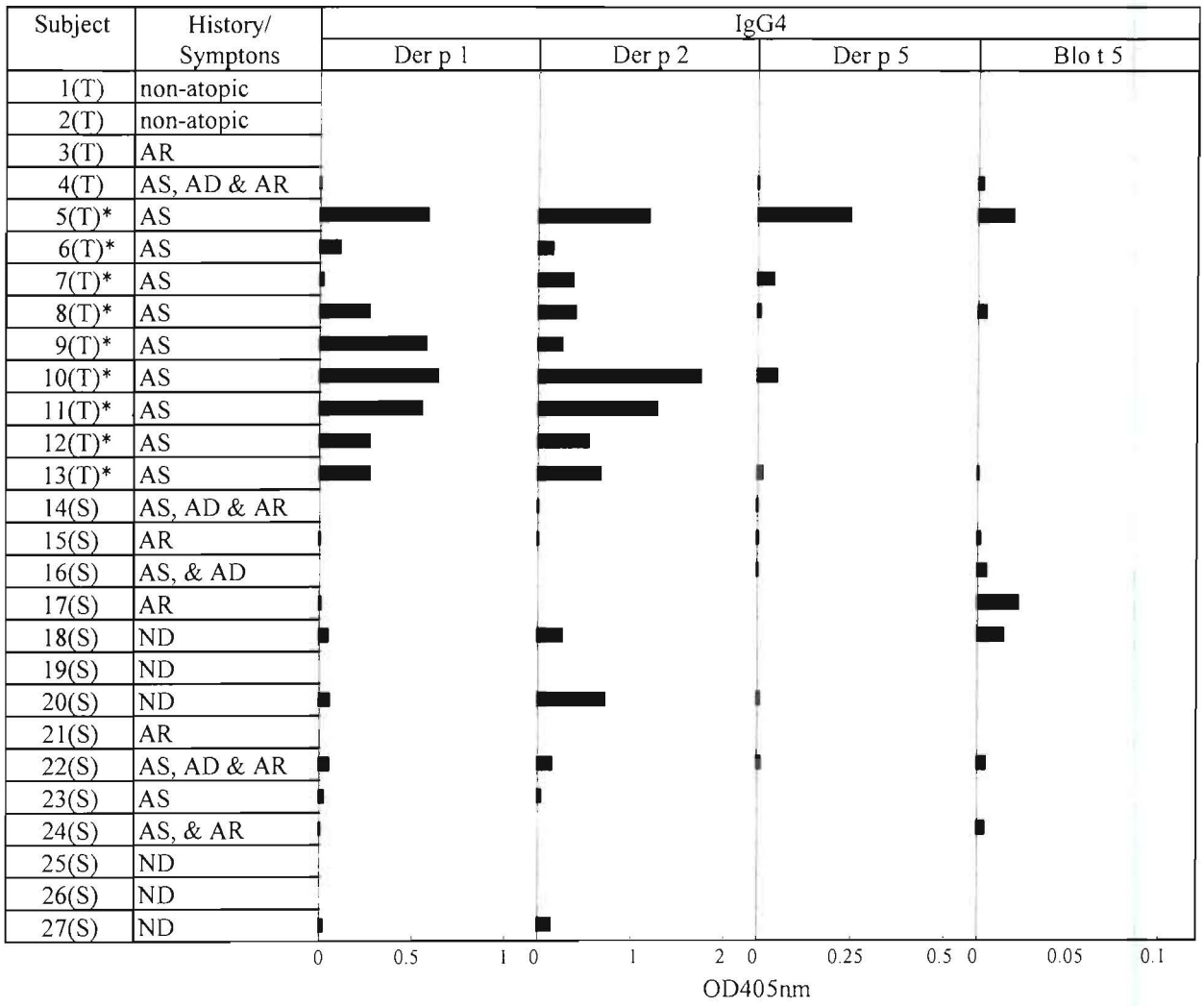

Fig. 3 Serum IgG4 reactivity to native Der p 1, recombinant Der p 2, Der p 5, and Blo t 5 allergens determined by ELISA. (S) and (T) represent Singapore and Taiwan, respectively. Patients that underwent immunotherapy with Dp extract were marked with astericks (*). AS= allergic asthma, AD= atopic dermatitis, AR= allergic rhinitis, ND= not determined.

amount of Der p 1 allergen reported to be present in the domestic environment for these two cities is comparable.[6,18] Further, a study in Taipei indicated that the amount of Der p 1 present in domestic dust is significantly higher (GM range for mattress, 1-10 μg/g of dust;[19] than that reported for Singapore[6] (geometric mean [GM] range for mattress 1-2 μg/g of dust), although the number of Dp mites in Singapore was several fold higher than that found in Taipei.[5,9] The precise explanation for the lack of correlation between mite counts and allergen level present in the domestic environment is unclear at this stage. It has been reported by Bischoff *et al.*[20] that the number of dead mites in various sampling sites of domestic homes were higher than those of live mites, in some cases there were 10-40 folds more dead mites, and the dead mites contributed significantly to the amount of environmental mite allergens. Therefore, one possible explanation for the poor correlation is that some published epidermiological data failed to reflect the total number of mites (live and dead) in the sampling sites. It is important to improve and standardize the methodology use for future epidermiological studies of environmental mite fauna. In

addition, methods and reagents use for the allergen extraction from the dust samples, ELISA method and reagents for allergen quantification and the storage and processing time of the dust samples after collection, require world wide standardization so that meaningful comparison of data from different laboratories can be performed.

The sensitization pattern of mite sensitized population in Singapore was assessed by skin prick tests using crude extracts prepared from Dp and Bt mites. As shown in Table 1b, greater than 90% of the tested subjects were skin test positive to Dp and Bt extracts. Although Bt mites were present in significantly higher number than Dp mites in Singaporean homes (Table 1a), there was no significant difference in the percentage of Bt positive subjects and Dp positive subjects in this test (97.5 *vs.* 93.5). As compared to the Singapore study, the skin prick test results from the Taiwanese study show lower percentages of positive skin prick tests reactivity to both mite species, 88.3 % for Dp and 73.3% for Bt. The pattern of sensitization was further analyzed by *in vitro* IgE immunoassay using mite allergic sera from Singapore and Taiwan. As indicated by the IgE immunodot assay results, both Singaporean and Taiwanese sera showed equal frequency of IgE reactivity to both mite extracts. However, a higher percentage of Taiwanese sera showed weaker IgE reactivity to Bt extracts, whereas all except one of the Singaporean sera showed moderate to strong IgE reactivity to Bt extract (Table 2). Eleven out 31 of the Singaporean sera reacted weakly to Dp extract, but these sera showed relatively strong IgE binding to Bt extract. The data clearly indicated that Bt-specific IgE titers are generally higher than those of the Taiwanese subjects. Both the skin prick tests and the *in vitro* IgE binding studies clearly indicate that dual sensitization to both species of mites occurs in high frequency in both countries. This is in accordance with the prevalence of the mite fauna found in the living environments of these countries.

Since there are some differences in the pattern of sensitization to both mite species, *in vitro* IgE reactivity of the sera from both countries to specific Dp and Bt allergens was analyzed. As shown in Fig. 2, the titers of IgE specific for Der p 1, Der p 2, and Der p 5 were generally higher in the Taiwanese sera. It is important to note that a number of the Taiwanese sera were from asthmatic patients that underwent immunotherapy. Such treatment may have boosted the titers of the allergen-specific serum IgE. The Blo t 5-specific IgE titer is much higher among the Singaporean sera and our previously published skin prick tests results also reveal that 76% of the Bt sensitized subjects are sensitized to Blo t 5.[21] These data clearly suggest that Blo t 5 is a major allergen in Singapore. Skin test results reported recently revealed that out of the Dp sensitized subjects, 74% 58% and 34% are positive for Der p 1, Der p 2, and Der p 5, respectively.[21] The present study also shows that Singapore allergic sera have high frequency of IgE reactivity to Der p 1 and Der p 2, whereas a lower frequency of reactivity to Der p 5. In addition to Blo t 5, our data also revealed that Der p 1 and Der p 2 are important major allergens in Singapore. A recent study from Colombia indicated that the *in vitro* IgE reactivity to Der p 1 and Der p 2 was 70% and 75%, respectively. The frequency of IgE reactivity to full length Blo t 5 was not assessed in the study.[22]

Analysis of IgG4 profiles indicated that the sera from the Taiwanese patients that underwent desensitization produced significant levels of allergen-specific IgG4 (Fig. 3). These patients had received immunotherapy that invoved multiple injections of Dp crude extract and the IgG4 detected was mainly Der p 1 and Der p 2-specific. All of these sera also had high specific IgE to both major Dp allergens. Allergen-specific IgG4 was also detected in a small number of atopic subjects that did not undergo allergen immunotherapy, extremely high titer of serum specific IgE was also detected in these subjects. Therefore, it is likely that the IgG4 production in the Taiwanese sera is induced by the desensitization procedure, and high IgE and IgG4 appear to coexist in these individuals. It has been reported that IgE and IgG4 are co-regulated in some pathological conditions such as allergic reactions,[23] IgA deficiency condition[24] and hyper IgE syndrome.[25] The precise mechanism involved in the co-regulation of both isotypes is unclear. It has been suggested that IgG4 antibodies do not fix complement, therefore the IgG4 may compete for allergens with complement-fixing IgG1 and suppress late reaction.[26] In the case of bee venom immunotherapy, the immunoprotection correlates with preferential expression of distinct IgG specificities, which appear equally distributed over the IgG1 and IgG4 subclasses.[27] A recent report of the results of allergen immunotherapy of pollen-allergic patients indicated that there were significant correlations between ratio of IgG4 to IgG1 with the symptom scores.[28] A recent report

on the long term study on the immunotherapy with crude mite extract showed that there were no clear cut correlations between any specific IgG subclass and clinical outcome.[29] Taken together, the clinical significance of serum specific IgG4 in patients undergoing allergen immunotherapy remains controversial. In the case of mite immunotherapy, it will be more informative to perform hypo-sensitization with purified, well-defined recombinant mite allergens and these purified specific allergens should be used for monitoring the treatment.

In summary, our data have further confirmed the dual sensitization to Dp and Bt mites in the Singapore population. There is no significant difference in the frequency of sensitization to both mites in Singaporean and Taiwanese allergic populations, but they differ in the pattern and magnitude of sensitization. The difference may due to the fact that there is a seasonal variation in mite numbers in Taiwan and the number of mites found is generally much lower than that of Singapore. Der p 1 and Der p 2 and Blo t 5 are important major allergens in both countries, but Blo t 5 appears to be a more potent allergen in Singapore as the magnitude of sensitization for Blo t 5 in Singapore population is higher than that for Der p 1 and Der p 2. The observed higher reactivity to *Blomia tropicalis* in Singapore may reflect higher level of exposure.

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