

Original article

Protein sequence analysis and mapping of IgE and IgG epitopes of an allergenic 98-kDa *Dermatophagoides farinae* paramyosin, Der f 11

Background: A 98-kDa mite paramyosin (Der f 11) from *Dermatophagoides farinae* (Df) is highly allergenic, and its cDNA (Df642) has been cloned. This paper describes the sequence characteristics and the mapping of the immunodominant human IgE and IgG epitopes of Der f 11.

Methods: The protein sequence analysis was performed with a combination of FASTA, GCG, and CLUSTAL W computing packages. The whole cDNA insert and its PCR-derived DNA fragments were generated and expressed in *E. coli*. These overlapping recombinant peptides (F1 to F5) were used for B-cell epitope mapping with 18 mite-allergic sera by dot immunoassays.

Results: Df642 cDNA encodes a partial sequence that contains the 2nd to 26th 28-residue repeats and lacks the N-terminus and the C-terminus. The sequence identity of Der f 11 with other known paramyosins is 34–60%. The dominant IgE epitopes are located in peptides F1 and F4, whereas the dominant IgG epitopes are located in peptides F1 and F2. These peptides are more reactive than whole rDf642.

Conclusions: Mite paramyosin is very similar to other known paramyosins. The human IgE and IgG epitopes are scattered throughout the entire molecule. Data also indicate the presence of unique IgE and IgG epitopes in Der f 11.

L.-C. Tsai, P.-L. Chao, M.-W. Hung

Department of Medical Research, Veterans General Hospital, Taipei, Taiwan

Y.-C. Sun, I.-C. Kuo, K.-Y. Chua

Graduate Institute of Immunology, College of Medicine, National Taiwan University, Taipei, Taiwan

S.-H. Liaw

Department of Life Science, National Yang-Ming University, Taipei, Taiwan

K.-Y. Chua, I.-C. Kuo

Department of Paediatrics, Faculty of Medicine, National University of Singapore, Singapore

Key words: Der f 11; *Dermatophagoides farinae*; epitopes; mite allergen; paramyosin.

Kaw-Yan Chua

Department of Pediatrics

Faculty of Medicine

National University of Singapore

Lower Kent Ridge Road

Singapore 119074

Accepted for publication 9 September 1999

Epidemiologic studies have revealed that allergy to house-dust mite is a worldwide health problem affecting about 10–20% of the world population (1). The two most important clinical mite species are *Dermatophagoides pteronyssinus* (Dp) and *D. farinae* (Df); the allergens from these closely related species show a high degree of IgE cross-reactivity.

In vitro IgE immunoassays have demonstrated that groups I and II allergens react with specific IgE present in 80–90% of mite-allergic sera (2–5) whereas the rest of the mite allergens showed up to 40–50% IgE reactivity *in vitro* (6–8). The mite

allergens identified to date are of relatively low molecular mass of 14–60 kDa (9). Although more than 50% of the mite allergens characterized so far are enzymes, the group 10 mite allergens have been identified as muscle-associated tropomyosins (10). In addition, our recently published report revealed that a 98-kDa Df mite paramyosin (designated as group 11 mite allergen, Der f 11) can bind IgE in allergic sera with high frequency and with strong potency (11). The cDNA coding for this allergen has been isolated and characterized (12). Like tropomyosin, paramyosin is a protein associated with muscles. Both tropomyosin and paramyosin are composed of two identical coiled-coil α -helices. Unlike tropomyosin, paramyosin is found only in invertebrates, and its distribution and function(s) are quite diverse. Paramyosin has been identified as a prominent immunogen in parasites and as a most promising vaccine candidate for parasitic diseases

Abbreviations. Df: *Dermatophagoides farinae*; Dp: *Dermatophagoides pteronyssinus*; mAb: monoclonal antibody; TBST: tris-buffered saline containing Tween 20; PBS: phosphate-buffered saline; PCR: polymerase chain reaction; GST: glutathione-S-transferase; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; rDf642: recombinant Df642 protein.

such as filariasis and schistosomiasis. For instance, *Taenia solium* paramyosin is a prominent antigen (antigen B) in human cysticercosis, and may play a role as a modulator of the host immune response (13).

The cDNA encoding Der f 11 allergen, designated as Df642, is 2134 bp long, coding for a polypeptide of 711 amino-acid residues. This cDNA insert was subcloned into pGEX-2T expression vector, and the resulting recombinant Df642 protein (rDf642) expressed as a fusion protein with glutathione-S-transferase (GST) was estimated as about 120 kDa in mass on SDS-PAGE gel (11). The recombinant Df642 protein, like its native counterpart, appeared to be very susceptible to degradation, giving rise to a number of smaller fragments of 45–110 kDa. Most of the derived fragments were reactive to human IgE (11). In order to investigate further the profile and distribution of human IgE and IgG epitopes of Df642, we generated a panel of overlapping recombinant peptides from cDNA fragments for this study. We report the amino-acid sequence analysis and the determination of human IgE and IgG epitopes of Df642 with the overlapping recombinant peptides.

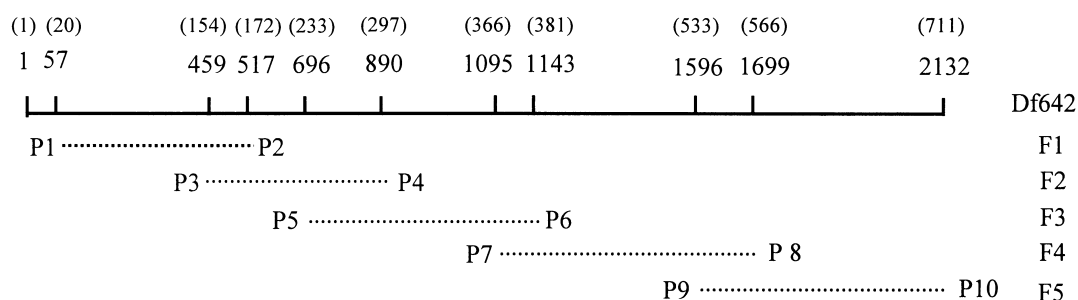
Material and methods

Sera

Human allergic sera were obtained from patients attending the Allergy Clinic of the National Taiwan University Hospital, Taipei, Taiwan. All these patients showed positive skin test reaction to *D. pteronyssinus* and *D. farinae* extracts.

SDS-PAGE and immunoblotting

SDS-PAGE was performed as previously described (12). The proteins were transferred from the gel to the polyvinylidene fluoride (PVDF) microporous membrane (Millipore Corp., Bedford, MA, USA) with a semidry blotting apparatus. For the specific antibody-binding capacity assessment of recombinant peptides expressed from the cDNA fragments, an immunoblot containing purified peptides was incubated with antinative Der f 11 mouse serum (1:100 000 diluted) for 1 h at 37°C. After extensive washing with TBS buffer containing 0.05% Tween 20, the blot was incubated with HRP-conjugated rabbit antimouse IgG (diluted 1:3000) for 1 h at 37°C. After washing, the blot was incubated with ECL reagent (Amersham, Buckinghamshire, UK)



Sequences of PCR primers (5' → 3')

P1: GCGGATCCATGAACAAAAAAGAGATTG
P2: GCGAATTCGAGTGTCTTCTAATTG
P3: GCGGATCCCAATTGGACAATGCAAAC
P4: GCGAATTCCTTGTGCATGTCGTGTCG
P5: GCGGATCCGAAGCTGAATTGCAGC
P6: GCGAATTCATGGATTCTACGGTAC
P7: GCGGATCCCTTGCCGAAGCTAAATC
P8: GCGAATTCGCTTCTTCATGCAATTG
P9: GCGGATCCCAACGACGATGCCAA
P10: GCGGATCCTTGCAACAACAATTCC

Figure 1. Schematic diagram showing strategy for generation of Df642-derived cDNA fragments by PCR. Numbers indicate nucleotide number of cDNA insert, and numbers in parentheses indicate amino-acid residue of deduced protein. Sequences for PCR primers (P1 to P10) are listed below. F1 to F5 represent five overlapping peptides generated.

for 1–2 min and exposed to radiograph film for 5–20 s at room temperature.

Human IgE and IgG immunodot analysis

The dot-blot immunoassay for IgE and IgG was performed by a protocol previously described (12). An amount of 2 µg of each purified protein was applied to nitrocellulose membrane in duplicate. The dot-blots were blocked with phosphate-buffered saline (PBS) containing 5% skim milk and 0.1% Tween 20. Allergic sera were diluted 1:1 in PBS and incubated with the dot-blots for 12–16 h at 4°C. The incubation with the secondary antibodies was performed with 1:1000 dilution of biotinylated mouse antihuman IgE (PharMingen) or biotinylated mouse antihuman IgG (PharMingen) for 1 h, followed by 1-h incubation with extravidin peroxidase (Sigma). The signal of the reaction was developed with enhanced chemiluminescent detection reagent (Amersham) and autoradiography. The dot-blots were extensively washed with PBS containing 0.1% Tween 20 after each treatment step throughout the immunoassay protocol. Western blots were prepared by separating protein samples on an SDS-PAGE gel and transferred to nitrocellulose membranes by protocols previously described (14).

Protein sequence analysis

The nucleotide and deduced amino-acid sequences were compared by FASTA with those in the Swiss-Prot and GeneBank databases, and the sequence analysis and alignments were performed with the GCG and CLUSTAL W packages (15). The hydrophilicity plot was computed with the Hopp and Woods algorithm with a program provided by the Bioinformatics Unit, Weizmann Institute of Science, Israel.

Expression of Df642 cDNA clone and its derived cDNA fragments in *E. coli*

A BamHI fragment containing the entire Df642 cDNA sequence was produced by polymerase chain reaction (PCR) amplification. In addition, five overlapping cDNA fragments were generated by PCR amplification. Fig. 1 shows the schematic diagram of the strategy for the generation of such cDNA fragments. These PCR products were subcloned into the expression vector pGEX-2T and expressed as GST fusion proteins in *E. coli* (16). Production and purification of the recombinant proteins were done by a protocol previously described (14). Briefly, a bulk culture of the recombinant protein was grown in Luria broth containing 100 µg/ml of ampicillin to log phase and

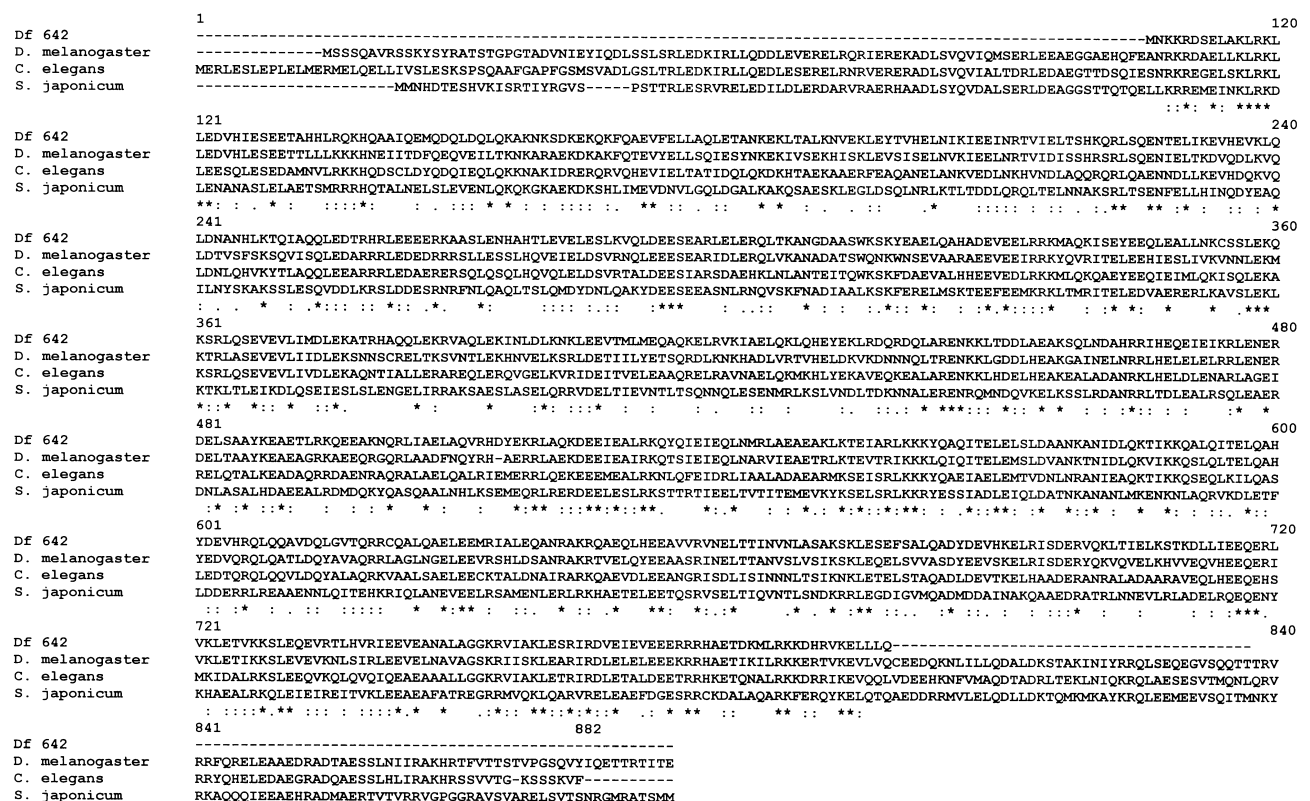


Figure 2. Amino-acid sequence alignments of Df642 and various paramyosins from invertebrates. Identical and similar residues are shown by (*) and (.), respectively. Gaps (- -) were introduced for optimal alignment.

then induced with 1 mM of isopropyl β -D-thiogalactopyranoside. After cell harvesting, the protein was purified from cell lysate supernatant by glutathione affinity chromatography.

Results

Clone Df642 codes for a fragment of *D. farinae* paramyosin

The homology search revealed that the Df 11 allergen (Df642) shows a high degree of sequence identity with paramyosin from invertebrates. The results of the sequence alignments shown in Fig. 2 indicate that the 711 amino-acid residues of Df642 protein shared 60% identity and 76% homology with the paramyosin of *Drosophila melanogaster*. It also shares 50% identity and 70% homology with paramyosin from *Caenorhabditis elegans*, and 34% identity, 55% homology with that of *Schistosoma japonicum*.

Although the sequence identity of the various paramyosins listed is 34–60%, their amino-acid compositions share a number of common characteristics. They all have high numbers of charged residues such as E, D, R, and K, and these charged residues make up about 45% of the protein sequence. Proline (helix breaker) and cysteine residues, as well as aromatic residues, are present in low numbers. Leucine is the most abundant hydrophobic residue present in all paramyosins under comparison. A characteristic feature of paramyosins is the presence of a serine-rich N-terminal region. Df642 contains a lower number of serine residues than other paramyosins. This is probably due to the truncation of the N-terminus of the cDNA. Hydrophobicity analysis of DF642 reveals that the molecule is highly hydrophilic and

therefore does not resemble the structure of typical globular proteins (data not shown).

Antibody-binding capacity of recombinant Der f 11 fragments

A DNA fragment containing BamHI restriction enzyme sites was PCR amplified from Df642 cDNA and subcloned into the pGEX-2T expression vector. The resulting recombinant Df642 protein (rDf642) was expressed as a fusion protein with GST and estimated to be about 120 kDa in mass on SDS-PAGE gel. The recombinant protein, like its native counterpart, is very susceptible to degradation, giving rise to a number of protein fragments of 45–120 kDa. Five overlapping DNA fragments were produced by PCR amplification with a combination of PCR primers, as described in Material and methods (Fig. 1). Each of these PCR products was expressed as a fusion protein with GST. The numbers of residues for the recombinant peptides F1 to F5 were 153, 144, 149, 201, and 179, respectively. The predicted molecular masses for these recombinant fusion peptides F1 to F5 were 44, 42, 43, 49, and 46 kDa, respectively. All five peptides reacted strongly with the anti-Der f 11 mouse serum that was raised with native Der f 11 (Fig. 3, lanes 3–7).

The reactivity of human IgE and IgG to rDf642 and the peptides (F1 to F5) was studied by immunodot assay. Fig. 4 shows the immunodot assay results obtained with a selected panel of four mite-allergic sera and a nonatopic control serum. Tables 1(a) and (b) summarize the *in vitro* IgE- and IgG-binding results for 18 mite-allergic sera and one nonatopic control serum. For the IgE reactivity, the recombinant peptides F1, F2, F3, F4, and F5 reacted with 72% (13/18), 55% (10/18), 61% (11/18), 78% (14/18), and 55% (10/18) of the 18 allergic sera, respectively, whereas whole rDf642 reacted with 55% (10/18) of the sera. For human IgG reactivity, both the recombinant peptides F1 and F2 reacted with approximately 94% (17/18) of the allergic sera. Peptides F3, F4, and F5 reacted with 67% (12/18), 89% (16/18), and 50% (9/18) of the tested allergic sera, respectively. Whole rDf642 reacted with 61% (11/18) of the sera tested. All the 18 allergic sera showed positive IgE and IgG reactivity to the recombinant Der p 2, and two of these sera showed negative IgE reactivity to the whole rDf642 protein and peptides F1 to F5.

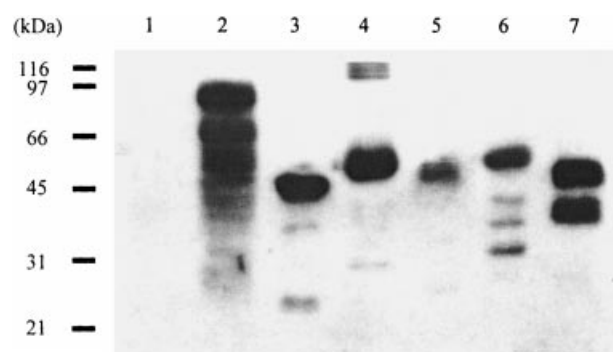


Figure 3. Western blot analysis of recombinant protein Df642 and peptides (F1–F5). All recombinant proteins were produced as fusion proteins with GST and affinity purified. IgG immunoassay was performed with Df642-specific mouse. Lane 1) GST control; lane 2) rDf642; lanes 3–7 correspond to peptides F1–F5, respectively.

Discussion

Paramyosin is known as a major structural protein of thick filaments in the muscle of most invertebrates, and contains a central α -helical coiled-coil rod, organized in 29 groups of 28-residue repeats

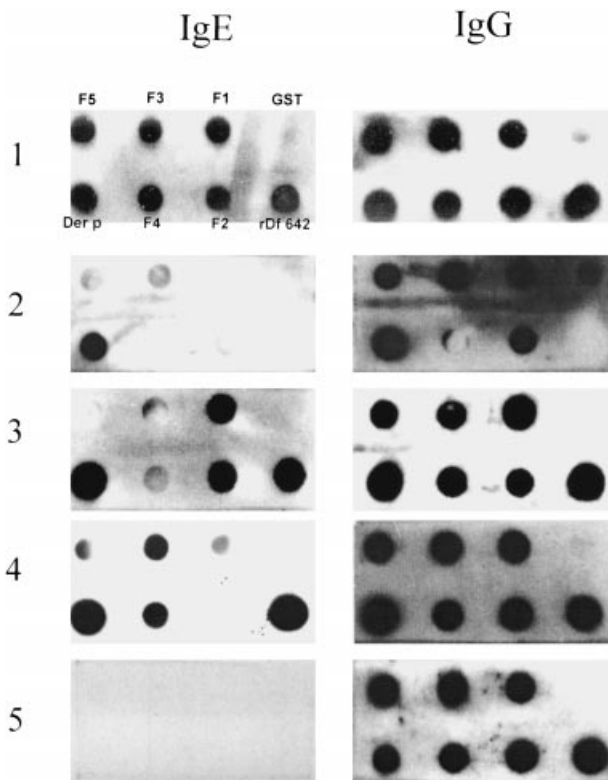


Figure 4. Human IgE and IgG immunodot assay. Panels 1–4 show IgE (left) and IgG (right) reactivity of four individual allergic sera to various proteins dotted on nitrocellulose membranes. Panel 5 shows IgE and IgG reactivity of nonatopic serum to proteins tested.

and flanked by two short, serine-rich, non- α -helical N- and C-termini (17). The sequence alignment of Df642 to other paramyosins suggests that the Df642 sequence contains the 2nd–26th 28-residue repeats and lacks both the N-terminus and the C-terminus and the 1st, 27th, 28th, and 29th repeats. The sequences of many α -helical coiled-coil proteins, especially the muscle proteins tropomyosin, myosin, and paramyosin, have been analyzed (17, 18). The α -helical coiled-coil rod displays a strong heptad repeat (abcdefg)_n, with the hydrophobic residues concentrated in the “a” and “d” positions and many charged residues clustered on the helix surface. Both myosin and paramyosin show 28-residue repeats, which have a characteristic pattern of hydrophobic residues and a remarkable distribution of charges. The hydrophobic residues at the “a” and “d” positions form a closely packed hydrophobic interface between two strands of the coiled coil, and the periodic distribution of charged residues could provide the driving force for the filament assembly (18, 19).

Although the sequence identity of the various paramyosins listed is 34–60%, their amino-acid composition shares a number of common char-

acteristics. The amino-acid composition usually contains high proportions of charged residues (D, E, R, and K) (40%), N plus Q (12%), and hydrophobic residues L (14%) and A (11%), but low contents of G, C, P, F, Y, and W (<1%). Most proline residues in paramyosin appear at the N-terminal fragment, which is unlikely to form a regular α -helical structure. Paramyosin contains very few cysteine residues. The two cysteine residues in the *D. melanogaster* and nematode (*C. elegans*, *O. valvulus*, *B. malayi*, and *D. immitis*), paramyosins, and the one cysteine residue in *Schistosoma mansoni* are also located in the “a” or “d” position and are predicted to form intermolecular disulfide bonds. It has been shown that the two cysteine residues of *D. melanogaster* are located in position “a” of the heptad repeat and contribute to dimerization under denaturing conditions in the absence of reducing agents (20). Therefore, it is deduced that the two cysteine residues found in Df642, which are located at the “a” position, might form intermolecular disulfide bonds for the protein’s stability and assembly. In addition, there are two putative N-glycosylation sites in Df642, and they are probably functional, because high-mannose N-glycan chains has been detected in native protein (11).

For the determination of IgE and IgG epitopes, the five overlapping recombinant peptides F1 to F5 were produced as fusion proteins with GST and used for epitope-mapping by immunodot assay. Out of the 18 mite-allergic sera tested, two (13 and 17) did not show any IgE reactivity to rDf642 and its five derived peptides F1 to F5. The remaining 16 sera reacted positively to either the whole rDf642 protein or one or more of the five overlapping peptides. Five out of the 16 sera that showed negative IgE binding to the whole rDf642 reacted positively to peptides F1 and F4, indicating that these peptides have higher IgE binding capacity than whole rDf642. This result is in accordance with the observation that the degradation products from the native and recombinant Df642 could bind human IgE (11, 12). In turn, the existence of these allergenic degradation products from mite paramyosin may account for some of the multiple IgE-binding proteins with molecular masses of 40–80 kDa that were commonly observed in the IgE immunoblot analysis of crude mite extract (21, 22). Absorption studies performed with rDf642 or its derived recombinant peptides will help to confirm such a hypothesis.

Because the Df642 is highly susceptible to degradation, and the degraded products have been shown to react strongly with IgE, it is conceivable that some subjects were sensitized by the peptides derived from the whole molecule. This may help to explain the fact that recombinant peptides F1 and F4

Table 1. Summary of reactivity of human IgE (a) and IgG (b) to recombinant Df642 and peptides F1 to F5 determined by immunodot assay. Positive and negative binding is shown by (+) and (—), respectively

Serum no.	Antigen							
	Df642	F1	F2	F3	F4	F5	GST	Der p 2
	IgE	IgE	IgE	IgE	IgE	IgE	IgE	IgE
a								
Nonatopic control	—	—	—	—	—	—	—	—
1	+	+	+	+	+	+	—	+
2	+	—	—	+	—	+	—	+
3	+	+	+	+	+	+	—	+
4	+	+	+	+	+	+	—	+
5	+	+	+	+	+	—	—	+
6	+	—	—	+	+	+	—	+
7	+	+	+	—	+	—	—	+
8	+	+	+	—	+	+	—	+
9	+	+	+	+	+	—	—	+
10	+	+	+	+	+	+	—	+
11	—	+	—	—	+	—	—	+
12	—	+	+	—	+	—	—	+
13	—	—	—	—	—	—	—	+
14	—	+	—	—	—	—	—	+
15	—	+	—	+	+	+	—	+
16	—	+	+	+	+	+	—	+
17	—	—	—	+	+	+	—	+
18	—	—	—	—	—	—	—	+
No. of positive reactions	10	13	10	11	14	10	0	18
b								
Nonatopic control	+	+	+	+	+	+	—	+
1	+	+	+	+	+	+	—	+
2	+	+	+	+	+	+	—	+
3	+	+	+	+	+	+	—	+
4	+	+	+	+	+	—	—	+
5	+	+	+	—	+	—	—	+
6	+	+	+	+	+	+	—	+
7	+	+	+	—	+	—	—	+
8	+	+	+	+	+	+	—	+
9	+	+	+	+	+	+	—	+
10	+	—	+	+	+	+	+	+
11	—	+	+	—	+	—	—	+
12	—	+	+	—	+	—	+	+
13	+	+	+	+	+	—	—	+
14	—	+	—	+	—	—	—	+
15	—	+	+	+	+	+	—	+
16	—	+	+	—	+	—	—	+
17	—	+	+	+	+	+	—	+
18	—	+	+	—	—	—	—	+
No. of positive reactions	11	17	17	12	16	9	2	18

showed higher frequency of IgE reactivity than whole rDf642. In addition, the recombinant peptides may have acquired conformations that made the IgE-binding epitopes more readily accessible to serum IgE antibodies. Our previous results showed that about 80% (55/67) of the mite-sensitive sera showed positive *in vitro* IgE reactivity with native Der f 11 (11), but this study revealed that only 62% of the sera tested reacted positively with rDf642 protein. The N- and C-terminal truncations in the rDf642 may have resulted in its lower frequency of *in vitro* IgE reactivity. The conformational changes in the recombinant protein may also account for its lower

IgE reactivity than its native counterpart. Table 1(b) summarizes the IgG reactivity of the 18 mite-allergic sera with rDf642 and peptides F1 to F5. Interestingly, certain sera, such as nos. 4, 5, and 16, showed positive IgE binding, but negative IgG binding to peptides F3 and F5. Furthermore, unlike IgE, where dominant epitopes are located in peptides F1 and F4, the IgG dominant epitopes are located in peptides F1 and F2. Taken together, these data suggest that IgE and IgG antibodies recognized distinct epitopes. In summary, the human IgE and IgG epitope-mapping data indicated that these epitopes were scattered throughout the entire molecule. The results were not

unexpected in view of the fact that the entire molecule is largely hydrophilic.

It is intriguing that paramyosin is a potent antigen of parasites such as *S. mansoni* (23, 24), and it is also a potent allergenic protein in mite. Both immune responses resulted in the production of IgE. This protein, therefore, provides an interesting model to study the structural characteristics of an antigen that can induce elevated levels of IgE

in susceptible hosts. This is a useful antigen to establish animal models to study the immunopathogenesis of allergic and parasitic diseases.

Acknowledgments

This study was supported by grants from the National Science Council and the Veterans General Hospital, Taipei, Taiwan.

References

1. CHAPMAN MD, PLATTS-MILLS TAE. Purification and characterisation of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980;**125**:587–592.
2. CHUA KY, KEHAL PK, THOMAS WR, VAUGHAN PR, MACREADIE IG. High-frequency binding of IgE to the Der p allergen expressed in yeast. *J Allergy Clin Immunol* 1992;**89**:95–102.
3. LIND P. Purification and partial characterisation of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1985;**76**:753–761.
4. CHUA KY, DOYLE CR, SIMPSON RJ, TURKER KJ, STEWART GA, THOMAS WR. Isolation of cDNA coding for the major mite allergen Der p II by IgE plaque immunoassay. *Int Arch Allergy Appl Immunol* 1990;**91**:118–123.
5. LIN KL, HSIEH KH, THOMAS WR, CHIANG BL, CHUA KY. Characterization of Der p V allergen, cDNA analysis, and IgE mediated reactivity to the recombinant protein. *J Allergy Clin Immunol* 1994;**94**:989–996.
6. SMITH WA, CHUA KY, KUO MC, ROGERS BL, THOMAS WR. Cloning and sequencing of *Dermatophagoides pteronyssinus* group III allergen, Der p III. *Clin Exp Allergy* 1994;**24**:220–228.
7. SHEN HD, CHUA KY, HSIEH KH, THOMAS WR. Molecular cloning and immunological characterization of the group 7 allergens of house dust mites. In: SCHON A, HAYGLASS KT, KRAFT D, editors. *New horizons in allergy immunotherapy*. New York: Plenum Press, 1996:241–242.
8. THOMAS WR, SMITH W. House-dust-mite allergens. *Allergy* 1998;**53**:821–832.
9. AKI T, KODAMA T, FUJIKAWA A, et al. Immunochemical characterization of recombinant and native tropomyosin as a new allergen from the dust mite, *Dermatophagoides farinae*. *J Allergy Clin Immunol* 1995;**96**:74–83.
10. TSAI LC, CHAO PL, SHEN HD, et al. Isolation and characterization of a novel 98kD *Dermatophagoides farinae* mite allergen. *J Allergy Clin Immunol* 1998;**102**:295–303.
11. TSAI LC, SUN YC, CHAO PL, et al. Sequence analysis and expression of a cDNA clone encoding a 98kD allergen in *Dermatophagoides farinae*. *Clin Exp Allergy* 1999 (in press).
12. LANDA A, LACLETTE JP, SHOEMAKER CB. cDNA cloning and recombinant expression of collagen-binding and complement inhibitor activity of *Taenia solium* paramyosin (AgB). *Mol Biochem Parasitol* 1993;**60**:343–347.
13. CHUA KY, GREENE WK, KEHAL PK, THOMAS WR. IgE binding studies with large peptides expressed from Der p II cDNA constructs. *Clin Exp Allergy* 1991;**21**:161–166.
14. THOMPSON JD, HIGGINS DG, GIBSON TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;**22**:4673–4680.
15. SMITH DB, JOHNSON KS. Single-step purification of polypeptides expressed in *Escherichia coli* as fusion with glutathione S-transferase. *Gene* 1988;**67**:31–40.
16. KAGAWA H, GENGYO K, McLACHLAN AD, BRENNER S, KARN J. Paramyosin gene (*Unc-15*) of *Caenorhabditis elegans*. Molecular cloning nucleotide sequence and models for thick filament structure. *J Mol Biol* 1989;**207**:311–333.
17. McLACHLAN AD, KAM J. Periodic charge distributions in the myosin rod amino acid sequence match cross-bridge spacings in muscle. *Nature* 1982;**299**:226–231.
18. MENG JJ, KHAN S, Ip W. Charge interactions in the rod domain drive formation of tetramers during intermediate filament assembly. *J Biol Chem* 1994;**269**:18679–18685.
19. VINOS J, DOMINGO A, MARCO R, CERVERA M. Identification and characterization of *Drosophila melanogaster* paramyosin. *J Mol Biol* 1991;**220**:687–700.
20. TOVEY ER, BALDO BA. Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. *J Allergy Clin Immunol* 1987;**79**:93–102.
21. BALDO BA, FORD SA, TOVEY ER. Toward a definition of the complete spectrum and rank order of importance of allergens from the house dust mite *Dermatophagoides pteronyssinus*. *Adv Biosci* 1989;**74**:13–31.
22. PEARCE EJ, JAMES SL, DALTON J, et al. Immunochemical characterization and purification of Sm-97, a *Schistosoma mansoni* antigen monospecifically recognized by antibodies from mice protectively immunized with a nonliving vaccine. *J Immunol* 1986;**11**:3593–3600.
23. FLANIGAN TP, KING CH, LETT RL, NANDURI J, MAHMOUD AAF. Induction of resistance to *Schistosoma mansoni* infection in mice by purified parasite paramyosin. *J Clin Invest* 1989;**83**:1010–1014.