

Protein sequence analysis of a novel 103-kDa *Dermatophagoides pteronyssinus* mite allergen and prevalence of serum immunoglobulin E reactivity to rDer p 11 in allergic adult patients

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Summary

Background House dust mites are regarded as important indoor allergens. While the most studies mite allergens are low molecular weight (mw), a high mw *Dermatophagoides farinae* mite paramyosin (Der f 11) has recently been cloned. We have also cloned a novel high mw *Dermatophagoides pteronyssinus* (Dp) mite allergen, Der p 11.

Objective The aim of this study was to isolate and express a cDNA gene coding for a Der p 11 allergen, to compare the sequence of Der p 11 with other antigens and to evaluate the presence of IgE reactivity to the recombinant protein (rDer p 11) in the sera of allergic adult patients.

Methods The full-length Der p 11 gene was isolated by cDNA library screening, 5'-3' rapid amplification of cDNA ends and PCR. The cDNA gene was expressed as a glutathione-S-transferase fusion protein in *Escherichia coli*. The allergenicity of rDer p 11 was tested by human IgE immunodot or immunoblot assay in a large panel of 100 allergic patients with bronchial asthma, allergic rhinitis or eczema.

Results Der p 11 is a 2965 bp cDNA gene with a 2625 bp open reading frame coding for a 875 amino acid protein. The deduced amino acid sequence of the Der p 11 showed significant homology with various invertebrate paramyosins. The prevalence of serum IgE reactivity to rDer p 11 on immunodot assay ranged from 41.7% to 66.7% in different allergic patient groups, whereas it was rare in non-atopic patients with urticaria (18.8%) and in normal individuals (8%). A high frequency (five out of eight) of MAST(Dp)⁺ allergic serum samples had specific IgE-binding activity to rDer p 11 or its fragments on immunoblot assay, even though their IgE-binding activity to Dp extract was either weak or negative.

Conclusion The 103-kDa Der p 11 appears to be major Dp mite allergen with a high frequency of IgE reactivity in sera of patients allergic to mites.

Keywords cDNA clone, *Dermatophagoides pteronyssinus*, mite allergen, paramyosin

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Introduction

Allergens derived from the dust mite *Dermatophagoides pteronyssinus* (Dp) and *D. farinae* (Df) have been recognized as the most important allergens in bronchial asthma (BA) and other allergic disease worldwide [1]. Since the first mite allergen (antigen P1) was identified in 1980 [2], major mite allergens have been studied intensively. To date, at least 19 groups of designated mite allergens have been characterized [3]. However, the great majority is of a relatively low molecular weight (mw) of 14–60 kDa or glycoproteins with characteristic structures and biochemical properties [3, 4]. Several high mw mite allergens such as Der f 11 and M177

from Df [5–7] and Blo t 11 from *Blomia tropicalis* [8] (antigenic in humans), and Der f 15 (antigenic in dogs) [9] have been cloned and identified. In a previous investigation, we identified an affinity-column purified 98-kDa paramyosin-like native protein (Df642), now designated Der f 11 [5, 6]. Its recombinant protein (rDer f 11) had frequent, strong allergic serum IgE-binding reactivity [10, 11]. Allergens from closely related mite species (such as Dp and Df), as well as other invertebrate species, frequently exhibit a high degree of IgE cross-reactivity [12–14]. Therefore, we used a specific monoclonal antibody (mAb642) against native Der f 11 to screen the Dp cDNA library for similar proteins and isolated a novel high mw Dp mite allergen. The Dp protein recognized by mAb642 is, therefore, quite likely to be an allergen similar to Der f 11. According to the WHO/IUIS allergen nomenclature subcommittee [15], we proposed that this novel paramyosin-like molecule designated as group 11 mite allergen, Der p 11. In this study, we developed a recombinant form of this newly

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recognized Dp mite allergen, sequenced it, and investigated the prevalence of serum IgE reactivity to the recombinant protein (rDer p 11) in allergic adult sera.

Materials and methods

Mite extracts

Dry Dp and Df (Allergon Inc., Angelholm, Sweden) were extracted with 0.01 M cold phosphate-buffered saline (PBS, pH 7.2) at a ratio of 1:100 (g/mL) as previously described [16]. In brief, the extract was dialysed and freeze-dried in aliquots for storage at -20°C . The protein content was determined with a Bradford protein assay kit (Bio-Rad, Richmond, CA, USA). Bovine γ -globulin and serum albumin were used as protein standards.

Preparation of rDer f 11

rDer f 11 was produced according to the method described previously [6]. In brief, a Df cDNA library was constructed in a lambda ZAPII vector and immunoscreened with a monoclonal antibody mAb642 [5]. The cDNA insert was subcloned into an M13 sequencing vector for single-stranded sequencing. The entire cDNA insert was expressed in an *Escherichia coli* system as a fusion protein with glutathione-S-transferase (GST). The protein content was also determined as previously described in a mite extract.

Subjects

Human allergic sera were obtained from atopic adults, 15–85 years of age, who attended the Allergy Clinic of Mackay Memorial Hospital (Taipei, Taiwan). Only patients with a history of moderate to severe allergic symptoms were included in this study. Normal sera were obtained from healthy volunteers. The MAST CLA Allergen-Specific IgE Assay (MAST Immunosystems, Inc., Mountain View, CA, USA) was employed to run serum-specific IgE antibody titre against Dp mite extracts. Positive and negative MAST reactions to Dp mite extract were denoted by MAST(Dp)⁺ and MAST(Dp)[−], respectively. The status of the subjects is

shown in Table 1. One hundred atopic subjects (31 men and 69 women) with BA, allergic rhinitis (AR) or eczema, 16 non-atopic individuals (three men and 13 women) with urticaria, and 25 healthy volunteers (eight men and 17 women) were included.

Immunoblot

Immunoblot was performed as previously described [17]. Briefly, Dp or Df extracts (5 $\mu\text{g}/\text{lane}$), rDer p 11-GST (or rDer p 11) (3 $\mu\text{g}/\text{lane}$), rDer f 11-GST (or rDer f 11), GST (3 $\mu\text{g}/\text{lane}$) and mw markers (Bio-Rad) were separated on 12.5% SDS-PAGE and electrotransferred onto the polyvinylidene fluoride (PVDF) membrane (Millipore Corp., Bedford, MA, USA) with a semidry blotting apparatus, followed by blocking with 5% skim milk. After blocking, blots were washed with PBS containing 0.1% Tween-20 (PBS-T) and then sequentially incubated with diluted patient sera (1:4) for 16 h at 4°C and diluted horseradish peroxidase (HRP)-conjugated rabbit anti-human IgE antibodies (1:1500) (DAKO Corp., Carpinteria, CA, USA) for 5 h at room temperature. Blots were extensively washed with PBS-T after each treatment step throughout the immunoassay protocol. They were then incubated with ECL reagent (Amersham, Pharmacia Biotech Co., Uppsala, Sweden) for 2 min and exposed to X-ray films for 5–30 s at room temperature.

Immunodot

The immunodot assay for IgE was performed according to a protocol previously described [10]. Briefly, rDer p 11, rDer f 11, GST (2 $\mu\text{g}/\text{dot}$) and Dp extract (3 $\mu\text{g}/\text{dot}$) were spotted on a PVDF membrane (Millipore Corp.). Each assay was performed twice, and GST and Dp extract were used as negative and positive controls, respectively. rDer f 11 was used as positive control only to compare with rDer p 11. The subsequent procedures for immunostaining of the dot blot were performed as described in the immunoblot assay. The intensity of the IgE-reacting dot was determined by transmission densitometry (ImageQuaNT Analyzer; Molecular Dynamics Inc., Sunnyvale, CA, USA) with ImageQuaNT software according to the user's guide. The mean

Table 1. Frequency of specific serum IgE reactivity to rDer p 11, Dp mite crude extract and GST by immunodot assay and commercial MAST analysis in allergic patients

Patient	Number	Age (mean)	Male/female	MAST ⁺ (%)	Positive IgE reactivity (%) [*]		
					Dp extract	rDer p 11	GST
Bronchial asthma	21	15–85 (53.9)	6/15	13 (61.9)	10 (47.6)	14 (66.7)	1 (4.8)
Allergic rhinitis	30	15–81 (40.3)	12/18	14 (46.7)	15 (50)	13 (43.3)	2 (6.7)
Asthma + rhinitis	25	15–83 (44.8)	6/19	16 (64)	13 (52)	13 (52)	2 (8)
Eczema	24	21–63 (43.2)	7/17	4 (16.6)	7 (29.2)	10 (41.7)	1 (4.2)
Urticaria	16	21–69 (42.1)	3/13	2 (12.5)	1 (6.3)	3 (18.8)	1 (6.3)
Healthy volunteer	25	22–71 (49.7)	8/17	2 (8)	3 (12)	2 (8)	1 (4)

^{*}IgE-binding reactivity of allergic patient and healthy sera to Dp extract (3 $\mu\text{g}/\text{dot}$), rDer p 11 and GST (2 $\mu\text{g}/\text{dot}$) were analysed with immunodot assay. Dp extract and GST were used as positive and negative controls, respectively. Samples were spotted on a PVDF membrane with negative pressure section. The subsequent procedures for immunostaining of the dot blot were performed as described in the immunoblot assay. The intensity of the IgE-reacting dot was determined by transmission densitometry (ImageQuaNT Analyzer). The mean of the controls plus 2 SD was used as a cut-off value for a positive reaction. The MAST CLA Allergen-Specific IgE Assay (MAST Immunosystem) was employed to run serum-specific IgE antibody titre against Dp mite extracts. GST, glutathione-S-transferase; PVDF, polyvinylidene fluoride; Dp, *Dermatophagoides pteronyssinus*.

of the controls plus 2SD was used as a cut-off value for the positive reaction.

Cloning of Der p 11 cDNA

Breeding Dp mites were kindly provided by Prof. E. L. Hsu (Department and Graduate Institute of Entomology, National Taiwan University, Taipei, Taiwan). The mites were bred at 25 °C and 75% humidity in 15-cm Petri dishes containing yeast extract and fish powder as a culture medium. Preparation of a Dp cDNA library was similar to that of Df cDNA library previously described [6]. In brief, total RNA from 1 g of live mites was isolated using RNeasyTM B kit (Qiagen Inc., Houston, TX, USA). A cDNA library was constructed by a standard method using lambda gt11 expression vector (Stratagene, La Jolla, CA, USA). MAb642 was used for plaque immunoscreening of the Dp cDNA library [18]. Phages were plated out with a Y1090 *E. coli* host on a 150-mm Petri dish containing broth medium supplemented with 50 µg/mL of ampicillin. After 6 h of incubation at 37 °C, the plate was overlaid with a nitrocellulose filter previously saturated with 10 mM IPTG (isopropyl β-D-thiogalactopyranoside) to induce antigen expression. The cDNAs of positive clones were primed with λgt11F (5'-TTGACAC CAGACCAACTGGTAATG-3') and λgt11R (5'-GGTGG CGACGACTCCTGGAGCCCG-3') as primers using *Tag* DNA polymerase. The PCR products were purified from 2% agarose gel with QIAquick gel extraction kit (Qiagen Inc., Valencia, CA, USA), ligated to pCRII-TOPO vector (3.9 kb) using the TOPO TA cloning kit (Invitrogen Co., Carlsbad, CA, USA) and subsequently transformed into the TOP10 competent *E. coli*. The plasmid DNA was analysed by restriction analysis (with *EcoRI*) or sequencing.

5'-3' RACE reaction to screen full-length cDNA sequence of Der p 11

The full-length cDNA of Der p11 was obtained by cDNA library immunoscreening, 5'-3' rapid amplification of cDNA ends (RACE, Gibco BRL Co., Gaithersburg, MD, USA), and PCR. For the 3'RACE reaction, oligo dT primed cDNA was obtained by reverse transcription of 1 µg of total Dp RNA with 25 pmol 3'RACE adapter primer (AP, 5'-GGCCACGCGTCGACTAGTACT-(dT)₁₆-3') and 200 U of SuperScriptTMII reverse transcriptase (Invitrogen Co.) in a 25 µL reaction volume for 1 h at 37 °C. The sequence of the abridged universal amplification primers (AUAP, 5'-GGCCACGCGTCGACTAGTACT-3') is a part of primer AP. The cDNA obtained was used as a PCR template with primers AUAP and in subsequent steps with the gene-specific primers 3'RACE-1 (5'-CAGGCTCTACAAATTACGGA-3'), 3'RACE-2 (5'-CCGATGAACGAGTACAGAAAC-3') and 3'RACE-3 (5'-GATCATCGTGTCAAAGAATTGTTG-3') according to the internal sequence of Der p 11. PCR was performed in a final volume of 50 µL using 2 µL of the oligo dT primed cDNA, 1 µL of 10 µM AUAP, 1 µL gene-specific primer, 2.5 µL of 4 mM dNTP, 5 µL 10 × buffer, 4 µL of 25 mM MgSO₄, 0.5 µL *Taq* DNA polymerase and 34 µL of ddH₂O. Amplification was performed over 35 cycles, at 94 °C for 30 s, 55 °C for 1 min and 72 °C for 3 min. The PCR product was purified with QIAquick PCR purification kit

(Qiagen Inc.), ligated into pCRII-TOPO vector (3.9 kb) using the TOPO TA cloning kit (Invitrogen Co.) and subsequently transformed into the TOPO competent *E. coli*. The plasmid DNA was purified and analysed by DNA sequencing.

In the 5'-RACE reaction, the template cDNA was synthesized by Moloney murine leukaemia virus (MMLV) reverse transcriptase using 1 µg of total Dp RNA and 2.5 pmol of gene-specific primer (5'RACE-1: 5'-TTCATCG GCATGTGCTTGC-3') according to the Der p 11 internal sequence at 42 °C for 45 min. After the reaction, reverse transcriptase was heat inactivated at 70 °C for 15 min. Afterward, RNA was degraded by adding of 2 U of RNase H and incubating the reaction at 55 °C for 10 min. The cDNA was purified by ethanol precipitation. Finally, 10 µL of the resulting single-stranded cDNA was tailed in 25 µL 1 × TdT buffer containing 200 µM dCTP and 10 U of terminal deoxynucleotidyl transferase (Gibco BRL) for 15 min at 37 °C. After heat inactivation for 10 min at 70 °C, the tailed cDNA was used for PCR. Primer 5'RACE-1 (5'-TTCATCG GCATGTGCTTGC-3'), 5'RACE-2 (5'-TCTTCTTCCAA ACGATGGCGAG-3'), 5'RACE-3 (5'-TGCATTTTCGTG TACGGCAG-3') and 5'RACE-4 (5'-CACCAGTGGATCG GTACAT-3') according to the internal sequence of Der p 11 and 5'RACE abridged anchor primer (AAP, 5'-GGCCAC GCGTCGACTAGTACGGGIIIGGGIIGGGIIG-3') were used in nested PCR. A 50 µL PCR reaction mixture composed of 10 µL 5'-RACE-ready cDNA, 1 µL of 2.5 pmol AAP primer, 1 µL of 25 pmol gene-specific primer, 2.5 µL of 4 mM dNTP, 15 µL 10 × buffer, 0.5 µL *Taq* DNA polymerase and 20 µL of ddH₂O. Amplification was performed as described above. The product from PCR was purified, subcloned, transformed and analysed as described above. The complete nucleotide of Der p 11 was then determined.

Construction of plasmid encoding Der p 11 cDNA

The expression vector in *E. coli* (TOP10) encoding cDNA for Der p 11 fused to a GST gene was constructed by insertion of the PCR-amplified cDNA fragment of 2628 bp (including stop codon) generated by digestion with *Bam*HI and *Sma*I to the pGEX-4T-1 expression vector (Amersham, Pharmacia Biotech Co.) [19].

Purification of recombinant Der p 11-GST and rDer p 11

Production and purification of the recombinant protein was prepared using the protocol previously described [20]. In brief, *E. coli* TOP10 containing pGEX-Dp 11 was grown overnight in Luria broth containing 50 µg/mL of ampicillin to log phase and then induced with 1 mM/L of IPTG. After cell harvesting, cell lysate was prepared by sonification and protein purification was performed with a one-step method using glutathione-affinity column chromatography (Amersham, Pharmacia Biotech Co.) to isolate the GST-fusion protein. Recombinant Der p 11-GST was eluted with 20 mM reduced form glutathione containing 50 mM Tris-HCl (pH 8.0). When cleaving a GST-fusion protein bound to glutathione sepharose, each millilitre of glutathione sepharose bed volume mix 100 µL of resuspended Thrombin Protease (Amersham Biosciences Co., Bucks, UK) and 950 µL of 1 × PBS. Gently shake the mixture at 4 °C overnight and collect the

reaction buffer that contain cleaved fusion protein (denoted rDer p 11 or rDer f 11). Purity and specificity of the recombinant protein were analysed with Western blotting and immunoblot analysis. The protein contents of rDer p 11 or rDer p 11-GST were also determined with a Bradford protein assay kit.

Protein sequence analysis

Homologues to the deduced amino acid sequence of Der p 11 were searched for in protein and gene databases by using the BLAST program. Sequence analysis and alignment were scanned with the SeqWeb Version 1.2 for use with the Wisconsin Package Version 10.1.

Results

Analysis of cDNA sequence

Twenty-two positive plaques were obtained after screening approximately 1×10^6 plaque-forming units from the Dp cDNA library with mAb642. Five of these plaques were purified to homogeneity by repeated screening. Nucleotide

sequence analysis showed that the five clones are related but have different degrees of truncation on the 5' and 3' ends. However, the nucleotide sequence alignment of the five clones exhibited significant homology to the Der f 11 nucleotide sequence. The longest clone (Dp23) had 1215 bp with a sequence from 897 to 2111 (data not shown). Even after several rounds of cDNA library screening, we still failed to isolate the full-length Der p 11 gene. The 5'- and 3'-RACE were further used step-by-step to isolate the 5' and 3' ends of Der p 11 gene using a gene-specific primer based on the nucleotide sequence of the truncated clone Dp23.

Briefly, the full-length Der p 11 cDNA gene was isolated by PCR using gene-specific primers based on the sequence of the clone Dp23 and the 5'- and 3'-RACE products. Der p 11 is a 2965 bp cDNA gene with a 2625 bp open reading frame coding for a 875 amino acid protein, containing 64% TA and 36% GC. The N-terminal ATG codon was located at nucleotide positions 182–184 bp, and no further upstream ATG was found (Fig. 1a). There was a typical polyadenylated site (TAA at nucleotide positions 2807–2809 bp) (Fig. 1a) at the C-terminal end of the cDNA insert, suggesting that Der p 11 is a full-length cDNA clone. The full 2628 bp sequence of Der p 11 cDNA was 550 bp longer than the incomplete

(a)

0001	a	ata	cga	tta	ttg	tgt	cag	ttg	ttg	ttt	gat	tgt	tgg	ttg	gtc	ttt	tgc	gtt	gtt
0056		ctg	ttt	ggt	aat	att	att	ttt	ttc	tcg	ttt	aaa	tat	cat	tca	tcg	att	ttg	tat
0110		aat	cca	tat	aca	acg	cga	tta	ttt	tca	caa	aca	aaa	aaa	acg	atc	caa	ttc	gaa
0164		aac	aga	taa	aga	gta	aca	atg	tcg	gca	cgt	aca	gca	aaa	tat	atg	tac	cga	tcc
0218		agt	ggt	gct	ggt	gct	tcg	ggt	gat	att	tcc	gtt	gaa	tat	ggt	acc	gat	tta	ggt
0272		gcc	tta	aca	cga	ctt	gag	gac	aaa	atc	cga	cta	tta	tcc	gat	gat	ctg	gaa	tcc
0326		gaa	cgc	gaa	atg	cga	caa	cgc	att	gaa	cgt	gaa	aag	gcc	gaa	tta	cag	atc	cag
0380		gta	atg	agc	cta	agt	gaa	cgt	ttg	gaa	gag	gct	gag	gga	tca	agc	gaa	agt	ggt
0434		acc	gaa	atg	aac	aaa	aaa	aga	gat	tca	gaa	ttg	gca	aaa	tta	aga	aaa	tta	ttg
0488		gaa	gat	ggt	cat	atg	gaa	tcg	gaa	gaa	act	gcc	cat	cat	tta	cga	caa	aaa	cat
0542		caa	gct	gcc	gta	cac	gaa	atg	caa	gat	caa	ttg	gat	caa	tta	caa	aaa	gca	aaa
0596		aat	aaa	tcg	gat	aaa	gaa	aag	caa	aaa	ttt	caa	gca	gaa	ggt	ttt	gaa	tta	tta
0650		tca	caa	ttg	gaa	aca	gct	aat	aaa	gaa	aaa	tta	aca	gca	ttg	aaa	agt	gtc	gaa
0704		Aaa	ttg	gaa	tac	act	gtc	cat	gaa	ttg	aat	att	aaa	att	gaa	gaa	atc	aat	cgt
0758		aca	gtt	att	gaa	tta	aca	tca	cat	aaa	caa	cgt	ttg	aca	caa	gag	aac	act	gaa
0812		ttg	atc	aaa	gaa	gtt	cat	gaa	gtt	aaa	tta	caa	ttg	gat	aat	gct	aat	cat	ttg
0866		aaa	caa	cag	att	gcc	caa	caa	ttg	gaa	gat	act	cgc	cat	cgt	ttg	gaa	gaa	gaa
0920		gaa	cga	aaa	cgt	tcc	agc	ctc	gag	aat	cat	gcc	cat	aca	ttg	gag	gtt	gaa	tta
0974		gaa	tca	ttg	aaa	gtg	caa	ttg	gat	gat	gaa	tct	gaa	gca	cgt	tta	gaa	ttg	gaa
1028		cgt	caa	ttg	acc	aaa	gct	aat	ggt	gat	gct	gca	tca	tgg	aaa	tca	aaa	tat	gaa
1082		gct	gaa	ttg	caa	gca	cat	gcc	gat	gaa	gtt	gag	gaa	ctt	cgt	cgc	aaa	atg	gct
1136		caa	aag	att	tct	gaa	tac	gaa	gaa	caa	llg	gaa	gct	tta	llg	aac	aaa	tgt	agt
1190		tca	ttg	gaa	aaa	caa	aaa	tcc	cgt	ctt	caa	agt	gaa	gtt	gaa	gta	ttg	att	atg
1244		gat	ttg	gaa	aaa	gca	gca	cat	gca	caa	caa	ttg	gaa	aaa	cgt	gtc	gca	caa	caa
1298		ttg	gaa	aag	att	aat	ttg	gat	ttg	aaa	gat	aaa	ttg	gaa	gaa	gtt	acc	att	ttg
1352		atg	gaa	caa	gca	cag	aaa	gaa	ctt	cga	atc	aaa	att	ggt	gaa	ttg	caa	aaa	ttg
1406		caa	cat	gaa	tat	gaa	aaa	gct	cgc	gat	caa	cgc	gat	caa	ttg	gca	cga	gaa	aac
1460		aaa	aaa	ctt	acc	gaa	gat	ctt	gct	gaa	gct	aaa	tca	caa	ttg	aac	gat	cat	cat
1514		cgt	aga	gct	cat	gaa	caa	gaa	att	gaa	atc	aaa	cga	ttg	gaa	aat	gaa	cgt	gat
1568		gaa	ttg	tcg	gct	gca	tac	aaa	gaa	gct	gaa	aca	tta	cga	aaa	caa	tta	gag	gct
1622		aaa	aat	caa	aga	ttg	att	gct	gaa	ttg	gca	cag	tta	cga	cat	gat	tat	gaa	aaa
1676		cgt	ttg	gca	caa	aaa	gat	gaa	gaa	att	gaa	gca	ttg	cgc	aaa	caa	tat	caa	att
1730		gaa	att	gaa	caa	ttg	aat	atg	cgt	ttg	gct	gaa	gct	gaa	gc	aaa	ttg	aaa	aca
1784		gaa	att	gca	cga	tta	aag	aaa	aaa	tat	caa	gca	caa	att	act	gaa	ttg	gaa	tta
1838		tca	ttg	gat	gcc	gct	aat	aaa	gct	aat	att	gat	tta	cag	aag	acg	att	aaa	aaa
1892		cag	gct	cta	caa	att	acg	gaa	tta	caa	gcc	cat	tat	gat	gaa	gta	cat	cgt	caa
1946		ttg	caa	caa	gct	gta	gat	caa	tta	ggt	gtt	aca	caa	cga	aga	tgt	caa	gaa	ttg
2000		caa	gcc	gaa	ttg	gaa	gag	atg	cgc	atc	gca	llg	gaa	cag	gct	agt	cgt	gct	aaa
2054		cga	caa	gct	gaa	caa	tta	cat	gaa	gaa	gct	gtt	gta	cgt	gtt	aat	gaa	ttg	aca
2108		aca	atc	aat	gtt	aat	cta	gca	tca	gct	aaa	agt	aaa	ttg	gaa	tct	gaa	ttc	tct
2162		gca	ctt	caa	gct	gat	tat	gat	gaa	gta	cat	aaa	gaa	ttg	aga	att	tcc	gat	gaa
2216		cga	gta	cag	aaa	ctt	aca	att	gaa	ctt	aaa	tca	aca	aaa	gat	tta	ttg	att	gaa
2270		gaa	caa	gaa	cga	ttg	gta	aaa	ttg	gaa	aca	gtg	aaa	aaa	tca	ttg	gaa	caa	gaa
2324		gta	cgt	aca	tta	cat	gtt	cgt	att	gaa	gaa	gtt	gaa	gct	aat	gct	ttg	gcc	ggt
2378		ggt	aaa	cgt	gtt	att	gct	aaa	ttg	gaa	agc	cgt	att	cgt	gat	gtt	gaa	att	gaa
2432		gtt	gag	gaa	gaa	cga	cga	cga	cat	gct	gaa	acg	gaa	aaa	atg	tta	cgt	aaa	aaa
2486		gat	cat	cgt	gtc	aaa	gaa	ttg	ttg	tta	caa	aat	gag	gag	gac	cat	aaa	caa	att
2540		caa	llg	tta	cag	gaa	atg	act	gat	aaa	llg	aat	gaa	aag	glt	aaa	glt	tat	aaa
2594		cga	caa	atg	caa	gaa	cag	gag	gga	atg	agc	caa	caa	aat	ttg	aca	cgt	gtc	aga
2648		cga	ttc	caa	cgt	gaa	ttg	gaa	gca	gcc	gaa	gat	cgt	gcc	gat	caa	gct	gaa	tcg
2702		aat	tta	tcg	ttt	atc	cgt	gct	aaa	cat	cgt	tca	tgg	gta	aca	aca	agc	gag	gtt
2756		cca	ggc	ggc	acc	cgt	caa	gtg	ttc	aca	acg	caa	gaa	gaa	aca	acc	aat	tat	taa
2810		tgt	tcg	aaa	atc	cgt	gcc	tat	acg	gcc	att	gct	aca	ttc	ctt	ggt	cca	att	cat
2864		aat	gac	gga	atc	ggc	gct	tac	gat	caa	tgg	aat	gga	tta	agt	aaa	aat	aaa	aaa
2918		att	caa	aat	gaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	2965

Fig. 1. The nucleotide (a) and deduced amino acid (b) sequence of rDer p 11. The numbers label the positions of the nucleotides and amino acid residues (in parentheses). An initiation (ATG) and a stop codon (TAA) are located at nucleotide positions 182–184 and 2807–2809, respectively. These two codons are shown with a double underline (a). Amino acid sequence homology between Der p 11 and other invertebrate species was compared (b). Identical residues are shown by (-). The predicted leucine zipper motifs are shown in bold letters. Four predicted N-linked glycosylation sites indicated by bold asterisks. The Der p 11, Der f 11, *Caenorhabditis elegans*, *Dirofilaria immitis* and *Drosophila melanogaster* sequence data are available in the GenBank sequence database under accession numbers AY189697, AF352244, T19296, A44972 and S22028, respectively.

(b)

	(1)		(70)
Der p 11	MSARTA KYMRSSGAG .ASGDISVEY GTDLGALTRL EDKIRLLSDD LESEREMRQR IEREKALQI		
Der f 11			
Blo t 11	-A--S- ---Q--R-- .HG----I-- -----E- ----L--- V----SDITV		
D.melanogaster	MSSSQAV-SS ---S--ATST- PGTA-VNI-- IQ--SS-S-- ---Q-- --V--L-- I----D-SV		
C.elegans	MSLYRSPS AALLK-PSQA AFGAPFGSMS VA---S-----QE- ----L-N- V---R-D-SV		
D.immitis	AFGSMS VA---S-----QE- ----L-N- V---R-D-SV		
	(71)		(140)
Der p 11	QVMSLSERLE EABGSSESVT EMNKKRDSLE AKLRKLEEDV HMESEETAH LRQKHQAAPHV EMQDQLDQLQ		
Der f 11			
Blo t 11	-L-N-T--- -T----- -I----- -IQ----- -V-		
D.melanogaster	--IQM----- --GA-HQP -A-R--A-- L-----TLL -KK--NEIIT DF-E-VEI-T		
C.elegans	--IA-TD--- D---TTD-QI -S-R--EG-- S-----ES QL---DAMNV --K---DSCL DY---IE---		
D.immitis	-LIA-TD--- D---TTD-QI -S-R--EA-- Q-----ES QL-N-DAMNV L-K---D-CL DYAE-IE---		
	(141)****		(210)****
Der p 11	KAKNKSDKEK QKQPAEVFEL LSQLETANKE KLTALKSVEK LEYTVHELNI KIEEINRTVI ELTSHKQRLT		
Der f 11			
Blo t 11	-----A----- -N----- -V-----V -V-A-R---S		
D.melanogaster	-N-ARAE-D- A---T--Y-- --I-SY-- --IVSE-HIS- --VSI--V ---L---DIS--RS--S		
C.elegans	-KNA-I-R-R -RV-H--I-- TATIDQLQ-D -H--E-AA-R F-AQAN--AN -V-DL-KH-N D-AQQR---Q		
D.immitis	-KNS-I-R-R -RL-H--I-- TATIDQLQ-D -HL-E-AA-R F-AQTI--SN -V-DL--H-N D-AQQR---Q		
	(211)		(280)
Der p 11	QENTELIKEV HEVKLQLDNA NHLKQQAQQ LEDTRHRLKE EERKRSSLEN HAHTLEVELE SLKVQLDDDES		
Der f 11			
Blo t 11	---S----- --Y-IS--- --G-----D----- -EE--		
D.melanogaster	--I---D- QDL-V---TV SFS-S-VIS- --A-R--D -D-R--L--S SL-QV-I--D -VRTN--EE--		
C.elegans	A--ND-L-- -DQ-V---L Q-V-YTL-- --EA-R--D A--E--Q-QS QL-QVQL--D -VRTA--E--		
D.immitis	A--ND-L--I -DQ-V---L Q-V-Y-L-- L-EA-R--D A--E--Q-QA QL-QVQL--D -VRTA--E--		
	(281)		(350)
Der p 11	EARLELERQL TKANGDAASW KSKYBAELQA HADEVEBLRR KMAQKISEYE EQLEALLNKC SLEKQKSRL		
Der f 11			
Blo t 11	-----V----- -G----- -A-----A-		
D.melanogaster	--ID---V--AD-T-- QN-WNS-VA R-E---I-- -YQVR-T-L -HI-S-IV-V NN--M-T--		
C.elegans	I--SDA-HK- NL--TEITQ-- --FD--VAL -HE--D--K -L--QA-- --I-IM-Q-I -Q---A----		
D.immitis	A--AEA-HK- AL--TEITQ-- --FD--VAL -HE--D--K -L--QA-- --I-IM-Q-I -Q---A----		
	(351)		(420)
Der p 11	QSEVEVLIMD LEKAAHAHQ LEKRVQLEK INLDLKDKE EVTMLMEQAQ KELRIKIGEL QKLHKEYEKV		
Der f 11			
Blo t 11	---T---A ---S--- -S--- -S-L--T- -D--V--AD- -----L		
D.melanogaster	A-----I-- --SNNSCRE -T-S-NT-- H-VE--SR-D -TII-Y-TS- RD-KN-HAD- VRTV--LD--		
C.elegans	Q-----V-- --QNTIAL -RARE--R QVGE--VRID -I-VEL-A-- R--AVNA-- --MK-L---A		
D.immitis	Q-----V-- --QNTIAL -RAKE--- TVNE--VRID -L-VEL-A-- R-A-AALA-- --MKNL---A		
	(421)		(490)
Der p 11	RDQRDQLARE NKKLTDLLAE AKSQLNDAHR RIHQEIEBIK RLENERDELS AAYKEAETLR KQBEAKNQRL		
Der f 11			
Blo t 11	--KEA---A----- -GAI-ELN- -L--L-L-LR -----T-----AG- -A--QRG--		
D.melanogaster	K-NNN--T-- --G--H-- --EA-A--N- KL--LDL-NA --AG-IR--Q T-L--DAQ- RDA-NRA--A		
C.elegans	VE-KEA--- --H-E-H- --EA-A--N- KL--LDL-NA --AG-IR--Q T-L--S-AA- RDA-NRA--A		
D.immitis	IE-KEA--- --Q--H-- --EA-A--N- KL--LDL-NA --AG-IR--Q T-L--S-AA- RDA-NRA--A		
	(491)		(560)
Der p 11	IAELAQRVHD YEKRLAQKDE EIEALRKQYQ IEIEQLNMRL AEAELAKLTE IARLKKKYQA QITELSLD		
Der f 11			
Blo t 11	T-----T--- -----E-----V-----		
D.melanogaster	A-DFN-Y-- A-R--E-- --I--TS- --A-V I--TR-- VT-I--L-I --M--		
C.elegans	L---QAL-IE M-R--QE-E- -M---NL- F--DR-TAA- -D--RM-S- IS-----E-A---MTV-		
D.immitis	L---QAL-IE M-R--QE-E- -M---NM- F--DR-TAA- -D--RM-A- IS-----E-A---MTV-		
	(561)		(630)
Der p 11	AANKANIDLQ KTIKKALQI TELQAHYDEV HRQLQQAQV DQ LGVTQRRCA LQAELEEMRI ALEQASRAKR		
Der f 11			
Blo t 11	-----G----- -ED- Q---ATL-- YAVAQRRLAG -NG---V-S H-DS-N---		
D.melanogaster	V--T---V--S-L -----SE-L KI--SLEDT Q---VL- YALAQRKVAA -S---CKT --DN-I---		
C.elegans	NL-R---EA- -----SE-L KI--SLEDT Q---TL- YALAQRKVSA -S---CKV --DN-I---		
D.immitis	NL-R---EA- -----SE-L KI--SLEDT Q---TL- YALAQRKVSA -S---CKV --DN-I---		
	(631)		(700)
Der p 11	QAEQLHEEAV VRVNELTTIN VNLASAKSL ESEFSALQAD YDEVHKLRI SDERVQKLTI ELKSTKDLI		
Der f 11			
Blo t 11	A---M----- -T-----N----- -V-----E		
D.melanogaster	TV-LQY--A S-I---A- -S-V-I--- -Q-L-VVAS- -E--S-----Y--VQV --HVVEQVH		
C.elegans	--VDL--N G-ISD-IS- N--T-I-N-- -T-L-TA-- L--T--HA A--ANRALA DAARAVEQ-H		
D.immitis	---IDL--N A-ITD-VS-- N--TAI-N-- -T-L-TA-- L--AT--HA A--ANRALA DAARAVEQ-H		
	(701)		(770)
Der p 11	EEQERLVKLE TVKKSLEQEV RTLHVRIEVE EANALAGGKR VIAKLESRI R DVEIEVEER RRHAEETKML		
Der f 11			
Blo t 11	S-T--VT-- -I---T-- -N-QI----- -L--V--S-- I--S--A-- -L-L-L--K -----I-I-		
D.melanogaster	---I---I---V-- KN-SI-L-- -L--V--S-- I--S--A-- -L-L-L--K -----I-I-		
C.elegans	---HSM-ID ALR---EQ- KQ-Q-Q-Q-A --A-L-----T--- -L-TALD--T --K--QNA-		
D.immitis	---HSM-ID ALR---EQ- KQ-Q-Q-Q-A --A-L-----T--- -L-TALD--T --K--QCA-		
	(771)		(840)****
Der p 11	RKKDHRVKEL LLQNEEDHKQ IQLQEMTDK LNEKVKVYKR QMQEQEGMSQ QNLTRVRRPQ RELEAAEDRA		
Der f 11			
Blo t 11	-----S--		
D.melanogaster	---ERT--V -VQC--QKN LI--DAL-- STA-INI-R- -LS--V-- -TT-----		
C.elegans	---R-I--V QQLVD-E--N FVMA-DTA-R -T--LNIQ-- -LA-S-SVTM --Q---Y- H--D--G--		
D.immitis	---R-I--V QM-V-D-E--M FVMA-DTA-R -L--LNIQ-W -LG-A-SLTM A--Q---Y- ---D--G--		
	(841)****		(883)
Der p 11	DQAESNLSPI RAKHRSWVTT SQVPGGTRQV FTTQEETNNY *		
Der f 11			
Blo t 11	-----V-EQ-SS-F *		
D.melanogaster	-T--S-NI- ---TF-- -T--S-- YI..Q--RT ITE		
C.elegans	---S-HL- ---S-V- GK...SSSK- F		
D.immitis	---S-HL- ---S-V- GK.NASASKI YVL		

Fig. 1. Continued.

nucleotide sequence of Der f 11 cDNA (2078 bp), since the latter was truncated slightly at the N- and C-termini.

Der p 11 encoding a protein similar to other paramyosins

A sequence homology research of protein databases revealed that Der p 11 shared 51%, 51%, 59% and 89% identity with paramyosin from *Caenorhabditis elegans*, *Dirofilaria immitis*, *Drosophila melanogaster* and *B. tropicalis*, respectively (Fig. 1b). Comparison of the amino acid sequence in the region of homology between Der p 11 (positions 88–779) and Der f 11 revealed a nearly identical (97%) composition (Fig. 1b). The amino acid composition of Dp paramyosin (Der p 11) thus shares some characteristics common to paramyosins from other mite species and variant invertebrates. In addition to the over 50% sequence identity, all have high numbers of charged residues such as D, E, H, K and R, which make up about 41% of the protein sequence. Proline and cysteine residues, as well as aromatic residues, are present in low numbers (less than 1%). In Der p 11, leucine is the most abundant hydrophobic residue (12.5 mol%) as is true of the other paramyosins (12–14 mol%). Mannose N-glycan chains have been found in the related native protein of Der f 11 [5] and Der p 11 has four putative N-glycosylation sites present at residues 139–142, 191–194, 817–820 and 841–844 (Fig. 1b). Blo t 11 [8] has four N-glycosylation sites similar to Der p 11 (in addition to one at position 69–71). A characteristic feature of paramyosins is the presence of a serine-rich N-terminal region. Der p 11 contains a higher number of serine residues (5.257 mol%) than Der f 11 (3.757 mol%) due to the truncation of the N-terminus of the latter. The presence of several relatively long leucine zipper motifs in Der p 11 as well as in Der f 11 and Blo t 11 may play a significant structural role in protein folding.

Antibody-binding capacity of recombinant Der p 11

A DNA fragment containing *Bam*HI restriction enzyme sites was PCR amplified from Dp cDNA and subcloned into the pGEX-4T-1 expression vector. The resulting rDer p 11-GST was expressed as a fusion protein with GST, which weighs 26 kDa. A Coomassie brilliant blue-stained SDS-PAGE showed that the mw of the rDer p 11 without GST and the related control allergen (rDer f 11) were approximately 103 and 85 kDa, respectively (Fig. 2, lanes 3 and 5). rDer p 11 was similar to rDer f 11, in that a portion of the protein appeared to be very susceptible to degradation, giving rise to a number of smaller fragments over 45 kDa in size (Figs 2 and 4). Fragments derived from rDer p 11-GST as well as rDer f 11-GST were reactive to mAb642 monoclonal antibody (Fig. 3) and allergic human IgE (Fig. 4). The protein profile of an experimental panel of 16 serum samples including the pooled patient (P) and pooled healthy (N) sera, MAST(Dp)⁺ (1–6) and MAST(Dp)[−] (7–14) sera binding to rDer p 11 is illustrated in Fig. 4. As shown, four out of six MAST(Dp)⁺ sera (No. 2, 3, 5 and 6) and pooled patient serum (P) had IgE reactivity with rDer p 11 or the degraded fragments (Fig. 4, lanes b). However, an unexpected result was finding positive IgE reactivity to these protein components in five out of eight MAST(Dp)[−] sera (No. 7, 8, 11, 12 and 13) from allergic patients. Weak and moderate IgE reactivity to the smaller

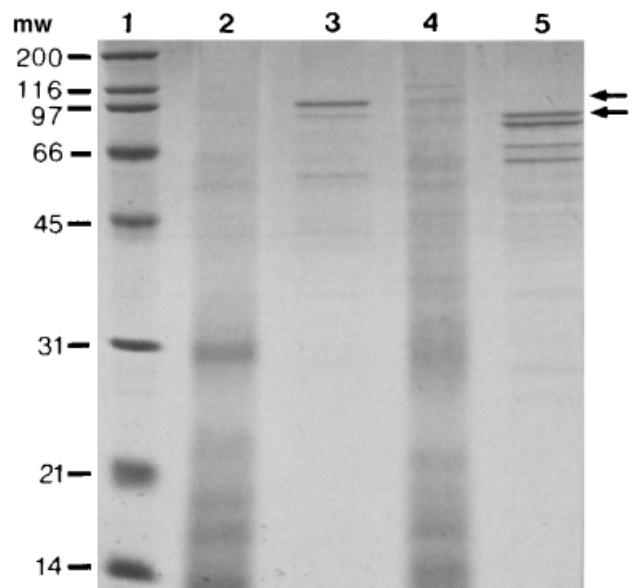


Fig. 2. Protein profiles of rDer p 11 (lane 3) or rDer f 11 (lane 5), *Dermatophagoides pteronyssinus* (Dp) mite (lane 2) and *Dermatophagoides farinae* (Df) (lane 4) mite crude extract in SDS-PAGE analysis. Dp or Df (5 µg/lane), rDer p 11 or rDer f 11 (3 µg/lane) and protein markers (lane 1) were separated under reducing condition on 12.5% PAGE. Separated proteins were visualized by staining with Coomassie blue. Arrows point to the component of rDer p 11 (the upper) and rDer f 11 (the lower).

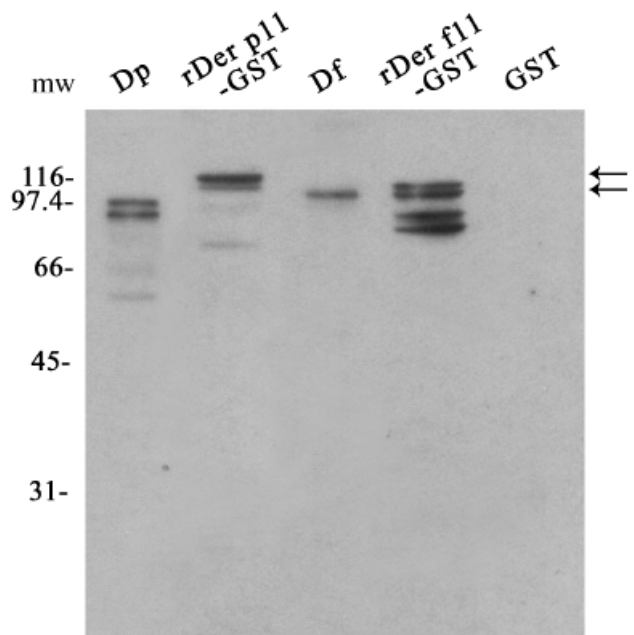


Fig. 3. Analysis of protein profiles of rDer p 11-GST, rDer f 11-GST and mite extracts using mAb642 monoclonal antibody in Western blotting. *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Df) mite extracts (5 µg/lane), rDer p 11-GST and rDer f 11-GST (3 µg/lane), and GST (3 µg/lane) were separated with SDS-PAGE chromatography and electrotransferred onto PVDF membrane. After blocking, blots were sequentially incubated with diluted mAb642 (1 : 10 000), HRP-conjugated rabbit anti-mouse IgG antibodies (1 : 2500) and ECL reagent. Lanes 1–4: reactivity of protein bands including original and degraded fragments reacted with mAb642. Lane 5: non-reactivity of the control protein GST with mAb642. Arrows pointed to the component of rDer p 11-GST (the upper) and rDer f 11-GST (the lower), respectively. GST, glutathione-S-transferase; PVDF, polyvinylidene fluoride; HRP, horseradish peroxidase.

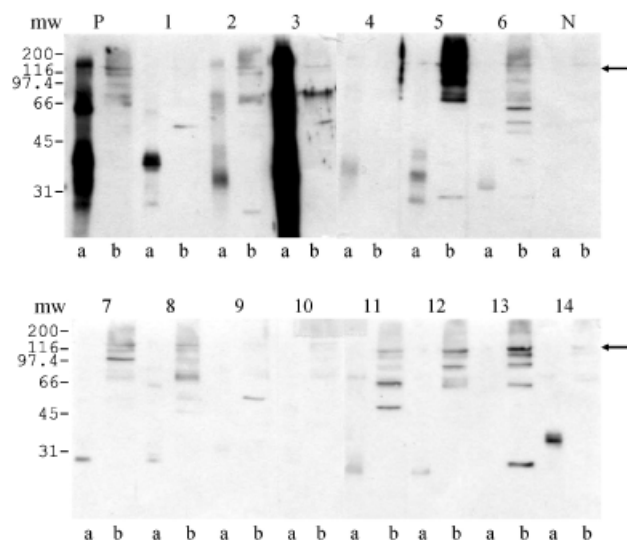


Fig. 4. Immunoblot analysis of IgE reactivity to rDer p 11-GST. Western blotting using patient sera after SDS-PAGE separation of *Dermatophagoides pteronyssinus* mite crude extract (lanes a, 5 µg/lane) and rDer p 11-GST (lanes b, 3 µg/lane) under reducing conditions (2-Me). Immunoblots were probed with pooled patient serum (P), MAST(Dp)⁺ (1–6) allergic patient sera MAST(Dp)[−] (7–14) allergic patient sera, and pooled normal serum (N). P and N pooled sera were used as positive and negative controls, respectively. Six of 47 MAST(Dp)⁺ and eight of 53 MAST(Dp)[−] sera were collected arbitrarily from the allergic sera tested. Sera were obtained from patients with bronchial asthma/allergic rhinitis (No. 1, 4, 5, 7, 8, 9, 10 and 11), bronchial asthma (No. 2, 6, 12 and 13) and eczema (No. 3 and 14). Reactivity of allergen components in No. 2, 3, 5 and 6 of MAST(Dp)⁺ sera, and in No. 7, 8, 11, 12 and 13 of MAST(Dp)[−] sera. Arrows point to the original protein band of rDer p 11. GST, glutathione-S-transferase.

components of Dp extract (lanes a) was also found in probing with MAST(Dp)[−] patient sera (No. 7, 8, 11, 12 and 14). No IgE reactivity to Dp extract or rDer p 11 was found in a pooled healthy serum (N).

The frequency of serum IgE reactivity to rDer p 11 and Dp extract was estimated by immunodot assay. The frequency of IgE reactivity to the rDer p 11 (52.8%) was similar to that to Dp extract (55.6%) in 36 random allergic serum samples (Fig. 5), while two sera (5.6%) with weak positive IgE reactivity to the control GST antigen were found (data not shown). There was no positive serum IgE reactivity to rDer p 11 or Dp extract in 12 healthy controls. Furthermore, the positive serum IgE reactivity to rDer p 11 was first compared with that to rDer f 11. In a large panel of 40 random allergic serum samples, the positive serum IgE reactivity to rDer p 11 (24 of 40, 60%) was slightly higher than that to rDer f 11 (21 of 40, 52.5%) (Table 2). Approximately 7% (three out of 40) of allergic sera reacted weakly to the control GST protein. In this study, IgE reactivity to rDer p 11, Dp extract and GST in all serum samples were estimating and summarized in Table 1. An estimation of the normal upper limits (mean + 2 SD) of IgE reactivity from 25 healthy adults by immunodot assay was 999 (mean ± SD; 871 ± 64) for Dp extract, 881 (833 ± 24) for rDer p 11, 839 (803 ± 18) for rDer f 11 and 818 (796 ± 11) for GST. Any value beyond these limits was regarded as positive. Table 1 shows that the positive IgE reactivity of 100 sera from allergic patients with eczema (41.7%), AR (43.3%), BA/AR (52%) or BA (66.7%) was much higher than that of sera from non-atopic patients with

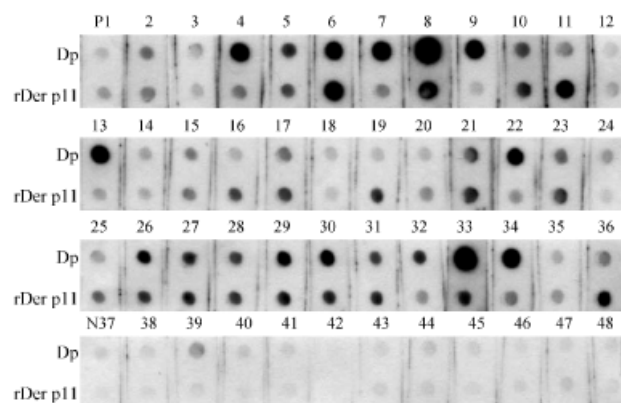


Fig. 5. Comparison of specific IgE reactivity between rDer p 11 and *Dermatophagoides pteronyssinus* (Dp) mite extract by immunodot assay. Thirty-six sera (P1–36) and 12 healthy sera (N37–48) were collected arbitrarily from allergic patients and healthy adults, respectively. Glutathione-S-transferase was used as a negative control (data not shown). Serum IgE reactivity to crude Dp extract (3 µg/dot) and rDer p 11 (2 µg/dot) were analysed in a dot blot immunoassay. The intensity of the IgE-reacting dot was determined by transmission densitometry. The mean of the controls plus 2 SD was used as a cut-off value for the positive reaction.

urticaria (18.8%) or healthy adults (8%). The frequency of serum IgE reactivity to Dp mite extract in patients with BA, BA/AR or AR was very close, ranging from 47.6% to 52%, but was also much higher than that with urticaria (6.3%) (Table 1).

In comparison of MAST and immunodot assays, 35 of 47 MAST(Dp)⁺ allergic sera were positive to Dp extract in immunodot assay, while 38 of 53 MAST(Dp)[−] sera were negative to Dp extract in immunodot assay (data not shown). Together, the frequency of agreement between MAST and immunodot assay to Dp mite crude extract was 73% (73/100).

Discussion

House dust mites (HDMs) are a major source of allergens in house dust in most temperate humid areas of the world [21]. Mite allergens sensitize and induce asthma, perennial rhinitis, or atopic dermatitis in a large portion of patients with allergic disease [21]. Exposure to mite allergens with criterias of a positive skin test and an increased specific IgE level in serum is associated with both diminished lung function and enhanced airway hyper-responsiveness, indicating that hypersensitivity to mites is a significant independent factors for the development of asthma [22]. Allergic patients exhibit individual differences in their allergen recognition spectra. In fact, the antigenicity of Dp is not due to one major allergen but to several different components. From a therapeutic point of view, there is considerable interest in elucidating the immunobiochemical characteristics and structure of allergen molecules and in defining new major allergens as a basis for designing effective interventions for allergic patients. Immunoblot, immunodot and cDNA techniques have enabled enormous progress in the characterization of mite allergens.

Like native Der f 11 [5], Der p 11 as well as Der f 11 is present in minimal amounts in crude mite extract (Fig. 2, lane 2 and 4) and is difficult to purify for clinical purposes. This is

Table 2. Comparison of specific IgE reactivity to rDer p 11 and rDer f 11 by dot blot immunoassay

Antigen					Antigen				
Serum number	Dp crude	rDer p 11	rDer f 11	GST	Serum number	Dp crude	rDer p 11	rDer f 11	GST
1	+	+	+	—	21	+	—	—	—
2	+	+	+	—	22	+	+	+	—
3	+	—	+	—	23	+	+	+	—
4	+	+	+	—	24	+	—	+	—
5	+	+	—	—	25	+	+	+	—
6	+	—	—	—	26	—	+	+	—
7	+	+	—	—	27	—	—	—	—
8	+	+	—	—	28	+	+	+	+
9	—	—	—	—	29	—	+	+	—
10	+	+	—	—	30	—	+	+	—
11	+	+	+	—	31	—	—	—	—
12	—	—	—	—	32	+	+	+	—
13	+	+	—	—	33	+	—	—	—
14	—	—	—	—	34	—	+	+	—
15	+	+	+	—	35	—	+	+	—
16	+	+	+	—	36	—	—	—	—
17	+	+	+	—	37	+	—	—	+
18	+	+	+	+	38	—	—	—	—
19	+	—	—	—	39	—	—	—	—
20	+	+	+	—	40	—	—	—	—

Positive reactions of 26 (65%) allergic sera to Dp extract, 24 (60%) sera to rDer p 11, 21 (52.5%) sera to rDer f 11 and three (7.5%) sera to GST are shown in 40 allergic patients. The negative reactions (—) are also shown. GST, glutathione-S-transferase; Dp, *Dermatophagoides pteronyssinus*.

why we prepared rDer p 11 for our study. rDer p 11 and its derived fragments were reactive to human IgE (Fig. 4), suggesting that rDer p 11-specific IgE in patient sera may consist of different populations of IgE antibodies: one that recognizes structural epitopes present on the surface of the intact form and others that recognizes epitopes on fragments produced by degradation.

The high frequency on immunodot of IgE binding to rDer p 11 (60%; 24/40) in the sera of allergic patients, higher even than to the major mite allergen - rDer f 11 (52.5%; 21/40) (Table 2), indicates that Der p 11 is also an important allergen. The presence of IgE reactivity to rDer p 11 in 5 out of 8 MAST(Dp)⁺ sera confirms our contention that Der p 11 is a discrete HDM allergen (Fig. 4). It may be that the native Der p 11 is such a minor component of crude mite extract that it is present in insufficient quantities for the MAST test. Another possibility is that the allergen's susceptibility to degradation (Figs 2 and 4) or the existence of different isoforms [5] may render it non-reactive or only weakly reactive in the MAST test. Regardless, the fact that five sera out of eight MAST(Dp)⁺ subjects responded to rDer p 11 suggests that commercial allergen tests may miss mite hypersensitivity in some individuals.

Previous studies have indicated that Dp and Df mite body and fecal extracts contain multiple allergens and share multiple allergenic determinants [13]. Our previous work showed a significant correlation between Dp- and Df-specific IgE [12]. A high correlation (82.5%, 33/40) between specific IgE reactivity to both of rDer p 11 and rDer f 11 was shown in the agreement of 19 positives and 14 negatives in an experimental panel of 40 allergic subjects (Table 2). These results confirm that these two mite species share common antigenic determinants and are cross-allergenic [12–14]. The slightly

higher binding of allergic sera to rDer p 11 (60%) than to rDer f 11 (52.5%) (Table 2) may reflect acquired conformational differences in the recombinant peptides, notably the N- and C-terminal truncations of rDer f 11, resulting in variable *in vitro* IgE binding.

Amino acid sequence analysis of rDer p 11 strongly suggests that it is a mite paramyosin, a muscle-associated protein. Parasite and mite paramyosins are strong immunogens. The former is regarded as having potential for use as a vaccine for parasitic disease [23], and the latter (Der f 11) has been defined as a major allergen in allergic patients, as well as being immunogenic in normal subjects [10, 11]. Native Der f 11 (Df642) is a glycoprotein with two putative N-glycosylation sites, which contain high mannose N-glycan in its carbohydrate moiety [5]. The similar Der p 11 has more than two putative N-glycosylation sites (Fig. 1b). Thus, it is possible that native Der p 11 is also a glycoprotein. Although many environmental allergens have been shown to have enzymatic activity that may enhance or be required for a persistent allergic response [24], glycoproteins containing abundant carbohydrates are also known to play a key role as internal adjuvant and may induce an allergic response [25, 26]. Induction of a specific IgE response *in vivo* has been demonstrated by sensitization with rDer f 11 absorbed onto alum in mice [27]. This suggests that Dp and Df mite paramyosin can efficiently induce an allergic immune response, even without enzymatic activity. However, the actual allergenicity of rDer p 11 still needs to be evaluated *in vivo*, not just by the use of *in vitro* IgE test. We believe that identification of Der p 11 is an important step in characterizing the complete spectrum of mite IgE specificities [28].

In conclusion, our studies have demonstrated that the Dp and Df mite paramyosins (Der p 11 and Der f 11) are two

major mite allergens. The allergenicity of Der p 11 has been demonstrated by the high incidence of *in vitro* human IgE reactivity to rDer p 11.

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