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Antigenic Analysis of Group I House Dust Mite Allergens Using Random Fragments of *Der p* I Expressed by Recombinant DNA Libraries

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Abstract. Antigenic regions of a major house dust mite allergen, *Der p* I, were identified by a recombinant DNA strategy employing the technique of random fragmentation. Fragments of cDNA coding for *Der p* I were produced by sonication and used to construct lambda gt11 expression libraries. Analyses of recombinant fragments reactive with a rabbit anti-*Der p* I antiserum showed that the B cell determinants expressed in *Escherichia coli* were limited, with the majority (86%) of antigenic clones isolated mapping to the region comprising amino acid sequence position 60-80. To define antigenic regions of *Der p* I more precisely, selected overlapping fragments were subcloned into the expression vector pGEX-1. Dot blot immunoassay and immunoabsorption studies using individual fusion proteins revealed five regions - 34-47, 60-72, 82-99, 112-140, and 166-194 - to contain B cell determinants responsible for the antigenicity of recombinant *Der p* I. Absorption of the antiserum with *Dermatophagoides pteronyssinus* extract removed reactivity to all fragments, whereas absorption with an extract from the related mite *Dermatophagoides farinae* removed reactivity to peptides containing residues 34-47, 60-72, and 166-194, but not 82-99 and 112-140. Similarly, rabbit anti-*D. farinae* reacted strongly with peptides containing residues 34-47, 60-72, and 166-194, but not residues 82-99 and 112-140 which again showed antigenic differences in these residues between the group I allergens.

Introduction

Allergens derived from the house dust mite, particularly those of the genus *Dermatophagoides*, are common triggers of allergic disease [1, 2] and several allergens from both *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* have been described [3-5]. Most emphasis, however, has been placed on the study of the major allergens *Der p* I and *Der p* II from *D. pteronyssinus* and their equivalents from *D. farinae* which are recognized by at least 80% of mite-allergic individuals [3, 4, 6]. Responses to *Der p* I, a protein of 25,000 daltons associated with mite faeces [7], are of additional interest because they can often comprise over 50% of the total anti-mite IgE antibodies represented in a serum sample [8, 9]; this response being highly cross-reactive with the homologous allergen *Der f* I [10]. Recent studies in our laboratory have resulted in the isolation and sequence analysis of cDNA clones encoding *Der p* I and *Der*

p II [11, 12]. The former demonstrates homology with cysteine proteases such as papain and actinidin, the three-dimensional structures of which have been determined to high resolution by X-ray crystallography [13, 14].

As well as facilitating the definition and characterization of allergens, it is possible to use recombinant DNA technology to map the determinants recognized by both the B and T cell compartments of the immune system. Although this technique has been used in the characterization of many antigens, it has not yet been used to our knowledge for epitope analysis of allergens. Thus far, the identification of allergenic determinants has been performed in relatively few studies using techniques such as the analysis of peptides produced by chemical or enzymatic cleavage [15], the screening of overlapping synthetic peptides [16], or the utilization of monoclonal antibodies as site-specific probes [17, 18]. With the recombinant DNA strategy of gene fragmentation [19] it is possible to

systematically examine large numbers of peptides of varying length and position within an antigen. In theory, this technique can facilitate not only the analysis of continuous epitopes formed by short, linear sequences, but also larger, topographical determinants demonstrating secondary and/or tertiary structure.

Here, we describe a study which had as its aims (1) a comparison of the antigenicity of recombinant *Der p* I with its native counterpart using a polyclonal antiserum as a probe, (2) a delineation of the sequences which, when expressed in *Escherichia coli*, retain reactivity with a hyperimmune antiserum, and (3) a determination of those regions containing *Der p* I and *Der f* I cross-reacting epitopes. The relative positions of defined antigenic regions were assigned using the conserved tertiary structures of the homologous enzymes papain and actinidin as models.

Materials and Methods

Construction of Der p I Random Fragment Lambda gt11 Libraries

Fifty micrograms of the 857-base pair (bp) *Eco*RI insert DNA fragment of the lambda gt11 clone 13T [11], encoding a portion of the proenzyme sequence (residues -23 to -1) in addition to the mature protein sequence (1-222) of *Der p* I, was isolated by agarose gel electrophoresis. The complementary *Eco*RI ends of the fragment were self-ligated overnight at 15°C and extracted twice with phenol/chloroform. The aqueous phase was then extracted with chloroform, sodium acetate was added to 0.3 M, and the DNA was precipitated with 2.5 vol of ethanol at -20°C. DNA was pelleted and washed with 70% (v/v) ethanol, resuspended in 400 µl of 10 mM Tris-HCl buffer (pH 7.5) containing 50 mM NaCl, 10 mM MgCl₂, and 1 mM dithiothreitol, then sonicated using a 150-watt sonifier (Branson Sonic Power, Danbury, Conn., USA) at 0°C for 30 1-min bursts with a 6-mm tapered microtip to produce fragments. The degree of shear was monitored by electrophoresis. Blunt-ended DNA fragments, produced by treatment with Klenow polymerase (Promega Biotec, Madison, Wisc., USA) for 1.5 h in the presence of dNTPs, were ligated to an 80-fold molar excess of phosphorylated *Eco*RI linkers (New England Biolabs, Beverly, Mass., USA). This mixture was then digested with *Eco*RI (Toyobo, Osaka, Japan), heated at 70°C for 5 min, and fractionated by 9% polyacrylamide gel electrophoresis (PAGE). DNA fragments in the range 72-118 and 118-281 bp were recovered by elution into 10 mM Tris-HCl buffer (pH 8.0) containing 0.5 M ammonium acetate and 1 mM EDTA at 37°C, extracted with phenol, and ethanol precipitated. *Eco*RI-linkered fragments were ligated to alkaline phosphatase treated lambda gt11 arms (Promega Biotec). The ligated DNA was packaged, and plaques were grown in *E. coli* Y1090. Cloned fragments were sized by adding 10 µCi of α^{32} P-dATP (Amersham International, UK) and 2 U of Klenow polymerase to phage DNA digested with *Eco*RI. After incubation at room temperature for 30 min, a sample of the labelled mix was examined by electrophoresis on a 7.5% PAGE gel. Gels were covered with

plastic wrap, loaded into a Kodak X-Omatic cassette (Eastman Kodak, Rochester, N.Y., USA) together with photographic film (Fuji RX, Tokyo, Japan), and exposed overnight.

Plaque, Colony, and Dot Blot Radioimmunoassays

Screening of libraries was performed by plaque radioimmunoassay [20], using a rabbit anti-*Der p* I antiserum. Phage were plated at 12,000 plaque-forming units per 150-mm Petri dish. For immunoassay with *E. coli* HB101 transformed with pGEX-derived constructs, ampicillin-resistant colonies were grown on nitrocellulose (0.45 µm BA 85, Schleicher and Schuell, Dassel, FRG) for 3 h at 37°C, then transferred to a plate with an agar overlay containing 10 mM isopropyl- β -D-thiogalactopyranoside. After a 2-hour induction period, lysis was achieved by placing the filters on 3MM paper (Whatman International, Maidstone, UK) saturated with 1% (w/v) sodium dodecyl sulphate (SDS) in an atmosphere of chloroform. For dot blotting, samples were transferred to nitrocellulose with a Bio-Dot apparatus (Bio-Rad Laboratories, Richmond, Calif., USA). Nitrocellulose was cut to size, soaked in 20 mM phosphate buffer (pH 7.4) containing 150 mM NaCl (phosphate-buffered saline), and placed on 3MM paper before being clamped into the apparatus. Samples containing 10 µg of recombinant or native protein were applied in a volume of 200 µl and drawn through the nitrocellulose by suction.

Each well was washed twice with 400 µl phosphate-buffered saline. For all procedures, the nitrocellulose was then washed with 10 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl and 0.05% w/v Tween 20 for 30 min and incubated with serum for 2 h at room temperature. All sera had been preabsorbed with *E. coli* lysate [21]. After incubation with serum, the filters were washed and bound rabbit IgG detected using ¹²⁵I-radiolabelled staphylococcal protein A [21] at 10⁶ cpm/ml. Autoradiography was carried out with intensifying screens at -70°C.

Sequence Analysis

Phage DNA, prepared by a rapid isolation procedure [11], was digested with *Eco*RI and ligated to M13 mp18 DNA. Transformation was carried out using *E. coli* JM107, and sequencing was performed by the dideoxynucleotide chain termination method [22].

Expression and Purification of Sj26-Der p I Fragment Fusions

DNA fragments were cloned into the unique *Eco*RI site of pGEX-1 [23], located at the 3' end of the Sj26 gene which codes for *Schistosoma japonicum* glutathione-S-transferase (GST). Vector DNA was treated with bacterial alkaline phosphatase (Toyobo) and the ligated mix used to transform *E. coli* HB101. Recombinant pGEX clones expressing *Der p* I fragments in the correct orientation were detected by colony radioimmunoassay using rabbit anti-*Der p* I. GST-*Der p* I peptide fusions were affinity purified from glutathione agarose [23]. Briefly, overnight cultures of transformed *E. coli* were diluted (1:10) into fresh medium and grown for 1 h at 37°C before adding isopropyl- β -D-thiogalactopyranoside to 0.1 mM. After a further 3-hour incubation, the cells were pelleted and resuspended in 0.01 culture volumes of 10 mM Tris-HCl buffer (pH 7.5) containing 150 mM NaCl. The cells were lysed by sonication after treatment with lysozyme (0.25 mg/ml) and three freeze/thaw cycles. Crude lysates were used for serum absorptions or after addition of Tween 20 (Sigma Chemical, St. Louis, Mo., USA) to 1% (v/v) were clarified by centrifugation at 10,000 g for 10 min at 4°C. Ten milliliters of supernatant was mixed with 1 ml of preswollen

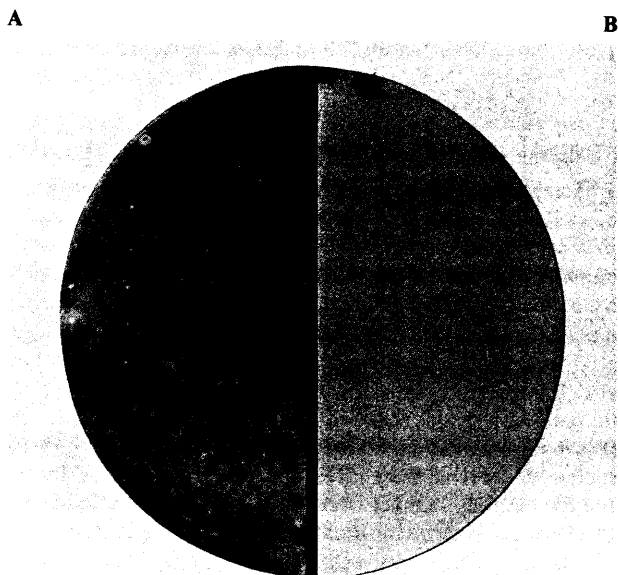


Fig. 1. Plaque radioimmunoassay. Nitrocellulose filters (150 mm), with antigen from 1.2×10^4 phage plaques produced by a *Der p* I cDNA random-fragment library (118–281 bp), were probed with a rabbit anti-*Der p* I antiserum absorbed with (A) an *E. coli* lysate containing protein expressed from the lambda vector itself or (B) a pool of lysates from clones expressing the antigenic *Der p* I peptides shown in figure 3. The results obtained indicated that 15% of plaques assayed with rabbit anti-*Der p* I were positive. No positive signals (in 48,000 plaques assayed) were found with the same antiserum after absorption with all of the antigenic fusions.

glutathione agarose beads (Sigma) and rotated at room temperature for 15 min. Beads were collected by centrifugation and washed with 3×10 ml Tris-HCl buffer containing 150 mM NaCl. Fusion proteins were eluted by adding 4 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 5 mM reduced glutathione (Sigma). Protein concentrations were determined from optical density measurements employing the algorithm of Waddell [24].

SDS-PAGE

SDS-PAGE [25] was performed using 8–18% (w/v) polyacrylamide gradient gels stained with 0.06% (w/v) Coomassie blue R250 (Sigma).

Antisera

The rabbit anti-*Der p* I antiserum was obtained by immunization with *Der p* I in Freund's complete adjuvant [26]. The rabbit anti-*D. farinae* antiserum, kindly provided by Mr. D. Hordle (Beecham Research Laboratories, Epsom, U.K.), was raised against whole *D. farinae* extract. Both antisera were absorbed with *E. coli* lysate (see below) and diluted to 1:100 (v/v) in phosphate-buffered saline containing 5% (w/v) skim milk powder and 0.1% (w/v) sodium azide.

Serum Absorptions

Antisera were absorbed with a lysate of *E. coli* HB101 transformed with pGEX or pGEX constructs expressing fusions with

Der p I fragments or with spent mite growth medium (SMM) extracted at 20% (w/v). SMM were obtained from pure cultures of either *D. pteronyssinus* or *D. farinae* kindly provided by Commonwealth Serum Laboratories (Parkville, Vic., Australia). Rabbit antisera were diluted 1:50 in the sonicate/extract, incubated with rotation overnight at 4°C, and then clarified by centrifugation (10,000 g, 15 min).

Affinity Purification of Native *Der p* I and *Der f* I

Der p I/*Der f* I-specific monoclonal antibody 4C1 B8 3F8, a generous gift from Dr M. Chapman and Prof. T. Platts-Mills (Department of Allergy and Clinical Immunology, University of Virginia, Charlottesville, Va., USA) was precipitated from ascites by 50% ammonium sulphate and coupled to 5 ml of Sepharose 4B (Pharmacia LKB Biotechnology, Uppsala, Sweden) using cyanogen bromide. SMM extract (10% w/v) derived from cultures of either *D. pteronyssinus* or *D. farinae* was passed through the column. The column was washed with phosphate-buffered saline and *Der p* I/*Der f* I eluted with distilled water adjusted to pH 11.0 with ammonium hydroxide.

Results

Construction of Random Fragment Libraries

To examine peptide antigens specified by linear segments of *Der p* I cDNA, DNA from the clone 13T was randomly sheared by sonication. Random breakage was optimized by ligating complementary *Eco*RI ends before sonicating. Two size ranges of fragments (72–118 and 118–281 bp) were subcloned into lambda gt11 and expressed as fusions with the lacZ gene which codes for *E. coli* β -galactosidase (116 kD). This enabled the examination of peptides comprising 24–40 and 40–90 residues. The libraries obtained comprised 51,000 and 149,000 recombinant phages for the 72–118 and 118–281 fractions, respectively. Size ranges were verified by PAGE of end-labelled DNA inserts from 20 clones.

Screening Libraries in Lambda gt11 for Antigenic Activity

The libraries were probed by plaque radioimmunoassay with rabbit anti-*Der p* I. Positive signals of varying strength (fig. 1A) were demonstrated by 6% of plaques producing β -galactosidase-fused *Der p* I peptides in the size range of 20–40 amino acid residues and 15% in the size range of 40–90 amino acids (corrected for orientation and reading frame). Twenty-two of these clones were purified and sequenced. Figure 2 illustrates the position of the fragments with respect to the full-length *Der p* I coding sequence. Their distribution was non-random, with most clones ex-

Fig. 2. Map showing the location of inserts from the 22 lambda gt11-*Der p I* fragment clones expressing fusion proteins reactive with rabbit anti-*Der p I* antiserum. The solid bar represents the full-length coding region of mature *Der p I*. Positive clones, from both sublibraries of 72-118 and 118-281 bp were isolated and plaque purified. Phage DNA was digested with *EcoRI* to release inserts which were ligated to M13 mp18 and sequenced by the dideoxy chain termination method.

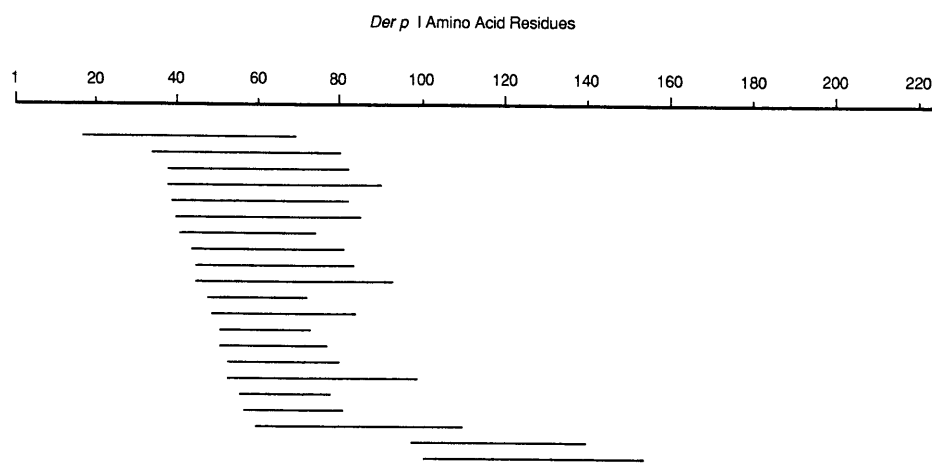


Table 1. Cross-absorption of rabbit anti-*Der p I* reactivity using overlapping *Der p I* fragments

Serum absorbed with peptides comprising residues	Serum reactivity with peptides comprising residues						
	34-80	48-72	53-99	57-81	60-111	98-140	101-154
34-80	-	-	+	-	+	++	++
48-72	+	-	+	-	+	++	++
53-99	+	-	-	-	-	++	++
57-81	+	-	+	-	+	++	++
60-111	+	-	-	-	-	++	++
98-140	++	++	++	++	++	-	-
101-154	++	++	++	++	++	-	-

The rabbit antiserum was absorbed with each of seven fusion proteins containing overlapping peptides (see figure 3). The changes in reactivity of antigenic peptides after absorption was assessed by dot blot radioimmunoassay and the results used to deduce regions responsible for rabbit antibody reactivity. Region 34-47 was inferred because the peptides 34-80 retained antigenicity after absorption of rabbit anti-*Der p I* by the peptides 48-72 and 57-81; region 60-72 because absorption with 60-111 abrogated reactivity to the cross-absorptive peptides 48-72 and 57-81; region 82-99 since the peptide 53-99 retained reactivity after absorption with 48-72 and 57-81, and region 112-140 by the non-reactivity of peptides 60-111 and 101-154 following absorption with 53-99 and 98-140, respectively. ++ = Positive reaction; + = reduced reaction; - = no reaction.

pressing peptides containing residues 40-80, centered on a core sequence between residues 60 and 72. There were two fragments in the region 100-140 and one comprising residues 166-194. No positive signals were detected when normal rabbit serum was used as a control (data not shown).

Definition of Der p I Antigenic Regions Using Fusion Proteins Expressed by pGEX

To define antigenic regions more precisely, selected overlapping fragments were subcloned into a

plasmid (pGEX) capable of producing large quantities of relatively soluble fusion proteins. A cross-absorption analysis was performed by absorbing aliquots of the rabbit anti-*Der p I* antiserum with each of seven GST-*Der p I* peptide fusions contained in bacterial lysates. Each aliquot of absorbed serum was then tested by dot blot to assess changes in reactivity to the fusion proteins expressed by the seven clones. The results are summarized in table 1 and figure 3. Based on a linear model, five regions, 34-47, 60-72, 82-99, 112-140, and 166-194, contained residues responsible

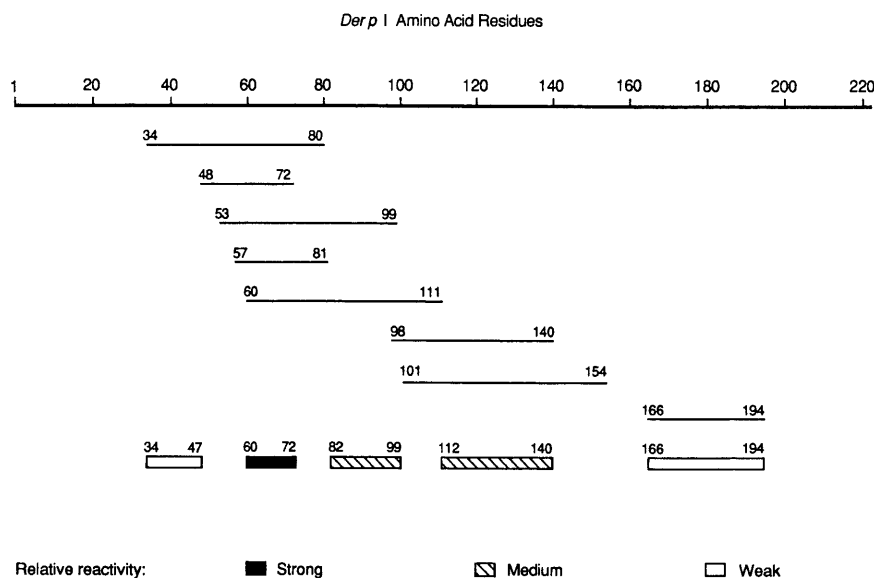


Fig. 3. Antigenic peptides encoded by *Der p I* cDNA fragments subcloned into pGEX-1. These peptides were used in a cross-absorption analysis of rabbit anti-*Der p I* antiserum (see table 1). Boxes correspond to regions of *Der p I* deduced to be responsible for reactivity of the rabbit antiserum (explained in table 1 legend). Relative strengths of binding as measured by dot blot radioimmunoassay are indicated.

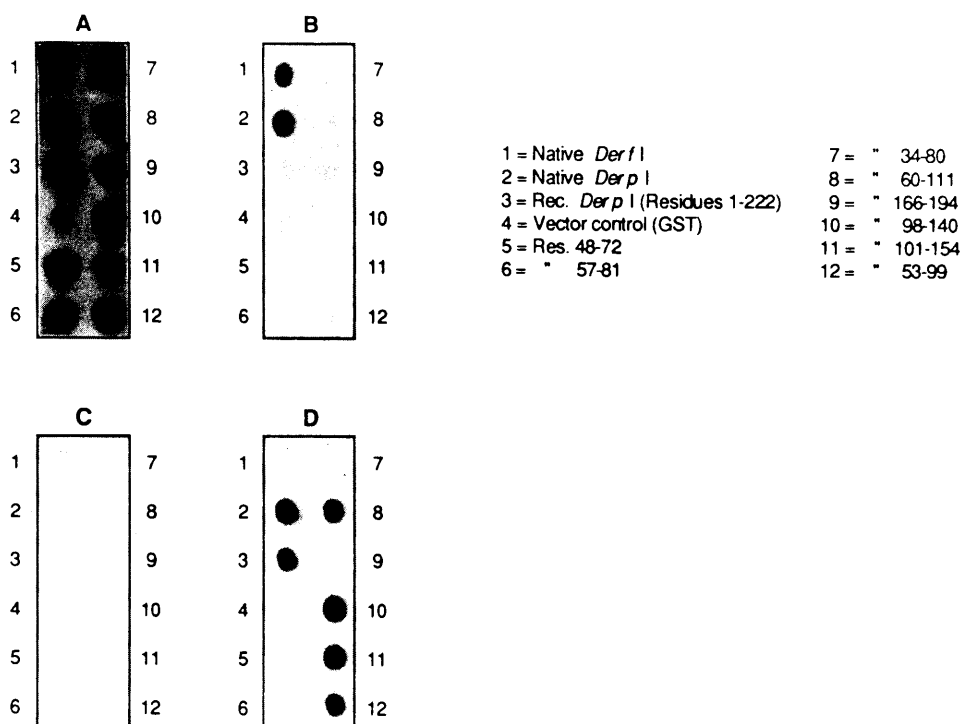
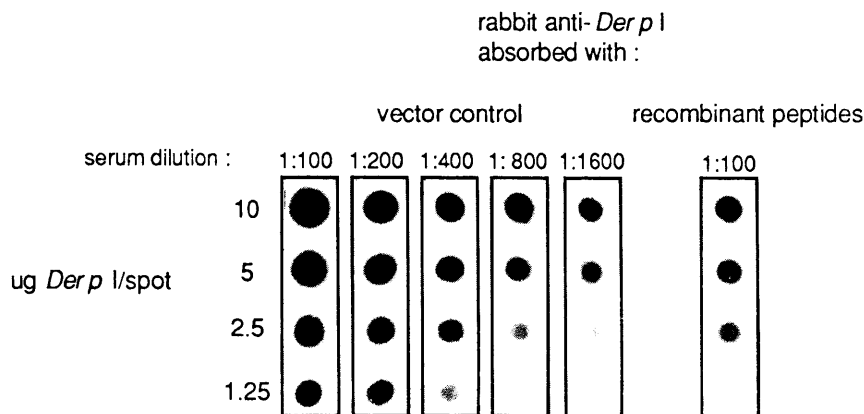


Fig. 4. Dot blot radioimmunoassay. Bacteria containing recombinant pGEX-1 plasmids were grown and induced as described in Materials and Methods. Affinity-purified recombinant antigens were filtered onto nitrocellulose membranes presoaked with phosphate-buffered saline 10 µg of fusion protein from all clones was filtered through separate wells to create antigen dots which included a dot of the full-length *Der p I* clone, protein expressed from the vector itself (GST), and native *Der p I*/*Der p I* isolated from SMM. The reactivity with rabbit anti-*Der p I* (A) or this same antiserum after absorption with *E. coli* lysate containing fusion proteins expressed by all eight clones (B), *D. pteronyssinus* SMM extract (C) or *D. farinae* SMM extract (D) is shown.

for antigenic reactivity. For example, the region comprising residues 34-47 was presumed to account for part of the reactivity of peptide 34-80 because absorption with fusion protein from clone 48-72 removed all reactivity to itself and 57-81, but not 34-80. No positive clones could be detected when the libraries were screened with antiserum absorbed with a pool of ly-

sates containing fusion proteins expressed by all eight subclones (fig. 1B). Absorption also abrogated reactivity to recombinant *Der p I*, but not all reactivity to native *Der p I* isolated from mite faeces (fig. 4A, B). The reactivity of the absorbed serum with the native molecule was, however, reduced by approximately 75% when compared to the control serum (fig. 5).

Fig. 5. Dot blot radioimmunoassay showing reactivity of native *Der p* I with rabbit anti-*Der p* I absorbed with an *E. coli* lysate containing GST expressed from the vector itself (vector control) or a pool of fusion proteins from all 8 clones (recombinant peptides). To estimate the degree of absorption, 1:100 to 1:1,600 dilutions of serum were tested against graded amounts (1.25–10 μ g) of antigen. A 1:100 dilution of serum absorbed with the peptides had a reactivity equivalent to a 1:400–1:800 dilution of serum absorbed with GST control.



Cross-Reactivity between *Der p* I and *Der f* I

To examine whether or not the epitopes defined were restricted to *Der p* I or shared with the homologous allergen *Der f* I, the rabbit anti-*Der p* I was tested by dot blot radioimmunoassay against individual fusion proteins after immunoabsorption with SMM extract from either *D. pteronyssinus* or *D. farinae*. The results show (fig. 4C, D) that whereas absorption with *D. pteronyssinus* extract abrogated reactivity to all of the peptides as well as the full-length recombinant molecule, serum absorbed with *D. farinae* extract retained reactivity to peptides containing regions 82–99 and 112–140. Reactivity attributed to regions 34–47, 60–72, and 166–194 was, however, completely removed by *D. farinae*. A similar pattern was found when the peptides were tested for reactivity to hyper-immune rabbit anti-*D. farinae*. Strong reactivity was found with most peptides containing antigenic residues 34–47, 60–72, and 166–194, but not 82–99 and 112–140 (fig. 6). Thus, it appeared that rabbit antibodies specific for epitopes on regions 34–47, 60–72, and 166–194 were cross-reactive and those directed toward regions 82–99 and 112–140 species specific.

Discussion

Isolation and sequencing of a cDNA clone coding for *Der p* I has facilitated not only the identification of this important allergen, but should also provide recombinant material for a range of immunological investigations. The lacZ fusion protein produced by *Der p* I cDNA cloned into *E. coli* with lambda gt11 is, however, insoluble and unsuitable for serological studies. Nevertheless, given some knowledge of the

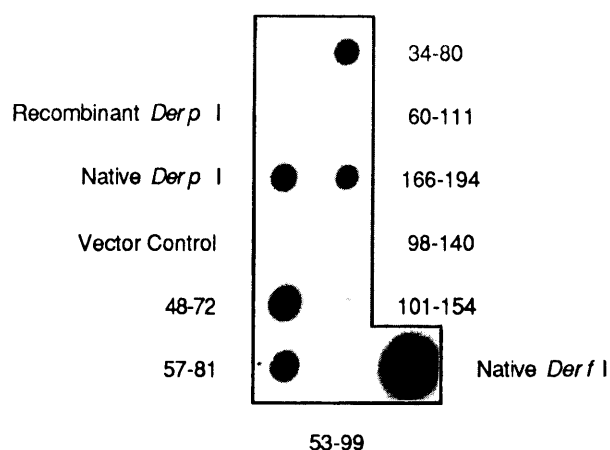


Fig. 6. Dot blot radioimmunoassay performed as described in figure 4 legend, demonstrating the reactivity of the eight recombinant peptide clones together with the full-length clone, vector control (GST), and native *Der p* I / *Der f* I with rabbit anti-*D. farinae*.

antigenic structures expressed on the recombinant protein, it should be feasible to engineer constructs useful for IgE epitope mapping and other investigations. In this study, random fragment libraries produced by mechanically shearing cDNA coding for *Der p* I were used to examine those regions of this molecule which retain antigenicity after expression in *E. coli*. The results showed that the reactivity of a rabbit anti-*Der p* I antiserum was limited to a small fraction of the recombinant clones in the lambda gt11 libraries. This suggests that only a small proportion of the peptides expressed in *E. coli* are capable of forming detectable epitopes. Indeed, only five antigenic regions were identified which comprise amino acid residues 34–47, 60–72, 82–99, 112–140, and 166–194. The

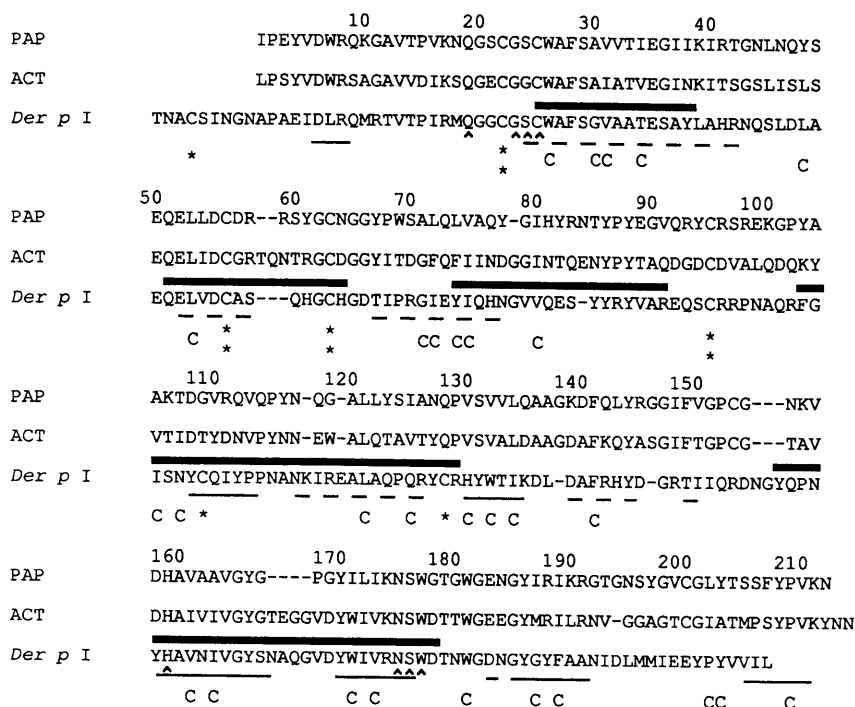


Fig. 7. Composite alignment of the amino acid sequences of *Der p I*, papain (PAP), and actinidin (ACT). The numbering above the sequences refers to papain. Arrowheads indicate those residues constituting the active site of papain and actinidin. C represents residues in the hydrophobic cores of papain and actinidin. - - - = α -Helices; - = β -pleated sheet. Double asterisks denote homologous 1/2 cysteine residues involved in disulphide bond formation in papain and actinidin. The single asterisks indicate cysteines in *Der p I* for which there are no homologous counterparts in either papain or actinidin. Solid bars indicate those regions determined to be responsible for reactivity with the rabbit anti-*Der p I* antiserum (see figure 3) and correspond to residues 34-47, 60-72, 82-99, 112-140, and 166-194.

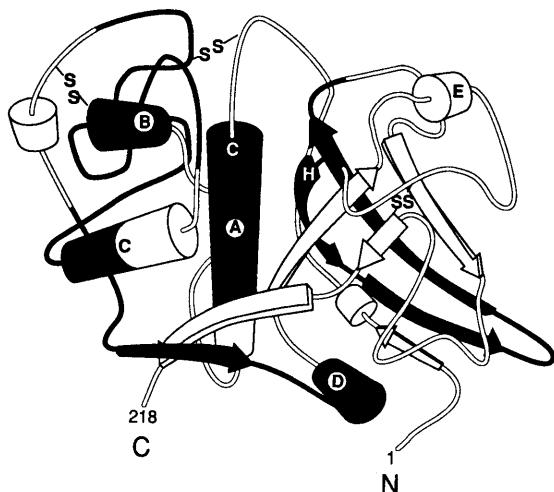


Fig. 8. Schematic representation of the polypeptide folding in papain and actinidin, modified from Baker and Drenth [28]. α -Helices are represented by cylinders, and strands of β -sheet by arrows. C and H represent the catalytic residues cysteine 34 and histidine 170, respectively. Areas in black indicate those regions of *Der p I* deduced to be responsible for reactivity of the rabbit antiserum.

limits of these sequences were defined using absorption studies with selected *Der p I* peptides that had been subcloned and expressed as smaller and more soluble fusions by the plasmid vector pGEX-1.

Because *Der p I* is a thiol protease [27] homologous

to papain and actinidin, the antigenic residues have been illustrated in figure 7 which shows a composite alignment of the sequence of these enzymes and the conserved residues which comprise the active site and hydrophobic cores. Tertiary structure for papain and actinidin is conserved, and a schematic representation of the polypeptide folding of a cysteine protease has been constructed [28]. Using this model (fig. 8) as a reference, the major antigenic region 60-72 corresponded to α -helix B and some of the loop connecting the B and C helices. Region 34-47 consisted of most of helix A and contained residues that line the active-site cleft. Antigenic determinants involving active-site regions have been observed in several enzymes, possibly because of the mobility required for substrate binding [29]. However, it has yet to be determined whether the rabbit antibody used in this study is capable of inhibiting proteolytic activity.

Also recognized by the rabbit antiserum was region 82-99 which comprised part of helix C and an adjoining loop structure. The antigenic region in the right domain (166-194) consisted of a β -turn connecting a largely buried antiparallel β -pleated sheet. The remaining antigenic sequence (112-140) was located in the extended surface loop that links the left and right domains and includes helix D. The rabbit antiserum did not detect any N- or C-terminal peptides of *Der*

pI, a result which has been confirmed using peptides expressed from cDNA fragments generated by restriction endonuclease digestion [unpubl. observations]. This is at variance with the observation that many antigenic determinants are located in relatively mobile amino and carboxyl termini [30]. However, the presence of a cysteine at residue 4 indicates a possible covalent attachment and thus reduced mobility of the amino terminus, whereas the C terminus is hydrophobic and may be buried or inaccessible after folding.

Native mite extracts were able to inhibit binding of anti-*Der p I* antibodies to the recombinant peptides. This result indicated that the epitopes described are not cryptotopes expressed only in *E. coli*. It is also likely that these fragments contain all of the epitopes which account for the rabbit anti-*Der p I* reactivity of recombinant *Der p I*. This is because absorption of the rabbit antiserum with all of the subclones abrogated reactivity to both the recombinant antigens expressed by the lambda gt11 random fragment libraries and the full-length clone. However, the number of B cell epitopes present on *Der p I* clearly exceeds the five identified in this report because after absorption of rabbit anti-*Der p I* with the recombinant peptides about 25% of the reactivity to native *Der p I* remained.

Previous studies [10, 31] have shown that the closely related allergens *Der p I* and *Der f I* are highly cross-reactive with respect to antibodies present in mouse, rabbit, and human sera. This cross-reactivity is not unexpected given the high homology (80%) between the known sequence of these two allergens [32]. Not all epitopes are shared between *Der p I* and *Der f I*, however, since 40% of the reactivity of a rabbit anti-*Der p I* antiserum was shown to be accounted for by epitopes unique to *Der p I* [31]. In addition, species-specific epitopes represent the major class of determinants recognized by the monoclonal antibodies of Chapman et al. [17]. In agreement with these observations, 3 of the 5 antigenic regions defined in this study comprised epitopes shared with *Der f I* (34–47, 60–72, and 166–194), and two (82–99 and 112–140) appeared specific for *Der p I*. The latter two regions that were not cross-reactive appeared to be located on surface loops and are perhaps less highly conserved than those sequences associated with defined secondary structures or the active-site cleft.

The structural basis for the species-specific reactivity will become more apparent when the full primary sequence of *Der f I* is determined and, if it extends to

IgE reactivity, may provide serological markers for determining exposure to the different mite species. Ultimately, this may have a bearing on the allergen used for immunotherapy. The pGEX clones described in this study are presently undergoing further examination because they may retain some structure and display reactivity with mite-specific human IgE and IgG.

Acknowledgements

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