

EPITOPE MAPPING OF THE *DERMATOPHAGOIDES* *PTERONYSSINUS* HOUSE DUST MITE MAJOR ALLERGEN *Der p* II USING OVERLAPPING SYNTHETIC PEPTIDES

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Abstract—Fourteen synthetic peptides of 15 amino acid residues length, overlapping by five residues and spanning the entire sequence of the major allergen *Der p* II from the house dust mite *Dermatophagoides pteronyssinus* were synthesized. These peptides were coupled to CNBr-activated Sepharose-4B and used as solid-phase antigens in epitope mapping studies using human IgE antisera. These antibodies bound predominantly to the peptide comprising residues 65–78, the binding of which was inhibited by native *Der p* II. In addition these antisera bound, to a lesser extent, to the peptide that comprised residues 1–15, which binding was not inhibited by native *Der p* II. Thus, we found one sequential epitope for a number of IgE sera.

INTRODUCTION

Epitope mapping with synthetic peptides can be an efficient technique to elicit the primary structure of linear antigenic determinants (Berzofsky, 1985; Lerner, 1982; Geysen *et al.*, 1987b; Getzoff *et al.*, 1987). Epitope mapping of several proteins with synthetic peptides has been reported: myohemerythrin (Geysen *et al.*, 1987a), sperm-whale myoglobin (Geysen, 1985), the outer structural protein of Foot-and-Mouth Disease virus (Meloan and Barteling, 1986; Geysen *et al.*, 1984), BCG (Van der Zee *et al.*, 1989) and several proteins from HIV (Meloan *et al.*, 1989; Mathiesen *et al.*, 1989). Some of these cases concern IgE-antibodies against allergens: *Chi t* I (Mazur *et al.*, 1988), *Cor a* I and *Bet v* I (Elsayed *et al.*, 1989), *Amb a* III (Atassi and Atassi, 1986),

Gad c I (Elsayed *et al.*, 1980) and 0.28 wheat α -amylase inhibitor (Walsh and Howden, 1989).

For *Dermatophagoides pteronyssinus*, an important allergen source for house-dust mite allergy, a number of allergens have been reported (Carswell, 1988), from three of which the cDNA has been sequenced. The major allergen *Der p* I (Thompson *et al.*, 1988) is a glycoprotein of 222 amino acid residues with a molecular mass of 25,371 Da with one glycosylation site (Chua *et al.*, 1988; Thomas *et al.*, 1988; Stewart *et al.*, 1989). *Der p* I is an excretory product, a cysteine-protease predominantly found in fecal particles (Chapman and Platts-Mills, 1980; Thompson and Carswell, 1988; Lowenstein *et al.*, 1986). The major allergen *Der p* II is a protein of 129 amino acid residues with a molecular mass of 14,131 Da, devoid of glycosylation sites and with no homology to any sequenced protein except for the *D. farinae* equivalent *Der f* II (Thomas *et al.*, 1989). The major allergen *Der p* III is a serine-protease of which only a partial sequence has been published (Stewart *et al.*, 1989).

Der p II is a very important major allergen as group II allergens are best recognized by allergic patients [over 90% of the patients (Van der Zee *et al.*, 1988; Heymann *et al.*, 1989)] and the rate of crossreactivity with major allergens from other mite species is the highest for group II allergens (Yasueda *et al.*, 1989). These properties implied that eventual results from our study, like a new diagnostic procedure for house dust mite allergy or a therapeutic application, would have a broad range of application. Denaturing conditions have little effect on the antibody binding

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Abbreviations: BOP, benzotriazol-1-yl-oxy-tris-(dimethyl-amino) phosphonium hexafluorophosphate; DCM, dichloromethane; DIPEA, *N,N*-diisopropyl-ethylamine; DMF, *N,N*-dimethylformamide; Fmoc-, 9-fluorenylmethoxycarbonyl-; HObt, 1-hydroxybenzotriazol; IPA, isopropylalcohol; PBS, phosphate buffered saline; PBS-AT: phosphate buffered saline with 0.3% bovine serum albumin and 0.1% Tween-20; PBS-ATS, PBS-AT with 4.5% normal bovine serum and 0.5% normal sheep serum; PITC, phenylisothiocyanate; Pmc-, 2,2,5,7,8-pentamethylchroman-6-sulphonyl-; TFA; trifluoroacetic acid; Trt-, trityl- (triphenylmethyl-); RAST, radio allergosorbent test.

capacity of group II allergens (Lombardero *et al.*, 1990). On immunoblots under denaturing conditions *Der p* II is better recognized by antibodies than *Der p* I, suggesting that linear epitopes must be exposed on *Der p* II relatively well. This, together with its smaller size, makes *Der p* II a good object for epitope mapping studies with overlapping synthetic peptides.

We synthesized a panel of overlapping peptides taken from the sequence of *Der p* II and tested their antibody-binding ability in order to localize the linear epitope(s) and found two binding regions.

MATERIALS AND METHODS

Preparation of the synthetic peptides

The T-bag method of Houghten (Houghten, 1985; Ruggeri *et al.*, 1986; Appel *et al.*, 1990) was adapted for Fmoc-chemistry. T-bags (3.5 × 3.5 cm), containing 70 mg of the *p*-benzyloxybenzyl alcohol-resins (Wang, 1973; Lu *et al.*, 1981) with the first *N*-Fmoc-protected amino acids already linked to them, were made by sealing 64 µm polypropylene mesh (Chicopee Industries, Gainesville, GA). All peptides, except peptide 1–15 (the *N*-terminus of *Der p* II) were acetylated at the *N*-terminus and all peptides except peptide 1–15 and peptide 116–129 were synthesized with an additional lysine at the *C*-terminus in order to facilitate the coupling to Sepharose. The *C*-terminal peptide 116–129 was synthesized with an additional lysine at the *N*-terminus rather than with a *C*-terminal extra lysine (see Table 1).

Coupling reactions were performed at room temp in fresh PA-grade DMF for 1 hr with a 10-fold excess of *N*-Fmoc-protected amino acid with respect to the loading of the first amino acid on the resin, a 10-fold excess of BOP (Richelieu Biotechnologies Inc., Quebec, Canada) as coupling reagent, a 10-fold excess of HOBt (Fluka, Buchs, Switzerland) to suppress racemization and a 13-fold excess of DIPEA. Deprotection was performed with 30% piperidine in DMF. After each coupling and deprotection step the T-bags were extensively washed with DMF, DCM and IPA. For deprotection and washings LAB-quality solvents were used. All resins and amino acid derivatives

were purchased from Novabiochem (Läufelfingen, Switzerland). Solvents and reagents, if not indicated otherwise, were purchased from Merck (Darmstadt, Germany). Semi-permanent side chain protection of arginine was achieved with the Pmc-group (Ramage and Green, 1987), of histidine (Sieber and Riniker, 1987) and cysteine (McCurdy, 1989) with the Trt-group. Cleavage from the resin and simultaneous deprotection of the side chains was performed with TFA containing 5% thioanisole within 4 hr. The TFA was evaporated with a stream of nitrogen. The resin was extracted with acetic acid (3 ml per T-bag) and the peptides precipitated by mixing the acetic acid extracts with ice-cold ether (9 ml). The peptides were dissolved in water and lyophilized. Yields were 25–50 mg, which is about 25–40% of theoretical yields. On reversed-phase HPLC (Delta Pak C-18, gradient from 0.1% TFA in water to 0.1% TFA in acetonitrile, u.v.-detection at 214 and 280 nm) all peptides showed a single main peak (no peaks larger than 15% of the major peak) except for peptides 20–33, 116–129 and 65–78. The former two peptides showed a second large peak (60 and 70% of the major peak), the latter peptide showed two smaller peaks (27 and 13% of the major peak), possibly due to degradation of the peptide at the Asp–Pro bond 69–70 during cleavage with TFA. A small amount of this crude peptide was purified by reversed-phase HPLC. The fraction corresponding with the main peak was hydrolyzed with 6 N HCl for 48 hr at 110°C and derivatized with PITC. HPLC analysis of the PITC derivatives gave the following amino acid composition (the values expected from the structure of the peptide are given in parentheses): Ala: 1.16 (1), Asx: 1.75 (2), Cys: detectable (2), Gly: 1.28 (1), His: 0.90 (1), Ile: 1.05 (1), Lys: 1.98 (2), Met: 0.79 (1), Pro: 2.10 (2), Tyr: 0.90 (1), Val: 1.08 (1), indicating that the main peak corresponded with the sequence 65–78 of *Der p* II with an additional lysine. The size of the peptide and the synthetic procedures used make it improbable that the peptide would have a wrong amino acid sequence.

Preparation of the solid-phase antigens and testing

After lyophilization the peptides were coupled to CNBr-activated Sepharose-4B without further purification. The coupling was performed in 0.1 M bicarbonate, pH 8.5 (10 ml), to which the peptides, dissolved in DMF (0.5–2.0 ml, depending on the solubility of the peptide), were added. Each peptide (4 mg) was coupled to a batch of 500 mg of CNBr-activated Sepharose-4B (Pharmacia). After 4 hr remaining binding sites on the Sepharose were blocked with 0.5 M glycine, pH 8.5. The Sepharose was suspended in PBS-AT (250 ml). In the RIA-test this suspension (250 µl) was incubated with 50 µl of antibody sample under shaking for 16 hr at room temp. After washing the Sepharose five times with PBS anti-Ig reagent (1 ng), radiolabelled with 400 Bq ¹²⁵I, was added in PBS-ATS (500 µl). After 16 hr of

Table 1. Sequences of the *Der p* II peptides

<i>N</i> -terminus residue No.	<i>C</i> -terminus residue No.	Sequence
1	15	H-NH-DQVDVKDCANHEIKK
11	24	Ac-NH-HEIKKVLVPGCHGS-K
20	33	Ac-NH-GCHGSEPCIHHRGK-K
29	42	Ac-NH-IHRGKPFQLEAVFE-K
38	51	Ac-NH-EAVFEANQNTKTAK-K
47	60	Ac-NH-TKTAKIEIKASIDG-K
56	69	Ac-NH-ASIDGLGVDVPGID-K
65	78	Ac-NH-VPGIDPNACHYMKC-K
74	87	Ac-NH-HYMKCPLVKGQQYD-K
83	96	Ac-NH-GQQYDIKYTNVVK-K
92	105	Ac-NH-WNVPKIAPKSENVV-K
101	114	Ac-NH-SENVVVTVKVMGDD-K
110	123	Ac-NH-VMGDDGVLCALAT-K
116	129	Ac-NH-K-VLACAIATHAKIRD

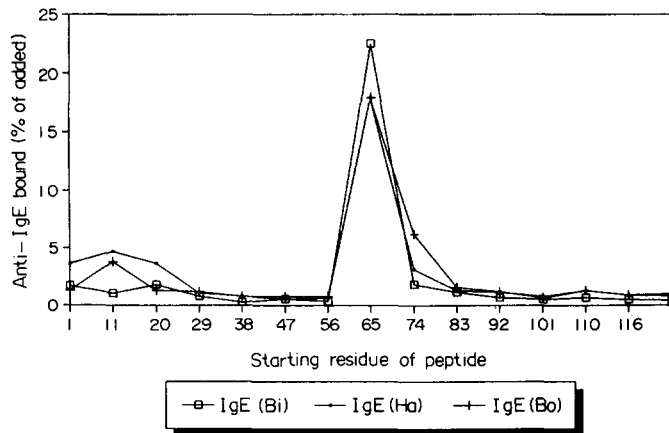


Fig. 1. Binding of the three IgE sera to the 14 Sepharose-coupled synthetic peptides.

incubation the Sepharose was washed four times with PBS and the gamma-radiation was counted. Results are given as percent of added label bound by the solid-phase antigen.

Inhibition studies

IgE antibody binding to Sepharose-coupled peptides 1–15 and 65–78 was studied. As inhibitor a *D. pteronyssinus* extract (Commonwealth Serum Laboratories, Australia; whole-body extract), a *D. farinae* extract, a gift from Dr M. D. Chapman (UVA, Charlottesville, VA), and peptide 65–78 were used. As negative control an irrelevant peptide [GVTHDQLNNFRAGFD, from the non-biting midge allergen *Chi t* I (Mazur *et al.*, 1988)] was used. The *Der p* II content of the 2% *D. pteronyssinus* extract was determined by comparison with the international reference NIBSC 82/518, which contains 100,000 U/ml *Der p* II (0.5 µg/ml or 40 nM). It contained 6880 kU/ml (34.4 mg/l or 2750 nM). The *Der f* II content of the *D. farinae* extract related to NIBSC 82/518 was 58 kU/ml (0.29 mg/l or 16 nM). The antibody samples (50 µl) were preincubated with 50 µl of the inhibiting solution for at least 2 hr.

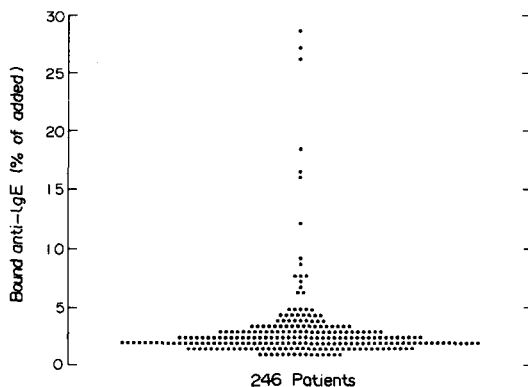


Fig. 2. Distribution of percentage binding to Sepharose-coupled peptide 65–78 in the 246 sera that gave >20% binding in the mite-RAST.

Depletion studies

Patients' serum (1 ml) was incubated with Sepharose-coupled peptide 65–78 (80 µg peptide coupled to 10 mg Sepharose) at room temp for 16 hr. Incubation with the Sepharose-coupled irrelevant peptide from *Chi t* I served as a negative control, whereas incubation with mite-Sepharose (200 µg of the *D. pteronyssinus* whole-body extract directly coupled to 100 mg Sepharose) served as positive control. After elution from the Sepharose the depleted sera were tested with "*Der p* II"-Sepharose, i.e. *Der p* II bound to Sepharose-coupled monoclonal antibody against *Der p* II (Van der Zee *et al.*, 1988), and Sepharose-coupled peptide 65–78. Dilution and non-specific losses were corrected taking into account decrease of binding to *Der p* I-Sepharose i.e. purified *Der p* I directly coupled to Sepharose.

Antisera

(a) *IgE panel*. Sera were obtained from our diagnostic department. A panel of patients whose serum bound more than 20% of the added anti-IgE reagent in the mite-RAST (Aalberse *et al.*, 1981) were tested. Binding was detected with ¹²⁵I radiolabeled sheep-anti-human IgE antibody.

(b) *IgG₄ panel*. Fifteen patients' sera with high titers of IgG₄-anti-mite-antibodies (15–65 U/ml) were tested. With ¹²⁵I radiolabeled monoclonal-anti-IgG₄ no binding was detected to any of the Sepharose-coupled peptides.

RESULTS

IgE panel

A panel of five sera from individuals with a high level of house dust mite sensitization (more than 20% of the added anti-IgE reagent bound in the mite-RAST) was tested with the 14 Sepharose-coupled peptides. As a blank we used Sepharose inactivated with 0.5 M glycine, pH 8.5. Of these patients one patient (Ha) gave strong binding (Fig. 1a), predominantly to Sepharose-coupled peptide 65–78.

Table 2. Inhibition assay, conditions

		Peptide 65-78 Sephacrose 0.5 mg/test	Peptide 65-89 Sephacrose 1.0 mg/test	Peptide 69-77 Sephacrose 0.5 mg/test
Patient Bi	μ l serum	6.3	12.5	NT
(2100 U/ml)	uninhibited %	13.1	11.5	4.0 (50 μ l)
Patient Bo	μ l serum	50.0	50.0	NT
(465 U/ml)	uninhibited %	12.9	10.3	1.8 (50 μ l)
Patient Ha	μ l serum	12.5	50.0	25.0
(2300 U/ml)	uninhibited %	13.9	10.9	10.4

Amounts of serum (for each serum the IgE concn is given in U/ml) and Sephacrose-coupled peptides used in the inhibition assays and the uninhibited percentage of radiolabeled sheep-anti-human IgE bound. Due to low binding of IgE from patients Bi and Bo to Sephacrose-coupled peptide 69-77 their sera were not tested (NT) in inhibition studies with this solid-phase antigen.

Because of lack of supply of serum we screened a panel of 195 patients' sera with this Sephacrose-coupled peptide only. Of these sera 122 bound to this solid-phase antigen, although generally the binding was very low (2-10%) (Fig. 2). From one of the patients whose serum gave binding in the range 10-25% (Bi) we obtained more serum from further studies (Fig. 1a). The 3.6% binding of IgE-anti-mite from IgE(Ha) to Sephacrose-coupled peptide 1-15 was not inhibited with whole-body extract from house dust mites. Preincubation with mite-extract (10^{-11} - 10^{-9} M *Der p* II) yielded 102-106% of the uninhibited binding. We screened another 46 patients with high house dust mite sensitization with Sephacrose-coupled peptides 1-15 and 65-78. Of this panel 18 sera bound to Sephacrose-coupled peptide 65-78. All sera showed less than 4% binding to Sephacrose-coupled peptide 1-15 except for one serum (24% compared to 16% binding to Sephacrose-coupled peptide 65-78, preincubation with mite-extract (10^{-11} - 10^{-9} M *Der p* II) yielded 98-120% of the uninhibited binding to peptide 1-15). As we could not obtain more serum this serum was not used for further analysis of the peptide 1-15. Of these sera the serum Bo bound in the 15-20% binding range and we obtained more serum for further studies (Fig. 1). The binding of all 246 sera to Sephacrose-coupled peptide 65-78 is represented in Fig. 2.

With the three sera (Bi, Bo and Ha) inhibition assays were performed. As inhibitors for the binding

of IgE-antibodies to Sephacrose-coupled peptide 65-78 the peptide itself, a longer peptide variant (peptide 65-89) and a shorter peptide variant (peptide 69-77), as well as the two house dust mite extracts, were used. As inhibitors for the binding of IgE-antibodies to Sephacrose-coupled peptide 65-89 we used the same reagents. Inhibition of IgE-binding to Sephacrose-coupled peptide 69-77 with these reagents was only performed with serum Ha, as the other sera did not bind significantly to this solid-phase antigen (2% binding of radiolabeled sheep-anti-human IgE for Bo, 4% for Bi). The Sephacrose-coupled peptides were tested in two concns and for each serum dilutions were used that yielded an uninhibited binding to each solid-phase antigen in the range of 10-15% of added sheep-anti-human IgE (see Table 2). The lines representing inhibition by *D. pteronyssinus* extract and *D. farinae* extract were parallel, and the ratio *Der f* II/*Der p* II needed for 50% inhibition was close to the expected ratio (see Table 3 and Fig. 3). The lines representing inhibition by peptide 65-89 and peptide 65-78 were parallel; the shorter peptide was slightly less potent: 28-56% of the longer peptide (Fig. 4). On a molar basis 200,000 times more peptide than *Der p* II was required for 50% inhibition. Binding of IgE-antibodies from patients Bi and Bo to the Sephacrose-coupled peptides 65-78 and 65-89 was not inhibited by peptide 69-77, which is consistent with the finding that these IgE-antibodies did not bind well to Sephacrose-coupled

Table 3. Inhibition assay, results

	Inhibitor	Peptide 65-78 Sephacrose	Peptide 65-89 Sephacrose	Peptide 69-77 Sephacrose	Peptide 1-15 Sephacrose
IgE(Bi)	<i>Der p</i> II	0.44	0.36	NT	NT
	<i>Der f</i> II	0.83	0.88	NT	NT
	65-78	83,000	100,000	NT	NT
	65-89	110,000	100,000	NT	NT
	69-77	$\geq 1,000,000$	$\geq 1,000,000$	NT	NT
IgE(Bo)	<i>Der p</i> II	0.53	0.36	NT	≥ 7.08
	<i>Der f</i> II	1.00	0.64	NT	NT
	65-78	88,000	46,000	NT	NT
	65-89	220,000	120,000	NT	NT
	69-77	$\geq 1,000,000$	$\geq 1,000,000$	NT	NT
IgE(Ha)	<i>Der p</i> II	0.11	0.38	0.13	≥ 7.08
	<i>Der f</i> II	0.20	0.73	0.38	NT
	65-78	150,000	150,000	77,000	NT
	65-89	320,000	320,000	100,000	NT
	69-77	$\geq 1,000,000$	1,000,000	410,000	NT

Concentration (nM) of added inhibitor to effectuate 50% inhibition of antibody binding to the various solid-phase antigens (NT = not tested).

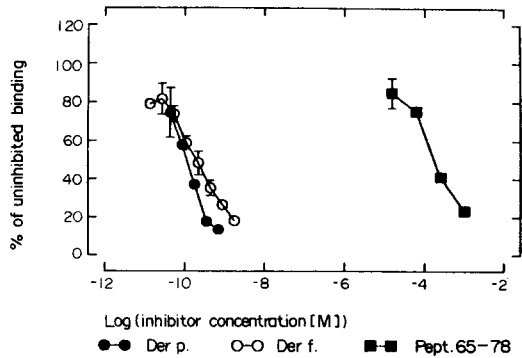


Fig. 3. Inhibition of IgE-binding (Ha) to Sepharose-coupled peptide 65-78 by *D. pteronyssinus* extract, *D. farinae* extract and peptide 65-78 as function of the concentration of *Der p* II, *Der f* II and peptide.

peptide 69-77 (Fig. 5). These data show that there was a large difference in affinity for anti-*Der p* II-IgE antibodies towards *Der p* II and these three synthetic peptides (Fig. 3).

In order to estimate which fraction of the anti-*Der p* II-IgE antibodies reacted with this Sepharose-coupled peptide we compared titration curves of these sera with "*Der p* II"-Sepharose and Sepharose-coupled peptide 65-78. For Bo and Ha we found that 25 and 7% of the anti-*Der p* II-IgE antibodies in these sera reacted with this Sepharose-coupled peptide. Binding of IgE(Bi) to the Sepharose-coupled peptide was higher than binding to "*Der p* II"-Sepharose. We therefore estimated that at least 75% of the IgE antibodies against *Der p* II recognized this peptide.

After depletion of the sera with Sepharose-coupled peptide 65-78 binding to this solid-phase antigen was reduced by more than 90%. Binding to "*Der p* II"-Sepharose was hardly reduced for Bo and Ha after depletion with Sepharose-coupled peptide 65-78 (less than 10%); for Bi we found that binding to "*Der p* II"-Sepharose was slightly increased.

For all three sera depletion with mite-Sepharose gave more than 90% reduction of binding to

Sepharose-coupled peptide 65-78 and "*Der p* II"-Sepharose, depletion with Sepharose-coupled *Chi t* I-peptide less than 10%.

DISCUSSION

Peptide 65-78

This peptide contains two of the six cysteines of *Der p* II. The two cysteines are separated by four amino acid residues, thus allowing an intrachain disulfide bridge. We do not know whether the disulfide bridge 73-78 is formed in the native *Der p* II molecule, but (as no special precautions were taken to avoid air oxidation) formation of this disulfide bridge in the synthetic peptide is a distinct possibility.

Binding of IgE antibodies to Sepharose-coupled peptides

When testing the Sepharose-coupled peptides with patients' sera we found two different binding regions in the *Der p* II molecule. Binding to Sepharose-coupled peptide 65-78 appeared to be specific as it was inhibited by *Der p* II. Until now we found no patients with IgG₄-anti-mite-antibodies that reacted with any of the synthetic peptides. Other groups found that different antibodies may recognize the same epitope (Atassi and Atassi, 1986), although minor individual and inter-species variations were observed (Rodda *et al.*, 1986; Appel *et al.*, 1990; Geysen *et al.*, 1986; Chapman *et al.*, 1987). We found a distinct intra-species variation: binding of IgE(Ha) to Sepharose coupled peptides 65-78, 65-89 and 69-77 was inhibited by peptide 69-77 (Fig. 4), binding of IgE(Bi) and IgE(Bo) was not.

For amino acid analysis a small amount of peptide 65-78 was purified by reversed-phase liquid chromatography. Fractions of the effluent containing the major component with the expected amino acid composition were collected, freeze-dried, coupled to CNBr-activated Sepharose and tested in a peptide-RAST. We found that the major component

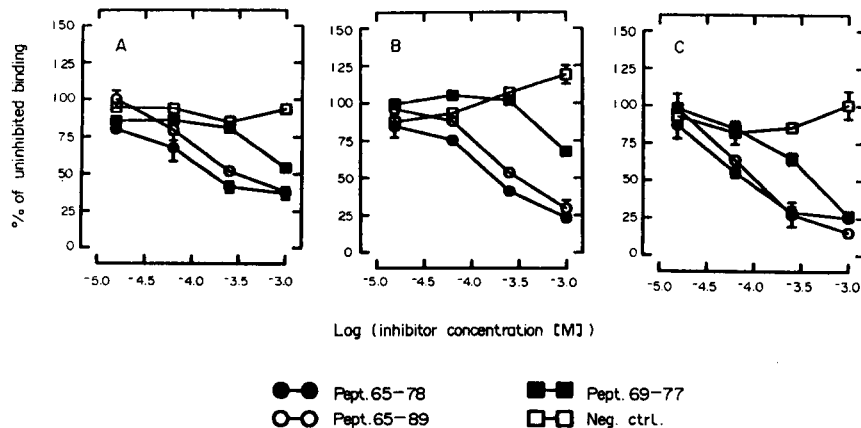


Fig. 4. Inhibition assay with IgE (Ha) with the three different solid-phase antigens: (A) Sepharose-coupled peptide 65-89, undiluted serum; (B) Sepharose-coupled peptide 65-78, serum dilution 1:2; (C) Sepharose-coupled peptide 69-77, serum dilution 1:2. The bars represent the deviation between duplicate assays.

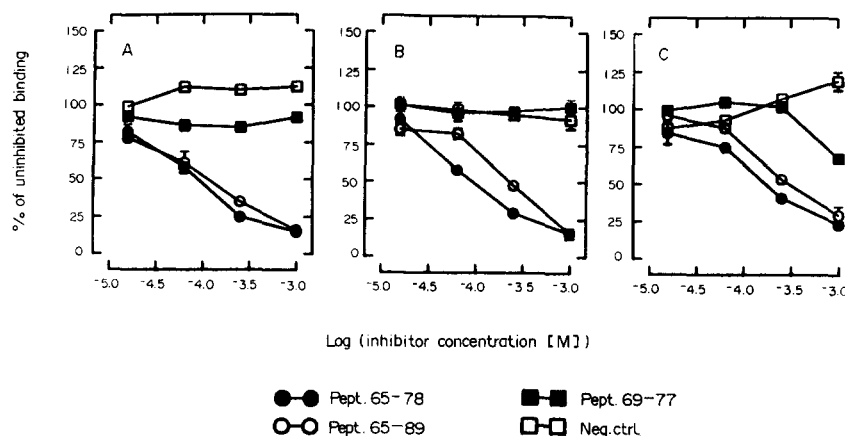


Fig. 5. Inhibition of binding of (A) IgE(Bi) 1:8, (B) IgE(Bo) and (C) IgE(Ha) 1:2 to Sepharose-coupled peptide 65-78. IgE binding to this solid-phase antigen is inhibited by *D. pteronyssinus* extract, *D. farinae* extract, peptide 65-78 and peptide 65-89 for all sera and by peptide 69-77 for IgE(Ha) only. The bars represent the deviation between duplicate assays.

containing peptide 65-78 bound the three patients' sera. This binding was inhibited by mite-extract. The amount of peptide purified by reversed-phase HPLC was too small to actually measure the amount coupled to Sepharose and therefore we are not able to compare quantitatively the responses obtained with crude and purified peptide. Binding to the purified peptide was smaller than to the crude peptide.

Although we established specificity by inhibition by *Der p* II, aspecific binding to these Sepharose-coupled peptides also may have occurred to a minor extent. We tested a panel of 15 sera with high total IgE levels (2000-70,000 U/ml) with less than 2% binding in the mite-RAST. These sera yielded 2-5% (one serum even 10%) binding to Sepharose-coupled peptide 65-78. The amount of binding showed no correlation to the total IgE level. In our inhibition studies we sometimes noticed a somewhat higher binding in the presence of a large amount of inhibitor (Figs 4 and 5). Presumably this was due to aspecific binding of mite-allergen to the Sepharose-coupled peptides.

The depletion studies indicated that for Bo and Ha the amount of anti-*Der p* II-IgE antibodies that reacted with Sepharose-coupled peptide 65-78 was less than 5%; this is lower than was indicated by titration of the sera. For IgE(Bi) we found that binding to Sepharose-coupled peptide 65-78 was stronger than to "*Der p* II"-Sepharose and that, after depletion with this Sepharose-coupled peptide, binding to "*Der p* II"-Sepharose was slightly increased rather than decreased. We assume that this serum contains non-IgE anti-mite antibodies that react better with the peptide than anti-mite IgE. With "*Der p* II"-Sepharose this competition led to incomplete IgE binding, with Sepharose-coupled peptide this effect was less pronounced as the loading of peptide on the Sepharose on a molar base was much higher than that of *Der p* II. Depletion with the Sepharose-coupled peptide reduced the amount of these antibodies and

therefore led to more binding of anti-mite IgE to "*Der p* II"-Sepharose. This hypothesis will be subject of further investigation.

We realize that antibody binding to a Sepharose-coupled peptide might be influenced by an extra C- or N-terminal lysine or even by the agarose matrix; this might result in false positive or false negative results. Since the binding of IgE to peptide 65-78 is inhibited by native protein we consider this binding specific. In peptide 65-89, which binds IgE antibodies to the same extent, there is no additional C-terminal lysine. This also makes us conclude that for the sera described the additional C-terminal lysine is not important. We cannot exclude that for other antibodies or Sepharose-coupled peptides such an effect exists.

Applicability of peptide 65-78

Only very few patients have appreciable amounts of IgE-anti-mite directed against peptide 65-78 (Fig. 2); this makes it unsuitable for the determination of anti-*Der p* II IgE-antibodies in patients' sera. Although 90% of the sera from mite-allergic patients recognize group II allergens (Van der Zee *et al.*, 1988; Heymann *et al.*, 1989; Chapman *et al.*, 1987; Yasueda *et al.*, 1989), only 60% of the most positive sera we tested bound to Sepharose-coupled peptide 65-78, only 7% yielded more than 5% binding.

Nevertheless the peptide may well be useful for a crossreactive mite allergen assay, e.g. in house dust extracts (Chapman *et al.*, 1980; Wood *et al.*, 1988) as binding to the Sepharose-coupled peptide was inhibited by both *D. pteronyssinus* extract and *D. farinae* extract. Alternatively, it may be possible to obtain antibodies suitable for such a crossreactive mite allergy assay by raising antibodies to this peptide in experimental animals.

Finally, it is of interest to establish whether the frequency of IgG₄ antibodies to this peptide is much

lower than that of IgE antibodies. This might help to elucidate the interrelatedness of the immune regulation of these two antibody isotypes.

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