

Analysis of sequence polymorphism of a major mite allergen, Der p 2

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Summary

Background The major house dust mite allergen Der p 2 has been regarded as an important allergen involved in the immunopathogenesis of allergic asthma and eczema.

Objectives To determine the degree of sequence polymorphism exists in Der p 2 at both the genomic DNA and cDNA levels.

Methods Isolation of cDNA clones was performed by screening the cDNA libraries with Der p 2 cDNA probe and the genomic sequences for Der p 2 were obtained by PCR amplification from environmental dust mites with Der p 2-specific primers. DNA sequencing was carried out by the dideoxynucleotide chain termination method. The study of Der p 2 isoforms was performed by 2-D gel immunoblot analysis using mouse anti-Der p 2 serum.

Results In this study, we have characterized the sequences of three different gene alleles coding for the major house dust mite allergen Der p 2 at the cDNA level. The translated polypeptides from these clones differed from each other by 3–4 amino-acid residues. These polymorphic residues determined were also found in Der f 2 and they were located in regions containing T-epitopes. In addition, the genomic DNA sequences of Der p 2 which were obtained by PCR amplification using the environmental mites isolated from Taiwan and Australia have helped to confirm the authenticity of the polymorphisms detected in the cDNA clones generated from CSL cultured mites. Furthermore, 2D- immunoblot analysis indicated that there were at least 10 different isoforms (p 1 values range from greater than 7.0–5.9) of Der p 2 proteins produced by CSL cultured mites.

Conclusion The results showed that there was a small but significant degree of sequence polymorphisms exists in Der p 2 gene alleles. Interestingly, the polymorphic residues were found in regions containing previously determined T-epitopes. The polymorphism data reported here will be important for the understanding of the immune responses of mite allergens as well as for the development of the peptide-based immunotherapeutic reagents for mite allergy.

Keywords: group II mite allergens, sequence polymorphism, isoforms

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Introduction

House dust mites allergy has been established as a cause of allergic asthma, rhinitis and atopic dermatitis [1]. These allergic diseases affected ≈10–20% of the world populations. In the past decade, significant progress has been made in the studies of mite allergens at both the biochemical and

molecular levels [2]. cDNA clones coding for important mite allergens such as the well-recognized major allergens Der p 1 and Der p 2 from *Dermatophagoides pteronyssinus* have been isolated and fully characterized [3,4]. Further analysis of cDNA sequences encoding one of the major allergens Der p 1 revealed that there is a significant degree of sequence polymorphism [5]. It is important to determine that the sequence polymorphism exist in the mite allergens due to the following reasons: first, a full understanding of the degree of sequence polymorphism of the allergens,

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Der p 2	(1) CACAAATTCCTCTTTCTTTCTTCTACTACTGATCATTAATCTGAAAAACAAA	49
	(2)	
	(3)-A.....T.....	
	-11	
	M M Y K I L V L	
(1)	ACCAAACAAACCATTCAAAA ATG ATG TAC AAA ATT TTG TGT CTT	92
(2)		
(3)		
	1	
	S L L V A A V A R→A D Q V D V	
(1)	TCA TTG TTG GTC GCA GCC GTT GCT CGT GAT CAA GTC GAT GTC	134
(2)		
(3)	C GC.....	
	10	
	K D C A N H E I K K V L V P	
(1)	AAA GAT TGT GCC AAT CAT GAA ATC AAA AAA GTT TTG GTA CCA	176
(2)		
(3)		
	20 30	
	G C H G S E P C I I H R G K	
(1)	GGA TGC CAT GGT TCA GAA CCA TGT ATC ATT CAT CGT GGT AAA	218
(2)		
(3)		
	40	
	P E Q L E A V→L F E A N Q N T→S	
(1)	CCA TTC CAA TTG GAA GCC GTT TTC GAA GCC AAC CAA AAC ACA	260
(2)		
(3)	T T-A.....T.....T..	
	50 60	
	K T A K I E I K A S I D G L	
(1)	AAA ACG GCT AAA ATT GAA ATC AAA GCC TCA ATC GAT GGT TTA	302
(2)	C.....T.....	
(3)	A.....T.....G	
	70	
	E V D V P G I D P N A C H Y	
(1)	GAA GTT GAT GTT CCC GGT ATC GAT CCA AAT GCA TGC CAT TAC	344
(2)		
(3)	T.....T	
	80	
	M K C P L V K G Q Q Y D I K	
(1)	ATG AAA TGC CCA TTG GTT AAA GGA CAA CAA TAT GAT ATT AAA	386
(2)	T.....	
(3)	T.....	
	90 100	
	Y T W N V P K I A P K S E N	
(1)	TAT ACA TGG AAT GTT CCG AAA ATT GCA CCA AAA TCT GAA AAT	428
(2)	A.....CG.....	
(3)		
	110	
	V V V T V K V M G D D→N G V L	
(1)	GTT GTC GTC ACT GTT AAA GTT ATG GGT GAT GAT GGT GTT TTG	470
(2)		
(3)	A.....A.....	
	120 129	
	A C A I A T H A K I→L R D	
(1)	GCC TGT GCT ATT GCT ACT CAT GCT AAA ATC CGC GAT TAAAT-	512
(2)	C.....T	
(3)	..T.....	
	560	
(1)	-----CAAACAAAATTT-A-TTGATTTTGTAAATCACAATGATTAT	
(2)	CAGCAAA.....C.....	
(3)	-----T.T.....C	
	608	
(1)	TGATTTTCTTTCCAAAAA-----TAAATAAAATTTTGG	
(2)	-----TAAATAAATAAATAAAATTTTGGAAAT	
(3)	-----	
	655	
(1)	AAAATTTTTTACTTTTTCTTTAAAAA	
(2)	AAAATTTTTTACTTTTTCTTTAAAAA	
(3)	AAAATTTTTTACTTTTTCTTTAAAAA	

Table 1. Nucleotide and amino acid changes in Der p 2 cDNA and genomic DNA clones. The table shows the codon and amino acid replacements (in parentheses) caused by the transversion mutations in the coding regions of the various Der p 2 alleles. The nucleotide changes are underlined

cDNA amino-acid residue no.	Residue with polymorphic codon						
	cDNA clone			Genomic DNA clone			
	1	2	3	a	b	c	d
–1	<u>C</u> G T (R)	<u>C</u> G T (R)	<u>G</u> C T (A)	<u>G</u> C T (A)	NA	NA	NA
40	<u>G</u> T <u>T</u> (V)	<u>G</u> T <u>T</u> (V)	<u>T</u> T <u>A</u> (L)	<u>T</u> T <u>A</u> (L)	<u>T</u> T <u>A</u> (L)	<u>G</u> T <u>T</u> (V)	<u>T</u> T <u>A</u> (L)
47	<u>A</u> C A (T)	<u>T</u> C A (S)	<u>T</u> C A (S)	<u>T</u> C A (S)	<u>T</u> C A (S)	<u>A</u> C A (T)	<u>T</u> C A (S)
111	<u>A</u> T G (M)	<u>A</u> T G (M)	<u>A</u> T G (M)	<u>T</u> T G (L)	<u>A</u> T G (M)	<u>A</u> T G (M)	<u>A</u> T G (M)
114	<u>G</u> A T (D)	<u>A</u> A T (N)	<u>A</u> A T (N)	<u>A</u> A T (N)	<u>G</u> A T (D)	<u>G</u> A T (D)	<u>G</u> A T (D)
127	<u>A</u> T C (I)	<u>C</u> T C (L)	<u>A</u> T C (I)	<u>A</u> T C (I)	<u>A</u> T C (I)	<u>A</u> T C (I)	<u>A</u> T C (I)

NA. Not available.

especially of those exist in the environmental dust mites, is essential for the development of effective therapeutic and diagnostic reagents for mite allergy; second, the various naturally occurring isoforms can serve as useful reagents for the study of immunopathogenesis of mite allergy. Besides Der p 1, the degree of sequence polymorphism existing in other important allergens and the impact of such sequence polymorphism on the immune responses of the allergens have not been investigated previously. We are now presenting the polymorphism data obtained from another major mite allergen Der p 2 which has been regarded as an important allergen involved in the immunopathogenesis of allergic asthma and eczema [6].

Materials and methods

Dermatophagoides pteronyssinus mites for cDNA library construction were purchased from Commonwealth Serum Laboratories, Parkville, Australia. Environmental mites used for polymerase chain reaction (PCR) amplification of Der p 2 genomic sequences were kindly supplied by Dr E. R. Tovey (Sydney, Australia) and Dr S.C. Tsai (Taiwan).

Screening of cDNA libraries for Der p 2 cDNA clones

The lambda gt11 and gt10 cDNA libraries were constructed as previously described [7,8]. Screening of the lambda gt10 library was performed by DNA hybridization with Der p 2

cDNA probe by a procedure previously described [8]. The probe was radiolabelled with ^{32}P by nick translation. Phages were plated at 20 000 pfu per 150 mm Petric dish and plaques were lifted onto nitrocellulose (Schleicher & Schull, Dassel, Germany) and denatured and baked. Prehybridization was performed for 2 h at 42°C in 50% formamide/5 × SSCE (with 0.01 M ethylenediamine-tetraacetic acid [EDTA])/1X Denhardt's/poly U (0.1 mg/mL). Hybridization was performed at 42°C with one million cpm of probe solution for 24 h. Washings were performed at high stringency.

Amplification of genomic DNA from environmental mites by PCR

Genomic DNA sequence for Der p 2 was obtained by polymerase chain reaction amplification from mite isolated from the house dust. *Dermatophagoides pteronyssinus* mites were identified by an acarlogist and picked under the microscope. Mite was then transferred to an Eppendorf tube containing 50 µL of 1X PCR reaction buffer (20 mM Tris-HCl, pH8.2, 10 mM KCl, 6 mM (NH₄)₂SO₄, 2 mM MgCl₂, 0.1% Triton X-100, 10 µg/mL nuclease-free (BSA) and boiled at 100°C for 10 min. The sample was cooled on ice for 10 min. The PCR reaction was performed in 1X PCR buffer (as detailed above) containing 100 µM of dNTP, 20 pmoles of each sense and antisense primers and 2.5 units of pfu DNA polymerase (Stratagene, La Jolla, CA, USA) in a final volume of 100 µL. The

Fig. 1 Nucleotide and translated amino acid sequences of Der p 2 cDNA clones. Number on the right indicates the nucleotide position. The amino acid position is indicated by number at the top of the sequence. Sequences underlined are the stop codons and polyadenylated sites.

	-17	-1	1	10	20	
Der p 2 (1)	MMYKILCLSLLVAAVARDQVDVKDCANHEIKKVLVPGCHGSEP					
(2)						
(3)	A.....					
Der f 2 (1)				N	M	D
(2)						
	30	40	50	60		
Der p 2 (1)	CIIHRGKPFQLEAVFEANQNTKTAKIEIKASIDGLEVDVPGID					
(2)	S.....					
(3)	L.....S.....					
Der f 2 (1)	T.....L.....D.....T.....L.....					
(2)						
	70	80	90	100	110	
Der p 2 (1)	PNACHYMKCPLVKGGQYDIKYTWNVPKIAPKSENVVVTVKVMG					
(2)						
(3)						
Der f 2 (1)	T.....F.....A.....LV.....					
(2)	V.....I.....					
	120					
Der p 2 (1)	DDGVLACAIATHAKIRD					
(2)	N.....L.....					
(3)	N.....					
Der f 2 (1)	N.....					
(2)	G.....					

Fig. 2 Comparison of the deduced amino acid sequences for three Der p 2 and two Der f 2 cDNA variants. Number at the top of the sequence indicates the position of amino acid residue.

primers 5'GCGGATCCATGTACAAAATTTTGTGTC 3'(P1) and 5' GCGGA ATCCGATCAAGTC GATGT-CAAA 3'(P2) corresponding to the sense strand of the signal peptide and the sequence coding for amino-acid residues 1–6 of the Der p 2 gene, respectively, were used as the sense primers. The antisense primer 5'GCGAATTCTTGTTTGATTTAATCGCG 3'(P3) was derived from the 3' end of the antisense strand of Der p 2 gene [4]. The underlined sequence represents the engineered restriction enzyme site for cloning. The reaction was heated to 95°C for 5 min, annealed at 57°C for 2 min, and polymerized at 72°C for 2 min followed by 38 cycles of amplification, each consisting of 95°C for 1 min, 57°C for 1 min, and 72°C for 2 min. The 40th cycle had the final step extended to 10 min. In order to perform the ligation of PCR product to the pCRTM vector (Invitrogene, San Diego, CA, USA), the PCR product was reamplified using AmpliTag DNA polymerase (Perkin Elmer, New Jersey, USA). The reamplification was performed in a final volume of 50 µL containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl₂, 100 µM of dNTP, 20 pmoles of each primer and 2.5 units of Taq DNA polymerase. The cycling reactions were the same as described above except that the total number of cycles performed was reduced to 35 cycles. The PCR product was purified on a 2% low melting point agarose gel (FMC SEAKEN, USA), the excised DNA

band was extracted with phenol and chloroform before use for ligation.

Preparation of DNA for molecular cloning and DNA sequencing

Phage DNA from gt10 clones was prepared by a rapid isolation procedure [3] and subcloned into M13-derived mp18 and mp19 sequencing vectors by standard methods [9,10]. The PCR-amplified genomic DNA was subcloned into the pCRTM vector according to the cloning procedure recommended in TA Cloning Kit (Invitrogene, San Diego, CA, USA). Plasmid DNA was prepared by WizardTM minipreps kit (Promega, Madison, USA). For DNA sequencing, ≈4 µg of plasmid DNA was denatured by alkaline solution (2 mol/L NaOH, 2 mmol/L EDTA) for 5 min and then neutralized with 2 µL of 2 mol/L ammonium acetate, pH 4.6 [11]. DNA sequencing was carried out by the dideoxynucleotide chain termination method [12] using the Sequenase Version 2.0 DNA sequencing kit (U.S.B., Cleveland, USA)

Two-dimensional gel electrophoresis and immunoblotting

The two dimensional (2-D) gel electrophoresis of Der p 2 was performed according to a previously published procedure [13] Der p 2 protein used for 2-D gel analysis was

purified using monoclonal antibody (MoAb 7A1 which is kindly supplied by MD Chapman, USA) affinity column from the spent mite medium (a gift from CSL, Australia) by the published method [14]. Twenty microgram of Der p 2 mixed with an equal volume of sample buffer containing 0.05 M CHES (cyclohexylamino ethane sulfonic acid pH 9.5), 2% (w/v) dithiothreitol and 10% (v/v) glycerol) was loaded on the first dimension gel which was performed at 14 000 V h in a 3.5% polyacrylamide, 8.8 M urea, 2% carrier ampholytes (pH 3–10), and 1.9% of Nonidet P–40. At the end of the isoelectric focusing step, the gel was equilibrated in a Tris buffer containing glycerol (10%), SDS (2.1%) and Bromophenol Blue, pH 6.8. The second dimension gel electrophoresis was carried out on a 13% polyacrylamide slab gel. The molecular weight of the protein was determined using the low molecular weight standards (Pharmacia, Uppsala, Sweden). The protein profile was developed by silver staining. Carbamylated creatinine kinase (BDH, Poole, UK) was used as the charge standard for calculation of isoelectric point of the resolved proteins. The immunoblotting was performed as previously described [13]. Briefly, the protein components separated by 2-D gel electrophoresis were transferred electrophoretically onto the polyvinylidene difluoride membrane (0.45 μ m, Millipore, Bedford, MA, USA). After blocking with 1% skimmed milk, the blot was incubated with mouse (C57) anti-Der p 2 polyclonal antibodies. The blot was then incubated with affinity purified horseradish peroxidase-labelled goat anti-mouse IgG (H + L) (Bio-Rad) for 1 h at room temperature. After washing, the blot was developed in a substrate solution of acetate buffer (50 mM, pH 5.0) containing 1 mM 3-amino-9-ethyl-carbazole (Sigma) and 0.015% (v/v) hydrogen peroxide for about 5 min and the red stain positive signal was recorded by photography. MoAb anti-Der p 1 (MoAb 4C1 supplied by MD Chapman, USA) was used as a negative control in the immunoassay.

Results

Sequence polymorphism of Der p 2 cDNA clones

Eight cDNA clones were isolated from the lambda gt10 library and analysed by DNA sequencing. The sequence data revealed that these clones can be divided into three distinct cDNA variants (Fig. 1). The length of the cDNA clones is 587 bp, 608 bp, and 526 bp for clones 1, 2, and 3, respectively. All clones contained the complete coding sequence for a 17-residue signal peptide and the 129-residue mature Der p 2 protein. The 5' untranslated regions of these clones were truncated at various positions, there are two point mutations and five deletions in the region between nucleotide number 17 and 68 of clone 3. There was also a significant degree of sequence diversity in the 3'

untranslated regions of these clones (Fig. 1). There were 17 nucleotide changes in the sequence coding for the mature protein, seven out of the 17 single-base substitutions are transversions which resulted in five amino-acid conversions (Table 1). The remaining 10 nucleotide changes are transition mutations which showed an excess (eight out of 10) of pyrimidine transitions, and none of these mutations causes amino acid replacement. Three additional nucleotide changes were observed in the leader sequence. The transversion of C to G and G to C at nucleotide positions 117 and 118, respectively, resulted in a change of residue arginine (R) to alanine (A) at the residue position (-1) which constitutes part of the signal peptidase cleavage site (Fig. 1). Figure 2 shows the deduced amino acid sequences of the cDNA clones 1, 2 and 3. The translated polypeptide sequence of the cDNA clone 1 (Fig. 2) is essentially identical to the previously characterized lambda gt11(C1) clone [4]. Sequence coding for the mature protein in clones 2 and 3 differed from that of clone 1 by 2 and 3 amino acids, respectively. The amino-acid changes at residues number 40, 47 and 127 are all conservative changes. However, the transition of G to A resulted in the conversion of residue aspartic acid (at position 114) which is uncharged to the negatively charged asparagine (N), resulted in a change of the polypeptide's calculated pI value from 6.6 to 7.3. Interestingly, two of the above mentioned polymorphic amino-acids (residues 40 and 114) can be found in Der f 2 [15,16].

Sequence analysis of Der p 2 genomic DNA in the environmental mites

Environmental *Dermatophagoides pteronyssinus* mites in the dust samples obtained from Australia and Taiwan were used for the PCR-amplification of Der p 2 genomic DNA and the nucleotide sequences were determined by DNA sequencing. The sequences of clones a, b and c shown in Fig. 3 represent the genomic sequences of Der p 2 obtained from Taiwanese mites by three independent PCR amplifications (two mites per amplification); clone 4 represents the Der p 2 gene sequence from Australian mites (10 mites per amplification). The sequence of clone (a) was obtained by PCR-amplification using P1 and P3 primers with a total length of 518 bp. The first 19 nucleotides were derived from the P1 sense primer. The sequences for clones (b) (c) and (d) were amplified using primers P2 and P3, and the first six amino-acid residues (residues 1–6) were primer-determined. The amino-acid residues 128 and 129 of the four clones were determined by the sequence of the antisense primer P3. The Der p 2 gene consists of a type 0 intron located between codons 8 and 9. The intron is small with its size ranges from 80 bp to 83 bp, but conforms to the 'GT-intron-AG' splice-site consensus and there were several

[illegible]

mutations in the intron. There are five residues with polymorphic codons at residue positions 40, 47, 111, 114 and 127 (Table 1). All except the polymorphic residue at position 111 (methionine [M]) to leucine [L]) correspond to those found in the cDNA clones isolated from the *Dermatophagoides pteronyssinus* cDNA library. Most of the silent mutations observed in the cDNA clones were also found in the genomic DNA clones.

2-D Immunoblot analysis

In order to address the question of Der p 2 variants at the protein level, affinity-purified natural Der p 2 proteins were subjected to two-dimensional gel electrophoresis and immunoblot analysis. About 10 isoforms of Der p 2 with pI values ranging from greater than 7.0 and 5.9 were observed (Fig. 4).

Discussion

In this study, we have examined the degree of sequence polymorphism in both the cDNA and genomic DNA of the major house dust mite allergen Der p 2. The sequence analysis of the cDNA clones isolated from the gene library constructed using the cultured mites supplied by the Commonwealth Serum Laboratory (CSL) revealed the presence of a small but significant degree of polymorphism (Table 1). Each clone differed from the others by 2–4 residues, similar to that reported previously for Der p1[5]. Unlike Der p 1, both the 5' and 3' untranslated regions of the Der p 2 cDNA clones show substantial sequence divergence indicating that there are several alleles of Der p 2 gene coding for different variants of Der p 2. The amino-acid substitutions at positions 40, 47, 114 and 127 in these Der p 2 cDNA clones were to residues found in Der f 2 of *Dermatophagoides farinae* mites (Fig. 2) suggesting that the sequence variations observed were not resulted from the replication errors that may have introduced during cDNA synthesis. It is interesting to note that all except one of the transversion mutations in the coding region of Der p 2 resulted in the amino-acid replacements (Table 1). This mutation profile is in contrast to that of Der p 1 reported previously where the five amino-acids replacements were caused by three and two transitional and transversal mutations, respectively, [5].

The frequency of polymorphism described for both Der p 1 and Der p 2 is unusual because there is a high number of

amino-acid replacements in the coding region of all three alleles of Der p 2. This is in contrast to the findings reported by Kreitman that most of the mutations in the coding regions of several alleles encoding alcohol dehydrogenase of *Drosophila melanogaster* were silent polymorphisms and that the natural selection seems to operate against coding replacement mutations [17]. In addition, it has been reported that the low frequency of amino acid replacement mutations appears to be the general feature which is common to the other eukaryotic genes [18].

To investigate the degree of sequence polymorphism of Der p 2 gene from the environmental mites, we adopted the strategy of polymerase chain reaction amplification of Der p 2 genomic DNA from two to 10 mites in the house dust samples collected from Taiwan and Australia. As shown in Fig. 3, the Der p 2 gene is divided into two exons. The first exon codes for a signal peptide and part of the mature protein. The second exon codes for the remainder of the mature protein. A small intron interrupted the coding region between codons 8 and 9. The size of the intron is smaller than the average eukaryotic intron [19], but it is of similar size to those present in the genes encoding group I mite allergens [20] and such small introns (less than 90 bp) are also commonly found in genes of *Drosophila melanogaster* [21]. All except one of the polymorphic codons observed in the coding region of these genomic clones are identical to those of the cDNA clones. This result has therefore further confirmed the authenticity of the polymorphic residues observed in the different variants of Der p 2. The amino-acid residue number 111 is a methionine (M) in all three cDNA clones as well as in three out of four of the genomic clones reported here, whereas the corresponding residue in clone (a) is a leucine (L). It is uncertain whether the point mutation is a PCR-generated artifact or a natural mutation present in the environmental but not in the CSL cultured mites. However, in view of the fact that amino-acid residue at the corresponding position in Der f 2 sequence is also polymorphic, it is plausible that the conversion of methionine (M) to leucine (L) is an authentic sequence divergence of Der p 2 in the environmental mites. We need to analyse more sequences from a greater number of mites isolated from different geographical locations in order to address the question fully. This PCR method described in this report greatly facilitated such study on the environmental mites. The 2-D gels reported here suggest that some of the amino-acid changes be responsible for variations in the pI. On the other hand, even though Der p 2 has no N-glycosylation

Fig. 3 Nucleotide and deduced amino acid sequences of Der p 2 genomic DNA obtained from the environmental mites. Intron is indicated in lower case. Nucleotide and amino acid positions are indicated by numbers on the right and top of the sequence, respectively.

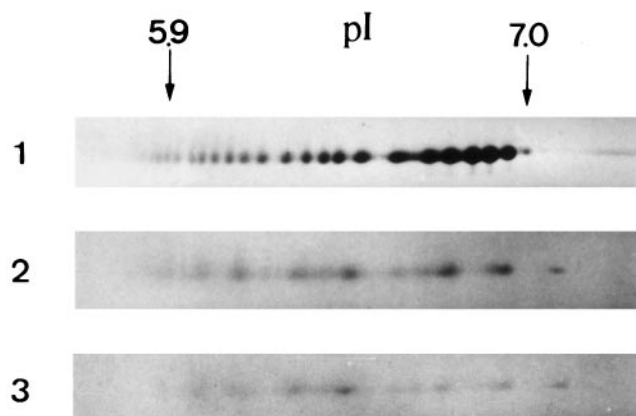


Fig. 4 2D-immunoblot analysis of mouse polyclonal anti-Der p 2 antibodies to the purified natural Der p 2. Panel 1 shows the silver-stained carbamylated creatine kinase (CPK) charge standard. The relative pI values corresponding to spots numbered 1–20 are assigned as 7.0 and 5.9, respectively, according to supplier (BDH, Poole, UK). Panel 2 shows the silver-stained 2D protein profile of the purified Der p 2. At least 10 different isoforms with pI values ranging from greater than 7.0–5.9 can be detected. Panel 3 shows the immunoblot reactivity of mouse polyclonal antibodies to these different isoforms of Der p 2.

sites and a molecular weight corresponding well to that determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the possibility of o-glycosylation cannot be excluded.

It is intriguing that the polymorphisms of Der p 2 alleles defined in this study were found in the regions containing epitopes recognized by T cells [22–24]. This has prompted us to perform studies to investigate the possible immunological impacts of the various polymorphic residues on the T and B epitopes of Der p 2 recognized by *Dermatophagoides pteronyssinus* sensitive asthmatic patients. Our unpublished data clearly demonstrated that the defined sequence polymorphisms of Der p 2 exhibit a profound influence on both the T and B epitopes recognized by the allergic patients (manuscripts in preparation). In summary, our studies performed at both the gene and protein levels have demonstrated that there are several variants of Der p 2 present in *Dermatophagoides pteronyssinus* mites. These polymorphism data are potentially important for the design and development of effective immunotherapeutic reagent for the treatment of mite allergy.

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