

Molecular cloning and immunological characterization of the house dust mite allergen Der f 7

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Summary

Background The allergen Der p 7 from *Dermatophagoides pteronyssinus* has been defined by molecular cloning and shown to be an important specificity in 50% of mite-allergic patients.

Objective This study compares the cDNA sequence and serological reactivity of Der f 7 from *D. farinae* with Der p 7.

Method cDNA encoding Der f 7 was amplified by polymerase chain reaction, sequenced and expressed as a fusion with glutathione-S-transferase for IgE and monoclonal antibody binding studies.

Results Der f 7 cDNA encoded a 213 polypeptide containing a predicted 17 amino acid leader sequence, no cysteines and a single *N*-glycosylation site similar to Der p 7. The predicted 196 residue mature polypeptide had 86% identity to Der p 7 and a calculated molecular weight of 22 348Da. No homologues were found in searches of the data banks. The Der f 7 fusion protein showed a single band of 46 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and reacted with IgE antibodies in 19/41 (46%) of sera from asthmatic children. The degree of binding was usually 30% of that to Der p 7 consistent with the exposure of the patients to *D. pteronyssinus*. Monoclonal antibodies (WH9 and WH22) against Der p 7 reacted with Der f 7 but inhibition studies showed a 10-fold difference in reactivity.

Conclusion Der f 7 has a predicted 213 residue polypeptide with 86% homology and serological crossreactivity to Der p 7.

Keywords: house dust mite, Der f 7, cDNA cloning, allergen

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Introduction

House dust mites of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* have been recognized as important causative agents of respiratory allergy for a long time in many countries [1,2]. At least seven different allergens of *D. pteronyssinus* with known cDNA or *N*-terminal amino acid sequences have been identified and characterized [3]. These are also outlined in the recent guidelines for the new nomenclature which is used here

[4]. For allergens of *D. farinae*, sequence analysis reveals that Der p 1 and Der f 1 share 81% identity in their primary structure [5]. Der p 2 and Der f 2 allergens share 88% identity while the other groups have not been investigated [6]. Serological investigations have shown a major component of the IgE response is crossreactive for both mite species [7,8]. Recently, a cDNA clone of a newly identified allergen of *D. pteronyssinus*, Der p 7 was isolated and sequenced [9]. Skin tests with recombinant Der p 7-GST (glutathione S-transferase) fusion protein showed reactivity in 16/30 (53%) allergic patients [9] and serological data [10] show that the degree of IgE binding in the responders is often as large as that to Der p 2.

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Apart from its IgE binding activity Der p 7 is of interest because it corresponds to components of a molecular weight of 31, 30 and 26 kDa in extracts of *D. pteronyssinus* [9]. This reduce to two bands with molecular weights of 27 and 26 kDa after treatment with N-glycosidase F suggesting that the 31 and 30 kDa reactivities may be different glycosylation products of Der p 7 with a molecular weight of about 27 kDa [11]. Since the *D. farinae* mite species also predominates in certain countries, for example North America and Japan [12], we report here characterization of cDNA clone coding for the allergen Der f 7 from *D. farinae*.

Materials and methods

Isolation of Der f 7 cDNA by polymerase chain reaction and DNA sequencing

To isolate Der f 7 cDNA, an oligonucleotide primer Df1 (GCGAATTCGATCCAATTCACCTATGAT-3') based on the predicted N-terminal sequence of Der p 7 was made and used as upstream polymerase chain reaction (PCR) primer. The first GCGAATTC encodes an EcoRI site (GAATTC) and the following sequence GAT... encodes the first six residues of Der p 7. For the other primer, the λ gt11 GGTGGCGACGACTCCTG-GAGCCCG-3' forward primer (Df 2) flanking the EcoRI cloning site was used (New England Biolabs, Beverly, USA). The PCR reactions were carried out in a final reaction volume of 50 μ L containing 20 mM Tris-HCl pH8.2, 10 mM KCl, 6 mM (NH₄)₂SO₄, 2 mM MgCl₂, 0.1% Triton X-100, 10 ng/ μ L nuclease-free BSA, 10 mM dNTPs, 20 pmol of each primer and 2.5 units of Pfu DNA polymerase. This was obtained as a kit from Stratagene (La Jolla, CA, USA). Target DNA (0.001 μ g of *D. farinae* cDNA ligated into λ gt11 [5]) was added and the reaction overlaid with paraffin oil. The tubes were initially denatured at 95°C for 5 min, then annealed at 55°C for 2 min and extended at 72°C for 2 min. Thereafter for 38 cycles, denaturing was carried out for 1 min at 94°C and annealing for 1 min at 55°C and extension for 1 min at 72°C. In the final (40th) cycle, the extension reaction was increased to 10 min to ensure that all amplified products were full length. Ten microlitres of the reaction was checked on a 1% agarose gel and the remainder of the reaction was ethanol precipitated and purified of the amplified product on a low melting point agarose (Bio-Rad, Richmond, VA, USA). The purified PCR product was digested with EcoRI and ligated into M13mp18 for transformation into *Escherichia coli* strain TG1 cells. DNA sequencing was performed with the dideoxynucleotide chain termination using Sequenase version 2.0 (USB Corporation,

Cleveland, USA) according to the supplier's protocol. The primers used for sequencing include the M13 sequencing primer, and primers corresponding to nucleotides 94-122, 247-263 and 559-582. To isolate cDNA containing the 5' untranslated region a reverse primer GGAATTCCTTAATTTTTTCCAATTCACG-3' based on the C-terminal sequence of Der f 7 was used. The GGAATTC preceding the complementary stop codon TTA... is an EcoRI cloning site. The other primer was the λ gt11 TTGACACCAGACCAACTGG-TAATG-3' reverse. The PCR reactions were performed as described above and the product was ligated into pUC19 digested with EcoRI and transformed into *E. coli* strain TG1. Plasmid DNA was isolated for sequencing with the dideoxynucleotide chain termination method using Sequenase version 2.0. Before sequencing, the double-stranded templates were denatured with NaOH and neutralized with sodium acetate and then ethanol precipitated according to the supplier's protocol. To obtain the 5' untranslated sequences, the primer used for sequencing was ATGACGTTTGAATTTATC-3' (Df8) corresponding to the reverse of nucleotide no. 225-208.

Preparation and serological analysis of recombinant Der f 7

The construct expressing Der f 7 as fusion protein from the pGEX vector was prepared as described [9]. The polypeptide fused to glutathione S-transferase (GST) encoded by the Sj26 gene of the pGEX were affinity purified from glutathione agarose [13]. The reactivity of recombinant Der f 7 to monoclonal antibodies (MoAbs) WH9 and WH22 which were generated by immunizing with Der p 7 [11] was analysed by the enzyme-linked immunosorbent assay (ELISA) and SDS-PAGE immunoblotting as described before [11] with the antibodies at 20 μ g/mL.

Radioimmunoassay (RIA)

The presence of Der p 7 and Der f 7-specific IgE antibodies in sera of 41 children known to be allergic to mite extracts by previous skin test was analysed by RIA. Serum samples tested were added individually into wells of flexible microtitre plates (Costar Corporation, Cambridge, Mass., USA) coated with recombinant Der p 7, Der f 7 or the fusion partner GST (glutathione S-transferase) at 0.05 μ g/well. After incubation overnight at 4°C, the presence of specific IgE antibodies was detected by incubation with ¹²⁵I-labelled monoclonal anti-human IgE antibodies (monoclonal 2.1.5 available from Silenus Laboratories, Mt Hawthorn, Victoria). The radioactivity of each well was then counted in a gamma

GATCTTATATCAATAACAATCCAAAAACATATCTTACAAAATG ATG AAA TTT TTG 57
Met Met Lys Phe Leu

TTG ATT GCT GCC GTG GCA TTT GTC GCC GTT TCG GCT GAT CCA ATT 102
Leu Ile Ala Ala Val Ala Phe Val Ala Val Ser Ala Asp Pro Ile

CAC TAT GAT AAA ATC ACC GAA GAA ATC AAC AAA GCT ATT GAT GAT 147
His Tyr Asp Lys Ile Thr Glu Glu Ile Asn Lys Ala Ile Asp Asp

GCC ATT GCT GCT ATT GAA CAA TCC GAA ACA ATA GAT CCA ATG AAA 192
Ala Ile Ala Ala Ile Glu Gln Ser Glu Thr Ile Asp Pro Met Lys

GTA CCT GAT CAT GCC GAT AAA TTC GAA CGT CAT GTT GGT ATT GTG 237
Val Pro Asp His Ala Asp Lys Phe Glu Arg His Val Gly Ile Val

GAT TTC AAA GGT GAA TTA GCC ATG CGA AAC ATT GAG GCT CGA GGA 282
Asp Phe Lys Gly Glu Leu Ala Met Arg Asn Ile Glu Ala Arg Gly

TTG AAA CAA ATG AAA CGT CAA GGT GAT GCT AAT GTC AAA GGT GAA 327
Leu Lys Gln Met Lys Arg Gln Gly Asp Ala Asn Val Lys Gly Glu

GAG GGT ATT GTT AAA GCT CAT TTG TTG ATC GGT GTT CAC GAT GAT 372
Glu Gly Ile Val Lys Ala His Leu Leu Ile Gly Val His Asp Asp

ATC GTC TCG ATG GAA TAT GAT TTA GCA TAC AAA TTG GGT GAT CTT 417
Ile Val Ser Met Glu Tyr Asp Leu Ala Tyr Lys Leu Gly Asp Leu

CAT CCA ACC ACT CAT GTC ATT TCG GAT ATT CAA GAT TTT GTT GTT 462
His Pro Thr Thr His Val Ile Ser Asp Ile Gln Asp Phe Val Val

GCC TTG TCC CTT GAA ATT TCT GAT GAA GGT AAC ATA ACA ATG ACA 507
Ala Leu Ser Leu Glu Ile Ser Asp Glu Gly Asn Ile Thr Met Thr

TCT TTT GAA GTA CGA CAA TTC GCT AAT GTT GTC AAC CAT ATT GGT 552
Ser Phe Glu Val Arg Gln Phe Ala Asn Val Val Asn His Ile Gly

GGT CTT TCA ATC TTG GAT CCA ATT TTT GGC GTT TTA TCT GAT GTA 597
Gly Leu Ser Ile Leu Asp Pro Ile Phe Gly Val Leu Ser Asp Val

TTG ACC GCT ATT TTC CAA GAC ACC GTA CGT AAG GAA ATG ACC AAA 642
Leu Thr Ala Ile Phe Gln Asp Thr Val Arg Lys Glu Met Thr Lys

GTA TTG GCA CCA GCA TTT AAA CGT GAA TTG GAA AAA AAT TAACCAA 688
Val Leu Ala Pro Ala Phe Lys Arg Glu Leu Glu Lys Asn

TAGACATCATTTTCCAACGTGACAACTCTATTCTCACTGACAAATAAAATAAAATTTT 747
ATTTTTATTCTCC 761

Fig. 1. Nucleotide and inferred amino-acid sequence of Der f 7 cDNA. Numbers to the right are nucleotide positions. The first 17 amino acid residues are a putative leader sequence followed by a 196 residue polypeptide of a molecular weight of 25 348 Da starting with Asp. Underlined sequences are the Asn, Ile, Thr *N*-glycosylation site, the TAA stop codon and the AATAAA polyadenylation signal.

counter (LKB, Turku, Finland). An average of two readings from two separate determinations was calculated. The result of RIA was expressed as count index which was calculated by dividing the average reading of individual test serum by the average reading of the cord serum sample included in the same assay.

Results

Isolation of Der f 7 cDNA and sequence analysis

D. farinae cDNA ligated into λ gt11 vector was used to amplify a sequence using an oligonucleotide primer based on the predicted *N*-terminal sequence of Der p 7 and a lambda gt11 forward primer. One band of about 650 bp was obtained and ligated into M13mp18. Two clones were sequenced, both of which produced data reading from the predicted *N*-terminal primer with only one-base difference from G to T at position 235 of the nucleotide sequence of Der f 7 (Fig. 1) resulting in one amino acid change from Val to Leu. To isolate cDNA containing the 5' untranslated region of Der f 7, PCR using primers based on the *C*-terminal sequence of the newly derived Der f 7 sequence and the λ gt11 reverse primer (Df7) was performed. The PCR product was ligated into pUC19 and sequenced. A complete match of the sequence from the two directions was found in the overlap around the predicted *N*-terminal region of Der f 7. The cDNA encoded a 213 residue protein which contained a predicted 17 amino acid residue leader sequence, no cysteines and a single *N*-glycosylation site (Fig. 1). The 196 residue mature protein which was two amino acids less than that of Der p 7 from the *C*-terminal (Fig. 2) would have a predicted molecular weight of

	10	20	30	40
Der p 7	DPIHYDKITEEINKAVDEAVAAIEKSETFDPMKVPDHS DK			
Der f 7	I.D.I....Q...I.....A..			
	50	60	70	80
Der p 7	FERHIGIIDLKGELDMRNIQVRGLKQMKRVGDANVKSE DG			
Der f 7	V...V.F....A....EA.....Q.....G.E.			
	90	100	110	120
Der p 7	VVKAHLLVGVDHDDVVSMEYDLAYKLGLDHPNTHVISDIQ D			
Der f 7	I.....I.....I.....T.....			
	130	140	150	160
Der p 7	FVVELSLEVSEEGNMTLTSFEVRQFANVNVNHIGGLSILDP			
Der f 7	...A....I.D...I.M.....			
	170	180	190	
Der p 7	IFAVLSVDVLTAFQDTVRAEMTKVLAPAFKKELERNNQ			
Der f 7	..G.....K.....R...K.			

Fig. 2. Composite alignment of the amino-acid sequences of the predicted mature Der p 7 [9] and Der f 7 proteins. The symbol . is used to indicate that the amino-acid residue of Der f 7 at the position is identical to the corresponding amino-acid residue of Der p 7.

22 348 Da. No homology was found when compared with sequences of proteins on the GenBank database (83.0 release). It showed 86% amino-acid residue identity with Der p 7 shown in the alignment in Fig. 2. Many of the nucleotide differences in the two sequences occurred at the third position of the codon, often resulting in no amino acid change. Twenty out of the 28 amino acid changes were conservative. Among these conservative changes, eight were between Val (V) and Ile (I), three were between (Asp) D and (Glu) E and 2 were between (Lys) K and (Arg) R which occurred at the C-terminal of both sequences (Fig. 2). Both a potential N-glycosylation site was present in both sequences of Der p 7 and Der f 7 at predicted amino acid residues 133-135. Although a polyA tail was not detected in the cDNA clone of Der f 7, the AATAAA polyadenylation signal was still found in position close to that in sequences of Der p 7.

Characterization of recombinant Der f 7

PCR was used to produce DNA encoding a product of

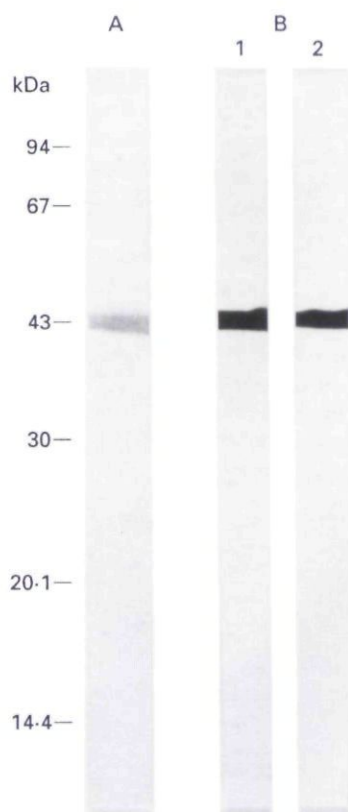


Fig. 3. SDS-PAGE-immunoblotting activity of MoAbs to the recombinant Der f 7. Panel A shows the Coomassie blue-stained SDS-PAGE protein profile of the affinity-purified Der f 7-GST fusion protein. Panel B shows the reactivity with MoAbs WH9 (B-1) and WH22 (B-2), respectively.

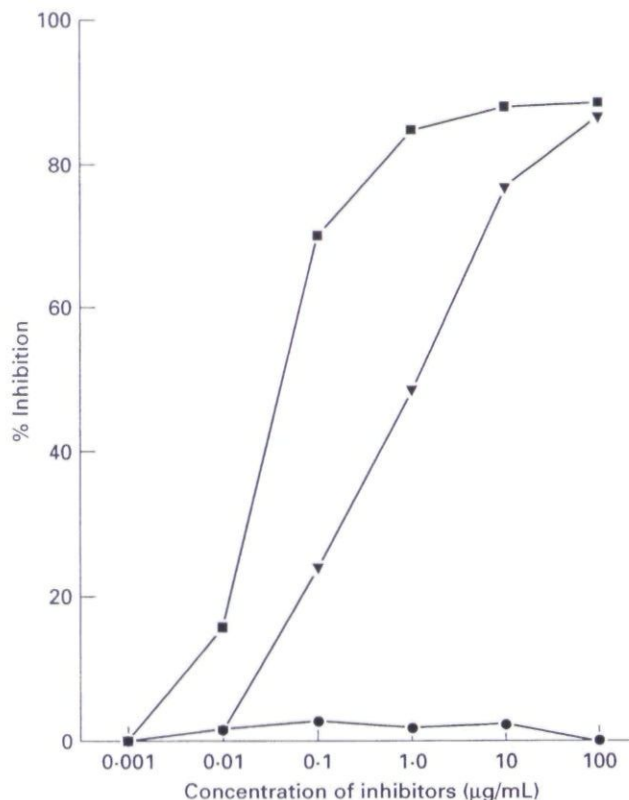


Fig. 4. Dose-dependent inhibition of ELISA activity of MoAb WH9 to recombinant Der p 7 by Der p 7-GST and Der f 7-GST fusion proteins but not by the Der p 5-GST fusion protein. The % inhibition was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{A415 without inhibitors} - \text{A451 with inhibitors}}{\text{A415 without inhibitors}} \times 100$$

Der f 7 for cloning in the expression vector pGEX. It started at the predicted N-terminal Asp encoded by nucleotide 94-96. The fusion protein from this construct was produced at a similar yield of soluble protein to the Der p 7-GST fusion protein (2 mg/L) described before [9]. In SDS-PAGE, recombinant Der f 7 showed a molecular weight of about 46 kDa (Fig. 3a). The MoAbs WH9 and WH22 which had previously been produced against Der p 7 showed a strong positive ELISA and immunoblotting activity to recombinant Der f 7 (Fig. 3b) with similar intensity to Der p 7. No colour reactivity was detected when a control MoAb P40 produced against the 68 kDa allergen of *P. notatum* was used in the same immunoblotting and ELISA (data not shown). A dose-dependent inhibition of ELISA activity of MoAb WH9 to recombinant Der p 7 by both Der p 7 and Der f 7 but not by the unrelated recombinant allergen Der p 5-GST [14] was also observed (Fig. 4).

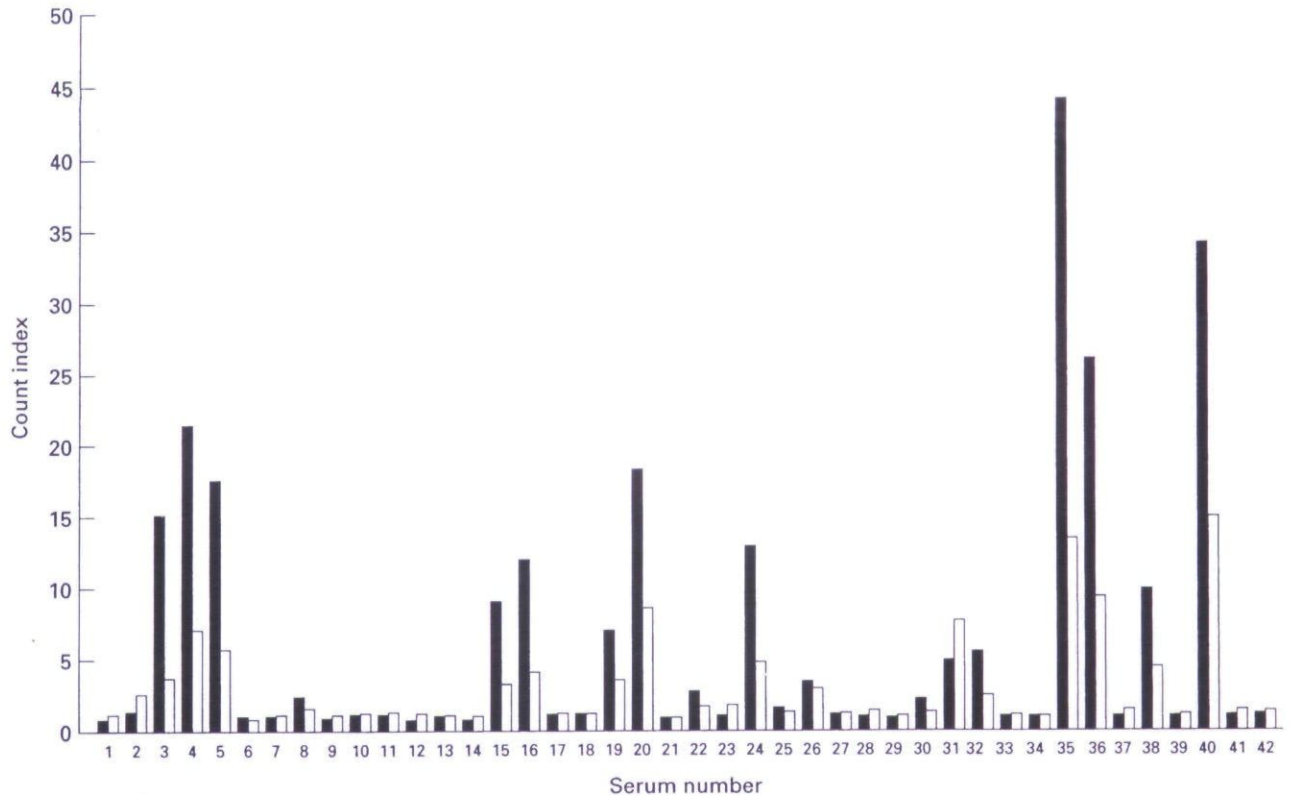


Fig. 5. The distribution of specific IgE count indices to Der p 7 and Der f 7 by 41 asthmatic sera tested. Serum no. 42 is the grass-only control. The results are expressed as an index of a cord serum control. The absolute binding activity of cord serum was 39 cpm to Der p 7 and 40 cpm to Der f 7.

In the dose response regions of the curve the homologous allergic Der p 7 was, however, 10-fold more effective.

The reactivity of IgE antibodies in sera of mite-allergic asthmatic patients to recombinant Der p 7 and Der f 7 was analysed by RIA. The binding values detected varied from an average of 39 cpm for cord serum and 47 cpm in grass-only allergic serum to 1718 cpm in mite allergic serum. The results in Fig. 5 have been expressed as an index of binding to cord sera. The value of 1.5 which was

equivalent to the mean + 3 sd of the cord value has been taken as a positive. None of the 41 sera tested showed binding to the GST fusion partner glutathione-S-transferase protein (data not shown). Seventeen (41%) showed some binding to both Der p 7 and Der f 7, respectively (Fig. 5) and 13/41 (32%) of these had indices > 5. Two samples (nos 25 and 30) showed indices ≥ 1.5 to Der p 7 only and another two samples (nos 2 and 23) showed indices ≥ 1.5 to Der f 7 only. These samples

Table 1. Oligonucleotides used for PCR amplification and sequencing

Name	Sequence	Derived from Der f 7 nucleotide positions
Df1	GCGAATTCGATCCAATTCACATGAT-3'	94–111
Df2	GGTGGCGACGACTCCTGGAGCCCG-3'	λ gt11 forward primer
Df3	GTTTTCCCAGTCACGAC-3'	M13 sequencing primer (-40)
Df4	GGTGAATTAGACATGCG-3'	247–263
Df5	TCAATCTTGGATCCAATTTTGGC-3'	559–582
Df6	GGAATTCCTTAATTTTTTCCAATTCACG-3'	684–664
Df7	TTGACACCAGACCAACTGGTAATG-3'	λ gt11 reverse primer
Df8	ATGACGTTCTGAATTTATC-3'	225–208

were, however, at the bottom of the binding scale and in general, sera with high binding activity to Der p 7 also bound Der f 7, at about one-third of the activity.

Discussion

Although mites of *D. pteronyssinus* are the most common *Dermatophagoides* species in house dust in western Europe and Australia, the species of *D. farinae* is also widespread and predominates in other countries, for example North America and Japan [12]. Sequence identities of 81% and 88%, respectively have been found between group 1 and group 2 major allergens of both mite species (Der p 1 and Der f 1, Der p 2 and Der f 2) [5,6]. However, in addition to crossreactive epitopes, there are still species-specific epitopes on corresponding allergens of both groups at T and B cell levels [7,8,17–20].

A cDNA of a newly-identified allergen of *D. pteronyssinus* (Der p 7) has been cloned, sequenced and expressed [9]. By using a PCR strategy, it has been possible to deduce the complete amino acid sequence of its *D. farinae* equivalent Der f 7 and compare it to Der p 7. The homology was 86%, which is between the 81 and 88% homology found comparing Der p 1 and Der f 1, and Der p 2 and Der f 2, respectively. Although 213 residue protein was found in Der f 7 which is 2 residues less than that of Der p 7, similar findings in both sequences are a predicted 17 amino acid residue leader sequence, no cysteine, and a single *N*-glycosylation site. Like Der p 2 and Der f 2 [6], the Der f 7 cDNA clone obtained did not have a polyA tail although the 3' non-coding region was rich in adenosine and had a polyadenylation signal. Although a primer based on the predicted *N*-terminal sequences of Der p 7 was used to clone the cDNA of Der f 7, data obtained from sequencing of PCR products using a primer based on the *C*-terminal sequence found the correct sequence of the *N*-terminal which in fact was identical probably to the 15th *N*-terminal amino acid sequence of Der p 7. The one-base difference from G to T at position 235 of the nucleotide sequences of Der f 7 found between two different cDNA clones obtained may represent sequence polymorphism of cDNA clones of Der f 7 or may be due to the copy error of PCR. However, Pfu is a high fidelity polymerase and GTG is a rare codon for *Dermatophagoides* allergens representing 2.7% of Val, and TTG is quite a common codon for mite allergens representing 64.5% of codon usage of Leu [21]. Similar to the finding in Der p 1 and Der f 1 [5], the potential *N*-glycosylation site in Der p 7 is also present at the equivalent position in Der f 7 with conservation of the first and last residues of the tripeptide site (Asn-x-Thr/Ser).

An immediate skin-test reactivity of 53% has been

detected in 30 asthmatic children using Der p 7-GST fusion protein [9]. In the present study, among 41 asthmatic sera tested, 19 (46%) showed the presence of specific IgE antibodies to Der p 7. Furthermore, 17 of the 19 serum samples also showed IgE antibodies to Der f 7. This is in accordance with findings to group 1 and group 2 major allergens of house dust mite which showed that human IgE response is usually crossreactive for both mite species [7,8]. The amount binding to Der f 7 was usually about 30% of that to Der p 7. This is consistent with the distribution of *D. pteronyssinus* which is more prevalent than *D. farinae* in northern Taiwan which is the place for collection of serum samples tested. It should be noted that the IgE binding to the group 7 is quite strong. In a separate comparison [10] it was found that the average IgE binding of responders to Der p 7 was not infrequently almost identical to the average binding of Der p 2 and the top binding values for Der p 7 (patients 35 and 40) were equal to the top values for Der p 2, although Der p 2 was recognized by more people. The allergen Der p 2 along with Der p 1 has been found to have the highest IgE-binding activity by several studies [15,16].

Both MoAbs WH9 and WH22 against Der p 7 cross-reacted with recombinant Der f 7, which contrasts with the group I allergens where only 2/53 bound to both allergens Der p 1 and Der f 1 [17]. MoAbs specific for Der f 2 but not for Der p 2 have also been reported [18]. It is, however, probable that specific Der p 7 and Der f 7 MoAbs could be found because inhibition studies showed a 10-fold difference. With the availability of the complete amino acid sequence of Der p 7 and Der f 7, it will also be feasible to attempt to map species-common and species-specific epitopes on group 7 allergens recognized by both the B and T cell compartments of the immune system.

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