

Differences between specificities of IgE and IgG4 antibodies: studies using recombinant chain 1 and chain 2 of the major cat allergen *Felis domesticus* (*Fel d*) I

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Summary

IgE- and IgG4 antibodies were compared for reactivity with recombinant chain 1 and chain 2 of the cat allergen *Felis domesticus* (*Fel d*) I. Recombinant chain 1 and chain 2 were coupled to sepharose and tested in IgE- and IgG4 radioallergosorbent test (RAST) experiments. Substantial IgE- and IgG4 binding was found. The fraction of *Fel d* I-specific antibody that bound to the recombinant chains was calculated. For chain 1, the mean value of this fraction was 0.30 for IgE and 0.23 for IgG4 ($P = 0.05$). For chain 2, the mean value of this fraction was 0.19 for IgE and 0.13 for IgG4 ($P = 0.02$). These results indicate that differences in fine specificity exist between IgE and IgG4 antibodies. Moreover, these findings support our results with chemically prepared peptides derived from these two chains and suggest that the B cells producing IgE antibodies are more likely to recognize a less 'native' form of *Fel d* I, compared with IgG4.

Clinical and Experimental Allergy, Vol. 25, pp. 247–251. Submitted 7 April 1994; revised 22 August 1994; accepted 5 September 1994.

Introduction

IgG4 antibodies are often found to antigens that are known to be efficient inducers of IgE antibodies. This is found not only with allergenic extracts (for a review on IgG4 in atopy, see [1]), but also with parasite extracts [2]. In the present study we investigated whether this similarity between IgE and IgG4 found at the level of the total antigen is also found at the level of the epitopes on these antigens.

As a model system we used a single, well-characterized allergen, the major allergen of the cat *Felis domesticus* (*Fel d*) I [3–9]. This allergen consists of four chains: a non-covalent dimer of a covalently linked heterodimer: chain 1 (70 amino acid residues) and chain 2 (92 residues). We compared the effect of variation in allergen structure on the reactivity of IgE and IgG4 antibodies to the native

allergen. In studies reported elsewhere, the allergen variants studied were either synthetic peptides derived from the *Fel d* I structure [10] or a phylogenetic variant of *Fel d* I, the homologous protein found in the ocelot [11]. In the paper presented here, we compared the reactivity of IgE and IgG4 antibodies to the native allergen with the reactivity to recombinant chain 1 and chain 2.

Materials and methods

Sera

Twenty-two cat allergic patients (aged 17–56 years) with high levels of IgE and IgG antibodies against the cat allergen *Fel d* I, were selected for this study. The patients did not receive immunotherapy, except for one patient who received cat immunotherapy more than 10 years ago. Seventeen non-cat-allergic individuals with IgG4 antibodies against *Fel d* I were also included. Of this group, 11 persons were known to keep cats in their

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homes. As negative controls, sera obtained from seven non-allergic individuals without detectable IgG to *Fel d I* were used. In each serum IgE and IgG4 against *Fel d I* were determined and expressed as arbitrary units. A serum with known IgE concentration, tested with anti-IgE-sepharose and ^{125}I -anti-human IgE, was used as standard curve. Units IgG4 against *Fel d I* were deduced from the IgE units in an ^{125}I -*Fel d I* binding assay with MoAb anti-human IgE-sepharose and MoAb anti-human IgG4-sepharose respectively, for a single reference serum. Using this serum as a standard in an IgG4 RAST for *Fel d I*, units IgG4 against *Fel d I* were calculated for the other sera.

Monoclonal antibodies

Monoclonal antibody (MoAb) Fd1a (anti-native *Fel d I*) [4] was a protein A purified preparation from mouse ascitic fluid. The concentration of IgG was 1.2 mg/ml, derived from A280. On an sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblot of reduced *Fel d I*, MoAb Fd1a reacts with chain 1 [12]. MoAb FdR-1a (anti-R&A *Fel d I*) was a protein A purified preparation of hybridoma culture supernatant, and reacts with chain 1 in immunoblotting experiments [12]. MoAb FdR-2b (anti-R&A *Fel d I*) was a hybridoma culture supernatant, precipitated with 50% ammonium sulfate. On an SDS-PAGE immunoblot of reduced *Fel d I* this MoAb reacts with chain 2 [12]. The concentration of chain 1-specific IgG of MoAb FdR-1a was 1.35 mg/ml; the concentration of chain 2-specific IgG of MoAb FdR-2b was 0.54 mg/ml. All MoAbs were of the IgG1 subclass.

Allergen sources

Fel d I was affinity purified from house dust with MoAb Fd1a [4]. The genes coding for chain 1 and chain 2 [7] were cloned into the expression vector pET11d [13] and transfected into the *Escherichia coli* strain BL21 (DE3). Fusion proteins were purified by Ni^{2+} metal ion affinity chromatography using their polyhistidine sequence as a purification tag [14]. *Fel d I* chain 2 has two alternative forms, designated long and short [15]. The recombinant (rec) chain 2 examined here corresponds to the long form of 92 amino acid residues [6, 15]. Both recombinant chain 1 and chain 2 have 30 non-relevant residues at the N-terminus that are comprised of a leader sequence, polyhistidine and a thrombin cleavage site [6].

Solid phase antigen

Five hundred micrograms recombinant chain 1 (46

nmole/l) or chain 2 (38 nmole/l), or 125 U (250 μg , 7.1 nmole/l) affinity purified *Fel d I* were coupled to 500 mg CNBr-activated sepharose 4B (Pharmacia), according to the manufacturer's instructions. Remaining binding sites were blocked with 0.5 M glycine pH 8.5 and the Sepharose was suspended in 250 ml of PBS containing 10 mM EDTA, 0.3% BSA, 0.1% Tween-20 and 0.05% NaN₃ (PBS-AT). The Ab-binding capacity [16] of solid-phase Ag was measured by determining the amount of MoAb, specific for *Fel d I* (MoAb Fd1a), chain 1 (MoAb FdR-1a) or chain 2 (MoAb FdR-2b), bound by a low concentration of Ag-sepharose, under saturating conditions.

RIA procedures

Binding of MoAbs against *Fel d I* to chain 1 and chain 2
Serial dilutions of MoAb were incubated overnight with serial dilutions of Ag-sepharose in a final volume of 500 μl . After washing of the sepharose 1.2 ng of ^{125}I -goat anti-mouse IgG and 500 μl of PBS-AT, containing 4.5% normal bovine serum and 0.5% normal sheep serum (PBS-ATS), were added. After another incubation overnight the sepharose was washed and radioactivity bound to the sepharose was measured.

Binding of IgE and IgG4 to chain 1 and chain 2 in a RAST
Fifty microlitres of serum dilution was incubated overnight with 0.5 mg of Ag-sepharose in a final volume of 500 μl . After washing the sepharose, IgE and IgG4 antibodies, bound to the sepharose, were detected using 1 ng of ^{125}I -sheep anti-human IgE (M1294, CLB, Amsterdam, the Netherlands) and 1 ng of ^{125}I -mouse anti-human IgG4 (M1278, CLB), respectively. Non-specific binding of IgE and IgG4 to chain 1 and 2-sepharose was determined in two ways: (i) each serum was tested in an IgE- and IgG4 RAST with glycine-inactivated sepharose to determine non-specific binding of each serum; (ii) seven negative control sera were tested in an IgE- and IgG4 RAST with chain 1- and chain 2-sepharose respectively, and average binding results were taken as measure for non-specific binding to polypeptide-sepharose. The standard procedure was to subtract binding found for control sera to chain-sepharose as background from the binding result found. However, when the glycine-sepharose binding of a patient exceeded this background value, the glycine blank was subtracted instead. Units of IgE and IgG4, binding to chain 1 and chain 2, were calculated using *Fel d I*-sepharose with serum 16 [10] (55 RAST units IgE anti-*Fel d I*, 351 RAST units IgG4 anti-*Fel d I*) as reference serum.

Results

The results of RAST experiments reflect not only the titre of IgE antibodies, but also the amount of allergen available for the test. Because we anticipated a lower avidity of the isolated chains relative to the intact allergen molecule, we used more of the recombinant peptides: 92 and 76 pmole/test for chain 1 and chain 2, respectively, vs 14.2 pmole/test for the intact allergen (an antibody-binding capacity of 1 pmole/test corresponds to 180 ng IgE per test, or 3600 ng/ml serum). As judged by reactivity to a saturating excess of appropriate monoclonal antibodies, the actual available molar antibody-binding capacity of the allergens was substantially lower than the molar amount of allergen that was used for coupling to the solid phase: 1.1% of expected for chain 1, 2.4% for chain 2 and 2.4% for the native

allergen. This means that 1.0 and 1.8 pmole/test of chain 1 and chain 2, respectively, vs 0.34 pmole/test of the intact allergen, was actually available. 'Unavailable' allergen as defined by the procedure used includes allergen that does bind the first antibody (mouse monoclonal antibody or human antibody) but does not bind the labelled anti-immunoglobulin, e.g. for steric reasons.

Percentage of *Fel d* I-specific IgE and IgG4 antibodies binding to chain 1 and chain 2 in a RAST

Figures 1 and 2 show the results of IgE and IgG4 binding to the two recombinant polypeptides; in Fig. 1 the results are presented in RAST units, Fig. 2 as fraction of *Fel d* I-specific IgE and IgG4 antibodies, respectively. For chain 1, the mean value of this fraction was 0.301 ± 0.106 (SD) for IgE and 0.226 ± 0.169 (SD) for IgG4. For chain 2, the mean value of this fraction was 0.187 ± 0.101 (SD) for IgE and 0.131 ± 0.095 (SD) for IgG4. For each patient the difference of this ratio (recombinant polypeptide/native allergen) between IgE- and IgG4 binding was calculated (Fig. 3). The difference for chain 1 was 0.075 (95% confidence interval: 0.001–0.150), for chain 2: 0.056 (95% confidence interval: 0.009–0.103). In terms of statistical probability of a two-tailed Student's *t*-test: IgE was in both cases found to be more reactive than IgG4 with $P = 0.05$ for chain 1 and $P = 0.02$ for chain 2. Chain 1 was more reactive than chain 2, both for IgE (mean relative reactivity 1.73, 95% confidence interval: 1.26–2.39) and for IgG4 (mean relative reactivity 1.60, 95% confidence interval: 0.98–2.64).

The fraction of *Fel d* I-specific IgG4 antibodies in non-

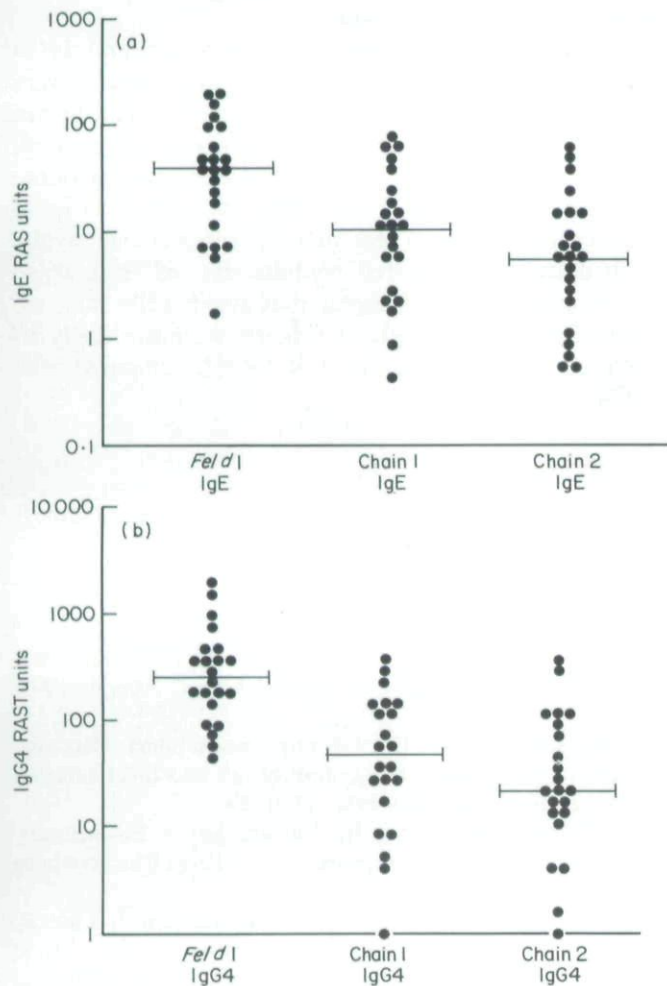


Fig. 1. RAST scores for the native allergen (left), recombinant chain 1 (middle) and recombinant chain 2 (right). (1a) IgE antibodies. (1b) IgG4 antibodies.

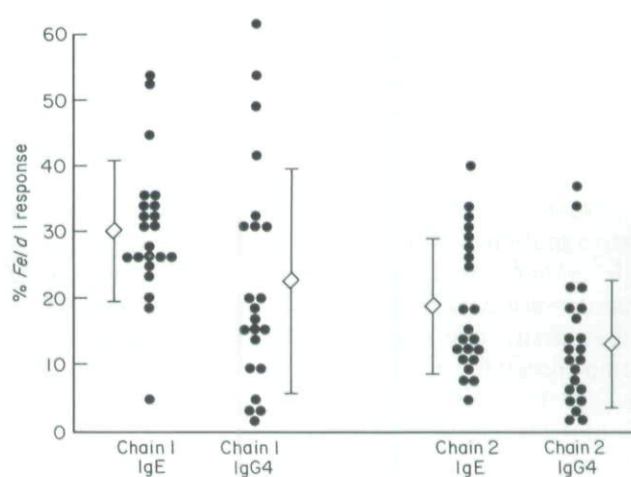


Fig. 2. Percentage of *Fel d* I-specific IgE and IgG4 reacting with chain 1 and chain 2. Mean $\pm$ SD are indicated. For every serum the paired difference (IgE – IgG4) was calculated and plotted in Fig. 3.

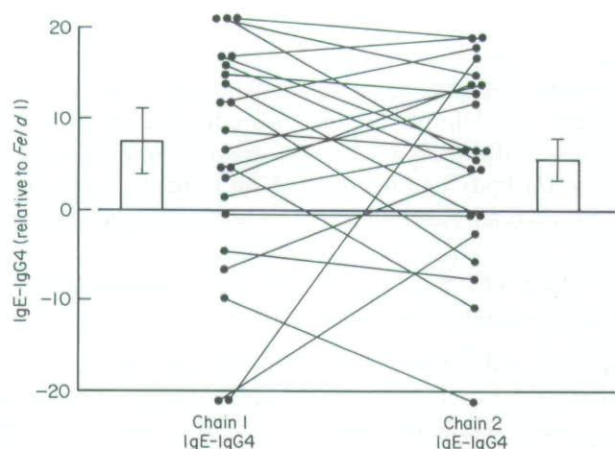


Fig. 3. Differences between chain-specific IgE and IgG4. For each patient the percentage of *Fel d I*-specific IgE reactive with either chain 1 (left) or chain 2 (right) was compared with the percentage of *Fel d I*-specific IgG4 reactive with the respective chains (see Fig. 2). The mean values and standard errors of the means of the differences (IgE minus IgG4) are indicated. The difference for chain 1 was 7.5% (95% confidence interval: 0.1%–15.0%), for chain 2: 5.6% (95% confidence interval: 0.9%–10.3%).

atopics reactive with chain 1 and chain 2 was also calculated and found to be very close to the IgG4 values found for the atopic patients: 0.31 for chain 1 and 0.15 for chain 2 (results not shown).

Discussion

These investigations were not aimed at an evaluation of the potential use of recombinant allergen fragments for serodiagnosis. Our results as well as those presented by Bond *et al.* [9] indicate that the reactivity of the isolated chains is clearly lower than that of the native whole molecule, but that the amount of IgE binding activity (approximately 50% in our hands) found in the isolated recombinant chains is still quite high. This indicates that the isolated chains are to some extent folded as in the native molecule. However, in calculating the amount of IgE binding activity recovered it has to be kept in mind that the reactivity recovered with the isolated chains is of substantially lower avidity. This was to some extent compensated for by using a larger excess of recombinant antigen. Furthermore, it has to be kept in mind that it is presumably not fully correct to add the reactivity of chain 1 to that of chain 2, because a fraction of the antibodies might be reactive with both chain 1 and chain 2. We observed this dual-specificity phenomenon with some monoclonal antibodies. Some epitopes appear to include parts of both chain 1 and chain 2.

In only a few studies a comparison is made between the specificity of human IgE and IgG antibodies. Chemically produced fragments of rye grass pollen extract lost their ability to bind to human IgE (individual patients tested in RAST inhibition experiments) whereas they largely retained their reactivity towards human IgG (serum pool tested; IgG antibodies measured by inhibition of binding of a labelled antigen) [17]. Large peptides expressed from cDNA fragments of a *Dermaphagoides pteronyssinus* (*Der p*) I clone showed a more diverse IgG binding than IgE binding [18]. For ovalbumin variants (e.g. partial denaturation) differences between IgE and IgG profiles were found [19]. For the ragweed allergen Ra3, synthetic peptides recognized by human IgE coincided with those recognized by human IgG, but maximum IgE and IgG binding varied between the peptides [20]. In these studies reactivity of IgG did not match that of IgE. A complicating aspect of some of these studies is, that different assays were used to measure IgE and IgG binding.

In our studies, differences between IgE and IgG4 antibodies for reactivity with chain 1 and chain 2 were found. Striking inter-individual differences were found in the patterns for IgE and IgG4 reactivities. In general, however, the fraction of IgE antibodies reactive with the allergen fragments was higher than the fraction of IgG4 antibodies (Fig. 3). These findings support our results with chemically prepared peptides derived from these two chains [10] and suggest that the B cells that are selected to produce IgE antibodies are more likely to recognize a less 'native' form of *Fel d I*, compared with IgG4.

Acknowledgement

This study was supported by a grant from the Netherlands Asthma Foundation (grant no 87.32).

References

- 1 Aalberse RC, Van Milligen FJ, Van't Hof W, Stapel SO. Allergen-specific IgG4 in atopic disease. *Allergy* 1993; 48:559–69.
- 2 Hussain R, Ottesen EA. IgE responses in human filariasis. IV. Parallel antigen recognition of IgE and IgG4 subclass antibodies. *J Immunol* 1986; 136:1859.
- 3 Leitermann K, Ohman JL. Cat allergen. I. Biochemical, antigenic and allergenic properties. *J Allergy Clin Immunol* 1984; 74:147–53.
- 4 De Groot H, Van Swieten P, Van Leuwen J, Lind P, Aalberse RC. Monoclonal antibodies to the major feline allergen *Fel d I*: I. Serologic and biologic activity of affinity-purified *Fel d I* and of *Fel d I*-depleted extract. *J Allergy Clin Immunol* 1988; 82:778–86.
- 5 Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TAE.

- Monoclonal antibodies to the major feline allergen Fel d I II. Single step affinity purification of Fel d I, N-terminal sequence analysis and development of a sensitive two site immunoassay to assess Fel d I exposure. *J Immunol* 1988; 140:812-8.
- 6 Rogers BL, Morgenstern JP, Garman RD, Bond JF, Kuo M. Recombinant Fel d I: Expression, purification, IgE binding and reaction with cat-allergic human T cells. *Mol Immunol* 1993; 30:559-68.
 - 7 Morgenstern JP, Griffith IJ, Brauer AW et al. Amino acid sequence of Fel d I, the major allergen of the domestic cat: Protein sequence analysis and cDNA cloning. *Proc Natl Acad Sci USA* 1991; 88:9690-4.
 - 8 Kleine-Tebbe J, Kleine-Tebbe A, Jeep S et al. Role of the major allergen (Fel d I) in patients sensitized to cat allergens. *Int. Arch Allergy Immunol* 1993; 100:256-62.
 - 9 Bond JF, Brauer AW, Segal DB et al. Native and recombinant Fel d I as probes into the relationship of allergen structure to human IgE immunoreactivity. *Mol Immunol* 1993; 30:1529-41.
 - 10 Van Milligen FJ, Van't Hof W, Aalberse RC. IgE and IgG4 binding to synthetic peptides of the cat (*Felis domesticus*) major allergen Fel d I. *Int Arch Allergy Immunol* 1994; 103:274-9.
 - 11 Van Milligen FJ, Van Swieten P, Aalberse RC. IgE and IgG4 to phylogenetic variants of the cat (*Felis domesticus*) major allergen Fel d I. *Allergy* (in press).
 - 12 Van Milligen FJ, Van Swieten P, Aalberse RC. Structure of the major cat allergen Fel d I in different allergen sources: An immunoblotting analysis with monoclonal antibodies against denatured Fel d I and human IgE. *Int Arch Allergy Appl Immunol* 1992; 99:63-73.
 - 13 Studier FW, Rosenberg AH, Dunn JJ, Dubendorff JW. Use of T7 RNA polymerase to direct expression of cloned genes. *Enzymology* 1990; 185:60.
 - 14 Hochuli E, Bannwarth W, Dobeli H, Gentz R, Stuber D. Genetic approach to facilitate purification of recombinant protein with a novel chelate absorbent. *Biotechnology* 1988; 6:1325.
 - 15 Griffith IJ, Craig S, Pollock J, Yu X-B, Morgenstern JP, Rogers BL. Expression and genomic structure of the genes encoding Fel d I, the major allergen from the domestic cat. *Gene* 1992; 113:263-8.
 - 16 Van Milligen FJ, Van Etten L, Aalberse RC. Calculation of the affinity constant K_{ASS} for solid phase antigen. A model system using monoclonal antibodies against the cat allergen Fel d I. *J Immunol Methods* 1993; 162: 165-73.
 - 17 Cottam GP, Wheeler AW, Cook RM, Moran DM. Immunological properties of chemically produced fragments of rye grass pollen extract. *Immunol Lett* 1988; 17:345-50.
 - 18 Greene WK, Cyster JG, Chua KY, O'Brien RM, Thomas WR. IgE and IgG binding of peptides expressed from fragments of cDNA encoding the major house dust mite allergen Der p I. *J Immunol* 1991; 147:3768-73.
 - 19 Honma K, Kohno Y, Saito K et al. Specificities of IgE, IgG and IgA antibodies to ovalbumin. Comparison of binding activities to denatured ovalbumin or ovalbumin fragments of IgE antibodies with those of IgG or IgA antibodies. *Int Arch Allergy Immunol* 1994; 103:28-35.
 - 20 Atassi H, Atassi MZ. Antibody recognition of ragweed allergen Ra3: localization of the full profile of the continuous antigenic sites by synthetic overlapping peptides representing the entire protein chain. *Eur J Immunol* 1986; 16:229-35.

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