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IgE and IgG4 Binding to Synthetic Peptides of the Cat (*Felis domesticus*) Major Allergen *Fel d I*

Key Words

Cat
IgE antibody
IgG4 antibody
Peptide
Epitope

Abstract

Specificities of IgE and IgG4 antibodies in 12 cat-allergic patients were compared with respect to their reactivity towards 3 IgE-binding synthetic peptides of *Felis domesticus* allergen 1 (*Fel d I*): peptides 25–38 and 46–59 of chain 1 and peptide 15–28 of chain 2. Peptides were coupled to Sepharose and anti-*Fel d I* antibodies were isolated by affinity chromatography. *Fel d I*-specific IgE- and IgG4 antibody activity in the peptide eluates was measured using *Fel d I* binding assays. *Fel d I*-specific IgE/IgG4 ratios in the eluates from peptide-Sepharose were determined and compared with the IgE/IgG4 ratios in the eluates from *Fel d I*-Sepharose. The mean ratio *Fel d I*-specific IgE/IgG4 in the peptide eluates (0.84; range 0.06–4.6) was significantly higher than the mean ratio in the eluates from *Fel d I*-Sepharose (0.31; range 0.13–1.1), demonstrating a higher reactivity of IgE antibodies with the peptides, compared to IgG4. These results indicate differences between the B cells producing IgE antibodies and the B cells producing IgG4 antibodies.

Introduction

In vitro the switch towards IgE expression (murine and human) depends on the presence of IL-4 [1–6], produced by the T_{H2} subset of CD4+ T cells [7, 8] and by mast cells [9]. This IL-4-induced IgE synthesis can be suppressed by interferon-gamma (IFN- γ) [4–6, 10], produced by the T_{HI} subset of CD4+ T cells and natural killer cells. In a simplified model for the development of allergy, the balance between the concentrations of IL-4 and IFN- γ will determine the induction of an allergic response or a 'normal' IgG response. However, the development of an atopic

response appears to be more complicated. In atopic patients usually also antigen-specific antibodies of other, less IL-4-dependent isotypes are markedly increased, like IgG4, IgG1 and IgA [11–14], pointing to a more generally upregulated immune response. The production of IgG4 also depends on the presence of IL-4 [1, 2], but IgG4 is certainly not specific for allergic patients. Moreover, under normal conditions the IgE response is suppressed very efficiently [15–18], suggesting that IgE is only produced under special conditions.

If the same B cells produce either IgE or IgG, dependent on the environment in which contact between Ag-

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1018–2438/94/1033–0274
\$5.00/0

presenting cells, T cells and B cells takes place (balance between IL-4 and IFN- γ), the IgE and IgG antibodies found in allergic patients will have the same specificity for the allergen. However, if a separate regulation mechanism is involved in the IgE response, different B cells will produce IgE and IgG antibodies, causing differences between specificities. In this study specificities of IgE and IgG4 for the major allergen of the cat, *Felis domesticus* allergen 1 (*Fel d I*) are studied. IgG4 was selected for this study because the regulation of IgG4 antibodies shows several similarities with that of IgE. *Fel d I* is a 37-kD homodimer of which each monomer consists of 2 polypeptide chains. On an SDS-polyacrylamide gel of reduced *Fel d I* the apparent molecular weights of chain 1 and chain 2 are 5 and 13 kD, respectively [19, 20]. Amino acid sequence analysis and cDNA cloning *Fel d I* revealed that chain 1 is composed of 70 amino acid residues and chain 2 of 92 residues [21]. Each chain contains 3 cysteine residues and preliminary analysis indicates that chains 1 and 2 are connected by disulfide bonds in an anti-parallel topology. Despite the sensitivity of the allergen to reduction and alkylation [19, 20, 22, 23], both chains bind human IgE [19, 24, 25]. Moreover, at least 3 synthetic peptides of 14 amino acid residues length were found that bind human IgE antibodies: peptides 25–38 and 46–59 of chain 1 and peptide 15–28 of chain 2 [26].

In this study reactivity of IgE and IgG4 antibodies with these 3 synthetic peptides of *Fel d I* were compared. Using radioallergosorbent test (RAST) experiments high non-specific binding of IgG4 to the peptides was found and therefore a more specific procedure was performed to compare IgE and IgG4 binding. By affinity-chromatography on peptide-Sepharose *Fel d I*-specific antibodies were isolated. *Fel d I*-specific IgE and IgG4 antibodies in the peptide eluates were measured by antigen binding experiments with monoclonal anti-IgE and anti-IgG4 Sepharose, respectively, and radiolabelled, purified *Fel d I*. Specificity of this procedure was confirmed by testing the sera and the peptide eluates for irrelevant antibody activity, IgE antimitic [26]. The ratios IgE/IgG4 in the eluates were determined and compared with the IgE/IgG4 ratios in the sera after affinity purification on *Fel d I*-Sepharose.

Materials and Methods

Peptides

Peptides 25–38 and 46–59 of chain 1 and peptide 15–28 of chain 2 [26] were synthesized by the T-bag method of Houghten [27–29], adapted for Fmoc chemistry [30]. The peptides were synthesized with an additional lysine residue at the C-terminus in order to facilitated

coupling to Sepharose. On reversed-phase HPLC (Delta Pak C18, gradient from 0.1% TFA in water to 0.1% TFA in acetonitrile, UV detection at 214 nm) the peptides showed one main peak, comprising 57–74% of the total profile.

Preparation Solid-Phase Antigen

Eight milligrams of peptide and 125 U (250 μ g) of affinity purified *Fel d I* from house dust [31], respectively, were coupled to 500 mg of CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's procedure. Remaining binding sites were blocked with 0.5 M glycine, pH 8.5, during 2 h. After an extensive washing procedure, the Sepharose was suspended in 500 ml of PBS containing 10 mM EDTA, 0.1% Tween-20, 0.3% BSA and 0.05% NaN₃ (PBS-AT). Excess of monoclonal antihuman IgE (MH25-01, CLB, Amsterdam, The Netherlands) and of monoclonal antihuman IgG4 (M1278, CLB), 5 and 3 mg, respectively, was coupled to 300 mg of Sepharose. After the coupling procedure, the Sepharose was suspended in 150 ml of PBS-AT.

Patient Sera

Twelve cat-allergic patients, with high levels of IgE (RAST score $\geq 4+$) and IgG4 antibodies against *Fel d I*, were selected for this study. In these patients binding of IgE antibodies to at least 1 of the 3 synthetic peptides was found. The patients did not receive immunotherapy, except for 1 patient who received cat immunotherapy more than 10 years ago. Units of *Fel d I*-specific IgE and IgG4 were calculated in RAST experiments.

IgG4 Peptide RAST

Serial dilutions of serum were incubated overnight with 0.5 ml of peptide-Sepharose. After an extensive washing procedure (7 steps) with PBS containing 0.1% Tween-20, 160 ng of anti-IgG4 biotin were added and 0.5 ml of PBS-AT. After an incubation of 6 h the Sepharose was washed, 1 ng of ¹²⁵I-streptavidin and 0.5 ml of PBS-AT were added and an incubation overnight followed. In negative control experiments peptide-Sepharose of a nonrelevant peptide, GVTHDQLNNFRAGFD from the non-biting midge allergen *Chironomus thummi thummi* (*Chi t I*) [32], was used.

Isolation of Anti-*Fel d I* Antibodies by Affinity-Chromatography with Peptide-Sepharose and *Fel d I*-Sepharose

For the isolation of anti-*Fel d I* antibodies from peptide-Sepharose and *Fel d I*-Sepharose we added 300 μ l of 0.5 M EDTA and 150 μ l of 10% Tween-20 to 15 ml of serum. Ten milliliters of this serum was incubated with 150 mg of peptide-Sepharose for 4 h. Five milliliters was incubated with 50 mg of *Fel d I*-Sepharose. Elution of anti-*Fel d I* antibodies with glycine-BSA buffer (0.1 M glycine, 0.2 mg/ml BSA, adjusted with HCl to pH 2.5) was performed as reported previously [26]. Specificity of this procedure was confirmed by testing the sera and the peptide eluates for irrelevant antibody activity, IgE antimitic. Antibody yield after affinity-purification on *Fel d I*-Sepharose ranged from 6 to 17%.

IgE/IgG4 Ratios in Eluates from Peptide-Sepharose and *Fel d I*-Sepharose

To measure the IgE- and IgG4 anti-*Fel d I* activities in the eluates from peptide-Sepharose and *Fel d I*-Sepharose, antigen binding assays with ¹²⁵I-*Fel d I* were performed. Two hundred and fifty microliters of mAb anti-IgE and mAb anti-IgG4 Sepharose, respectively, were incubated overnight with serial dilutions of eluate. After washing of the Sepharose with PBS containing 0.1% Tween-20, 0.1 U of

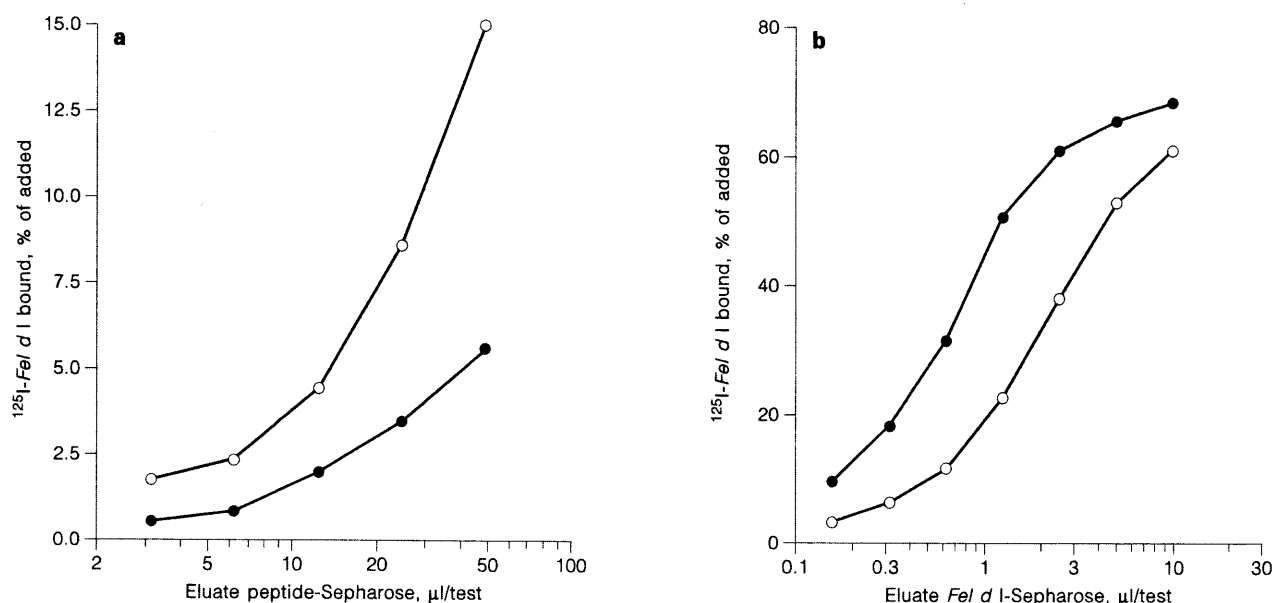


Fig. 1. *Fel d I* binding assays of eluates from *Fel d I*-Sepharose (a) and peptide 15–28 (chain 2)-Sepharose (b). Serial dilutions of eluates were incubated with mAb anti-IgE Sepharose (○) and mAb anti-IgG4 Sepharose (●), respectively. *Fel d I*-specific antibody bound to the Sepharose was detected with ^{125}I -*Fel d I*.

^{125}I -*Fel d I* (4630 Bq/U) and 500 μl of PBS-AT were added and another incubation overnight followed. From the binding curves found (fig. 1), the ratios for *Fel d I*-specific IgG4/IgE were calculated. In negative control experiments eluates from a nonrelevant peptide-Sepharose, GVTHDQLNNFRAGEFD from the non-biting midge allergen *Chi t I* [32], were similarly tested for *Fel d I* binding.

Results and Discussion

In this report, reactivity of IgE and IgG4 antibodies, of 12 cat-allergic patients, with 3 synthetic peptides of *Fel d I* was studied: peptides 25–38 and 46–59 of chain 1 and peptide 15–28 of chain 2. IgG4 binding to the peptides was first studied in RAST experiments. IgG4 binding to the control peptide was high, binding of ^{125}I -streptavidin (% of added) ranged from 1.0 to 16.7%, and for only 1 patient did IgG4 binding to the *Fel d I* peptide exceed this background value. Therefore, a more specific procedure was performed to compare IgE and IgG4 binding to the peptides. Sera of cat-allergic patients were incubated with peptide-Sepharose and anti-peptide antibodies were isolated by affinity-chromatography. In antigen-binding experiments with mAb anti-isotype-Sepharose and ^{125}I -*Fel d I*, IgE and IgG4 activity anti-*Fel d I* in the eluates was measured. The ratios *Fel d I*-specific IgE/IgG4 in the eluates from peptide-Sepharose

pharose were determined and compared with the IgE/IgG4 ratios in the eluates from *Fel d I*-Sepharose.

To exclude preferential loss in activity of one of the isotypes during the isolation procedure, the ratio *Fel d I*-specific IgE/IgG4 after affinity-chromatography on *Fel d I*-Sepharose in every patient was compared with the ratio in serum. The mean ratio of *Fel* specific IgE/IgG4 antibody in the sera was 0.31 (range 0.07–2.0). After affinity purification on *Fel d I*-Sepharose, no statistically significant differences were found between the IgE/IgG4 ratios (fig. 2; paired, two-tailed Student's *t* test). Nevertheless, IgE/IgG4 ratios in the eluates from *Fel d I*-Sepharose were used as reference value.

Six sera were affinity purified on peptide 25–38 (chain 1)-Sepharose, 3 sera on peptide 46–59 (chain 1)-Sepharose and 3 sera on peptide 15–28 (chain 2)-Sepharose. For each patient the IgE and IgG4 activity and *Fel d I* of the peptide eluate was compared with the activity of the *Fel d I*-eluate (table 1). In 10/12 patients the relative activity of IgE in the peptide-eluate was higher than the IgG4 activity. The ratios *Fel d I*-specific IgE/IgG4 in the eluates from *Fel d I*-Sepharose and peptide-Sepharose are indicated in figure 3. The mean value of this ratio was 0.31 (range 0.13–1.11) and 0.84 (range 0.06–4.6) for the *Fel d I*-eluate and peptide-eluate, respectively. The IgE/IgG4 ratios in the

Table 1. Potency of peptide eluates compared with *Fel d* I-eluates

Patient No.	Serum <i>Fel d</i> I-specific U/ml		<i>Fel d</i> I-eluate 10% binding μ l		Peptide	Ratio peptide-eluate/ <i>Fel d</i> I-eluate, %	
	IgE	IgG ₄	IgE	IgG ₄		IgE	IgG ₄
6	8	171	0.5	0.06	25-38 (ch1)	1.6	6.0
12	39	57	14.0	3.8	25-38 (ch1)	1.6	0.16
15	50	198	45.0	8.4	25-38 (ch1)	3.8	1.6
16	55	351	24.2	9.7	25-38 (ch1)	2.5	1.4
20	112	155	6.0	7.0	25-38 (ch1)	39.2	2.5
22	191	2000	1.4	0.7	25-38 (ch1)	0.17	1.6
11	39	221	12.5	3.4	46-59 (ch1)	0.4	0.2
13	48	91	7.0	2.3	46-59 (ch1)	27.0	3.1
23	213	287	0.34	0.09	46-59 (ch1)	0.5	0.2
7	23	351	1.9	1.3	15-28 (ch2)	1.6	1.2
17	98	47	3.6	0.54	15-28 (ch2)	12.5	2.4
18	98	368	0.53	0.17	15-28 (ch2)	1.8	0.2

Of 12 patients Units of *Fel d* I-specific IgE and IgG₄ in serum are indicated in column 2. Potencies of the *Fel d* I eluates were compared by calculating the amount of serum required for 10% binding of radiolabelled *Fel d* I (% of added) in IgE and IgG₄ Ag binding assays (column 3). The eluates from peptide- and *Fel d* I-Sepharose were tested for IgE- and IgG₄ activity anti-*Fel d* I in antigen binding tests. For each patient activity of the peptide eluate was compared with activity of *Fel d* I-eluate and expressed as percentage activity of *Fel d* I-eluate (column 5).

eluates from peptide-Sepharose were statistically significantly higher than the ratios in the eluates from *Fel d* I-Sepharose (fig. 3, $p = 0.01$; paired, two-tailed Student's t test). The same was found when ratios in eluates from peptide-Sepharose were compared with the ratios in serum (fig. 4). No IgE- or IgG₄ anti-*Fel d* I activity was found in the eluates from *Chi t* I peptide-Sepharose [data not shown]. For each patient the IgE/IgG₄ ratio in the peptide-eluate was divided by the ratio in the *Fel d* I eluate (fig. 5). The mean value of this ratio was 2.7.

The higher reactivity towards the peptides of IgE antibodies, compared to IgG₄, suggests that at least part of the B cells producing IgE antibodies are different from B cells producing IgG₄ antibodies. Higher reactivity of IgE with allergen fragments was also found in RAST experiments with recombinant chain 1 and chain 2 of *Fel d* I [25]. These results suggest that a larger fraction of the B cells producing IgE antibodies recognize a partly unfolded or 'processed' form of *Fel d* I, compared to IgG₄-producing B cells. It is not likely that the B cells synthesizing IgE or IgG₄ antibodies went through an extra affinity-maturation step, because in previous studies the avidity of IgG₄ for native *Fel d* I and for recombinant chain 1 and chain 2 was close to that of IgE [25].

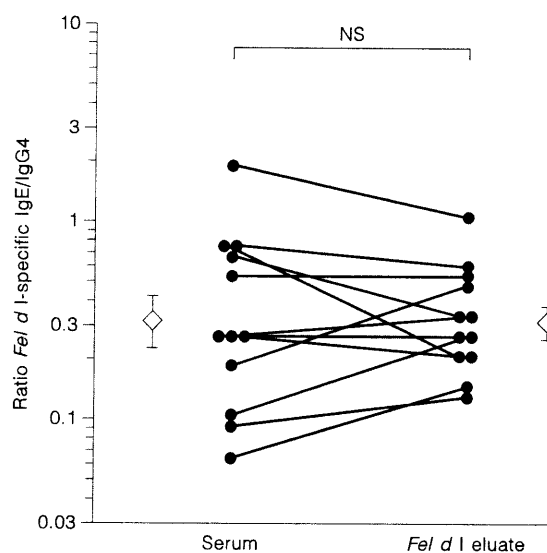


Fig. 2. Ratios *Fel d* I-specific IgE/IgG₄ antibodies in sera of 12 cat-allergic patients and in the eluates from *Fel d* I-Sepharose.

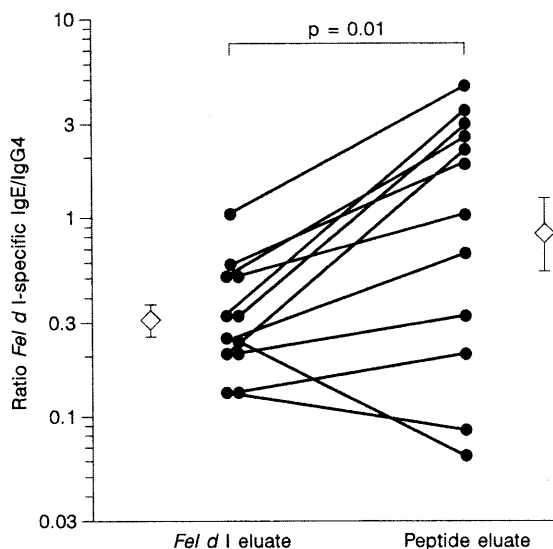


Fig. 3. Ratios *Fel d I*-specific IgE/IgG4 antibodies in eluates from *Fel d I*-Sepharose and peptide-Sepharose.

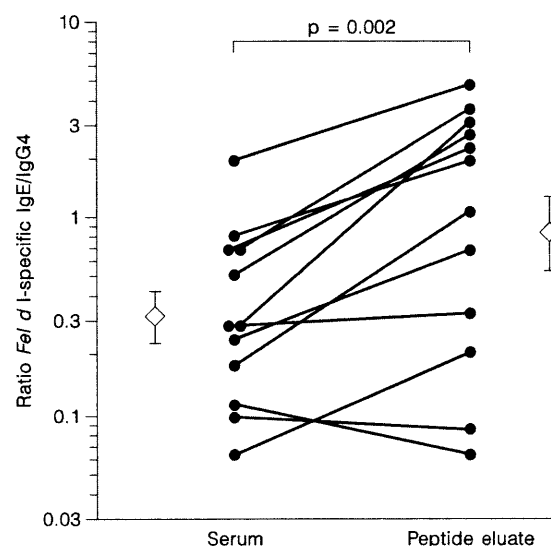


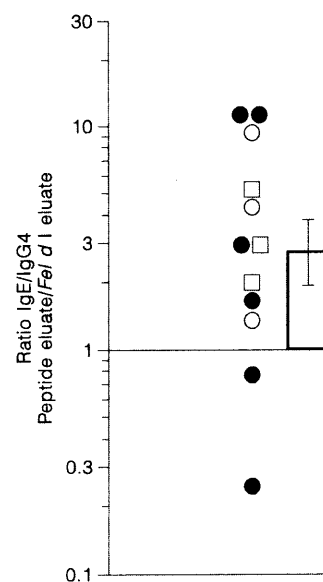
Fig. 4. Ratios *Fel d I*-specific IgE/IgG4 antibodies in sera of cat-allergic patients and in the eluates from peptide-Sepharose.

The difference between B cells producing IgE and B cells producing IgG4 is probably not *absolute*, i.e. epitopes inducing only IgE, others only IgG4. Much more likely this difference is *relative*, i.e. B cells to some epitopes are more likely to switch to IgE than B cells to other epitopes. Our working hypothesis is, that this difference is related to the microenvironment where the B cell encounters the antigen. This microenvironment affects the degree of antigen processing as well as the type of cytokines (and thus the spectrum of immunoglobulin isotypes) induced.

Acknowledgements

We thank Marjan van den Berg for synthesizing the synthetic peptides. This work was supported by a grant from the Netherlands Asthma Foundation (grant No. 87.32).

Fig. 5. *Fel d I*-specific IgE/IgG4 ratios in peptide eluates divided by the ratios in *Fel d I* eluates for peptide 25–38 of chain 1 (●), peptide 46–59 of chain 1 (□) and peptide 15–28 of chain 2 (○). Mean $\pm$ SE.



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