

IMMUNOGENICITY AND ANTIGENICITY OF SYNTHETIC PEPTIDES DERIVED FROM THE MITE ALLERGEN *Der p I*

PASCALE JEANNIN,* YVES DELNESTE,* ERIC BUISINE,† JOËLLE LE MAO,‡
ALAIN DIDIERLAURENT,§ GEOFFREY A. STEWART,|| ANDRÉ TARTAR,†
ANDRÉ-BERNARD TONNEL* and JOËL PESTEL*¶**

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¶URA CNRS 624, Institut Pasteur, BP 245, 59019 Lille, France; †Chimie des Biomolécules, URA
CNRS 1309, Institut Pasteur, BP 245, 59019 Lille, France; ‡Immuno-Allergie, Institut Pasteur, Rue
du Dr. Roux, 75724 Paris, France; §Laboratoire des Stallergènes, Allée des Platanes, 94264 Fresnes,
France; and ||The Western Australian Research Institute for Child Health, Princess Margaret
Children's Hospital, Subiaco, Perth, Western Australia 6008, Australia

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Abstract—As an immunogen must contain both B- and T-cell epitopes, small peptides are usually reported as non-immunogenic unless coupled to a protein carrier. In this study, the immunogenicity of the *Der p I* synthetic uncoupled peptides (p52–71, p89–104, p117–133 and p176–187) previously reported as B-cell epitopes, was evaluated. Different schedules of immunization were used. Results indicated that by using the Vaitukaikis' method three injections of the same peptide without protein carrier was sufficient to induce an specific anti-peptide IgG antibody response (evaluated by ELISA). Indeed, the 16–20 amino-acid long peptides p52–71, p117–133 and p89–104 were revealed highly immunogenic in rabbits. Furthermore anti-peptide p52–71 and p117–133 antibodies were shown by Western-blotting or by neutralization assay to recognize the *Der p I* molecule either in denaturated or native form as well as *Der f I* (major allergen of *Dermatophagoides farinae*). Finally, taking into account the location of *Der p I*-derived peptides in the three-dimensional model of *Der p I*, the antigenicity and immunogenicity of peptides were discussed.

INTRODUCTION

The association between the house dust mite and allergic diseases such as asthma and rhinitis is now well established (reviewed by Platt-Mills *et al.*, 1987). Mites of the genus *Dermatophagoides* [species *farinae* (*Df*) and *pteronyssinus* (*Dpt*)] are a source of various allergenic molecules (Lind and Lowenstein, 1983; Le Mao *et al.*, 1983; Krilis *et al.*, 1984). *Der p I*, a 25 kD cysteine protease found in mite faecal particles (Tovey *et al.*, 1987; Thompson and Carswell, 1988) is considered as a major allergenic molecule of *Dpt*; indeed about 80% of *Dpt*-sensitive patients may have specific anti-*Der p I* IgE (Chapman *et al.*, 1983). The recent cDNA cloning of *Der p I* (Chua *et al.*, 1988; Thomas *et al.*, 1988) has allowed a more precise analysis of the immune response to *Der p I* including the definition of *Der p I* T- and B-cell epitopes. For example *Der p I*-recombinant peptides as well as *Der p I* or *Dpt* allergens were shown to induce the proliferation of *Dpt*-T cell clones (Yssel *et al.*, 1992) and peptides binding IgE (residues 1–56, 53–99, 98–104, 166–194 and 188–222) have been described (Greene *et al.*, 1991, 1992). In similar studies, four *Der p I*-synthetic peptides (p52–71, p117–133, p176–187, p188–199) have been identified as potential B-cell epitopes on the basis of their capacity to activate basophils and platelets from

Dpt-sensitive patients (Cardot *et al.*, 1992; Jeannin *et al.*, 1992).

It is known that an immunogen must contain both B- and T-cell epitopes and that small peptides are not usually immunogenic unless coupled to a carrier (Beachey *et al.*, 1984; Schulze-Gahmen *et al.*, 1986; Good *et al.*, 1987) or possessing all these conjugates features (Francis *et al.*, 1987; Tam and Lu, 1989). As different reports have focused on the *in vitro* analysis of B- and T-cell responses to *Der p I*-derived peptides, we have evaluated, in this study, the immunogenicity of four *Der p I*-derived peptides, p52–71, p89–104, p117–133, p176–187. We report that peptides p52–71, p89–104 and p117–133, which were previously described as potential *Der p I* B- and T-cell epitopes, are immunogenic in rabbits and in rats. Moreover, anti-peptide p52–71 and p117–133 antibodies were shown to recognize native *Der p I* and, in addition, to cross-react with *Der f I*, one of the major allergens from *Dermatophagoides farinae* belonging to the group I house dust mite allergens which present homology with defined cysteine proteases.

MATERIALS AND METHODS

Allergens and proteins

Enriched *Der p I*-containing *Dpt* antigen (100 µg/ml of *Der p I*) and *Der f I*-containing *Df* antigen

**To whom all correspondence should be addressed.

(100 µg/ml of *Der f* I) (quantified as described by Luczynska *et al.*, 1989) were prepared by the Laboratoire des Stallergènes (Fresnes, France). Purified *Der f* I was obtained as described by Dandeu *et al.* (1982). Standardized hymenoptera venom antigen (Laboratoire de Stallergènes), purified papain (EC 3.4.22.2) and human cathepsin B (EC 3.4.22.1) (Sigma, Saint-Louis, MO) were also used.

Molecular modelization of *Der p* I molecule

The HOMOLGY and the DISCOVER modules of the BIOSYM software package (BIOSYM Technologies, San Diego, CA) were used to build a three-dimensional (3D) model of the *Der p* I structure. By using the FASTA program (Pearson and Lipman, 1988) the *Der p* I sequence was shown to be similar to two structurally known proteases: actinidin (Baker and Dodson, 1980) and papain (Kamphuis *et al.*, 1984). Thus, the sequence of *Der p* I (except the first nine residues) was optimally aligned on the structurally conserved regions and on the key-residues of these enzymes. For each conserved region, atomic coordinates of one of the two reference-structures were transferred to the corresponding part of the *Der p* I sequence. The structurally variable regions at the junction of the different conserved domains were built with geometrically appropriate similar fragments of protein structures found by scanning the Protein Data Bank (Brookhaven National Laboratory, NY). Finally, molecular mechanics procedures were used to eliminate steric overlaps and to refine the variable regions. Calculations were performed on a 4D35 Silicon Graphics workstation.

Der p I-derived peptides

Using conventional predictive methods (Cardot *et al.*, 1992) and the modeled *Der p* I, *Der p* I sequences were selected as potential B-cell epitopes and corresponding peptides synthesized as described (Jeannin *et al.*, 1992). The homogeneity of each peptide was checked by analytical reverse phase-HPLC and the amino-acid com-

position by total acid hydrolysis. The sequences of peptides were: NQSLDLAEQELVDCASQHGHC for p52–71; VQESYYRYVAREQSCR for p89–104; CQIY-PPNANKIREALAQ for p117–133; (acm)CGYSNA-QGVDYWIC (acm: acetamidomethyl protection) for p176–187. A peptide derived from the protein P28 of *Schistosoma mansoni* (KPQEEKEKITKEILNGK) was used as a control.

Immunization protocols

Peptide (100 µg) or *Dpt* Ag (containing 0.2 µg *Der p* I) in 250 µl of 0.1 M phosphate buffer pH 7.4 (PBS) were emulsified with CFA (v/v) and injected in rabbits subcutaneously as described by Vaitukaitis *et al.* (1981). At one month intervals, rabbits were boosted with the same amount of Ag first in CFA and then in IFA. Thus, some rabbits were injected either three times with peptides: p52–71 (rabbit A), p117–133 (rabbit B), p89–104 (rabbit C), or once with *Dpt* and twice with peptides: p52–71 (rabbit A1), p117–133 (rabbit B1), p176–187 (rabbit D1). One rabbit (B2) was injected twice with *Dpt* and once with p117–133. Sera, obtained two weeks after the last injection and which presented the highest levels of anti-peptide Ab, were analysed here.

Similarly, six Fisher rats (4–6 weeks old) injected subcutaneously with 100 µg of p89–104 ($n = 3$) or p117–133 ($n = 3$) emulsified in CFA, were further boosted twice with the same dose of peptide at two-week intervals. Sera obtained one week after the last injection were studied here.

ELISA

Microtitre plates (Nunc, Roskilde, Denmark) were coated with *Dpt* Ag (0.2 µg *Der p* I/ml), peptides (20 µg/ml) or purified *Der f* I (1 µg/ml) in 0.1 M carbonate-bicarbonate buffer, pH 9.6 (100 µl) overnight at 4°C and then saturated with 1% (w/v) BSA in PBS. Sera diluted in PBS/BSA containing 0.1% (v/v) Tween 20 were incubated for 4 hr at room temperature. After washings, peroxidase-conjugated goat anti-rabbit IgG

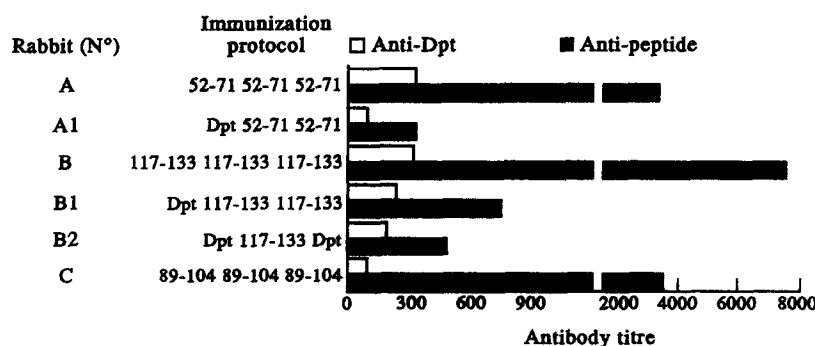


Fig. 1. Specific anti-peptide and anti-*Dpt* antibodies in rabbits immunized with *Der p* I peptides. Some rabbits were injected three times either with p52–71 (rabbit A) or with p117–133 (rabbit B) or with p89–104 (rabbit C); other rabbits were primed with *Dpt* and then injected twice with peptides: p52–71 (rabbit A1), p117–133 (rabbit B1). Rabbit B2 was injected twice with *Dpt* and once with p117–133. For each rabbit serum, an anti-peptide Ab titre (anti-p52–71 for rabbit A and A1, anti-p117–133 for rabbits B, B1, B2 and anti-p89–104 for rabbit C) (■) as well as an anti-*Dpt* Ab titre (□) was defined in ELISA.

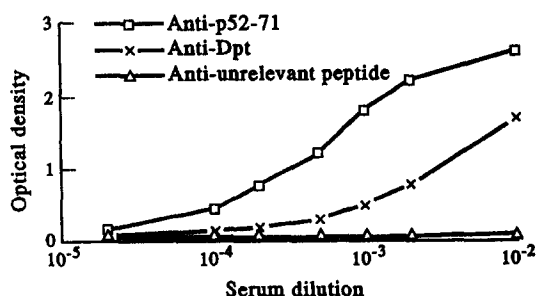


Fig. 2. Specificity of the antibody response induced by p52-71. Serum from rabbit A (immunized with p52-71) was tested at various dilutions (from 10^{-5} to 10^{-2}) in ELISA for the presence of IgG Ab against p52-71 or against a *Der p* I-containing *Dpt*. Wells coated with irrelevant peptides were used as negative controls.

Ab (Diagnostic Pasteur, France) or rabbit anti-rat IgG Ab (ICN, Lisle, Israel) (at 1/1000) in PBS/BSA/Tween 20 were incubated at room temperature for 3 hr. After washings, *o*-phenylene diamine (2 mg/ml) in 0.1 M citrate buffer pH 5 was added and 30 min later the absorbance was determined at 492 nm. ELISA titre was defined as the reciprocal of the antiserum dilution giving an OD = 1. In our conditions, the incubation of non-immune sera with peptide-coated plates or of rabbit immune sera with BSA-coated plates always resulted in OD lower than 0.05.

Western-blot analysis

The antigens were boiled for 2 min with the sample buffer (0.1 M Tris-HCl pH 7, 0.1% SDS (sodium dodecyl sulphate) containing 2-mercaptoethanol and were electrophoresed on a 13% polyacrylamide gel. After transfer onto nitrocellulose membranes (0.45 μ m; Schleicher & Schuell, Germany) and saturation, nitrocellulose strips were incubated with rabbit immune sera (at 1/1000) overnight at 4°C under constant agitation. *Der p* I was located using a polyclonal rabbit anti-*Der p* I serum (Stewart *et al.*, 1989). After washings, bound rabbit IgG were detected by peroxidase-conjugated goat anti-rabbit IgG Ab (at 1/1000) overnight at 4°C under constant agitation. After a final washing, bands were revealed with 0.06% (w/v) 4-chloro-1-naphthol/0.075% (v/v) H_2O_2 in PBS (Bio-Rad, Richmond, CA).

Neutralization assay

To determine the ability of anti-peptide Ab to react with the non-denatured *Der p* I, *Der p* I-containing *Dpt* was used to inhibit Ab binding to *Dpt* immobilized on microtitre plates or onto nitrocellulose sheets. Neutralization ELISA was identical to direct ELISA, except that sera were pre-incubated overnight at 4°C with a soluble *Dpt* (at *Der p* I concentrations ranging from $25-25 \times 10^{-5}$ μ g/ml) before incubation with coated *Dpt* Ag. In Western-blot analysis, sera were pre-incubated with *Dpt* (corresponding to 50 μ g *Der p* I/ml), hymenoptera venom or peptide (200 μ g/ml) before testing.

RESULTS

1. Immunogenicity of *Der p* I peptides

As presented in Fig. 1, immune sera from rabbits A, B and C easily recognized peptides (anti-p52-71 Ab titre of 3333 for rabbit A, anti-p117-133 Ab titre of 7500 for rabbit B and anti-p89-104 Ab titre of 3500 for rabbit C). One or two injections of *Der p* I-containing *Dpt* led to a lower binding to peptide (anti-p52-71 Ab titre of 1000 for rabbit A1 and anti-p117-133 Ab titre of 500 and 750 for rabbits B1 and B2, respectively). Only p176-187 was weakly immunogenic (titre of 100) (not shown). In addition these rabbit immune sera also recognized *Dpt* Ag (Fig. 1). Binding to *Dpt* was higher for sera from rabbits A and B (titre of 333) than for sera from rabbits A1, B1 and B2 injected once or twice with *Dpt* (titres ranging from 100 to 250). Nevertheless, for rabbit C a lower response to *Dpt* is observed (titre of 100). In rats, p89-104 and p117-133 were also immunogenic; indeed the anti-peptide Ab titres ranged from 450 to 2000 for anti-p89-104 and from 400 to 1000 for anti-p117-133.

Then, without any previous *Dpt* injection, *Der p* I-peptides p52-71, p89-104, p117-133 are highly immunogenic and able to induce an anti-*Dpt* response.

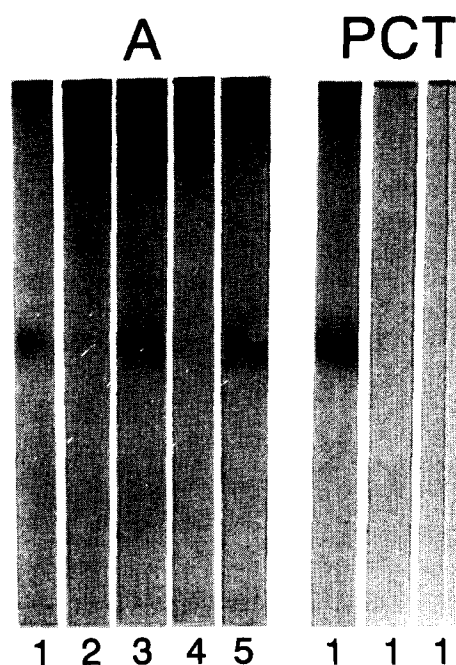


Fig. 3. Recognition of *Der p* I by anti-peptide rabbit Ab. *Der p* I-containing *Dpt* was separated on SDS-PAGE and transferred onto nitrocellulose membrane. Sheets were first incubated with rabbit serum (diluted at 1/2000) and secondly with peroxidase-conjugated anti-rabbit IgG antiserum. After enzyme reaction, the 24-26 kD band corresponding to *Der p* I was evidenced. Lane A1: rabbit A serum; lane A2: rabbit A serum after pre-incubation with p52-71; lane A3: rabbit A serum after pre-incubation with unrelated peptide; lane A4: rabbit A serum after pre-incubation with *Der p* I containing *Dpt*; lane A5: rabbit A serum after pre-incubation with hymenoptera venom Ag; lane P1: rabbit anti-*Der p* I serum; lane C1: rabbit C serum; lane T1: non-immunized rabbit serum.

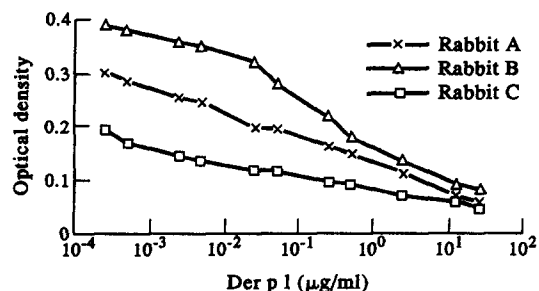


Fig. 4. Specific inhibition of ELISA. *Der p* I-containing *Dpt* soluble Ag was used to inhibit the binding of anti-peptide IgG Ab to *Der p* I-containing *Dpt*-coated plates. Diluted rabbit serum (A, B or C) (1/1000) was previously incubated with different concentrations of a *Der p* I-containing *Dpt* and further tested by ELISA using a *Dpt* solid phase antigen.

2. Specificity of the immune response

Rabbit immune sera A, B and C appeared specific for the injected peptide and, in some extent, for a rich *Der p* I-containing *Dpt* Ag. Representative results are reported for rabbit A (Fig. 2). Indeed, by ELISA, Ab from the serum A bind to *Dpt* and to p52–71 but not to other *Der p* I-derived peptides or to unrelated allergens (hymenoptera venom).

Then, by Western-blotting analysis (Fig. 3), the capacity of these Ab to recognize *Der p* I molecules (apparent mol. wt = 24–26 kD) (Fig. 3; P1) was evaluated. No binding was observed with the serum from non-immune rabbits (Fig. 3; T1) or from a rabbit

immunized with p89–104 (Fig. 3; C1). However IgG Ab from rabbits A (Fig. 3; A1) and B bound to *Der p* I and the Ab binding was inhibited after pre-incubation of the sera with, respectively, p52–71 (Fig. 3; A2) and p117–133, but not after incubation with an irrelevant peptide.

To investigate the ability of the anti-peptide Ab to bind to native *Der p* I, the Ab binding of sera from rabbits A, B and C to *Dpt* immobilized on nitrocellulose strips (Fig. 3) or on ELISA plates (Fig. 4) was studied, before and after pre-incubation with the *Der p* I-containing *Dpt*. By Western-blotting analysis, a decrease in binding of Ab from rabbits A, B and C to *Der p* I was observed after serum absorption with *Dpt* (Fig. 3; A4) but not after incubation with hymenoptera venom (Fig. 3; A5). By ELISA, the binding of Ab from sera A, B to the *Dpt* was progressively reduced as the concentration of neutralizing *Der p* I increased (Fig. 4). Pre-incubation of sera with hymenoptera venom was without significant effect (data not shown). Then, *Der p* I-derived peptides elicit production of anti-peptide Ab able to bind to the native *Der p* I molecule.

3. Cross-reactive specificity of *Der p* I-derived peptides

The capacity of anti-peptide Ab present in rabbit immune sera A, B and C to react with other cysteine proteases (papain, human cathepsin B and *Der f* I) which demonstrate sequence homology with *Der p* I (Chua *et al.*, 1988; Dilworth *et al.*, 1991) was evaluated by Western-blotting (Fig. 5). For the three sera, no binding

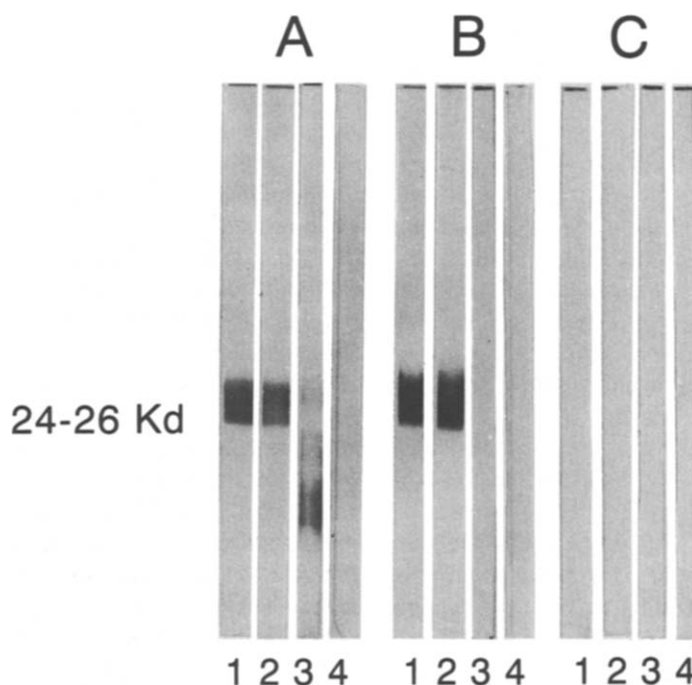


Fig. 5. Western-blot analysis of anti-p52–71 and p117–133 IgG Ab binding to *Der f* I, papain and human cathepsin B. *Der p* I-containing *Dpt* (lane 1), *Der f* I containing *Df* (lane 2), papain (lane 3) or human cathepsin (lane 4) were separated on SDS-PAGE and transferred onto nitrocellulose membranes. Strips were first incubated with diluted serum (1/1000) either from rabbit A (anti-p52–71), B (anti-p117–133) or C (non-immunized) and secondly with a peroxidase-conjugated anti-rabbit IgG antiserum. After enzyme reaction, the 24–26 kD band corresponding to *Der p* I (A1 and B1) or to *Der f* I (A2 and B2) was evidenced.

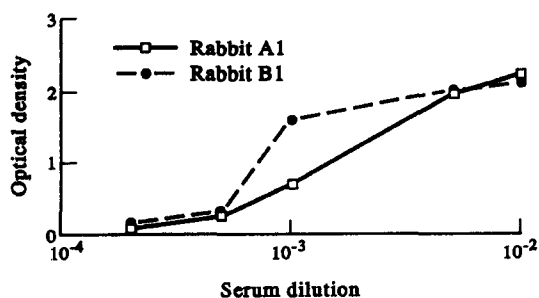


Fig. 6. Recognition of *Der f* I by anti-*Der p* I peptide rabbit antibodies. Using ELISA, binding curves of rabbit anti-peptide IgG Ab to purified *Der f* I were obtained. Various dilutions (from 10^{-4} to 10^{-2}) of sera from rabbit A1 (immunized with p52–71) and rabbit B1 (immunized with p117–133) were added to *Der f* I-coated wells.

to cathepsin B was observed and only serum from rabbit A reacted weakly to papain (Fig. 5; A3). However, Ab in rabbit immune sera A and B highly bound to *Der f* I (Fig. 5; A2 and B2). These results were confirmed by ELISA using purified *Der f* I; indeed anti-*Der f* I Ab titres of 700 and 4000 were respectively detected (Fig. 6).

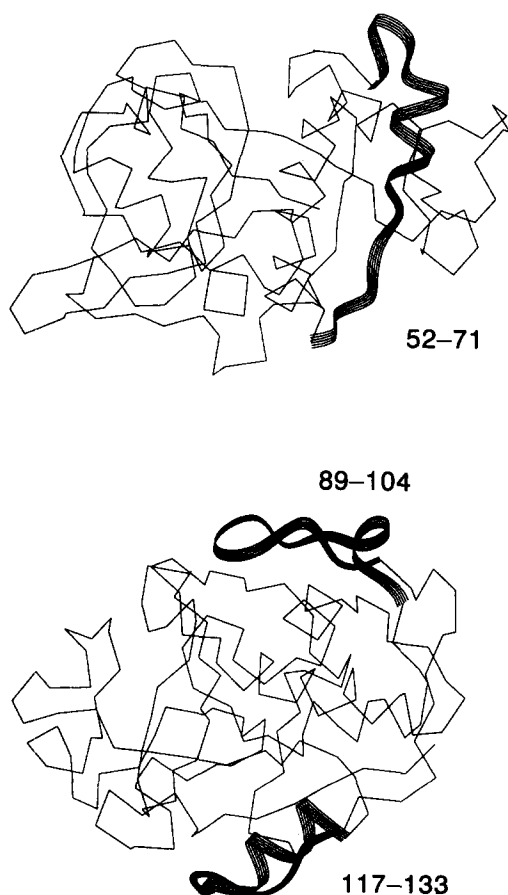


Fig. 7. Peptide location in the 3D model of *Der p* I. The diagrams show the solvent accessibility of the three selected peptides 52–71, 89–104 and 117–133. Whereas these peptides are displayed in a ribbon representation, only the carbon alpha backbone is represented for the remaining part of the *Der p* I 3D structure.

4. The 3D model of *Der p* I and location of *Der p* I peptides

The 3D model of *Der p* I was determined by using the corrected amino-acid sequence of *Der p* I (Dilworth *et al.*, 1991). According to this model, several observations can be made. First of all, it is important to notice that residues implicated in the catalytic site of homologous cysteine proteases, actinidin and papain, are strictly conserved (Gln 28, Cys 34, His 170, Asn 190, Trp 192). With regard to disulphide bridges, only two are present in the modeled *Der p* I (C31–C71 and C65–C103) and correspond respectively to the conserved disulphide bridges (C22–C65 and C56–C98) in actinidin and (C22–C63 and C56–C95) in papain. Nonetheless, according to our calculations, Cys 4 and Cys 117 residues of *Der p* I (which are not present in actinidin and papain) could be involved in the formation of a third disulphide bridge structurally different to those found in the two other enzymes, respectively (C156–C206) and (C153–C200). In such a situation, the presence of this disulphide bridge could be responsible for a decrease in accessibility of the peptide 117–133.

Concerning the position of the synthesized peptides (Fig. 7), it appears that all of them are located at the surface of *Der p* I and potentially accessible to Ab. The peptide p52–71 is parallel to the intraproteic α helix supporting the catalytic Cys 34 residue and its central part exhibiting an helicoidal conformation, is partially masked by the peptide p89–104. However, p89–104 shows very good accessibility to solvents and its predictable flexibility may allow better access to p52–71. In the present 3D model, p117–133 consists of a loop followed by an α helix (Asn 123–Gln 133). Finally the last peptide p176–187 is organized as an extended and well-exposed loop which is only present in actinidin.

DISCUSSION

It was demonstrated that a functional immunogen must contain both B- and T-cell epitopes to induce a T cell-dependent B cell activation. Indeed B cell epitopes are recognized by surface B cell immunoglobulins and T cell epitopes activate Th-cells after interaction with TcR receptor and MHC class II molecules. On the basis of this concept, different small peptides were reported as weakly or non-immunogenic (Schulze-Gahmen *et al.*, 1986; Good *et al.*, 1987; O'Hern, 1991; Marguerite *et al.*, 1992) and numerous presentations or constructions of effective synthetic peptides have been reported as immunogens (Borras-Cuesta *et al.*, 1988; Tam and Lu, 1989; Löwenadler *et al.*, 1990; Partidos *et al.*, 1992).

In our study, apart from the 12 amino-acid long p176–187, the three *Der p* I-derived peptides, p52–71, p89–104 and p117–133 appeared immunogenic in rabbits and in rats suggesting that they could contain both B- and T-cell epitopes. On this regard, peptides p52–71, p89–104 and p117–133 selected as potential B cell epitopes, specifically stimulated basophils and platelets from *Dpt*-sensitive patients (Jeannin *et al.*, 1992; Cardot

Table 1. Sequence alignment of *Der p* I, actinidin and papain used in the *Der p* I structural model elaboration. Eight structurally variable domains (in boxes) were identified as described in Material and Methods. The first nine residues of *Der p* I were not included in our model. X indicates the amino-acids of *Der p* I sequence which are found conserved in actinidin and/or in papain.

	1	10	20	30	40	50	
ACT	LPSYVDWRSAGAVVDIKSQGECGCWAFSAIATVEGINKITSGSLISLSE						
		x	x x	x x	xx xx xxxxx	x x	x x
DERP	TNACSGINGNAPAEIDLRQMRVTPIRMQGGCGSCWAFSGVAATESAYLAHRNQSLDLAE						
		x	x xx	xxx	xx xxxxxxxx	x x	x
PAP	IPEYVDWRQKGAVTPVKNQGGSCGCWAFSAVVTIEGIKIRTGNLNQYSE						
	60	70	80	90	100	110	
	QELIDCG	RTQNT	GCDGGYITDGFQFI	INDG	GINTEENYPYTAQDQDCD	VALQDQ	KYV
	xxx xx		xx x x x x		x x x x x	x	
	QELVDCA	SQH	GCHGDTIPRGIEYI	QHN	GVVQESYYRYVAREQSCR	RPNA	QRF
	xxx xx		xx x		x x x	xx	
	QELLDCD	RRSY	GCNGGYPWSALQLV	AQY	GIHYRNTYPYEGVQRYCR	SREKGP	YAA
	120	130	140	150	160		
	TIDTYENVPYNNEWALQTAVT	YQP	VSVALDAA	G	DAFKQYA	SG	IFTG
	x x	x	x		xxx x		x
	GISNYCQIYPPNANKIREALA	QTHSA	IAVIIGIK	DL	DAFRHYD	GRT	IIQR
	x x x	x	x		x x		x
	KTDGVRVQYPYNQGALLYSIA	NQP	VSVVLQAA	G	KDFQLYR	GG	IFVG
	170	180	190	200	210	220	
	VDHAIVIVGYGTEGGVDYIWVKN	SDTTWGE	EGYMRILR	NVGGAGT	CGIATMPSY	PVKY	
	xx	xxxx	xxxxxx	xxxx		x x	
	NYHAVNIVGYSDNAQGV	DYIWVRN	SDTNWGD	NGYGYFAA	NIDL	MMIEEYPY	VVIL
	xxx	xxx	x	xxx x xx	xxx		
	VDHAAVAVGYGP	NYILIKNSWGT	GWGENGYIRIKR	GTGNSYGV	CGLYTSSF	YPVKN	

et al., 1992; and unpublished data for p89–104). Our data are consistent with studies showing that *Der p* I-recombinant peptides p53–99 and p98–140 bind IgE and IgG (Greene *et al.*, 1991, 1992). With regard to T cell epitopes, *Dpt*-specific T cell clones have been shown to be stimulated by the *Der p* I-recombinant peptides p45–67, p94–140 and p117–143 (Yssel *et al.*, 1992). Moreover, as recent data showed that naturally processed peptides, eluted from MHC II molecules, are 13 to 17 amino-acid long (Rudensky *et al.*, 1991), the *Der p* I-derived peptides p52–71, p89–104 and p117–133 seem long enough to provide T-cell help.

Then, the *in vivo* data suggesting that these sequences could contain B- and T-cell epitopes were reinforced by the demonstration of their immunogenicity in various animals. Indeed, in mice, preliminary experiments indicated that p52–71 and p117–133 induce production of specific Ab but also a specific response to *Der p* I (data not shown). However, as the sequence of each peptide has a non-protected cysteine residue, we cannot yet exclude that dimerization of peptides by an intermolecular disulphide bond enhance their immune reactivity. Nevertheless, the main point of our experiments is that small peptides, without the need of carrier or synthetic construction, could be immunogenic.

Our studies also showed that *Der p* I-peptides, p52–71, p117–133 and p89–104, elicited rabbit Ab which bound to *Der p* I-containing *Dpt*. Interestingly, levels of anti-*Dpt* Ab were higher in rabbits immunized three times with p52–71 or p117–133 than in animals injected once

or twice with *Dpt*. In addition, by Western-blotting, anti-p52–71 and anti-p117–133 but not anti-p89–104 Ab from rabbit immune sera were shown to bind to *Der p* I molecules (apparent mol. wt = 24–26 kD). Many authors have already observed by Western-blotting or by solid-phase assays that anti-peptide Ab bind to the denatured form of the protein from which they derived, but usually failed to react to the native protein (Jemerson and Blankenfeld, 1989; Leonetti *et al.*, 1990). One explanation may be that linear synthetic peptides adopt various conformations in solution and then elicit different specificities of Ab; a small part of them might be able to bind to the native protein (Lerner, 1982). Nevertheless peptides of sufficient amino-acid chain length may assume in aqueous solution a secondary structure mimicking the organization of the native protein. Our results indicated that rabbit anti-p52–71, anti-p117–133 or anti-p89–104 Ab react with the native protein *Der p* I.

In the 3D model of *Der p* I, the three peptides studied are surface-located; the sequence 52–71 adopts an helix α conformation and the sequence 89–104 appears preferentially as a loop largely accessible on the surface. Circular dichroism analysis of peptides (100 μ g/ml) in 0.1 M Tris-HCl, pH 7 solution indicated that whereas p117–133 and p89–104 did not show any defined secondary structure, p52–71 was able to fold in an α helix structure (data not shown). Thus at least one of the peptides may adopt in aqueous solution a conformation similar to that found in the native molecule. However anti-p89–104 binds weakly to *Dpt* and fails to bind to

Der p I. Thus a precise knowledge of the organization of the native protein seems necessary to select more easily immunogenic peptides.

As *Der p* I shows homology with other cysteine protease sequences including *Der f* I (80% homology), papain (29% homology) and human cathepsin B (21% homology), the ability of anti-peptide Ab to bind to these proteins was evaluated. No binding to cathepsin B and a weak binding of anti-p52-71 Ab to papain was demonstrated; this result is probably related to the large number of amino-acid changes observed between p52-71, p117-133 and the two corresponding sequences in papain and cathepsin B (Table 1). By contrast, when the number of amino-acid residue changes between *Der p* I and *Der f* I is very limited, the antiserum directed against *Der p* I-derived peptide (i.e. anti-p52-71 and p117-153) binds very well to *Der f* I.

In attempt to prevent allergic disorders specific immunotherapy may be undertaken. Repeated injections of progressive amounts of specific allergen are required to favour a protective immune response against the responsible allergen. Purified and standardized sources of antigens are usually necessary. However, different observations indicate that use of peptides could be easier in immunotherapy than in vaccination. Indeed secondary B-cells, in contrast to primary B-lymphocytes, seem to require a lower binding affinity for activation and could be stimulated by linear peptide as well as by native protein (Klinman *et al.*, 1973). Thus, the study of these *Der p* I-derived synthetic peptides of sufficient length to be immunogenic and elicit IgG anti-native protein antibody, has to be performed, for example to evaluate the ability of a peptide to interact with a *Der p* I-primed B-cell.

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