

ORIGINAL PAPER

Characterization of glutathione S-transferase from dust mite, Der p 8 and its immunoglobulin E cross-reactivity with cockroach glutathione S-transferase

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Clinical and Experimental Allergy

Summary

Background Sensitization to mite and cockroach allergens is common, and diagnosis and therapy of allergy can be further complicated by the presence of allergen isoforms and panallergens. Purified recombinant and native allergens are useful for studies to resolve such problems.

Objective To assess the allergenicity of native and recombinant mite glutathione S-transferase (GST) (Der p 8) and study the IgE cross-reactivity between Der p 8 and cockroach GST.

Methods Der p 8 cDNA encoding a new isoform was isolated and expressed in yeast. Native Der p 8 was affinity purified from mite extract. IgE reactivity to native and recombinant Der p 8 was assessed by ELISA using sera from allergic subjects from Taiwan, Singapore and Malaysia. IgE cross-reactivity between Der p 8 and cockroach GST was examined by IgE inhibition assays.

Results Our Der p 8 cDNA encoded a basic isoform ($pI = 8.5$) containing six polymorphic residues located at positions 46, 106, 149, 160, 167 and 184. At least 8 isoforms of native Der p 8 were detected by two-dimensional gel and immunoblot analyses. Sera from Taiwanese asthmatics showed 96% and 84% IgE reactivity to native Der p 8 and recombinant Der p 8, respectively. Native Der p 8 showed 75% and 65% IgE reactivity with sera from Malaysia and Singapore, respectively.

Conclusions A high frequency of sensitization to mite GST among allergic subjects was observed but the titres of IgE reactivity were low. The IgE cross-reactivity between mite and cockroach GST suggests that GST is a panallergen.

Keywords *Dermatophagoides pteronyssinus* (dust mites), glutathione S-transferase, IgE cross-reactivity, *Periplaneta Americana* (cockroach)

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Introduction

Exposure to indoor allergens such as dust mites, cockroaches and animal dander is a risk factor for allergen sensitization and the development of allergic disease [1–3]. In subtropical and tropical countries, *Dermatophagoides pteronyssinus* mites have been recognized as a dominant species of clinical importance, and sensitization to *D. pteronyssinus* mite allergens could be as high as 80–90% among asthmatic and allergic patients [4, 5]. As the first cloning of the cDNA of major house dust mite allergen Der p 1 from *D. pteronyssinus* [6], 19 groups of dust mite allergen have been identified [7]. Sequence polymorphisms and isoforms have been reported [8–12].

The unique roles for the polymorphisms in T and B cell epitope binding have also been reported [12, 13].

Another risk factor associated with asthma and allergy in these regions is cockroach allergens [14]. The most commonly found domiciliary cockroach species are *Blattella germanica* (German cockroach) and *Periplaneta americana* (American cockroach), with a predominance of the latter in the subtropics such as Taiwan, tropical countries such as Brazil, Singapore and Malaysia [5, 15–17]. Several allergens from both species have been cloned and IgE cross-reactivity has been described. For example, the group 1 cockroach allergens, Bla g 1 and Per a 1, are the major cross-reactive cockroach allergens reported [18, 19], and tropomyosin from American, Per a

7, and German cockroach, Bla g 7, are also allergenically cross-reactive [20, 21].

The glutathione S-transferase (GST) from German cockroach, Bla g 5, is also a major allergen [22], but the allergenicity and IgE cross-reactivity of the GST homologues in American cockroach have not been reported. The group 8 allergen of Der p mites was shown to be a homologue of GST and reacts with IgE in 40% of sera from mite-allergic patients [23]. It has 27% sequence identity with Bla g 5 and 33% sequence identity with another IgE-eliciting GST from *Schistosoma japonicum* (Sj26). The IgE cross-reactivity among these GST allergens had been suggested but the question has not been addressed experimentally.

This study evaluated IgE binding to Der p 8 in three populations of Der p-sensitized subjects, and examined the IgE cross-reactivity between GST from the two most important sources of indoor allergens in these populations, namely Der p mites and American cockroach.

Methods

Cloning and expression of Der p 8

The λ phage DNA was purified from the *D. pteronyssinus* λ gt11 cDNA library [6] using the Wizard[®] Lambda Preps DNA purification system (Promega, Madison, WI, USA). To obtain the complete coding region of the Der p 8 gene, polymerase chain reaction (PCR) was performed using *pfu* DNA polymerase. According to the published Der p 8 cDNA [23], primers were designed as 5'-GCGGATCCATGAGCCAACCAATCCTA-3' (anti-sense) and 5'-GCGGATCCTCAGGCATATGAAGCATT-3' (sense). The cDNA was cloned into the pGEX-2T vector and nucleotide sequencing was analyzed using the ABI 377 DNA Sequencer (PE Applied Biosystems, Foster City, CA). To express the recombinant Der p 8 in *Pichia pastoris*, the full-length Der p 8 cDNA was amplified from pGEX-2T-Der p 8. The primers used were 5'-GCCTCGAGAAAAGAATGAGCAACCAATCCTA-3' (anti-sense) and 5'-GCGAATTCTCAGGCATATGAAGCATT-3' (sense). The cDNA was cloned into the pPIC9 vector (Invitrogen Corp, San Diego, CA). The pPIC9-Der p 8 construct was linearized with *Bgl* II and transformed into the *P. pastoris* strain GS115 by the lithium chloride transformation method as described in the manual of the *Pichia* expression kit (version A, Invitrogen Corp). For large-scale protein expression, a single colony from the positive clone was picked and inoculated into 4 mL of buffered glycerol-complex medium and grew at 30 °C. Twenty-four hours later, the small culture was further diluted into two litres and cultured until OD_{600nm} = 6.0 at 30 °C with vigorous shaking (250 r.p.m). After that, cells were centrifuged and transferred to 400 mL of buffered methanol-complex medium for methanol-induced expression. After 48 h, the supernatant was harvested.

Purification of native Der p 8 and native Der p 2

Dry *Dermatophagoides pteronyssinus* mites (Commonwealth Serum Laboratories, Parkville, Australia) were grounded by mortar and pestle in 10 mM Tris, 300 mM NaCl buffer in the presence of 1 mM phenyl-methyl sulfoxide (PMSF) for 30 min on ice and further stirred overnight at 4 °C. The crude mite extract was obtained after spinning at 14 000 g for 20 min. Native Der p 8 was affinity purified by glutathione agarose beads (Sigma Aldrich, St Louis, MO, USA). Native Der p 2 was also purified by affinity chromatography. Anti-Der p 2 monoclonal antibody C2 was coupled to cynogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's instruction. The crude mite extract was applied to affinity chromatography column and the unbound materials were washed with Tris buffered saline (TBS). The column was eluted with 0.1 M glycine, 0.15 M NaCl, pH 2.6 and neutralized with 1 M Tris-Cl, pH 8.8. The purified native Der p 2 was transformed to PBS immediately after elution by dialysis and stored in -20 °C.

Cockroach allergens

The crude extract of *P. americana* that was used for ELISA and absorption studies was a gift kindly provided by Dr Wu CH from Department of Medical Research, Taichung Veterans General Hospital, Taichung, Taiwan. Cockroach GST of *P. americana* was affinity purified from 20 mL of whole body extract (1 : 20 w/v, Greer laboratories, Lenoir, NC, USA) using glutathione agarose beads. To prevent the interference of 50% glycerine in the commercial extract, the extract was diluted 20 times in TBS before loading into the column. The unbound materials were washed with TBS and the column was eluted with 10 mM of reduced glutathione (Sigma Aldrich, St. Louis, MO).

Sera

Sera for testing IgE reactivity to Der p 8 were obtained from: (1) 55 asthmatic Taiwanese children (aged 7–15 years) who had undergone immunotherapy with Der p extract in the Allergy Clinic of the Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan, (2) 20 *D. pteronyssinus* mite-allergic Singaporeans (aged 20–50 years) and (3) 17 patients with allergic asthma (aged 1–44 years) visiting the Asthma Clinic at the University Hospital, University of Malaysia. All the subjects showed positive skin tests to Der p crude mite extract. For the negative control, sera from eight cord blood samples and two adults showing no IgE reactivity to Der p crude mite extract were included.

Two-dimensional electrophoresis and Western blotting

Isoelectric focusing was performed using a 7 cm long immobilized DryStrip[™] with linear pH ranging from 3

to 10 on an IPGphorTM isoelectric focusing system (Amersham Biosciences Corp. Piscataway, NJ) according to the manufacturer's instructions. Briefly, 2 µg of native Der p 8 was added to 125 µL of the rehydration solution (8 M urea, 2% CHAPS, 20 mM 1, 4-dithio-DL-threitol) and loaded into the strip holder, followed by placing the IPG strip. Gel rehydration was performed at 30 V for 18 h. Isoelectric focusing was performed at 50 mA/strip, 20 °C as follows: step 1 : 500 V for 250 V/hr, step 2 : 1000 V for 500 V/hr and step 3 : 8000 volt for 20 000 V hr. After equilibration, the second dimension electrophoresis was performed using 12.5% SDS-PAGE. For Western blotting, proteins were transferred onto a Hybond-C nitrocellulose membrane (Amersham Biosciences Corp.). The isoforms of Der p 8 were identified by polyclonal anti-Der p 8 mouse sera (generated by injecting recombinant Der p 8 into Balb/c mice) followed by biotinylated anti-mouse immunoglobulin (Sigma Aldrich) and ExtrAvidin-Peroxidase[®] (Sigma Aldrich). The result was developed using the SuperSignal chemiluminescence substrate (Pierce, Rockford, IL, USA). Der p 8 proteins were further characterized by MALDI-TOF mass spectrometry that was performed as described previously [24]. Peptide mapping was performed by comparison with the amino acid sequence deduced from the Der p 8 cDNA.

Determination of antigen-specific immunoglobulin E by enzyme-linked immunosorbent assay

ELISA was performed as described previously [25], except that the wells were coated with 50 µL of rDer p 8 (5 µg/mL), nDer p 8 (2 µg/mL), nDer p 2 (2 µg/mL), cockroach extract (50 µg/mL) or anti-human IgE (2 µg/mL) (Pharmingen, San Diego, CA, USA) diluted in coating buffer (0.1 M NaHCO₃, pH 8.2). For the semi-quantification purpose, partial purified human myeloma IgE (Chemicon, Temecula, CA, USA) in two times serial dilution were added to the anti-human IgE-coated wells of each plate. Readings of absorbance were referenced to these standards to obtain the relative Der p 8-specific IgE titre, unless otherwise specified.

Inhibition study

Inhibition study was performed using ELISA. Diluted sera were incubated with 100 µg/mL, 1 µg/mL or 0 µg/mL of cockroach extract or 1 µg/mL of GST of *P. americana* for 12–16 h before adding to the nDer p8-coated plates. ELISA was performed as described above. The percentage of inhibition was calculated as $(1 - OD_{405\text{ nm}} \text{ with inhibitor} / OD_{405\text{ nm}} \text{ without inhibitor}) \times 100$.

Statistic analysis

Student's *t*-test was used to compare the IgE titre between native and recombinant Der p 8. Pearson's correlation was used to analyse the IgE correlation.

Results

Sequence analysis of complementary DNA clone coding for Der p 8

An isoform of Der p 8 cDNA was identified from the *D. pteronyssinus* cDNA lambda gt11 expression library by PCR amplification. When we compared the nucleotide sequence with the previously published clone [23], 11 nucleotide differences were found. These nucleotide changes resulted in six polymorphic amino-acid residues located at positions 46(E → Q), 106(E → K), 149(I → V), 160(N → Y), 167(H → R) and 184 (R → K). The six polymorphic residues contributed to the change of the theoretical isoelectric point from 6.3 to 8.5. The molecular weight calculated was 25 704.69 Da.

SDS-PAGE analysis of native and recombinant Der p 8

Recombinant Der p 8 was produced in *P. pastoris*. In this yeast expression system, 24 h after the methanol induction, significant amounts of Der p 8 protein could be detected in the culture supernatant, and a continued increase in protein production was observed for up to 72 h (data not shown). The yeast-expressed recombinant Der p 8 (rDer p 8) showed three bands when the protein was analysed by SDS-PAGE (Fig. 1a, as indicated by arrow). These three bands were further analysed by N-terminal amino-acid sequencing, and the data demonstrated that they had identical sequences starting from the first amino acid methylene of the deduced sequence from cDNA. Native Der p 8 (nDer p 8) was obtained from the crude mite extract by affinity purification using glutathione agarose beads. The yield of native Der p 8 was 0.1 milligram per gram of dry mites. The nDer p 8 showed a single band on SDS-PAGE, which a the mobility similar to the lower band of the rDer p 8 (Fig. 1a).

Presence of isoforms in native Der p 8

A number of GST isoenzymes from the crude mite extract had been isolated by glutathione affinity purification. To determine whether all mite GSTs were related to Der p 8 and to evaluate the number of isoforms existing, two-dimensional electrophoresis was performed. The results showed that there were at least eight isoforms in the native protein that could be recognized by anti-Der p 8 mouse sera that had been generated by immunizing mice with the recombinant Der p 8 (Fig. 1b). The *pI* of these Der p 8 isoforms ranged from 5 to 10. There was an isoform at the isoelectric point similar to that of the recombinant Der p 8 described in this paper (as indicated by the arrow). The native protein was further analysed by MALDI-TOF MS. Four tryptic peptides from our native protein were found to match with the amino acid sequences deduced from the

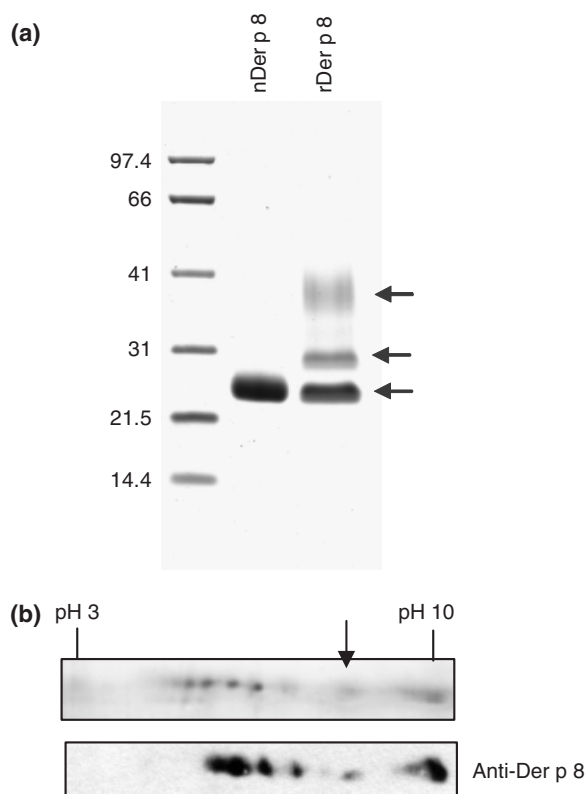


Fig. 1. SDS-PAGE analysis of recombinant and native Der p 8. (a) The nDer p 8 migrated as a single band, and the rDer p 8 showed three bands as indicated by arrows. (b) nDer p 8 showed multiple dots on two-dimensional electrophoresis (upper panel). The parallel gel was transferred onto a nitrocellulose membrane and blotted with anti-serum raised with rDer p 8 (lower panel).

cDNA clone. A total of 44 amino acids were identified from the four peptides with sequences from 34–44, 71–83, 170–183 to 189–194 (data not shown). The results confirmed that the native GST proteins purified from crude mite extract by glutathione affinity purification were indeed Der p 8 allergens.

Comparison of immunoglobulin E reactivity with recombinant Der p 8 and native Der p 8

The IgE reactivity to the recombinant and native Der p 8 was determined by ELISA. Among the 55 Taiwanese sera tested, IgE reactivity to nDer p 8 and rDer p 8 was 96% and 84%, respectively. The level of nDer p 8-specific IgE (mean: 615.37) was significantly higher than that of rDer p 8-specific IgE (mean: 95.61; $p < 0.05$). There was a poor correlation between the level of nDer p 8- and rDer p 8-specific IgE ($r = 0.037$, $P = 0.788$) (Fig. 2a). Although the tested sera showed high frequency of IgE reactivity to nDer p 8, the titer was much lower than that of nDer p 2-specific IgE (Fig. 2b). There was also a high frequency of IgE reactivity to nDer p 8 in the tropics, with fifteen of

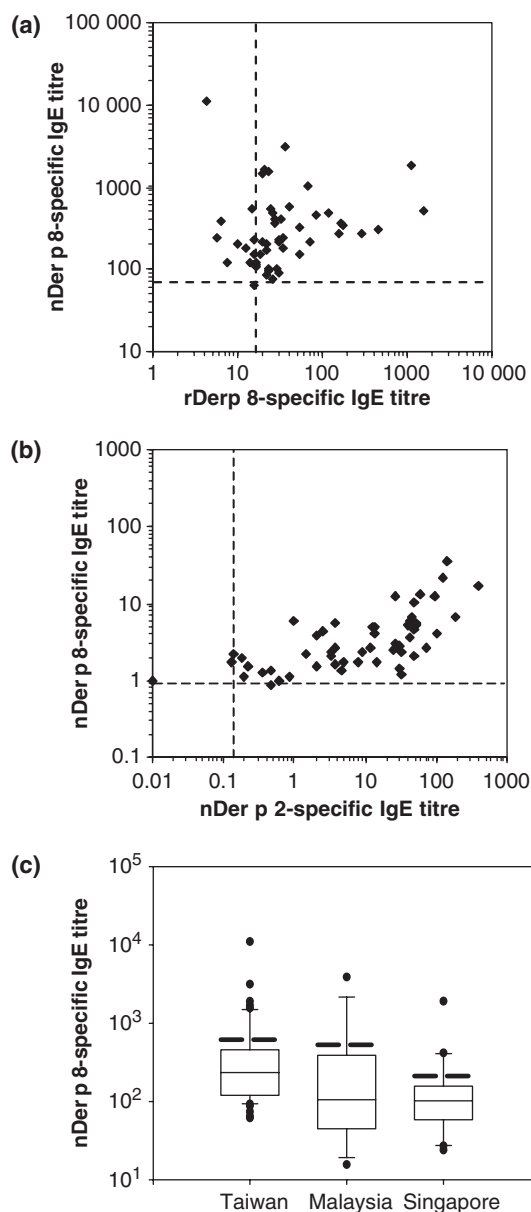


Fig. 2. IgE reactivity to rDer p 8 and nDer p 8. (A) Correlation of IgE titer to rDer p 8 and nDer p 8 in Taiwan sera ($n = 55$). (B) Correlation of the titre of nDer p 8- and nDer p 2-specific IgE. IgE titer was determined by $OD_{405\text{ nm}}$ reading $\times$ serum dilution factor. (C) Box plot showing the nDer p 8-specific IgE titres among Taiwanese, Malaysians and Singaporeans. The mean values are indicated with a dashed line. Each dot on the figure indicates an individual serum. In panels a and b, the cut-off values (dashed line) were determined by the mean plus threefold of standard deviation of Der p 8-specific IgE of control sera.

twenty (75%) Singaporeans and 11 of 17 (65%) Malaysians having detectable IgE to nDer p 8. In addition, the two asthmatic patient groups, Taiwanese and Malaysian, showed no difference in the level of nDer p 8-specific IgE. The level was slightly lower in the mite-sensitized Singaporeans (Fig. 2c).

Presence of IgE cross-reactivity between Der p 8 and cockroach GST

The IgE cross-reactivity between mite GST and cockroach GST was examined next. The American cockroach GST was chosen in these experiments as this species of cockroach is predominant in the tropics and subtropics and a high level sensitization in allergic subjects has been observed. Firstly, sera of positive IgE to Der p 8 were selected and tested for the IgE reactivity to extract of *P. americana*. Sixty-eight percent and 85% of selected sera from Taiwan ($n = 22$) and Singapore ($n = 7$), respectively, showed positive IgE to cockroach extract; however, the titres of cockroach-specific IgE were much lower than that of nDer p 8-specific IgE (mean: 103 vs. 661; $r = 0.09$) (Fig. 3a). Six sera showing positive IgE to both cockroach extract and nDer p 8 were then selected for inhibition studies. Sera preabsorbed with 100 $\mu\text{g/mL}$ cockroach extract inhibited 16–68% of IgE reactivity to nDer p 8 in five of the test sera (Fig. 3b). One serum showed almost no inhibition. Subsequently, GST of *P. americana* was purified from the whole-body extract (Fig. 3c) for inhibition studies. Because of the limited amount of purified cockroach GST obtained, only one subject showing high cross-reactive IgE to cockroach extract was selected for inhibition study. Data showed that cockroach GST inhibited 47% of IgE reactivity to nDer p 8 (Fig. 3d).

Discussion

This study has shown that Der p 8 consists of at least eight isoforms and has high-frequency IgE binding in both its native and recombinant forms to the sera of mite-allergic subjects in three populations (Taiwan, Singapore and Malaysia). We also report for the first time a high frequency of IgE cross-reactivity between GST from Der p mites and American cockroach. Der p 8 was cloned by O'Neill et al. [24] in 1994 and belongs to the μ class of the glutathione-S-transferase superfamily. Here, we report the isolation of a Der p 8 isoform consisting of six polymorphic residues with a theoretical pI of 8.5. This pI is quite different from the original clone that had a theoretical pI of 6.3. The presence of basic Der p 8 in mite was demonstrated by two-dimensional electrophoresis of purified native Der p 8, which showed a wide distribution of pI ranging from 5 to 10 for native Der p 8. There was indeed a native isoform with a pI of 8.5 similar to that of our rDer p 8 isoform, strongly supporting the true existence of our Der p 8 clone rather than the result of a technical artefact. The purified rDer p 8 showed three bands on SDS-PAGE analysis but they had identical N-terminal amino-acid sequences, suggesting that the difference in molecular weight was probably because of the varying degree of glycosylation of the same protein. In fact, there is one putative *N*-glycosylation site in the Der p

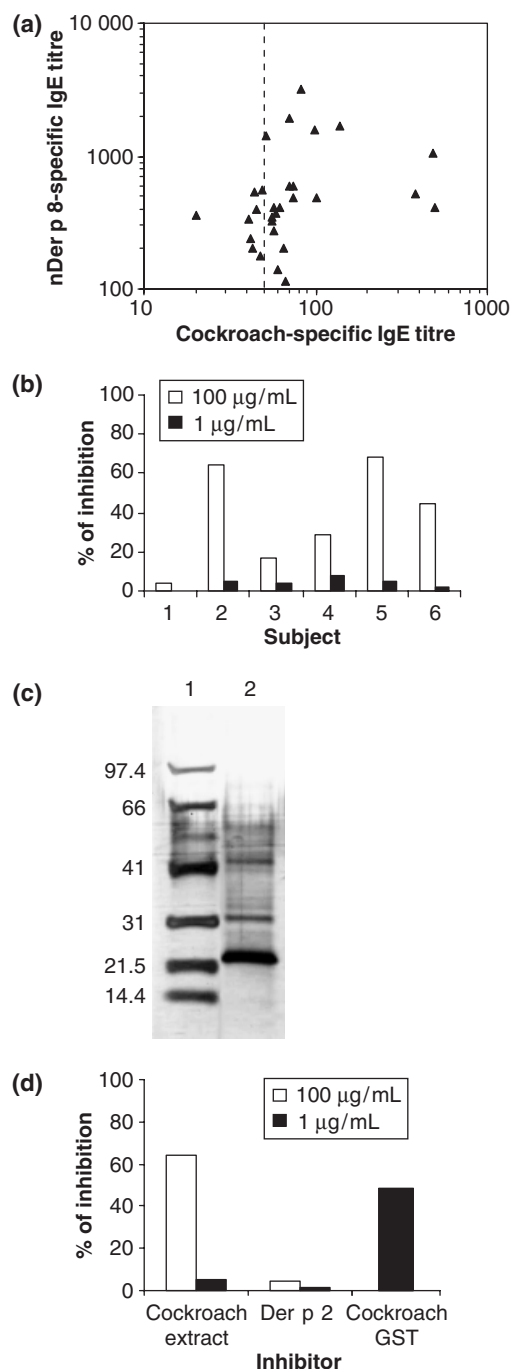


Fig. 3. Presence of cross-reactive immunoglobulin E (IgE) between nDer p 8 and cockroach glutathione S-transferase (GST). (a) Correlation of the titre of nDer p 8- and cockroach extract-specific IgE. Twenty-two nDer p 8 positive sera were selected for testing of cockroach extract-specific IgE. (b) Absorption assay showed various degrees of IgE cross-reactivity between Der p 8 and cockroaches. Sera were pre-absorbed with 100 or 1 $\mu\text{g/mL}$ of cockroach extract before determining the titer of nDer p 8-specific IgE. (c) The silver-stained SDS-PAGE shows the purified cockroach GST. Lane 1: molecular weight marker; Lane 2: cockroach GST. (d) Inhibition of IgE reactivity to nDer p 8 by cockroach GST. Sera were pre-absorbed with 100 or 1 $\mu\text{g/mL}$ of cockroach extract, nDer p 2 or 1 $\mu\text{g/mL}$ purified cockroach GST before determining the titer of nDer p 8-specific IgE.

8 sequences and post-translational modification by glycosylation is a common feature of yeast expression systems. It has been shown that Der p 1 and Der f 1 proteins expressed in *P. pastoris* are also heterogeneously glycosylated [26, 27].

The presence of at least eight isoforms, of Der p 8-related mite GST shown in this study is not unexpected because it is well known that GST is widely distributed in nature and often exists as multiple isoenzymes [28]. The existence of multiple isoforms for other major allergens has also been reported. The isoforms of Birch pollen allergen Bet v 1 and mite allergen Der p 2, which consist of up to 10 and eight isoforms, respectively, have shown differences not only in the extent of IgE-binding activity but also in T cell activation [13, 29]. More interestingly, isoforms of Der p 2 could induce different cytokine production patterns in allergic subjects [13]. It will be interesting to perform further studies to investigate the IgE binding to Der p 8 isoforms to determine whether all mite GSTs are allergenic.

When the allergenicity of native and recombinant Der p 8 was assessed by IgE ELISA, we found that 84% of mite-sensitized Taiwanese asthmatic subjects showed positive IgE reactivity to rDer p 8. This frequency of IgE reactivity was significantly higher than 40% and 10% reported previously by O'Neil *et al.* [23] and Pittner *et al.*, [30], respectively. The difference in isoform and sera used in the various studies may have contributed to the discrepancies in the results obtained. Furthermore, our data revealed that 96% of the same panel of Taiwanese sera showed a positive IgE reaction to native Der p 8. The presence of multiple isoforms in native Der p 8 could partly explain why the frequency of IgE reactivity to nDer p 8 was higher than that of rDer p 8 as the rDer p 8 used represented only one of the isoforms. In addition, the lower allergenicity of rDer p 8 could also be because of the differences in protein conformation between the rDer p 8 and its native counterpart as the recombinant form could not bind to the glutathione affinity column (data not shown). Although nDer p 8 showed an extremely high frequency of IgE binding (96%) to the Taiwanese sera, it may not be a major allergen as the titres of nDer p 8-specific IgE were much lower than that of nDer p 2-specific IgE. It should be noted that the Taiwanese sera used in this study were from asthmatic patients undergoing immunotherapy with *D. pteronyssinus* crude mite extract. It has been suggested that immunotherapy could actively boost the frequency and titre of IgE to Der p 5 [25]. To determine whether the high frequency of IgE reactivity to Der p 8 is because of natural sensitization or boosted by immunotherapy, we had then tested a panel of sera from allergic Singaporeans and Malaysians asthmatic patients who had never undergone immunotherapy. The frequency of IgE reactivity to native Der p 8 was 65% and 75% for Singaporeans and Malaysians, respectively. The reduction in the frequency

was expected, as unlike the subtropical Taiwan, *Blomia tropicalis* instead of *D. pteronyssinus* is the most important mite species in these two tropical countries. However, there was no difference in the Der p 8-specific IgE titres between immunotherapy and non-immunotherapy groups. Taken together, the results confirmed that the sera obtained from mite-sensitive subjects living in tropical and subtropical countries showed a high frequency of IgE reactivity with Der p 8 but the IgE titres were generally low.

The concept of panallergy that involved arthropods and other invertebrates had been discussed extensively; conserved proteins such as tropomyosin and paramyosin of shrimp, snail, cockroach and schistosomes, have been found to be allergenic [31]. Patients infected with *Schistosoma mansoni* and *Onchocerca volvulus* produced cross-reactive IgE antibody to paramyosin [32]. The IgE cross-reactivity of mite tropomyosin with other invertebrate was especially well documented [16, 20, 33, 34]. IgE cross-reactivity to GST from different organisms including mites had been postulated by Aalberse and colleagues [35], but there was no experimental evidence to support this notion. The high frequency of IgE reactivity to Der p 8 shown by the allergic sera tested prompted us to examine the possibility of IgE cross-reactivity between GSTs from mite and cockroach. This study was rationalized by the observation that mites and cockroaches are the most important source of indoor allergens associated with asthma in these regions [5, 15, 36]. Our results showed moderate cross-reactivity between Der p 8 and American cockroach, to both the crude extract and purified American cockroach GST. Six sera preabsorbed with 100 µg/mL cockroach extract inhibited 16–68% of IgE reactivity to nDer p 8 in five of the test sera (Fig. 3B) and one serum showed almost no inhibition, suggesting that not all IgE to Der p 8 were cross-reactive to its homologue in cockroach. It has previously been suggested that *P. americana* extracts could not inhibit IgE antibody binding to Bla g 5 using an RIA assay, suggesting that there was no significant IgE cross-reactivity between GSTs from these two species [22]. However, our data revealed that GST(s) in American cockroach is/are allergenic. We therefore propose that the Der p 8 cross-reactive allergenic GST of American cockroach may not be the direct counterpart of Bla g 5. The question of IgE cross-reactivity between cockroach GSTs should be further addressed by using purified native GSTs from both species and recombinant Bla g 5. More quantitative information including the relative affinity of such IgE cross-reactivity may help to shed some light on the verification of the clinical impacts of such cross-reactive IgE.

In summary, both native Der p 8, which exists in multiple isoforms, and recombinant Der p 8 showed a high frequency but a relatively low titer of IgE binding in the dust mite-allergic subjects studied, and inclusion of Der p 8 isoforms for diagnostic and therapeutic purposes

may be necessary. We have also demonstrated the presence of cross-reactive IgE between Der p 8 and GST in *P. americana* cockroach, suggesting that the GST in mites and cockroach may be considered a panallergen. The unresolved question of which GST is the primary sensitizing allergen in an environment where both mites and cockroaches are equally prevalent can be addressed more fully by using purified native or recombinant Der p 8 and its homologues.

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