

Characterization of *Der p V* allergen, cDNA analysis, and IgE-mediated reactivity to the recombinant protein

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Abstract

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A λ gt11 library for cDNA from *Dermatophagoides pteronyssinus* was screened by plaque immunoassay, and a number of related clones that produced IgE-binding proteins were isolated. Their sequences were homologous to that of a cDNA described previously, which contained a reading frame encoding a 17 kd polypeptide and which as determined by serologic analysis corresponded to a protein with relative molecular mass of 14 kd in mite extracts. The cDNA from the λ gt11 clones was truncated so it was used to obtain longer clones from a λ gt10 library. Analysis of the sequence of these clones showed that the allergen now designated *Der p V* is produced from a 132-residue polypeptide, which has a putative 19-residue leader peptide and a 113-residue mature protein. This would have a molecular weight of 14 kd, corresponding to that found in mite extracts. IgE binding studies with the λ gt11 clone and a fusion of the mature sequence in a pGEX construct showed that it reacted with 50% of allergic sera. Further studies with skin tests indicated that it caused reactions in about 60% of patients with asthma and 29% of those with allergic rhinitis alone. (J ALLERGY CLIN IMMUNOL 1994;94:989-96.)

GST	Glutathione-S-transferase
M_r	Apparent molecular weight
PCR	Polymerase chain reaction
SDS	Sodium dodecylsulfate
SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

Key words: [Dermatophagoides pteronyssinus](#), [cDNA sequence](#), [recombinant allergen \(Der p V\)](#)

The association between mite allergy and diseases such as asthma, allergic rhinitis, and atopic dermatitis has been established for over 2 decades.^{1,2} The predominant species in Taiwan, Australia, and Western Europe is *Dermatophagoides pteronyssinus*, whereas *D. farinae* is predominant in many areas of the United States, Japan, and continental Europe.³ Anti-mite IgE in the serum of allergic patients reacts with a variable number of components as determined by Western blotting, which can range from 1 to 2 bands to over 14.⁴ The recent study by Ford et al.⁵ shows that most persons react with 1 to 6 bands, although detailed studies relating the recognition of different mite allergens to antibody titers and clinical groups are still required. It has, however, been documented that there are about eight allergens that react with 40% or more of allergic sera. To assess their importance it is necessary to characterize these allergens and to quantitate their relative amounts of reactivity both with IgE antibody and T cells.

The *Der p* I and *Der p* II allergens react with about 80% of allergic sera, often in high titers,⁶ and have been characterized by cDNA cloning,^{7,8} as well as by monoclonal antibodies and other immunochemical procedures. Other allergens are less well characterized but include the *Der p* III allergen or mite trypsin, which reacts at high frequency but at a lower level.⁹ *Der p* IV, defined as amylase,¹⁰ and *Der p* VI,¹¹ which is chymotrypsin, react with about 40% of allergic sera, as does another allergen defined by a cDNA clone and provisionally designed *Der p* VII.¹² The designation *Der p* V has been adopted for an allergen identified by IgE reactivity with a cDNA clone described by Tovey et al.¹³ The sequence of the cDNA encoded a 17 kd protein and affinity-purified antibodies prepared with the cloned protein reacted with a protein having a relative molecular mass (M_r) of 14 kd in mite extracts. Even though the clone reacted strongly with IgE in 50% of allergic sera, this allergen has not been further characterized and amino acids comprising the 14 kd allergen have not been ascertained. We describe cDNA clones encoding *Der p* V isolated in our laboratories, which confirm and extend the previous data by using IgE binding and skin tests and significantly define the allergen as having a putative 19-residue leader peptide and a 113-residue mature protein with a molecular weight of 13,585 d. This information can be used for studies with peptides and recombinant constructs.

Methods

Sera

The serum used for the immunoscreening of the cDNA library was from a 12-year-old patient with asthma from the allergy clinic of National Taiwan University Hospital in Taipei. This serum has been shown to react with mite proteins ranging from 14 to 110 kd by IgE immunoblot analysis. The sera for frequency analysis were from the National Taiwan University Hospital and the Royal Children's Hospital in Melbourne (supplied by Dr. D. Hill).

cDNA library of *D. pteronyssinus*

Both the λ gt10 and λ gt11 cDNA libraries were constructed previously^{14,15} and stored in 7% dimethyl sulfoxide at -70°C .

IgE plaque immunoscreening and dot blot immunoassay

The λ gt11 cDNA library was plated out at 20,000 plaques per 14.5 cm plate with *Escherichia coli* Y1090 cells, and the immunoassay of the library lift with human allergic serum was performed according to method described by Chua et al.⁸ To develop IgE reactivity, iodine 125-labeled anti-human IgE monoclonal antibody (2.1.5) was used, and autoradiography was carried out at -70°C . Dot blot immunoassay was performed as described by Chua et al.¹⁶

Screening of plaques by in situ DNA hybridization

The λ gt10 cDNA library of *D. pteronyssinus* was plated out at 20,000 plaques per plate with *E. coli* JP777 cells. The preparation of filters for hybridization was done with a standard method. The DNA probe used in our experiments was a 0.4 kb cDNA insert from λ gt11 clone, which was phosphorous 32-P-labeled by nick translation¹⁷ and used at 10⁶ cpm/ml of hybridization solution (50% formamide, 5 × standard sodium citrate [SSC] with 0.1 mol/L ethylenediaminetetraacetic acid, 1 × Denhardt's reagent). The prehybridization and hybridization were performed as described by Dilworth et al.¹⁸ After hybridization, the filter was washed three times at 65° C in 2 × SSC/0.1% sodium dodecylsulfate (SDS), 0.5 × SSC/0.1% SDS, 0.1 × SSC/0.1% SDS for 15 minutes, respectively. The final wash was in prewarmed 0.1 × SSC/1% SDS at 50° C for 30 minutes. Autoradiography was at -70° C with intensifying screens.

Preparation and sequencing of insert DNA

DNA was prepared from λ gt10 and λ gt11 by Lambdabsorb Phage Adsorbent (Promega, Madison, Wis.) and digested with *Eco*RI enzyme to release the cDNA insert. The insert DNA was subcloned into Bluescript phagemid (Stratagene, La Jolla, Calif.) by standard procedure. The small-scale plasmid DNA was prepared by a modified alkaline lysis method.¹⁹ The large-scale preparations of plasmid DNA were isolated by a rapid Magic TM Maxipreps (Promega) or cesium chloride centrifugation. For DNA sequencing, 4 µg of double-stranded plasmid DNA was denatured by alkali solution (2 mol/L NaOH, 2 mmol/L ethylenediaminetetraacetic acid) for 5 minutes of room temperature and then neutralized with 2 µl of 2 mol/L ammonium acetate, pH 4.6. cDNA sequencing was performed by the dideoxynucleotide chain termination method²⁰ with Sequencing Version 2.0 kit (USB, Cleveland, Ohio).

Expression of *Der p V* cDNA in *E. coli*

To obtain purified recombinant protein, the cDNA sequence from the λ gt10 clone WM was used for expression as a fusion protein with glutathione-S-transferase (GST) in *E. coli* by using the expression vector pGEX-2T.²¹ The polymerase chain reaction (PCR) technique was used to remove the 5' noncoding region and the putative signal peptide sequence (nucleotides 1 to 118). This was achieved by using a 5' PCR primer (5' GCGGATCCGAAGATAAAAAACATGAT, 3') spanning nucleotides 119 to 136 and 1 3' primer derived from λ gt10 vector sequence (5' AGCAAGTTCAGCCTGGTTAAG 3'). These primers generated a PCR product with restriction enzyme sites that permit directional cloning into pGEX-2T vector cleaved by *Eco*RI and *Bam*HI. Approximately 250 ng WM DNA was amplified with 2.5 units of plaque-forming unit DNA polymerase (Stratagene) in 50 µl reaction mix containing 20 mmol/L Tris-HCl, pH 8.2, 10 mmol/L KCl, 6 mmol/L (NH₄)₂ SO₄, 2 mmol/L MgCl₂, 200 µmol/L deoxyribonucleoside triphosphate and 20 pmol each of the primers. The PCR mixture was denatured at 95° C for 5 minutes, annealed at 60° C for 2 minutes, polymerized at 72° C for 2 minutes and followed by 38 cycles of amplification; each cycle consisted of 95° C for 1 minute, 60° C for 2 minutes, and 72° C for 2 minutes. The final cycle had its polymerization step extended to 10 minutes. DNA ligation and transformation of TG1 cells were performed with the standard methods.²² The screening of the recombinant clones was by restriction enzyme analysis and by the size of the GST fusion produced by the clone. The fusion protein was purified, by a published method, from glutathione-coupled agarose,²³ and the thrombin cleavage of the fusion protein was as described previously.²¹

SDS-PAGE and Western analysis

SDS-PAGE was performed according to the method of Laemmli²⁴ with a 13% miniprotein II apparatus (Bio-Rad, Richmond, Va.). For dust mite extracts, samples were loaded at 200 µg protein/lane. For bacteria culture, a cell pellet from 100 µl of broth culture was resuspended in sample buffer and loaded at 5 µg per lane. Purified proteins were electrophoresed at 10 µg/lane. The proteins on gels either stained with Coomassie Brilliant Blue 250 (Imperial Chemical Industries, Ltd., Macclesfield, Cheshire, England) or were transferred to nitrocellulose²⁵ for Western blotting.

Skin test

Skin testing was done by injecting 0.02 ml of recombinant *Der p V* with purified *Der p I*, *Der p II*, and GST as controls at a concentration of 1 µg/ml into the volar surface of the forearm through a 26-gauge needle and

tuberculin syringe to raise a wheal of 3 mm in diameter. The result was read at 15 minutes. A wheal size of greater than 6 mm was considered to be a positive reaction.

Results

Isolation and sequences of clones encoding *Der p V*

Serum samples from patients allergic to the house dust mite were used to screen λ gt11 libraries by IgE plaque radioimmunoassay for clones producing allergens. A series of clones reacting strongly with IgE was isolated: at least four of these contained 400 bp inserts, and their DNA cross-hybridized. ■Fig. 1 shows some of the plaques selected from a high-density screen. The sequence of the cDNA from one of the clones was determined and found to be a truncated version of the sequence published by Tovey et al.¹³ for a cDNA clone that encodes all or part of an allergen with an M_r of 14 kd. To obtain longer clones that could be analyzed, the cDNA was used to screen a λ gt10 library by DNA hybridization; and clones containing 750, 600, and 500 bp were isolated. Initially the clone WM was sequenced with universal and internal primers. The 613 bp sequence (■Fig. 2) had an open reading frame, extending from nucleotide 2 to a TAA stop codon at 458-460. The predicted 3' untranslated region contained a polyadenylation site at 518-523 and a polyadenylic acid tail. The sequence reported by Tovey et al.¹³ was identical to nucleotides 15 to 459. Although the open reading frame began at nucleotide 2, it was noticed that the ATG at 62-64 was followed by a Lys and 18 hydrophobic residues and hence formed a classic leader peptide. According to this analysis, which is given in ■Fig. 2, the polypeptide would begin at nucleotides 62-64 with a 19-residue leader sequence, which would probably be cleaved between Gly 19 and Glu 20 with Glu as the N-terminal.^{26, 27} The mature polypeptide would then contain 113 residues with a molecular weight of 13,585 d in agreement with the serologic data of Tovey et al.¹³

FIG. 1. The high-density screening of *D. pteronyssinus* λ gt11 cDNA library by IgE plaque radioimmunoassay with allergic serum. The arrows indicate plaques that showed strong IgE binding and were subsequently found to encode *Der p V*.

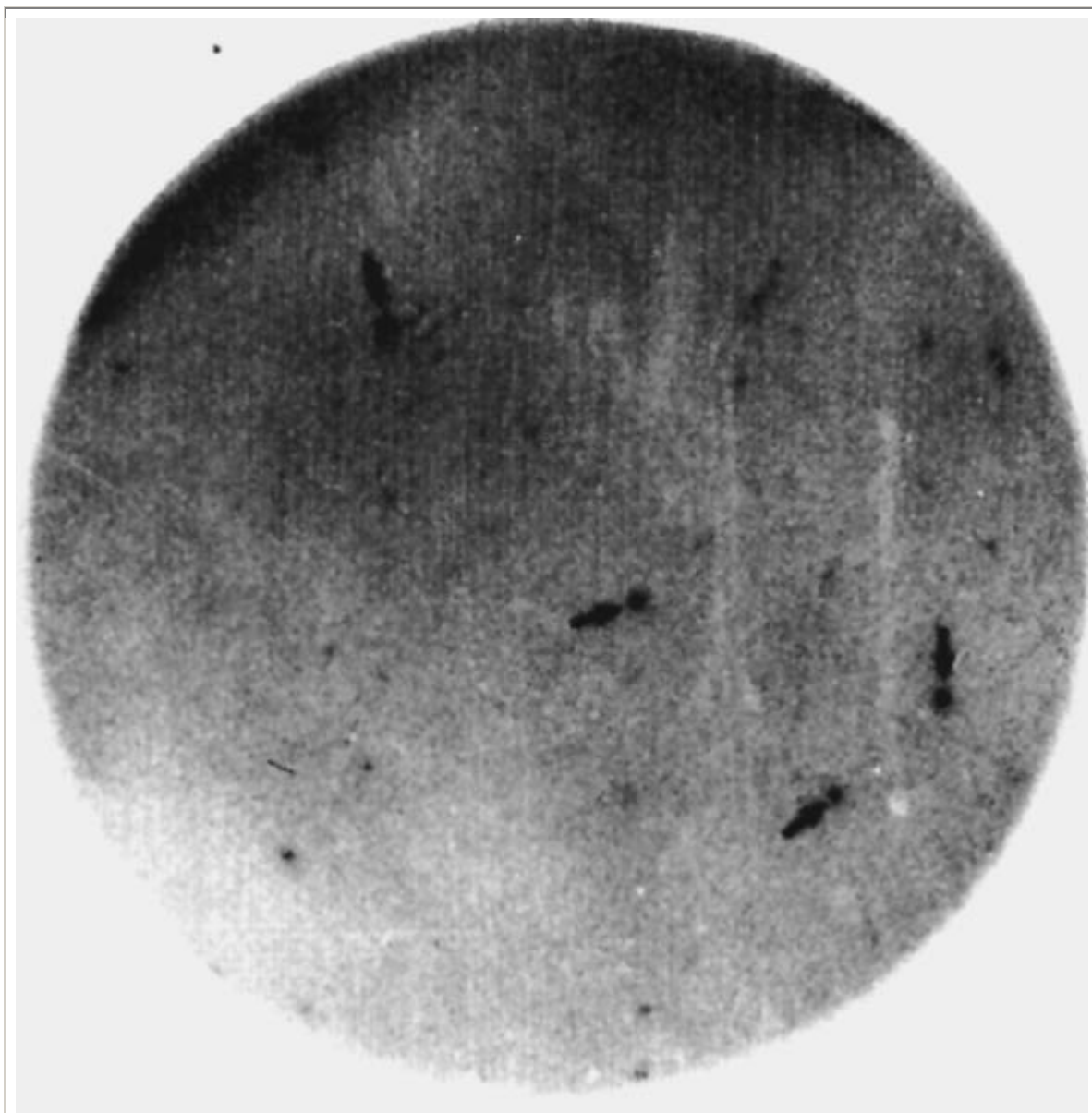


FIG. 2. cDNA sequence of λ gt10 clone WM. The predicted translation initiation ATG (nucleotide 62-64), the two consecutive stop codons TAA (nucleotide 458-460), and the polyadenylated site AATAAA (nucleotide 517-522) are *underlined*. The putative signal peptide cleavage site is between residues 19 (Gly) and 20 (Glu), which are typed in bold. The numbers on the left and right of the sequence indicate nucleotide and amino acid positions, respectively.

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1 TTTTTTTTTTTTTTTTTTCTCGAAAATAAAGATCCCAAACCG
44 TTAAAAAAATATCAATCATG AAA TTC ATC ATT GCT TTC TTT GTT GCC ACT TTG GCA 13
    Met Lys Phe Ile Ile Ala Phe Phe Val Ala Thr Leu Ala
101 GTT ATG ACT GTT TCA GGT GAA GAT AAA AAA CAT GAT TAT CAA AAT GAA TTT 30
    Val Met Thr Val Ser Gly Glu Asp Lys Lys His Asp Tyr Gln Asn Glu Phe
152 GAT TTC TTA TTG ATG GAA CGT ATT CAT GAA CAA ATT AAA AAA GGT GAA CTT 47
    Asp Phe Leu Leu Met Glu Arg Ile His Glu Gln Ile Lys Lys Gly Glu Leu
203 GCA TTG TTC TAT CTT CAA GAA CAG ATT AAT CAT TTT GAA GAA AAA CCA ACA 64
    Ala Leu Phe Tyr Leu Gln Glu Gln Ile Asn His Phe Glu Glu Lys Pro Thr
254 AAA GAA ATG AAA GAT AAA ATT GTA GCC GAA ATG GAT ACC ATT ATT GCT ATG 81
    Lys Glu Met Lys Asp Lys Ile Val Ala Glu Met Asp Thr Ile Ile Ala Met
305 ATC GAT GGT GTA CGT GGT GTA CTT GAT CGT CTT ATG CAA CGT AAA GAT TTA 98
    Ile Asp Gly Val Arg Gly Val Leu Asp Arg Leu Met Gln Arg Lys Asp Leu
356 GAT ATT TTT GAA CAA TAT AAT CTT GAA ATG GCT AAA AAA TCT GGT GAT ATT 115
    Asp Ile Phe Glu Gln Tyr Asn Leu Glu Met Ala Lys Lys Ser Gly Asp Ile
407 TTG GAA CGT GAT TTG AAA AAA GAA GAA GCA CGT GTT AAA AAG ATT GAA GTT 132
    Leu Glu Arg Asp Leu Lys Lys Glu Glu Ala Arg Val Lys Lys Ile Glu Val
458 TAA TTTAAT TGA ATTAAAAATTTCTTGTTTCTTGTTGCTTCATCAATTGACAAATAAAATGAATAAA
533 GAATTTTGAATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
608 AAAAAA

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A further clone WL was sequenced and compared with clone WM (■Fig. 3). It did not extend to the polyadenylic acid tail but did contain a longer 5' sequence, which was substantially different. The 15 base pairs preceding the proposed ATG start were the same as those in WM, but 17 of 46 of the nucleotides preceding these, which were available for comparison, were different. Another significant feature was that the extended 5' region in WL contained two stop codons TAA and TAG at nucleotides 69-71 and 122-124 in the same reading frame as the translated sequence. The coding region differed in only three positions, one of which resulted in an amino acid transition, glu to Ala at residue 61.

FIG. 3. Sequence alignment of WM and WL cDNA clones. The predicted translation initiation site, the stop codons, and the polyadenylated site in both sequences are as described in ■Fig. 2. WL clone also contains two stop codons TAA (nucleotide 71-72) and TAG (nucleotide 121-123) in the 5' end of its open reading frame. The two nucleotide changes at position 437 and 438 in WL resulted in amino acid conversion from Ala to Glu at residue 61. The nucleotide positions are indicated by the numbers to the right of the sequences.

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WL TTTTTTTTGCTTGTGGATGTATTGTCCAATAGATTAATGAACTGTAACTTTTTTTCAAGTGATCT 67
WM

WL TTGTAATTGGTAATTTTAAATCGATCAAAATGTAAACAACAACAAAAAAGAGATAGAAATACAATT 134
WM

WL TGATCGATATAAAAAATAAAGAACTGGAAGTTATAATTTTTTGAAAAGATTGATCGACAAAAGTGTC 201
WM T*T*TT 6

WL ATTTATATAATTTTTTCTAGAAAAAATCCCAAACCGTTTAAAAAATATCAATC ATG AAA TTC 265
WM T***T*T*TT**C*CGA*A*T***G***** 70

WL ATC ATT GCT TTC TTT GTT GCC ACT TTG GCC GTT ATG ACT GTT TCA GGT GAA 316
WM *** *** *** *** *** *** *** *** **A *** *** *** *** *** *** *** 121

WL GAT AAA AAA CAT GAT TAT CAA AAT GAA TTT GAT TTC TTA TTG ATG GAA CGT 367
WM *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** 172

WL ATT CAT GAA CAA ATT AAA AAA GGT GAA CTT GCA TTG TTC TAT CTT CAA GAA 418
WM *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** 223

WL CAG ATT AAT CAT TTT GAA GCC AAA CCA ACA AAA GAA ATG AAA GAT AAA ATT 469
WM *** *** *** *** *** *** **AA *** *** *** *** *** *** *** *** *** *** 274
Ala
Glu

WL GTA GCC GAA ATG GAT ACC ATT ATT GCT ATG ATT GAT GGT GTA CGT GGT GTA 520
WM *** *** *** *** *** *** *** *** *** *** *** **C *** *** *** *** *** 325

WL CTT GAT CGT CTT ATG CAA CGT AAA GAT TTA GAT ATT TTT GAA CAA TAT AAT 571
WM *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** 376

WL CTT GAA ATG GCT AAA AAA TCT GGT GAT ATT TTG GAA CGT GAT TTG AAA AAA 622
WM *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** 427

WL GAA GAA GCA CGT GTT AAA AAG ATT GAA GTT TAATTTAATTGAATTAAAAATTTTCTT 679
WM *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** 484

WL GATTTTTTTCTTGTTCCTTCATCAATTGACAAATAAAATGAATAAAGAATTTTGG 736
WM *C*****AAATAAAAAA 551

WL
WM AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 613

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IgE binding and skin tests of expressed *Der p V*

The allergen expressed from the original *Der p V* clone bound IgE in 40% of 100 serum samples from allergic children tested. This was performed by reacting the serum samples with segments of a nitrocellulose lift taken from plaques induced to produce the allergen. For a more controlled test, a construct was made producing the predicted 113-residue mature protein in Fig. 2 as a fusion with GST from the pGEX-2T vector. *E. coli* transformed with the construct produced large quantities of a protein with an M_r of 40 kd, which could be isolated by glutathione affinity chromatography. It could be isolated at a yield of about 2 mg/L of culture and was effectively cleaved by thrombin at the site engineered into this vector to produce an M_r of 27 kd glutathione transferase and a 14 kd molecule expected for the allergen (Fig. 4). For IgE tests, 2 μ g of the purified fusion protein was dotted onto nitrocellulose in 2 μ l, as well as crude mite extract, pGEX-2T lysate, and purified GST protein for controls. Serum samples were preabsorbed with pGEX *E. coli* lysate before use. The allergen fusion reacted with 52% of the 25 serum samples tested, and the mite extract with 96% (Fig. 5). A hyper IgE serum with 1000 IU of IgE/per milliliter had no reactivity. For skin tests, 0.02 ml of the purified *Der p V* fusion, affinity-purified *Der p I*, and recombinant *Der p II* at 1 μ g/ml were injected into 45 patients with known diagnoses of asthma, rhinitis, or asthma and rhinitis and 11 normal subjects (Table I). The overall frequency of reactivity to *Der p V* in the allergic groups was 51% compared with 80% for *Der p I* and

Der p II. The groups with asthma, however, had significantly more reactivity than the group with rhinitis alone, which only showed 29% reactivity compared with 67% and 58%. The 27% reactivity to recombinant *Der p* II of the normal control group could be in part attributed to the background reactivity of its GST fusion partner because the allergic groups showed 6% to 15% reactivity with the control GST alone.

FIG. 4. Fusion protein produced from pGEX-2T-*Der p* V. The DNA encoding the predicted 113-residue *Der p* V polypeptide was expressed as a fusion with GST in pGEX-2T. Lanes 1 and 2 show the lysate of *E. coli* transformant before and after isopropyl thiogalactose induction. Lane 3 demonstrates the fusion protein purified by glutathione affinity chromatography. The fusion protein was cleaved with thrombin to separate into a 28 kd GST and 15 kd protein (lane 4).

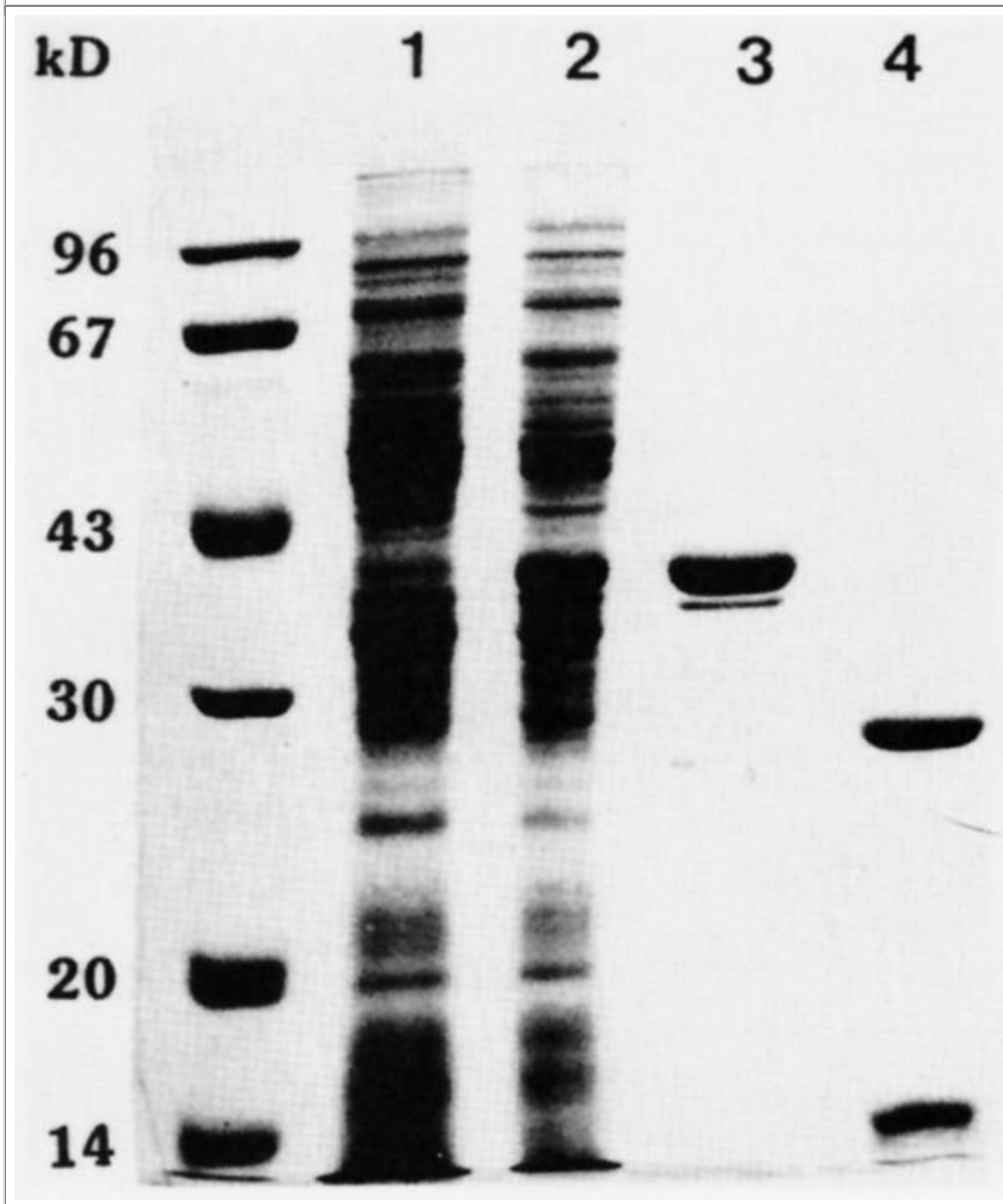


FIG. 5. IgE dot-blot radioimmunoassay. Nitrocellulose filters were divided into quadrants, and mite extract (2 µg) was applied to the upper left, recombinant GST-*Der p* V (2 µg) to the upper right, control pGEX-2T *E. coli* lysate to the bottom left, and purified GST (2 µg) to the bottom right. The results show 13 of 25 (52%) of the serum samples reacting with *Der p* V. Serum samples 1-4, 1-6

	1	2	3	4	5	6
I						
II						
III						
IV						
V						

Allergy	Asthma and rhinitis (n=15)	Rhinitis (n=17)	Asthma (n=13)	Total (n=45)	Normal (n=11)
Normal saline solution	—	—	—	—	—
Histamine (1 mg/ml)	100%	100%	100%	100%	100%
GST	13%	6%	15%	9%	—
House dust	80%	71%	75%	78%	27%
Crude mite	90%	82%	75%	85%	18%
<i>Der p I</i>	73%	65%	83%	78%	9%
<i>Der p II</i>	73%	82%	83%	80%	27%
<i>Der p V</i>	67%	29%	58%	51%	9%

The previous report on *Der p* V¹³ described a λ gt11 cDNA clone that produced a fusion protein, which reacted with IgE. Affinity-purified antibody eluted from the fusion reacted with a band with M_r of 14 kd as determined by Western blotting, whereas the published sequence encoded a reading frame for a 17 kd protein. Despite this significant advance, the authors were unable to conclude whether the sequence contained all or part of the allergen or whether it was a cleavage product from a larger molecule. The

additional sequence obtained in this study and our analysis allows us to conclude that the *Der p V* is produced from a polypeptide, which has 132 residues including a putative leader sequence of 19 residues and a mature protein of 113 residues with a molecular weight of 13,585 d. This agrees well with the size of the corresponding allergen found in mite extracts. As shown in ■Fig. 2, the sequence contained an ATG start codon at nucleotides 62-64, followed by Lys and 17 hydrophobic residues, which is a classic consensus sequence for a leader peptide. The predicted cleavage site would be between residues 19 and 20 to produce a mature protein with an N-terminal Glu. The sequence preceding the translation initiation ATG, CAATC, fits with a Kozak consensus sequence, and the entire 5' region is 82% adenine-thyrosine-rich, which is consistent with other mite 5' regions. A construct made to produce fusion protein, starting at the N-terminal Glu, produced a protein of the predicted size and with high IgE reactivity, which confirmed the reading frame. Further confirmation came from the sequence of the WL clone. This had a 5' region, which was extended beyond the previous clone, and it contained two stop codons in the same reading frame as the predicted peptide translation without any intervening start codons. The translation could not therefore be made upstream of the predicted initiation ATG at nucleotide 62-64.

The nucleotide sequence of the WM clone was identical to that published by Tovey et al.,¹³ as was the sequence of our initial *Agt11* cDNA clone, which corresponded to nucleotides 139-461. It therefore appears to be more conserved than *Der p I* for which the five cDNA sequences have differed by one to two amino acids.²⁸ The clone WL was, however, different, particularly in the 5' untranslated region, which had 17 of 61 disparate nucleotides. This was not a cloning artifact because the degree of homology, 72%, was highly significant, and the sequence was accordingly adenine-thyrosine-rich like other 5' sequences. Perhaps more significantly, this clone had three nucleotides in the coding region that were different from the other clones, one of which encoded an amino acid transition. It is conceivable that WL and WM are derived from two mRNAs, which could result either from the differential processing of a single primary transcript or from the use of different promoters.²⁹ They could also represent the result of a gene duplication. The conversion of Ala to Glu at residue 61 resulted in isoelectric point changes from 4.9 to 5.1 and a significant change in the hydrophilic profile of the protein (data not shown), suggesting that, like the example of alcohol dehydrogenase in *Drosophila melanogaster*.³⁰ The two cDNA clones probably coded for two different isoforms of *Der p V*.

The sequence for the *Der p V* allergen is now defined, except for the exact N-terminal, which will need to be determined from the natural protein. Although the allergen reacted with only 50% of sera, the reactivity appeared to be strong as determined by immunostaining of plaques and dot blots, and it will be interesting to perform quantitative measurements to determine whether those persons who react will react strongly. It could in this respect be different from *Der p III* and *Der p VI*, which usually react weakly. The pGEX *Der p V* construct produced a good yield of soluble protein, which could be cleaved from the GST fusion partner and is thus a useful reagent for further study. In this respect the skin test data showed that patients with asthma and allergic rhinitis had 67% reactivity with *Der p V* compared with 58% reactivity in patients with asthma alone and only 29% in patients with allergic rhinitis alone. At this stage it is not known whether the less frequent reactivity of the group with allergic rhinitis alone was because less mite allergen was recognized or whether there is a closer relationship.

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