

IgE epitopes on the cat (*Felis domesticus*) major allergen *Fel d* I: A study with overlapping synthetic peptides

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Abstract

Background: The major cat allergen *Fel d* I is composed of two disulfide-linked polypeptide chains, chain 1 (70 amino acid residues) and chain 2 (92 amino acid residues). Reduction and alkylation of *Fel d* I eliminates almost all antigenic and allergenic activity, and detection of linear epitopes with synthetic peptides is therefore not expected.

Methods: We synthesized synthetic peptides of both chains of about 14 amino acid residues, overlapping by 7 residues. The peptides were coupled to Sepharose (Pharmacia, Uppsala, Sweden) and tested with sera of patients with cat allergy.

Results: Three peptides showed specific binding of human IgE, residues 25-38 and 46-59 of chain 1 and residue 15-28 of chain 2. IgE binding was inhibited by *Fel d* I and the corresponding peptide. Of 61 patients with cat allergy tested, 65% showed IgE binding to at least one of the peptides; 46% showed IgE binding to peptide 25-38, 11% to peptide 46-59, and 28% to peptide 15-28. Each peptide was recognized by only one of the 78 patients with negative RAST results. By affinity chromatography with peptide-Sepharose anti-*Fel d* I antibodies were isolated, also confirming the specificity of IgE binding to the peptides. The percentage of IgE antibodies against *Fel d* I reactive with the peptides varied with the serum and the peptide-Sepharose used and ranged from 2% to 55%.

Conclusions: Because the affinity of IgE binding to the peptides was very low and only serum samples with high titers of *Fel d* I-specific IgE antibodies (RAST 4+/5+) showed significant binding, these peptides are not suitable for diagnostic purposes. However, the peptides are useful tools for comparing IgE and IgG responses and for studying the relationship to the T-cell epitopes on this molecule. (J ALLERGY CLIN IMMUNOL 1994;93:34-43.)

Der p II	<i>Dermatophagoides pteronyssinus</i> allergen 2
Fel d I	<i>Felis domesticus</i> allergen 1
PBS-AT	Phosphate-buffered saline pH 7.4, containing 10 mmol/L ethylenediaminetetraacetic acid, 0.3% bovine serum albumin, 0.1% Tween-20, and 0.05% NaN ₃
R&A	Reduced and alkylated

Key words: [Fel d I](#), [synthetic peptides](#), [IgE epitopes](#)

Felis domesticus allergen 1 (*Fel d I*) is the major cat allergen, ¹⁻³ found in cat saliva, ⁴⁻⁷ pelt, ^{5,6} and lacrimal fluid. ⁸ Affinity-purified *Fel d I* from house dust ⁹ and cat dander ^{10,11} has a molecular weight of approximately 37 kd. On sodium dodecyl-sulfate-polyacrylamide gel electrophoresis *Fel d I* is an 18 kd protein, which dissociates after reduction into a 13 kd chain and a 5 kd chain. ^{12,13} Amino acid sequence analysis and complementary DNA cloning of *Fel d I* ¹⁴ show that these two polypeptide chains consist of 70 amino acid residues (chain 1) and 92 residues (chain 2), respectively. Each chain contains three cysteine residues, and preliminary analysis indicates that chains 1 and 2 are connected by disulfide bonds in an antiparallel configuration. ¹⁴ Chain 2 is glycosylated, probably at asparagine 33, and treatment of the allergen with N-glycosidase in one study showed some reduction in the inhibition capacity of the RAST as compared with native *Fel d I*. ¹² However, other investigators have not found any decrease. ¹⁵ Heating of *Fel d I* or treatment at pH 12 or with 6 mol/L guanidine causes only a modest reduction in *Fel d I* activity. However, after reduction and alkylation, loss of activity of more than 80% in RAST inhibition experiments and allergen binding experiments is found. ^{10,12,16} Because of this decrease in *Fel d I* activity after reduction and alkylation (and perhaps after deglycosylation), detection of linear IgE epitopes of *Fel d I* with the use of synthetic peptides was not expected. However, group II mite allergens also completely lose their IgE-binding capacity after reduction and alkylation. ¹⁷ Nevertheless, a linear epitope of *Dermatophagoides pteronyssinus* allergen II (*Der p II*), comprising residues 65-78, was found for a number of IgE sera. ¹⁸ For several other allergens, IgE epitopes were found with the use of synthetic peptides. ¹⁹⁻²⁴

In this article we describe three IgE-binding peptides, two on chain 1 and one on chain 2 of *Fel d I*. Synthetic peptides of about 14 residues and with an overlap of 7 residues were synthesized, coupled to Sepharose (Pharmacia, Uppsala, Sweden), and tested with IgE sera. Specificity of IgE binding was studied in several ways. First, inhibition experiments were performed with native *Fel d I*, reduced and alkylated (R&A) *Fel d I*, and the corresponding peptides. Second, panels of subjects with positive and negative RAST results to cat dander were tested on the peptide-Sepharose; this study also provided information about the diagnostic value of these peptides. Third, anti-peptide antibodies were immunopurified with peptide-Sepharose and tested for their anti-*Fel d I* activity.

Methods

Peptides

Twenty-one synthetic peptides were made by the T-bag method of Houghten, ²⁵⁻²⁷ adapted for Fmoc chemistry. ¹⁸ All peptides were synthesized with an additional lysine at the C-terminus to facilitate coupling to CNBr-activated Sepharose (Table I). On reversed-phase high-performance liquid chromatography (Waters Delta Pak C18 [Millipore BV, Etten Leur, Holland], gradient from 0.1% trifluoroacetic acid in water to 0.1% trifluoroacetic acid in acetonitrile, ultraviolet detection at 214 nm) the IgE binding peptides 25-38 and 46-59 of chain 1 and 15-28 of chain 2 showed one main peak, comprising 57% to 74% of the total profile. Peptide 25-38 of chain 1 contains a tyrosine residue and showed one main peak at 280 nm.

TABLE I. Sequence of the *Fel d I* peptides

<u>Chain 1</u>			<u>Chain 2</u>		
<i>N</i> -terminus (res. no.)	<i>C</i> -terminus (res. no.)	Sequence	<i>N</i> -terminus (res. no.)	<i>C</i> -terminus (res. no.)	Sequence
1	16	EICPAVKRDVDLFLTG-K	1	14	VKMAETCPIFYDVF-K
9	24	DVDLFLTGTPDEYVEQ-K	9	21	PIFYDVFFAVANGN-K
18	31	PDEYVEQVAQYKAL-K	15	28	FAVANGNELLLDCS-K
25	38	VAQYKALPVLLEN-A-K	22	35	ELLLDLSLTKVNAT-K
32	45	PVVLLENARILKNCV-K	29	42	LTKVNATEPERTAM-K
39	52	RILKNCVDAKMTEE-K	36	49	EPERTAMKKIQDCY-K
46	59	DAKMTEEDKENALS-K	43	56	KKIQDCYVENGLIS-K
53	66	DKENALSLLDKIYT-K	50	63	VENGLISRVLDGLV-K
60	70	LLDKIYTSPLC-K	57	70	RVLDGLVMTTISSS-K
			64	77	MTTISSSKDCMGEA-K
			71	84	KDCMGEAVQNTVED-K
			78	92	VQNTVEDLKLNTLGR-K

res, Residue.

Preparation of solid-phase antigens

Lyophilized peptides were dissolved in water and coupled to CNBr-activated Sepharose 4B (Pharmacia) without further purification. Eight milligrams of each peptide were coupled to 500 mg of CNBr-activated Sepharose in 0.1 mol/L bicarbonate, pH 8.5. After an overnight incubation at 4° C, remaining binding sites were blocked with 0.5 mol/L glycine, pH 8.5, for 2 hours. The Sepharose was put through a low pH/high pH cycle of washes three times to desorb noncovalently bound peptide. Then the Sepharose was suspended in 500 ml PBS-AT (phosphate-buffered saline containing 10 mmol/L ethylenediaminetetraacetic acid, 0.3% bovine serum albumin, 0.1% Tween-20, and 0.05% NaN₃) (2 mg/ml). For the *Fel d I* RAST, 125 U (250 to 500 µg) of affinity-purified *Fel d I* from house dust was coupled to 500 mg of CNBr-activated Sepharose 4B. After the coupling procedure, the Sepharose was suspended in 500 ml of PBS-AT (2 mg/ml). Anti-IgE Sepharose was prepared by coupling 5 mg of monoclonal anti-human IgE ²⁸ (MH25-01, CLB, Amsterdam, Holland) to 300 mg of CNBr-activated Sepharose 4B. After the coupling procedure, the Sepharose was suspended in 150 ml of PBS-AT (2 mg/ml).

Human serum

Serum samples from patients with cat allergy were obtained from our diagnostic department. Only samples showing more than 20% binding of the added anti-IgE antibodies in the cat dander RAST (RAST score ≥4+) were used for screening the *Fel d I* peptides (total IgE, 67 to 4200 IU/ml). As negative control sera, samples from patients with negative RAST results to cat (also obtained from our department) with elevated total IgE levels (130 to 24000 IU/ml) were used.

Allergen sources

Cat dander extract (10% [wt/vol]) was obtained from Haarlems Allergenen Laboratorium (Haarlem, Holland) and contained 120 U/ml *Fel d I*. *Dermatophagoides pteronyssinus* source material (purified mite bodies) was

obtained from Commonwealth Serum Laboratories (Melbourne, Australia). A 2% extract contained 35 µg/ml *Der p* II.

IgE binding assays

RAST experiments were performed as previously described. ²⁹

RAST inhibition studies

The specificity of IgE binding to Sepharose-coupled peptides 1-16, 25-38, 46-59, and 60-70 of chain 1 and to peptides 1-14, 15-28, 36-49, 43-56, and 78-92 of chain 2 was studied by competitive inhibition experiments. Fifty microliters of IgE serum was preincubated with 50 µl of various concentrations of inhibitor. After 2 hours, 500 µl of peptide-Sepharose was added, and the rest of the procedure was performed as in the RAST. Inhibitors used were cat dander extract, affinity-purified *Fel d* I, R&A *Fel d* I, ¹³ or the corresponding peptides. In negative control experiments a *D. pteronyssinus* extract and an irrelevant peptide (GVTHDQLNNFRAGFD) from the nonbiting midge allergen *Chironomus thummi thummi* (*Chi t* I) ¹⁹ were used.

Comparison of the avidity of IgE binding to *Fel d* I and *Fel d* I peptides

The avidity of IgE binding to *Fel d* I and *Fel d* I peptides (residues 25-38 and 46-59 of chain 1 and residue 15-28 of chain 2) was compared in dissociation studies. Fifty microliters of IgE serum was incubated with 250 µl of *Fel d* I-Sepharose and peptide-Sepharose, respectively, overnight to reach a state of equilibrium. After extensive washing, the Sepharose was incubated at room temperature with an excess of *Fel d* I (2.4 µg) for 1, 2, 4, 8, and 16 hours, respectively, to block the dissociation of antibodies from the Sepharose. Then the Sepharose was washed again, and residual IgE antibodies were detected by an overnight incubation with 1 ng of radiolabeled sheep anti-human IgE in 500 µl of PBS-AT. For each peptide two serum samples were tested. IgE binding to *Fel d* I- and peptide-Sepharose was completely inhibited by 2.4 µg of *Fel d* I in RAST inhibition experiments.

Isolation of anti-*Fel d* I antibodies by affinity chromatography with peptide-Sepharose

For the isolation of peptide-specific antibodies, we added 100 µl of 0.5 mol/L ethylenediaminetetraacetic acid and 50 µl of 10% Tween-20 to 5 ml of cat RAST-positive serum (most serum samples were also mite RAST-positive). Then the serum was incubated with 150 mg of peptide-Sepharose (peptides 25-38 and 46-59 of chain 1 and peptide 15-28 of chain 2) for 4 hours. The Sepharose was washed extensively with PBS, and peptide-specific antibodies were eluted with 2 ml of 0.1 mol/L glycine pH 2.5 containing 0.03% bovine serum albumin. The eluate was neutralized immediately with 120 µl of 1 mol/L Tris pH 9 and dialyzed against phosphate-buffered saline. In the eluate specific IgE was determined and expressed as arbitrary units. A serum with known IgE concentration (determined relative to the World Health Organization standard), tested with sheep anti-human IgE-Sepharose and iodine 125-labeled sheep anti-human IgE, was used as a standard curve. Concentrations of IgE in the eluates ranged from 0.8 to 10.0 IU/ml. To measure *Fel d* I-specific IgE antibodies in the eluate from the peptide-Sepharoses, 50 µl of eluate was incubated overnight with 250 µl anti-IgE Sepharose. After washing, 500 µl of PBS-AT and 0.2 ng of ¹²⁵I labeled *Fel d* I (463 Bq) were added, and another incubation overnight followed. To verify the specificity of the peptides adsorbent, the eluates were also tested for anti-mite activity in a RAST. ²⁹

Percentage of IgE antibodies against *Fel d* I reacting with the synthetic peptides

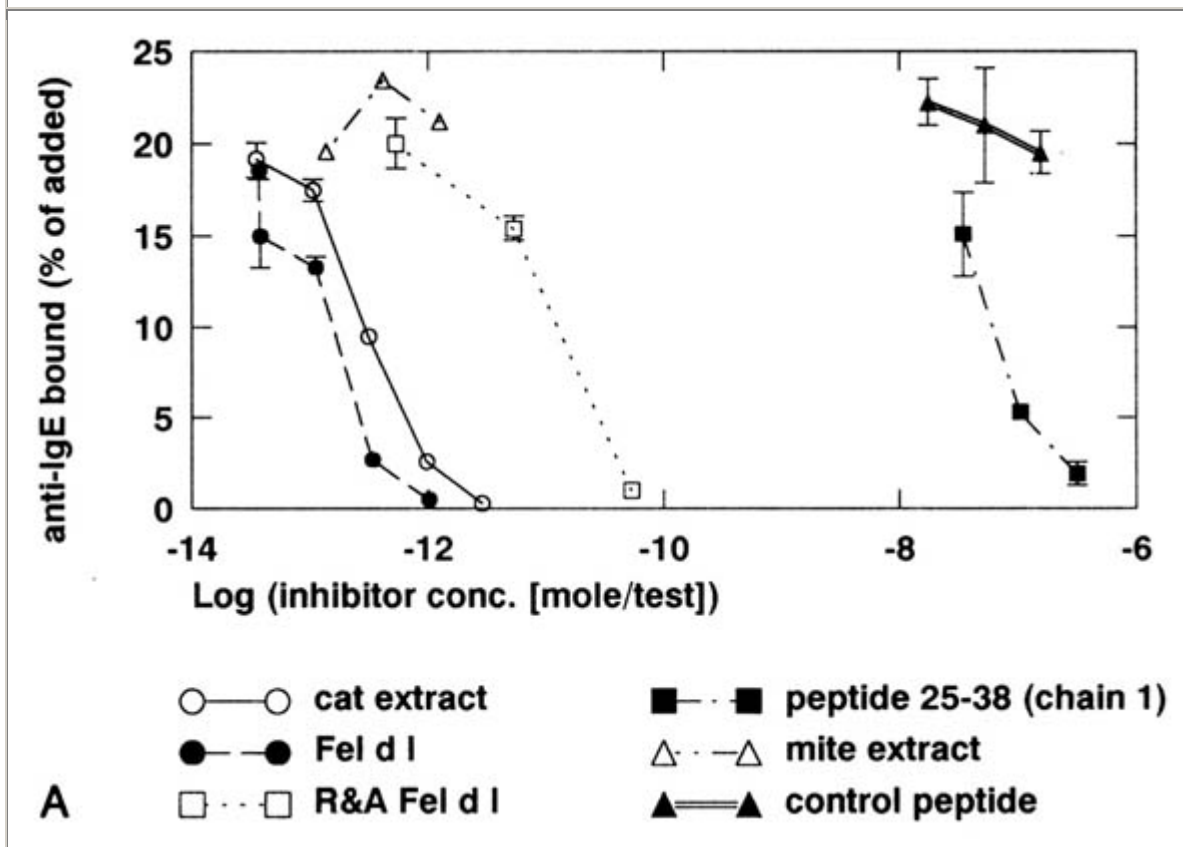
To estimate the percentage of IgE antibodies directed against *Fel d* I reacting with the different synthetic peptides, IgE binding to peptide-Sepharose and to *Fel d* I-Sepharose were compared. For each peptide two serum samples were tested. Because the concentration of peptide-Sepharose used in the binding assays (8 µg) was not a sufficient excess for binding all IgE antibodies, serum dilutions were tested with three concentrations of peptide-Sepharose (0.5, 1.0, and 2.0 mg per test, respectively). From the binding data obtained, the percentage IgE antibodies against *Fel d* I binding to peptide-Sepharose was calculated, and the maximal IgE binding was estimated by linear extrapolation of a double reciprocal plot. ³⁰ For all sera the highest binding found was at least 60% of the extrapolated value.

Results

A panel of 12 IgE sera (*Fel d I* RAST score $\geq 4+$) were tested on all 21 Sepharose-coupled synthetic peptides from chain 1 and chain 2 of *Fel d I*. Binding of serum samples was found to peptides 1-16, 25-38, 46-59, and 60-70 of chain 1 and peptides 1-14, 15-28, 36-49, 43-56, and 78-92 of chain 2.

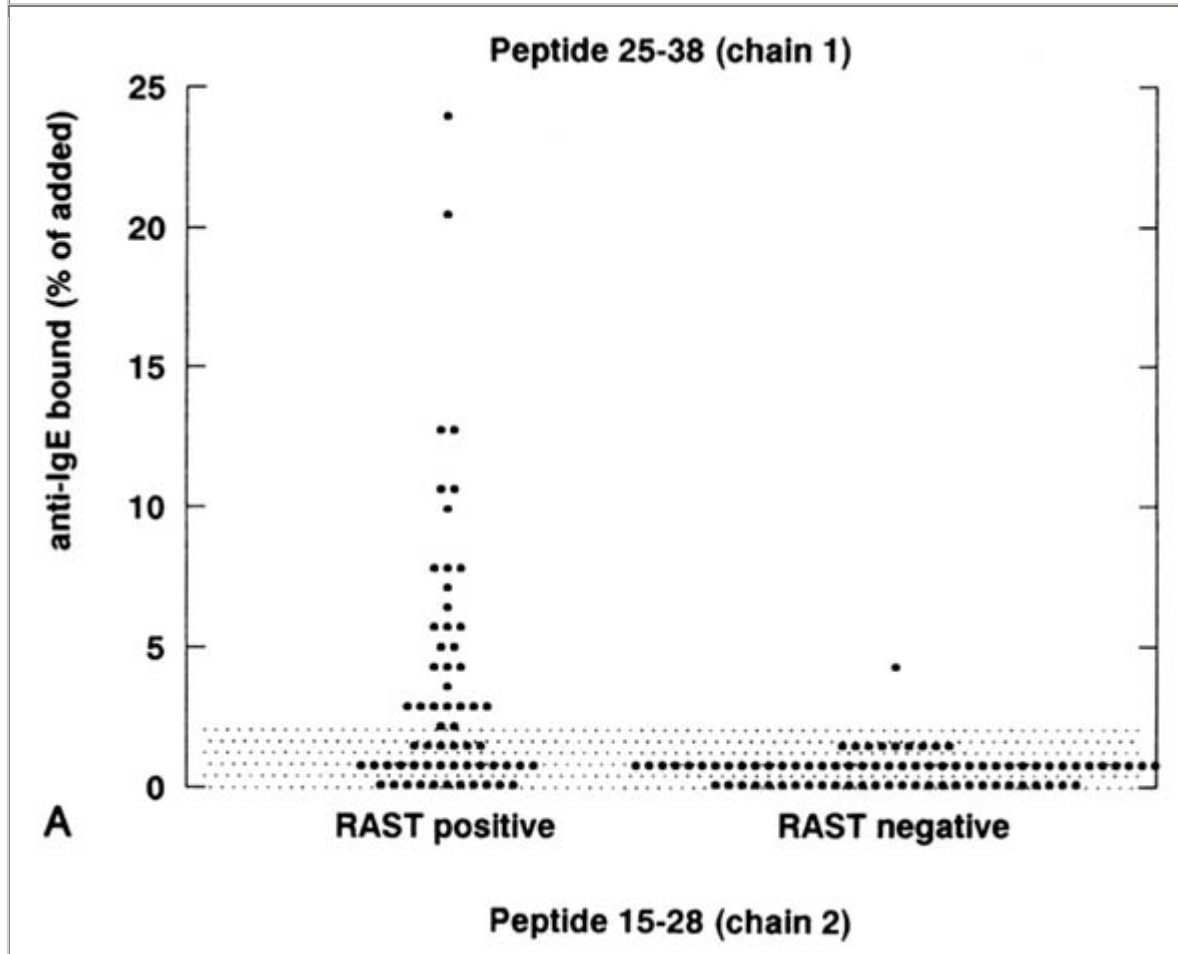
Specificity of IgE binding to these nine peptides was studied in competitive inhibition experiments. For each peptide-Sepharose preparation, serum samples from patients with cat allergy were tested, (four for peptide 25-38, three for peptide 78-92, and two for the other peptides). Preincubation of serum with cat dander extract, affinity-purified *Fel d I*, R&A *Fel d I*, or the corresponding peptide resulted in a dose-dependent inhibition of IgE binding to peptides 25-38 and 46-59 of chain 1 and peptide 15-28 of chain 2 (Fig. 1, A, B, C). IgE binding to peptide 1-16 of chain 1 and peptides 1-14, 36-49, 43-56, and 78-92 of chain 2 was only inhibited by the corresponding peptide and not by native or R&A *Fel d I* (Fig. 1, D), whereas IgE binding to peptide 60-70 of chain 1 was not inhibited at all. Preincubation of serum with mite extract or an irrelevant peptide from the nonbiting midge allergen *Chi t I* did not result in inhibition of IgE binding. Cross-inhibition studies were performed with peptides 25-38 and 46-59 of chain 1 and peptides 15-28 and 78-92 of chain 2 with four different serum samples. No cross-inhibition between the peptides was found, suggesting that each peptide is part of a different epitope.

FIG. 1. Specificity of IgE binding to **A**, peptide 25-38 of chain 1; **B**, peptide 46-59 of chain 1; **C**, peptide 15-28 of chain 2, and **D**, peptide 78-92 of chain 2. Inhibitors used were native *Fel d I* (cat dander extract and affinity-purified *Fel d I*), R&A *Fel d I*, and peptide.



To further investigate the specificity of IgE binding to the peptides (inhibited by *Fel d I* or peptide), panels of 61 cat dander RAST-positive sera (RAST score $\geq 4+$; total IgE: 67 to 4200 IU/ml) and 78 cat dander RAST-negative sera (total IgE: 130 to 24,000 IU/ml) were tested on the peptide-Sepharose preparations. Peptides 25-38 and 46-59 of chain 1 and peptide 15-28 of chain 2 were recognized by only 1 of 78 of the RAST-negative sera, whereas none of the RAST-negative sera recognized peptide 78-92 of chain 2. Of the RAST-positive sera, 46% showed IgE binding to peptide 25-38, 11% to peptide 46-59, 28% to peptide 15-28, and 46% to peptide 78-92 ($\geq 2.3\%$ binding of anti-IgE antibodies added; this cutoff value was selected for optimal discrimination between RAST-positive and RAST-negative sera) (Fig. 2, A, B, C, and D).

FIG. 2. IgE binding of cat dander RAST-positive and RAST-negative sera to **A**, peptide 25-38 of chain 1; **B**, peptide 46-59 of chain 1; **C**, peptide 15-28 of chain 2; **D**, peptide 78-92 of chain 2; **E**, peptide 1-14 of chain 2, and **F**, peptide 43-56 of chain 2.



IgE binding to peptide 1-16 of chain 1 and peptides 1-14, 36-49, and 43-56 of chain 2 was found for subjects with positive RAST results, as well as those with negative RAST results and was thus not specific for cat-allergic sera (■Fig. 2, E, F). On the basis of the inhibition studies and the finding that IgE binding correlated with the total IgE levels in the sera, peptides 1-16 and 60-70 of chain 1 and peptides 1-14, 36-49, and 43-56 of chain 2 were not further studied.

An additional experiment was performed to confirm the specificity of IgE binding to peptides 25-38 and 46-59 of chain 1 and peptides 15-28 and 78-92 of chain 2: *Fel d I*-specific IgE antibodies were isolated by affinity chromatography on peptide-Sepharose. Serum was incubated with peptide-Sepharose, and the eluates of the peptide-Sepharose were tested with anti-human IgE Sepharose and ^{125}I -labeled *Fel d I* (■Fig. 3); for each peptide two serum samples were tested. The eluates from peptides 25-38, 46-59, and 15-28 Sepharose showed anti-*Fel d I* activity. However, the eluate from peptide 78-92 Sepharose did not show anti-*Fel d I* activity. As a specificity control, the anti-mite activity of sera (allergic to cat and house dust mite) and their eluates of the peptide-Sepharose were tested (■Fig. 4).

FIG. 3. Anti-*Fel d I* activity of IgE antibodies isolated by affinity chromatography with peptide. The eluates and full sera were tested for anti-*Fel d I* activity with anti-IgE Sepharose and ^{125}I -labeled *Fel d I*. Serum 1 was incubated with peptide 25-38 (chain 1)-Sepharose and peptide 15-28 (chain 2)-Sepharose (**A**). Serum 2 was incubated with peptide 46-59 (chain 1)-Sepharose (**B**), and serum 3 was incubated with peptide 15-28 (chain 2)-Sepharose (**C**).

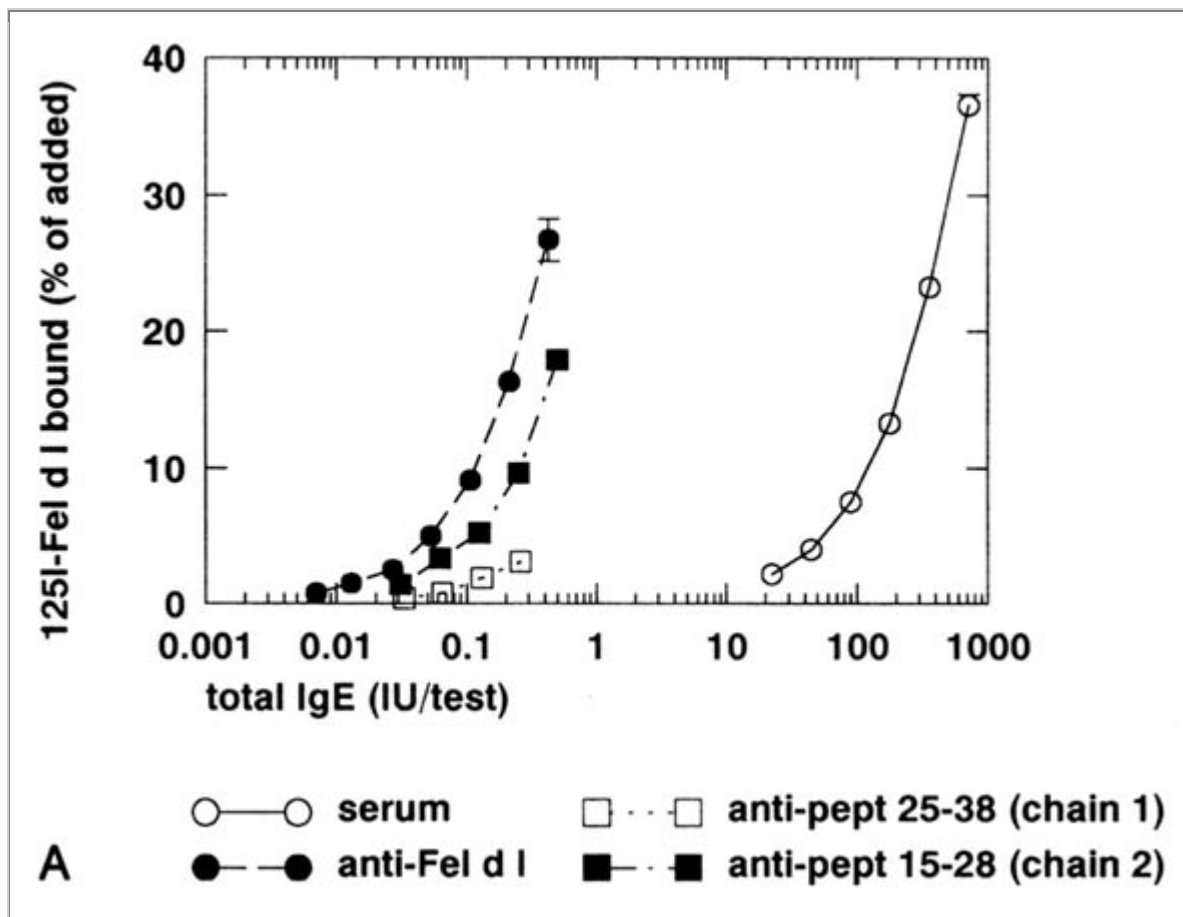
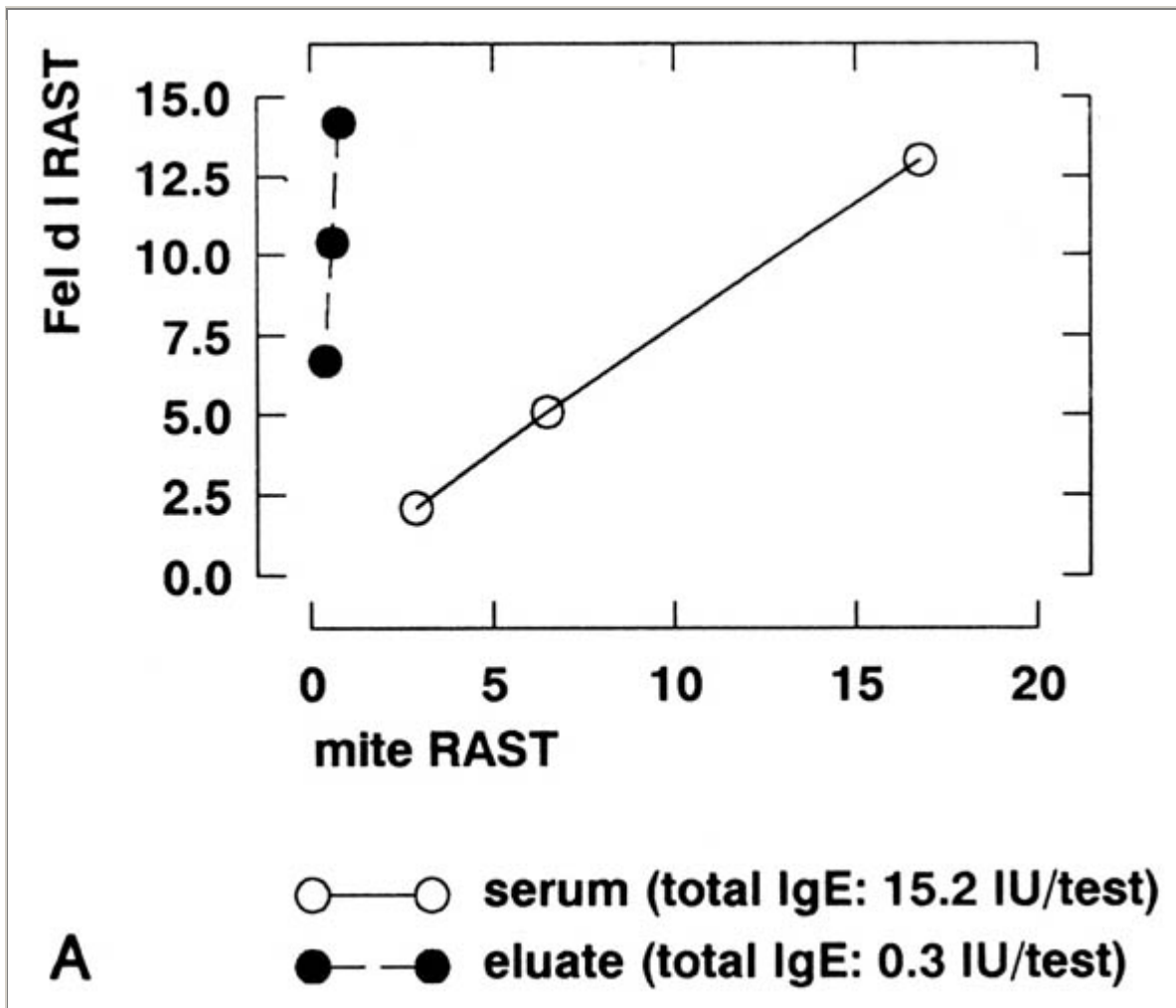
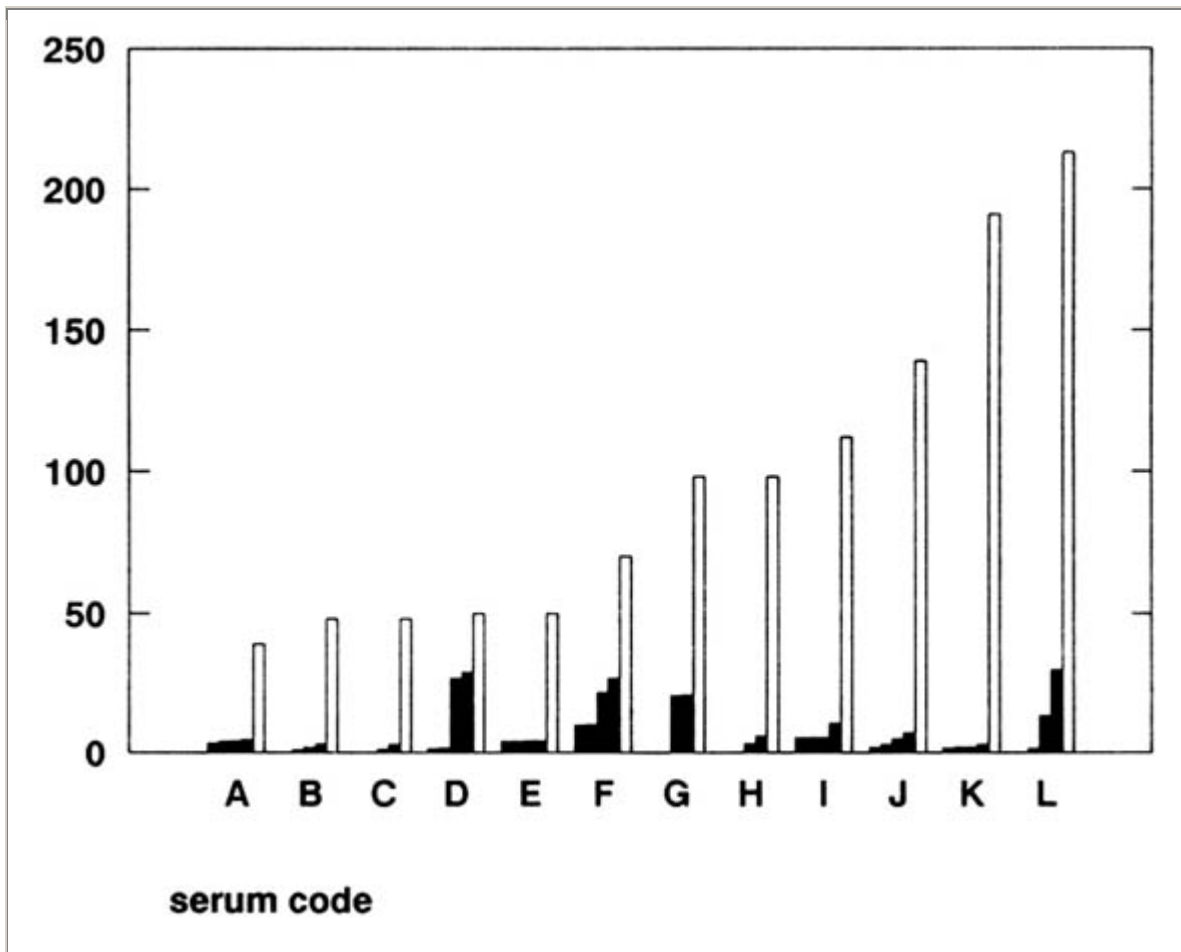


FIG. 4. Anti-*Fel d I* and anti-mite activity of sera and their eluates from peptide-Sepharose. Three sera and eluates from **A**, peptide 25-38 (chain 1)-Sepharose; **B**, peptide 46-59 (chain 1)-Sepharose; and **C**, peptide 15-28 (chain 2)-Sepharose were tested in several dilutions in the *Fel d I* RAST and the mite RAST. Threefold dilutions of the sera and twofold dilutions of the eluates were made. The highest IgE concentrations in the sera and eluates are shown.



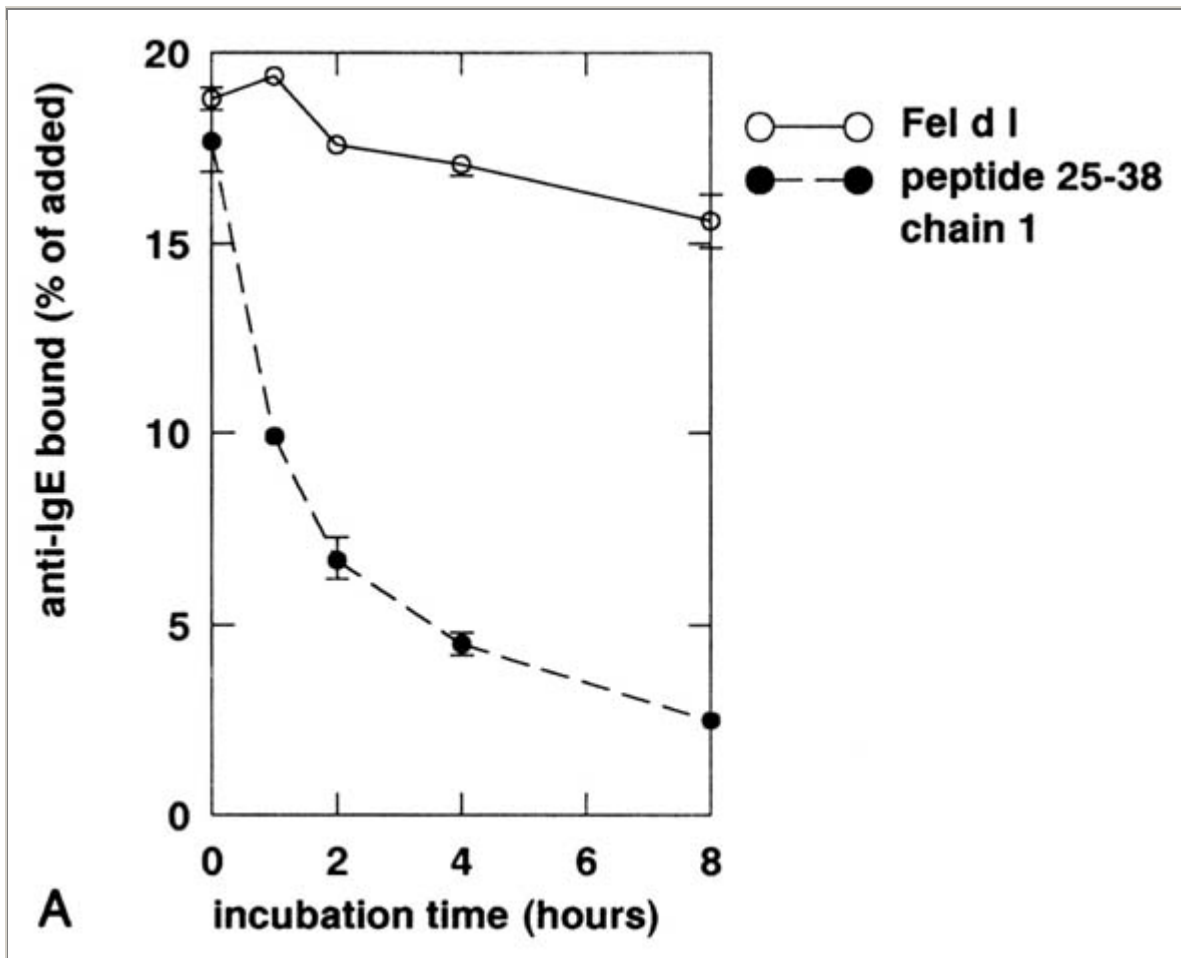
We estimated the contribution of the peptide-reactive IgE antibodies to the IgE anti-*Fel d I* response by calculating RAST units from the binding data. In [Fig. 5](#) binding of the 12 most reactive serum samples to the four peptides (25-38 and 46-59 of chain 1 and 15-28 and 78-92 of chain 2) is plotted in a cumulative way and compared with the RAST units to *Fel d I* in each serum. The cumulative percentage of IgE antibodies binding to the peptides varied with the serum and the peptide-Sepharose used and ranged from 1.5% to 58%.

FIG. 5. Cumulative IgE binding (expressed in RAST units) of IgE from 12 serum samples (labeled A to L) to, successively, peptides 25-38, 46-59, 15-28, and 78-92 (*filled bars*) compared with the concentration of *Fel d I*-specific IgE (*open bars*).



The binding of IgE to the peptides was much weaker than to *Fel d I*, as indicated by the markedly higher dissociation rate (■Fig. 6).

FIG. 6. Dissociation rate of IgE antibodies from *Fel d I*-Sepharose and peptide-Sepharose. Fifty microliters of serum dilution was incubated overnight with *Fel d I*-Sepharose and peptide-Sepharose. To *Fel d I*-Sepharose, a 1:10 dilution of serum 1 was added (A), a 1:40 dilution of serum 2 (B), and a 1:30 dilution of serum 3 (C). To peptide 25-38 Sepharose (A), peptide 46-59 Sepharose (B), and peptide 15-28 Sepharose (C), undiluted serum was added. After washing, the Sepharose was incubated with an excess (2.4 µg) of *Fel d I*.



Discussion

In this study we investigated IgE binding determinants on chain 1 and chain 2 of the *Fel d I* molecule. Serum samples from patients with *Fel d I* cat allergy were tested on peptide-Sepharose, and nine peptides showed IgE binding. Specificity of IgE binding was studied in several ways. First, panels of subjects with positive and negative RAST results to cat dander were tested on peptide-Sepharose. Second, the IgE antibodies binding to peptide were tested for their reactivity with *Fel d I*. IgE binding to only three of the synthetic peptides appeared to be specific, to peptides 25-38 and 46-59 of chain 1 and peptide 15-28 of chain 2. These peptides were distinct from the peptides recognized by monoclonal antibodies against native *Fel d I* or R&A *Fel d I*.³¹ Among the nonspecific IgE-binding peptides were the N-terminus and C-terminus of chain 1 and the N-terminus of chain 2.

The specificity of antibody binding to a peptide can be investigated by competitive inhibition experiments with peptide or native antigen.^{18-21, 23, 32} In our inhibition experiments we used three types of inhibitor: native *Fel d I*, R&A *Fel d I*, and peptide. IgE binding to peptides 25-38 and 46-59 of chain 1 and peptide 15-28 of chain 2 was inhibited by all three components. However, R&A *Fel d I* was found to be a less potent inhibitor than native *Fel d I*; the concentration of R&A *Fel d I* needed for 50% inhibition of IgE binding was 30 to 100 times higher than the concentration of native *Fel d I*. This inhibition is probably due to residual activity of native *Fel d I* after reduction and alkylation. This may seem unexpected because of the more linear configuration of R&A *Fel d I*. However, the results clearly indicate that the IgE antibodies reactive with peptide are in fact antibodies to native *Fel d I* and thus more strongly reactive with native than with unfolded *Fel d I*.

In the inhibition experiments no cross-inhibition between the peptides was found. The concentration of native *Fel d I* required for 50% inhibition of IgE binding was 0.02 to 1.6 pmol per test for the different peptides. The concentration of peptide required for 50% inhibition was much higher (ratio 10^5 to 3×10^6). Together with the finding that large amounts of peptide were needed to bind any IgE in the peptide RAST (although this is an overestimation because part of the peptide will not be accessible), this implies that the avidity of IgE antibodies for peptide is lower than that for native *Fel d I*. Indeed, comparing the dissociation rate of IgE from

Fel d I-Sepharose and peptide-Sepharose showed that IgE binding to a *Fel d* I peptide is relatively weak. Scatchard analysis of 17 *Fel d* I RAST-positive sera showed that association constant for native *Fel d* I = 4.4×10^9 to 5.3×10^{10} mol/L⁻¹ (unpublished results). This implies that association constant for *Fel d* I peptide can range from 1.5×10^3 to 5.3×10^5 mol/L⁻¹. This "poor fit" between IgE and the peptides again indicates that the peptides do not properly represent the native structures reacting with antibodies, as described by Melen et al. ³³ Detection of these low-affinity bindings in our studies was presumably possible because of the high ligand densities on the peptide-Sepharose.

More evidence for the specificity of IgE binding to peptides 25-38 and 46-59 of chain 1 and peptide 15-28 of chain 2 was obtained by testing panels of cat dander RAST-positive and RAST-negative sera on peptide-Sepharose preparations. Each peptide was recognized by only 1 of the 78 RAST-negative sera tested (for each peptide a different serum sample was used). The IgE binding from these exceptional "false-positive" sera was only inhibited by the corresponding peptide and not by native or R&A *Fel d* I (data not shown). Of the RAST-positive sera, 46% showed IgE binding to peptide 25-38, 11% to peptide 46-59, and 28% to peptide 15-28. No obvious pattern of reactivity was found. Also, 46% of the RAST-positive sera showed IgE binding to peptide 78-92 of chain 2, whereas none of the RAST-negative sera recognized the peptide. Moreover, the IgE antibodies binding to peptide 78-92 did not bind to native or R&A *Fel d* I, as was demonstrated by the negative results of the inhibition studies and affinity chromatography experiments with peptide-Sepharose. Assuming that IgE binding to this peptide is specific for patients with cat allergy, the antibodies may be directed against a C-terminal fragment of *Fel d* I, which is not exposed in the native molecule and not present in cat extract. In any case, we are cautious in classifying this peptide to the IgE epitopes of *Fel d* I, particularly because the peptide showed a poor high-performance liquid chromatography profile.

The third study we performed to confirm the specificity of IgE binding comprised affinity chromatography experiments with peptide-Sepharose. The eluates of peptide 25-38 Sepharose, peptide 46-59 Sepharose, and peptide 15-28 Sepharose showed considerable anti-*Fel d* I activity, whereas the anti-mite activity of the sera was eliminated during the isolation procedure.

Of the RAST-positive sera tested, 65% bound to one or more of the three peptides. This percentage is clearly still too low to use the peptides for diagnostic purposes. Moreover, only a small part of the IgE antibodies against *Fel d* I was reactive with the peptides. Besides, the overall response would be lower, because only sera with a RAST score of $\geq 4+$ were used.

From our study we conclude that with the use of synthetic peptides, IgE epitopes can be detected on an allergen, despite the sensitivity of the allergen to reduction and alkylation. Although the overall IgE response to the peptides was too low to use the peptides for diagnostic purposes and the avidity of the antibodies for the peptides was low, the peptides are suitable for analyzing individual variability in the immune response of patients with cat allergy, for comparing IgE responses with IgE responses, and for studying the relationship to the T-cell epitopes on this molecule.

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