

SPECIFIC HISTAMINE RELEASE CAPACITY OF PEPTIDES SELECTED FROM THE MODELIZED DER P I PROTEIN, A MAJOR ALLERGEN OF *DERMATOPHAGOIDES PTERONYSSINUS*

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Abstract—Dust mite allergens are considered as a major cause of allergic disease and as a risk factor for asthma. Der p I, a 222 amino-acid residue globular glycoprotein, is one of the major allergens from *Dermatophagoides pteronyssinus* (Dpt) mites. In this study, we have used predictive conventional algorithms (i.e. hydrophilicity, mobility, accessibility) and a three-dimensional model of Der p I derived from comparison to actinidin and papain to select continuous amino acid sequences as potential B cell epitopes. Four peptides, 52–71, 117–133, 176–187, 188–199 were synthesized. Their antigenic reactivity was investigated, mainly by measuring their capacity to induce *in vitro* histamine release. Results indicated that only Dpt-sensitive patients react specifically to Der p I-derived peptides and more frequently to 52–71 and 117–133. For each peptide, the intensity of response was dependent on the patient tested and on the peptide concn. The capacity of peptides to induce histamine release was demonstrated to be correlated with the serum level of anti-Der p I IgE ($r = 0.86$; $p < 10^{-2}$).

Taken together these data emphasize, in Dpt-sensitive patients, the heterogeneity of the specific response to synthetic Der p I-derived peptides and underline the possible variety of epitopes belonging to the allergen Der p I.

1. INTRODUCTION

Dust mite (*Dermatophagoides* sp.) allergens are a common cause of immediate hypersensitivity reactions such as asthma and perennial rhinitis and could play a role in atopic dermatitis (reviewed by Platt-Mills *et al.*, 1987). As *Dermatophagoides farinae* (Df), and *Dermatophagoides pteronyssinus* (Dpt) represent the more common species, many studies have been performed to identify more precisely their allergens. Indeed, immunoelectrophoretic investigations of house dust mite extracts have indicated the existence of a large number of antigens and allergens (Lind and Lowenstein, 1983; Le Mao *et al.*, 1983; Krilis *et al.*, 1984). Two major groups of mite allergens, structurally and antigenically unrelated, have been identified: the 25 kD group I allergens (Der p I, Der f I) found in faecal particles (Tovey *et al.*, 1981; Thompson and Carswell, 1988), and the 14-kD group II allergens (Der p II, Der f II) isolated from mite body extracts (Heyman *et al.*, 1989). Indeed, most dust mite allergic patients present positive immediate skin tests and have serum IgG and IgE antibodies to group I and group II allergens (Chapman and Platts-Mills, 1978; Chapman

et al., 1983). Moreover, specific proliferative T cell response to Der p I (Rawle *et al.*, 1984) and to *D. farinae* (O'Hehir *et al.*, 1987) have also been reported.

Der p I has been shown to react with IgE present in the sera of almost 80% of patients suffering from house dust allergy (Chapman *et al.*, 1983) and recent studies clearly suggest that Der p I levels indoors may be associated with an enhanced risk for a specific IgE response and for exacerbation of respiratory symptoms in asthma (Chapman *et al.*, 1988). Der p I has been purified (Chapman and Platts-Mills, 1980) and the secondary structure of the purified molecule was determined by circular dichroism (Stewart *et al.*, 1987). The recent cDNA cloning (Thomas *et al.*, 1988) and the deduced amino acid sequence analysis have revealed that the 222 residues of the mature protein show a partial homology with a group of cysteine proteases (Chua *et al.*, 1988). Subsequent studies have tried to characterize the B and T cell epitopes on Der p I. By using human IgG and IgE, rabbit polyclonal, and either polyclonal or monoclonal murine anti-Der p I antibodies, the presence of several epitopes has been demonstrated (Chapman, 1989). The murine monoclonal antibodies have been recently used to develop a two-site monoclonal antibody ELISA for Der p I (Luczynska *et al.*, 1989). Although

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the amino acid sequence of Der p I was determined, the nature of the sites recognized by these polyclonal or monoclonal antibodies remains unclear.

The mapping of epitopes on several proteins indicates that antigenic sites are generally located on the surface of proteins (Benjamin *et al.*, 1984) in regions of relatively high segmental flexibility (Tainer *et al.*, 1984) and hydrophilicity (Hipp and Woods, 1981). These data led to substantial and analytical information to predict the locations of continuous antigenic determinants on globular proteins. Moreover, in the present investigation we have taken advantage of the 29% homology shared between Der p I and actinidin, a protease obtained from the Chinese gooseberry (Chua *et al.*, 1988) whose three-dimensional structure was established by X-ray crystallographic analysis (Baker, 1977), to build a three-dimensional model of Der p I by a knowledge-based approach (Blundell *et al.*, 1987). Then, by studying the modelled Der p I molecule and analyzing its amino acid sequence using predictive algorithms (i.e. hydrophilicity, accessibility, mobility), four potential B cell epitopes were identified and synthesized. The three-dimensional model indicated that the amino acid sequence of the selected peptides, which were revealed as potential epitopes in our study, were surface-located.

Preliminary experiments demonstrated that platelets could be significantly triggered by Der p I-derived peptides and that IgE was implicated in this process (Cardot *et al.*, accepted for publication). Both basophils (reviewed by Marone *et al.*, 1989) and platelets (Capron *et al.*, 1987; Joseph *et al.*, 1986, 1988) express Fcε receptors, respectively of high and low affinity, and may participate in the pathophysiology of the allergic reaction by the release of proinflammatory products (Marone *et al.*, 1989; Page, 1990). We have investigated the potential antigenic reactivity of the four selected Der p I-derived peptides by quantifying the basophil histamine release (Morel and Delaage, 1988). Similar to results obtained previously with the IgE-dependent platelet-mediated cytotoxicity test, histamine release indicated that only Dpt-sensitive patients specifically responded to the synthetic peptides.

2. MATERIALS AND METHODS

2.1. Allergen and peptides

2.1.1. Criteria used for the selection of synthetic peptides

2.1.1.1. Conventional predictive methods

In order to predict the location of continuous antigenic determinants on the globular protein Der p I, conventional predictive methods were used initially as the three-dimensional structure was not available. Briefly, the conventional computerized algorithm described by Hopp and Woods (1983) was used to determine the solvent accessible sequence; indeed hydrophilicity parameters were averaged over 6 amino acids and mean values were then attributed to the first residue of each hexamer (Hopp and Woods, 1981). The same

calculation was performed using different exposure criteria, i.e. surface accessibility (Janin, 1979), mobility (Ponnuswamy and Bashkaran, 1984) and hydropathy (Chothia, 1976). Flexibility was determined by criteria proposed by Karplus and Schulz, 1985. For each parameter analyzed a precise profile was obtained as reported elsewhere (Cardot *et al.*, accepted for publication). Three amino acid sequences highly responding to at least two of these criteria were selected and the corresponding peptides synthesized. In fact peptide 52–71 was selected upon criteria of hydrophilicity and flexibility, peptide 117–133 was predicted using hydrophilicity, mobility, acrophily and hydropathy criteria and peptide 188–199 was determined on mobility, flexibility and hydropathy criteria.

2.1.1.2. Modelization

The COMPOSER program in SYBYL package (Tripos Associates Inc, St Louis, MO) was used to model Der p I on the basis of the three-dimensional structures of known homologous proteases: papain (Kamphuis *et al.*, 1985) and actinidin (Baker, 1977). The computational step was performed on a Vax 6320 and Evans and Sutherland graphic terminal. The modelization was achieved in several stages. After alignment of the sequences of Der p I and actinidin or papain, insertions or deletions were created in the known three-dimensional structure of actinidin or papain and amino-acid residues of Der p I were inserted in order to obtain a best fit alignment (Table 1) (reviewed by Blundell *et al.*, 1987). Finally to avoid steric clashes, the model was refined by minimizing the energy of junction points between structurally conserved regions and loops using the Kollman force field and the ANNEAL program in the SYBYL package. This Der p I modelization led us to synthesize an additional peptide 176–187 which appears in a β -turn on the surface of this molecule.

2.1.2. Peptide synthesis. Peptides were synthesized by solid phase method according to a Boc-benzyl strategy (Merrifield, 1963) using an automated synthesizer (Applied Biosystems model 430 A). Side chains protection was as follows: Lys (2-Cl-Cbz), Ser and Thr (Bzl), Asp (OcHex), Glu (OcHex), Arg (Tos), Cys (p-Me Bzl or AcM), Tyr (2,6-diCl Bzl). At the end of the synthesis, peptidyl resins were treated with hydrogen fluoride. Peptides were purified by preparative reversed phase HPLC on a 5 μ 300 Å C18 Vydac column. Elution was performed with a linear water (0.1% TFA)/acetonitrile (0.1% TFA) gradient (0–60% in 60 min). Purified peptides were checked for homogeneity by analytical reversed phase-HPLC and for identity by amino-acid analysis after total acid hydrolysis.

2.1.3. Peptide sequence. Amino acid sequences of the four selected peptides are reported in Table 2. Acetamidomethyl cysteine residues were added at extremities of peptide 176–187. A non-relevant peptide, corresponding to the 141–160 sequence of VP1 derived from the foot-and-mouth disease virus (VPNLR-GDLQVLAQAVARLP) was used as negative control throughout this study. In some cases, two other

[illegible]

peptides were used: one derived from the protein P28 of the helminth *Schistosoma mansoni* (115–131) (KPQEEKEKITKEILNGK) and one derived from a 85 kD surface protein of protozoan *Trypanosoma cruzi* trypomastigotes (DKKESGDSEDKKGSGDSL).

2.1.4. *Dpt extract.* The same Der p I-containing Dpt antigen preparation standardized and prepared by Laboratoire des Stallergènes (Fresnes, France) was used throughout this study. The dilutions of Dpt allergen were prepared from a stock solution (26 µg of

Table 2. Amino acid sequences of selected Der p I-derived peptides

Number	Sequence
52-71	N Q S L D L A E Q E L V D C A S Q H G C
117-133	C Q I Y P P N A N K I R E A L A Q
176-187	(acm)C G Y S N A Q G V D Y W I C(acm)
188-199	V R N S W D T N W G D N G Y

(acm: acetamidomethyl protection)

nitrogen/ml) corresponding to 100 IR/ml (biological units used by Laboratoire des Stallergènes). The presence of Der p I was detected by a two-site monoclonal antibody immunoassay and estimated to 2 µg/100 IR/ml.

2.2. Patients

Twenty-nine patients (aged 18–52 years) sensitized to *Dermatophagoides pteronyssinus* and in some cases to *Dermatophagoides farinae* were included in this study. All were atopic and presented asthma and/or perennial rhinitis, with usual clinical features of house dust mite sensitization. No sensitization to grass pollen or hymenoptera venom allergens was detected. Cutaneous prick-tests were positive towards dust mite allergens (diameter ≥ 9mm), mean total serum IgE level was 854.5 KUI/ml (± 812 SD) and all patients exhibited class 2 to 4 positive RAST against Dpt determinants (Pharmacia, Uppsala, Sweden).

Patients allergic to grass-pollens ($n = 3$) or exhibiting anaphylactic reactions to hymenoptera venom ($n = 3$) (defined by positive cutaneous prick tests and clinical or biological data), and healthy donors ($n = 10$) without any allergic disorder or atopy, were tested as negative controls.

2.3. Histamine release

Histamine release from human basophils was quantified by a specific radio-immuno assay (Immuno-tech, Luminy, France) according to the technique previously described (Morel and Delaage, 1988). Heparinized venous blood was diluted (1/4) in histamine release buffer. Diluted blood (100 µl) was incubated with 50 µl of related allergen at different concns in round-bottomed microtiter plates. After 30 min of incubation at 37°C each sample was centrifuged at 4°C at 1500g. Supernatants were collected and stored at –20°C until assayed. To increase the sensitivity and the specificity of the dosage, histamine was acylated just before incubation with monoclonal antibodies bound to the solid phase. Following competition between ¹²⁵I-histamine and cold histamine (performed for 18 hr at 4°C), the radioactivity specifically bound was determined by using a gamma counter (LKB, Uppsala, Sweden). Data were calculated from the standard curve and expressed as a percentage of the total releasable histamine (obtained after total cell lysis). The sensitivity of the assay was 0.1 nM. Background which did not exceed 5–7% was deducted from each value.

2.4. Detection of specific anti-Der p I IgE

The ELISA protocol previously described for quantification of Der p I (Luczynska *et al.*, 1989) was adapted to the detection of specific anti-Der p I IgE (Didierlaurent and Garcelon, 1991). In brief, polystyrene microtiter wells (Maxisorb, Nunc, Denmark) were coated with 5H8 (a specific anti-Der p I murine monoclonal antibody) (obtained from Dr M. D. Chapman, University of Virginia, Charlottesville, VA) at 1 µg/well in 50 mM carbonate-bicarbonate buffer pH 9.6 overnight at 4°C. After washings, plates were saturated with a 1% BSA solution in PBS (0.1M) pH 7.4 containing 0.5 M NaCl. After washings and drying, the plates were incubated with 0.1 ml of a diluted Der p I allergenic preparation (2 µg/ml), for 90 min at room temp and extensively washed in PBS/Tween 0.05% solution. Then, 0.1 ml of the Dpt-sensitive patient serum (at the appropriate dilution) was added for 3 h-incubation. After five washings, bound IgE Ab was detected by a horseradish peroxidase conjugated anti-human IgE monoclonal Ab (Stallerzym-Dye-bioMérieux). After 30 min of incubation at room temp and washings, the appropriate substrate (ortho phenylene diamine) was used to detect the bound anti-Der p I IgE. The reaction was developed in phosphate-citrate buffer containing H₂O₂ (bioMérieux) and, after 15 min, was stopped by adding 1.8 N H₂SO₄. The optical density was measured at 492 nm. Absorbance readings were directly proportional to the quantity of anti-Der p I IgE bound to Der p I and values were calculated from the standard curves. Results are given in arbitrary units (AU) (see Table 3) in reference to positive serum considered as equivalent to 100 Units.

3. RESULTS

3.1. Selection and localisation of peptides

The comparison of the mature Der p I with the corresponding homologous thiol proteases papain and actinidin by best fit sequence alignment (Table 1) has shown that Der p I probably follows the same general folding pattern. It is noteworthy that two cysteine residue pairs (31–71 and 65–103) are conserved in these proteins (Thomas *et al.*, 1988). The three-dimensional structure of actinidin and papain suggests that Der p I also has two similar disulfide bonds and consequently that most motifs or domains previously identified in these proteins appear to be present in Der p I.

Table 3. Relationship between the anti-Der p I IgE serum level and the histamine release induced by the synthetic peptides

Patients and controls	Skin-tests diameter (mm)	Total IgE (KUI/I)	Anti-Dpt specific IgE (U RAST)	Anti-Der p I specific IgE (AU)	HR induced by Dpt extract	Means of the HR induced by the four peptides
8	10	960	49	190	83	27
9	12	762	23	193	90	50
10	18	3743	58	86	97	36
11	10	246	38	42	81	15.5
12	9	136	22	43	94	17.5
13	10	141	2	14	63	3.5
14	10	271	10	14	92	17
15	0	707	<0.1	<1	5	1.4
16	0	194	<0.1	<1	3	1
17	0	13	<0.1	<1	4.5	0.9

Analysis of the location of the three selected peptides using conventional algorithms indicates that these peptides are located on the surface of the molecule, confirming the efficiency of the prediction and their potential accessibility (Fig. 1A and B). Moreover, the existence of a protuberant β -turn in the modeled Der p I molecule led us to select another amino acid sequence 176–187.

3.2. Reactivity of basophil containing cell population to Der p I-derived peptides

Basophils expressing high affinity receptors for IgE were shown to be associated with the hypersensitivity reactions observed in some allergic disorders and demonstrated to be the main accessible source of histamine (Saxena *et al.*, 1989, Marone *et al.*, 1989).

Possible basophils interaction with the synthetic peptides was evaluated by measuring the amount of histamine released following incubation of peripheral blood cells from Dpt-sensitive patients ($n=29$) with each peptide. In contrast to that was observed with irrelevant peptide, the selected peptides and Dpt extract induced significant histamine release (Fig. 2). However, for each peptide, the percentage of release differed according to the patients tested (for example for peptide 52–71 from 1.5% to 93% and for peptide 176–187 from 2.3% to 39%); the higher values were obtained with peptides 52–71 or 117–133. Indeed by regarding as positive the histamine release higher than 5%, it appeared that patients responded to both peptides with a high frequency (Fig. 3) and that the mean of response intensity was higher with peptides 52–71 and 117–133 than with peptides 188–199 and 176–187. These results suggest that basophils from Dpt sensitive patients could react to various epitopes of Der p I allergen and may be more sensitive to these two peptides (52–71 and 117–133) than to the others.

3.3. Allergen-dependency of histamine release

The capacity of histamine release appeared dependent on the allergen-sensitivity of the patient tested. As indicated above and illustrated in Fig. 4A, only Dpt-sensitive patients reacted with the selected peptides.

Indeed, no significant histamine release could be observed when cells from non-allergic (healthy) subjects or patients sensitive to other allergens were incubated with Der p I peptides. Moreover, peripheral blood cells from patients sensitive to grass pollen (with high levels of serum IgE) or to hymenoptera venom failed to respond to Dpt extract or to synthetic Der p I-derived peptides, although they released histamine after incubation with pollen or venom allergens respectively (Fig. 4B). These results emphasize that only Dpt-sensitive patients could specifically react to Der p I-derived peptides.

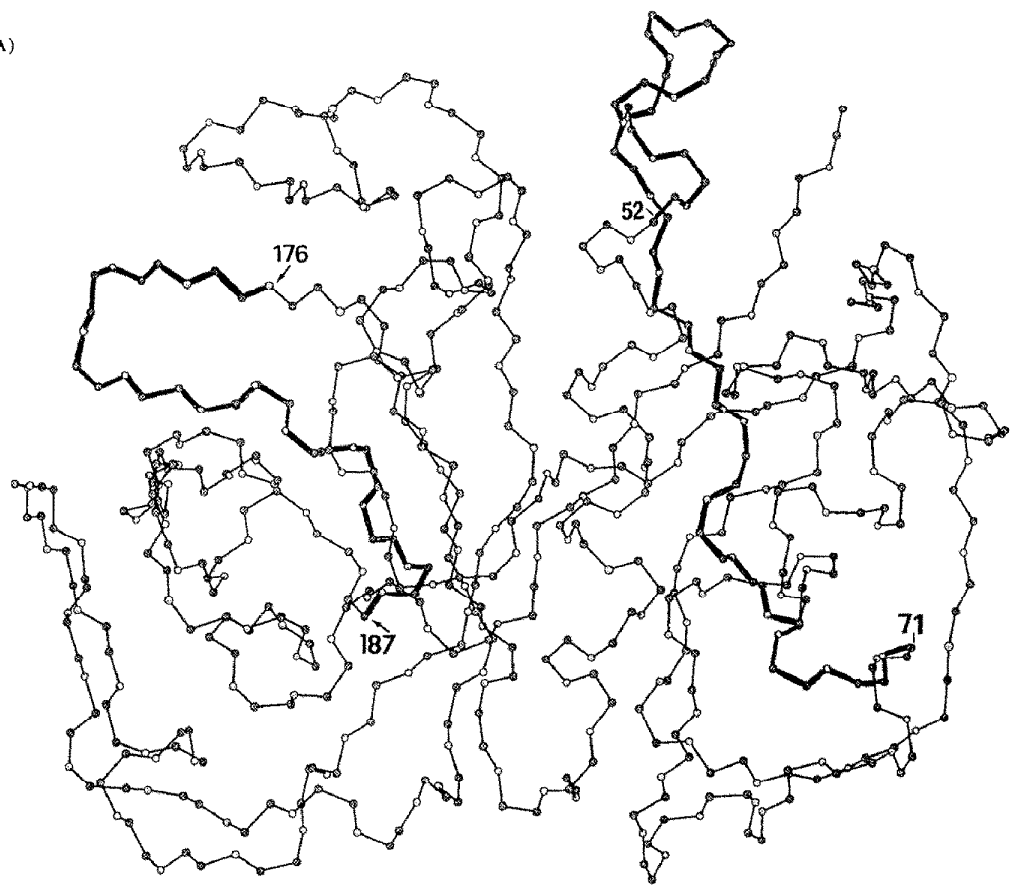
3.4. Influence of peptide concentration on histamine release

Though Dpt-sensitive patients could respond intensively to each of the four selected peptides, the percentage of histamine release varied according to the amount of peptide used during cell incubation. Basophil containing cell populations were always tested with peptide concns from 10^{-2} to 10^2 μ g/ml. Usually, following incubation with a peptide, maximal histamine release was achieved by using concns ranging from 1 to 50 μ g/ml. Representative curves of dose-response are given in Fig. 5 for four Dpt-sensitive patients tested with peptide 117–133; this pattern of response was frequently observed with peptides tested. We observed that a higher histamine release was more frequently observed with a lower concentration of peptide 117–133 than peptide 52–71 (data not shown).

3.5. Patient-specific pattern of peptide-mediated histamine release

The reactivity to Der p I-derived peptides is exclusively observed with cells from patients sensitive to Dpt. The analysis of the individual reactivity of patients indicated that each of them exhibited a typical pattern of response. Figure 6 shows three examples of pattern of histamine release obtained from Dpt-sensitive patients (patients 5, patient 6, patient 7). Indeed, whereas peptides 117–133 and 188–199 appeared as the best histamine release inducers for patient 5, peptides 52–71 and

(A)



(B)

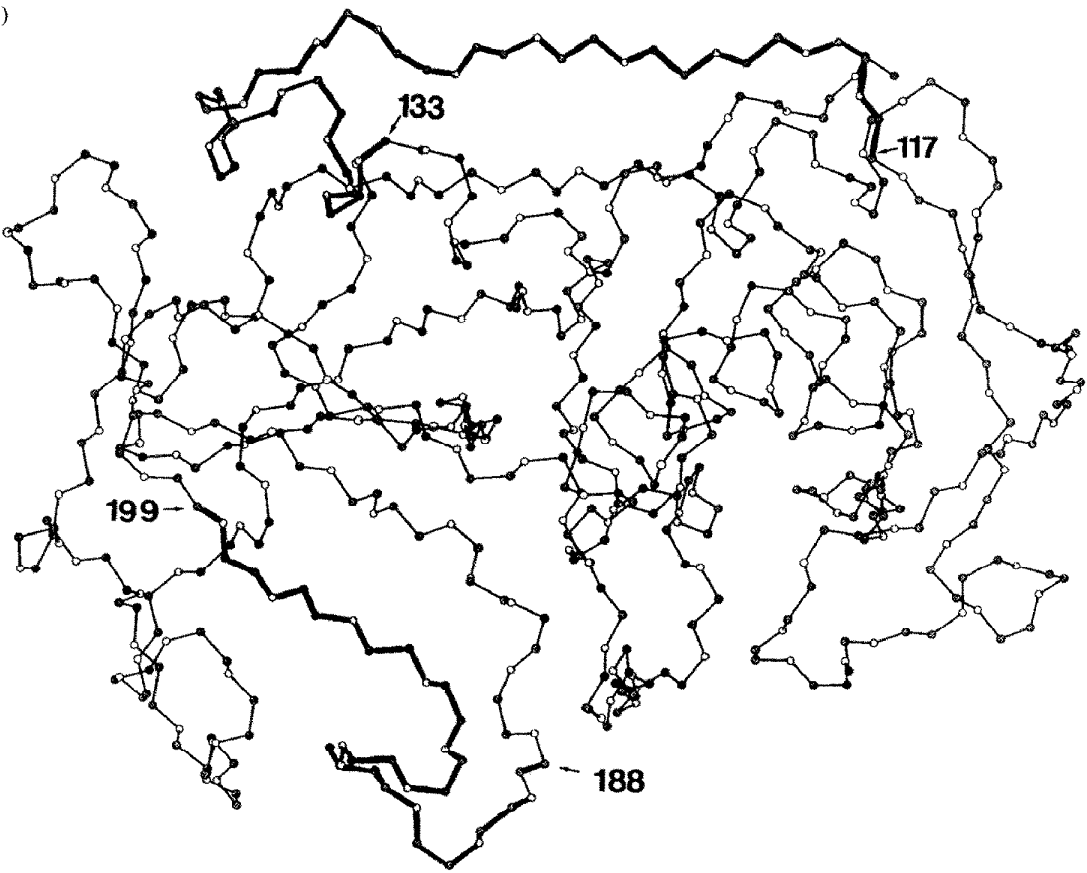


Fig. 1. Schematic folding of the main chain of the Der p I modelized molecule. The *N*-terminal amino acid is at the left and the *C*-terminal amino acid is at the right. The peptides 52–71 and 176–187 are indicated in (A); the peptides 117–133 and 188–199 in (B).

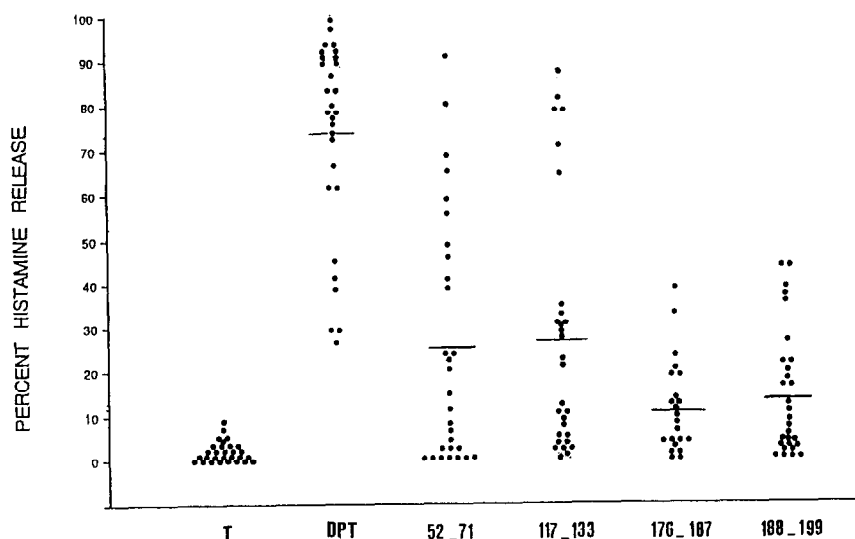


Fig. 2. Peptide-induced histamine release. Basophils containing peripheral blood cells from Dpt-sensitive patients ($n = 29$) were incubated with different concns of one of the four synthetic peptides (52-71, 117-133, 176-187, 188-199) (from 10^{-2} to $10^2 \mu\text{g/ml}$) or with the Dpt allergen (dilution from 10^{-4} to 10^{-1}). Each value represents, for each patient tested, the highest histamine release induced by one dose of each peptide. Positive control corresponds to the histamine release induced by Der p I-containing Dpt antigen. Negative control consists of cell suspension incubated with an unrelated peptide.

176-187 were more efficient for patient 6. However from the present results, we cannot conclude that a better response to peptide 117-133 is associated with a lower response to 176-187 or that a good reactivity to peptide 52-71 might be associated with a significant reactivity to peptide 176-187. In the case of patient 7 the positive response to a variable extent to four peptides (a pattern occurring more frequently than the other ones) confirmed the fact, already observed, that none of the synthetic peptides tested could be considered as immunodominant.

3.6. Reactivity to Der p I-derived peptides is related to Der p I IgE serum level

As the patients were selected on the basis of their specific reactivity to Dpt (as judged by clinical data,

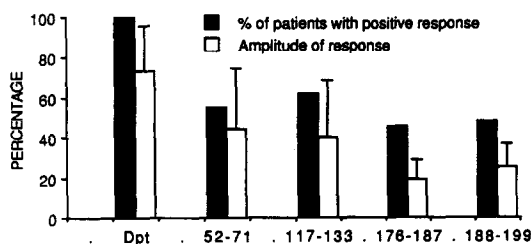


Fig. 3. Determination of the percentages of Dpt-sensitive patients responding to Der p I-derived peptides. The frequency of Dpt-sensitive patients responding to each peptide (histamine release $> 5\%$) is shown as the percentage of patients with positive response (■). The response amplitude corresponds to the mean ($\pm$ SD) of the higher percentages of histamine release obtained for the positive patients (□).

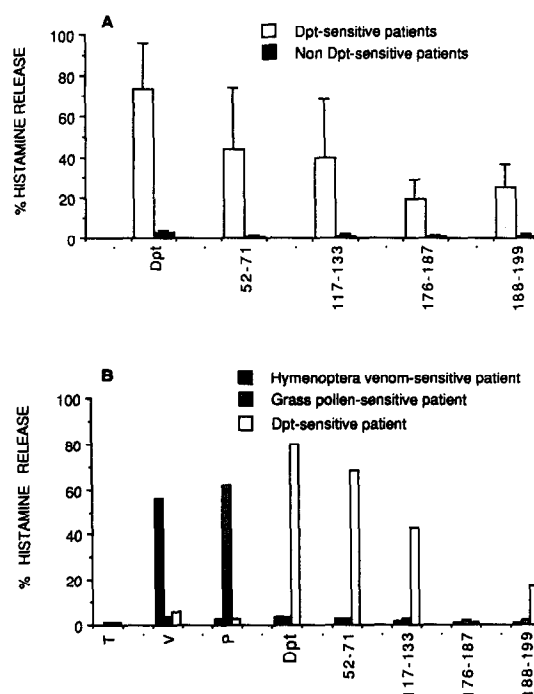


Fig. 4. Specificity of the histamine release induced by Der p I-derived peptides. (A) Percentage (expressed as mean $\pm$ SD) induced by peptides or Dpt allergen obtained from Dpt-sensitive patients (cf. Fig. 3) and non Dpt-sensitive patients $n = 16$ (healthy subjects and patients sensitive to pollen or hymenoptera venom) (■). (B) Per cent of histamine release induced by peptides or allergens such as Dpt extract, hymenoptera-venom (V) or grass-pollen (P) obtained from one representative patient allergic to Dpt (□), hymenoptera-venom (■) or grass-pollen (□). Different concns of peptide were used and the higher per cent of histamine release induced by one concn was taken into account.

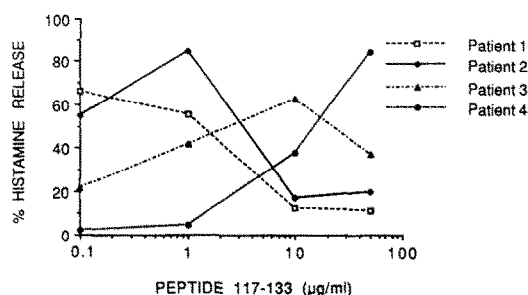


Fig. 5. Effect of peptide concn on histamine release. Percent of histamine release obtained after incubation of peripheral blood cells from 4 Dpt-sensitive patients (1–4) with different concns of the peptide 117–133 in a range from 10^{-2} to 10^2 $\mu\text{g/ml}$.

positive skin tests and RAST), the fact that the pattern of histamine release was heterogeneous might suggest that only highly Der p I-sensitive patients could react to Der p I-derived peptides. In order to verify this hypothesis, the specific IgE response against Der p I allergen was evaluated by using a two-site monoclonal antibody ELISA. From the present results (Table 3), it appeared that the intensity of the reactivity to Der p I-peptides was dependent on the level of specific anti-Der p I IgE present in the serum of Dpt-sensitive patients (patients 8–14 representative of Dpt-sensitive patients tested in this report). In fact, we observed that Dpt-sensitive patients exhibiting high level of anti-Der p I IgE, responded more intensively to synthetic peptides as expressed by the percentage of histamine release. With Dpt-sensitive patients who had a lower level of anti-Dpt specific IgE and of anti-Der p I specific IgE, a significant histamine release could be observed only for one or two peptides. In contrast, no significant histamine release was observed when patients without Dpt sensitivity (patients 15 and 16) or healthy control (17) were tested. Then, data shown on Table 3 indicated that the anti-Der p I level is correlated to the mean response to the four peptides ($r = 0.86$; $p < 10^{-2}$). Moreover, the intensity of cell reactivity was not correlated with the reactivity to Dpt allergen judged by the skin-tests or RAST.

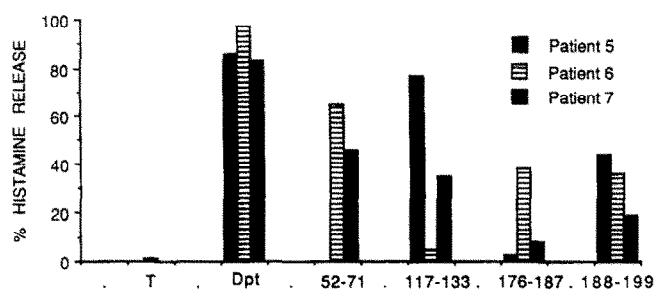


Fig. 6. Patient-specific pattern of peptides-induced histamine release. Percent of histamine release obtained after incubation of peripheral blood cells from 3 Dpt-sensitive patients with each of the four peptides and with Dpt. Different concns have been tested for each peptide (from 10^{-2} to 10^2 $\mu\text{g/ml}$) and for Dpt (dilution from 10^{-4} to 10^{-1}); only the maximal response obtained was taken into account. Controls (T) correspond to values obtained with an unrelated peptide.

DISCUSSION

The aim of the present report was to define sequential epitopes from the globular glycoprotein Der p I. Thus, peptidic sequences were selected using predictive algorithms and a computer-generated model Der p I molecule. In fact, sequence homologies between Der p I and X-ray crystallographied proteins, actinidin and papain, led us to construct a three-dimensional model of Der p I using a knowledge-based approach. In these conditions, precise analysis of the modelled protein indicated that the peptides selected using algorithm predictions were surface-located and allow us to select an amino acid sequence (176–187) (not revealed by predictive algorithms) which represents a β turn. Thus, all these characteristics emphasized the possibility that these peptides could be recognized by specific ligands such as anaphylactic antibodies. The synthetic peptides were further tested *in vitro*, in biological assays involving cell populations expressing Fc ϵ receptors, in order to give information on their potential *in vivo* allergenic activity.

It is known that addition of crude extracts of Dpt or purified Der p I allergen to mast cells or basophils from Dpt allergic patients can induce activation of these Fc ϵ RI bearing cells with a subsequent release of histamine (Casolaro *et al.*, 1990). In this study, we demonstrate that the biological effect (i.e. histamine release) induced by Der p I-derived synthetic peptides was only observed with Dpt-sensitive patients. Indeed, when cell populations from healthy donors or from patients exhibiting immediate hypersensitivity reactions to allergens unrelated to mites, such as hymenoptera venom or grass-pollens, were incubated with one of the four peptides or with Dpt extracts, no significant histamine release could be detected. Moreover, the Dpt sensitive patients did not react to other allergens (grass-pollen or hymenoptera venom) or to unrelated peptides demonstrating the allergen specificity of the mechanism involved in basophil activation. The fact that these basophil containing cell populations only react with the allergen to which patients are sensitive (hymenoptera venom, grass-pollen or Dpt) (Fig. 4B) emphasizes the specificity of the mechanism of cell activation triggered by the Der p I-derived peptides. Similar results were obtained with platelets (Cardot *et al.*, accepted for publication).

Different mechanisms could be involved in peptide induced histamine release. As the profile of histamine release as well as the dose response curve were different from one Dpt-sensitive patient to another, a direct and non specific basophil activation by peptides, as observed with some neuropeptides (reviewed by Mousli *et al.*, 1990) or peptidic fragments derived from bacteria (fMLP) (Siraganian and Hook, 1977) or fungi (such as pepstatin) (Marone *et al.*, 1984) appeared quite unlikely. However, we cannot yet exclude the triggering of histamine release by another mechanism (additive or not), requiring for example the participation of serum complement factors (C3a, C5a) (Hook *et al.*, 1975) and/or various mediators (HRF, IL1, IL6) (Thuesen *et al.*, 1979; Goetzl *et al.*, 1984; Subramanian and Bray, 1987).

Similar to basophils, platelets from patients sensitive to Dpt are likely to be activated by the specific allergen through low affinity IgE receptors present on their surface (Joseph *et al.*, 1983, 1986) and then are involved as effectors in hypersensitivity reactions (Joseph *et al.*, 1988; Capron *et al.*, 1991). Platelet reactivity induced by the four Der p I derived peptides was recently demonstrated to be mediated by an IgE-dependent mechanism (Cardot *et al.*, accepted for publication), since platelets from healthy subjects were sensitive to the Der p I-derived peptides after addition of serum IgE antibodies from patients allergic to Dpt. However, only indirect data implicated such a mechanism in peptide-induced basophil-associated histamine release. We demonstrated in this study that peptide-induced histamine release is correlated with the concentration of specific anti Der p I IgE (Table 3). It is known that the IgE receptor density on the basophil surface is strongly correlated with the serum IgE level and the intensity of the anti IgE-induced histamine release (Malveaux *et al.*, 1978). However, though haptens were considered unable to trigger an IgE-dependent histamine release, which requires at least divalent antigen (Kagey-Sobotka *et al.*, 1982), we can hypothesize, in our case, that the cell peptide-dependent triggering was favoured by a non-covalent binding of peptides to human serum albumin behaving as an hapten-carrier. The way by which the peptides were recognized and could induce the stimulation of the Fcε bearing cell populations requires further experiments to be fully understood. Nonetheless, the main point of our results was that only Dpt-sensitive patients were able to respond to Der p I peptides.

Different approaches to map the antigenic structure of the 25 kD Der p I glycoprotein allergen have previously been reported. Using a panel of murine monoclonal IgG antibodies, four non-overlapping, non-repeating, species-specific epitopes of Der p I, and a common epitope to Der p I and Der f I allergens were defined. In cross-inhibition studies, it was observed that the binding of human IgE antibodies directed against cross-reacting epitopes could be significantly inhibited (>40%) by monoclonal antibodies (Chapman *et al.*, 1989). However, until now, the specificity of these Mab has not identified to a known amino-acid sequence. Recently, an IgE ELISA profile of reactivity to 215 Der p I-derived overlapping octapeptides has been established with a pool of human sera from allergic patients (Stewart *et al.*, 1990). As observed in our study the analysis of this profile suggests that IgE from Dpt-sensitive patients were directed against numerous antigenic determinants of the Der p I molecule. By comparing the sequence of overlapping octapeptides most frequently recognized by IgE antibodies as analyzed by an ELISA technique (Stewart *et al.*, 1990) and the amino acid sequences of our synthetic peptides, it seems that peptides 117–133, 176–187 and 188–199 could correspond to IgE binding sites. It is noteworthy to indicate that, at variance with other synthetic peptides, which were chosen by using conventional algorithms, the peptide 176–187 was selected as potential epitope mainly following analysis of

the modeled Der p I protein. However this additional peptide (though associated with a β-turn, which could be easily recognized by antibodies) was not revealed in our study as an immunodominant epitope. In fact, the analysis of the reactivity to peptides demonstrated that each Dpt-sensitive patient shows an individual profile of response.

The individual antigenic sensitivity to some epitopes of the globular protein may suggest the existence of an individual B cell (and T cell) repertoire dependent on the presence of a defined CMH antigen and/or of a specific pathway of allergen processing. This leads to the conclusion that, according to the patient, different subpopulations of B cells specific to different sequences of Der p I could be selectively stimulated.

In conclusion, though some studies have underlined the importance of the allergen conformation on the antigenicity of the group I allergens (Lombardero *et al.*, 1990), conventional predictive algorithms and a three-dimensional modelization of the native molecule Der p I allow us to define peptides as sequential epitopes of the Der p I allergen. The four derived peptides selectively activate Fcε-bearing cells from Dpt-sensitive patients and, consequently, these epitopes could play a role in pathological reactions observed in the house dust mite allergy. Nevertheless, with the aim of potential therapeutic application to specific hyposensitization, peptides should cross-react with lymphocytes primed and stimulated by the Dpt allergens as observed in another model (Jemmerson *et al.*, 1987). Finally, the *in vivo* functional activities of the peptides, free or associated to a carrier, remain to be investigated by experiments such as skin tests or bronchial provocation tests to confirm their potential role in allergic diseases.

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