

Feline immunoglobulin E: induction of antigen-specific antibody in normal cats and levels in spontaneously allergic cats

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Abstract

Sera from 10 cats with symptoms consistent with atopy, from 15 normal household cats and from 11 laboratory maintained cats were assessed for allergen-specific IgE and IgG to *Der-matophagoides farinae* (DF) by enzyme-linked immunosorbent assay (ELISA). In addition, 10 normal cats were immunised with DF and intradermal skin tests (IDST) were performed weekly. Sera from the latter were also assessed for DF-specific IgE by ELISA and using Prausnitz–Küstner (PK) tests. Although DF-specific IgE was detectable in all the atopic cats, there was no significant difference between the levels in this group and in the clinically normal household cats. However levels in both these groups were significantly higher than those in the laboratory maintained cats. Detectable DF-specific IgE was induced in all of the 10 cats, but the levels were not correlated with the development of positive IDSTs, nor with the level of IgE as assessed by PK tests. These findings are consistent with a possible heterogeneity of IgE antibody in cats. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Allergic skin disease is a common reason for the presentation of cats to veterinarians. One important cause is believed to be feline atopy, although an inherited predisposition

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to this disease has not been proved and thus the true designation of atopy in this species remains uncertain. The existence of reaginic antibody in cats has already been shown by passive cutaneous anaphylaxis (PCA) tests and by demonstrating heat or mercapto-ethanol sensitivity (Walton et al., 1968; Powell et al., 1980; Foster et al., 1995). Further evidence was provided by DeBoer et al. (1993) who also demonstrated shared antigenicity between canine immunoglobulin E (IgE) and the feline homologue. Cat bladder tissue was sensitised with sera from a parasitised cat and contraction noted following challenge with monoclonal anti-canine IgE antibody. The development of in vitro tests for the measurement of allergen-specific IgE is thus an important goal as an aid to the practice of feline dermatology.

Although enzyme-linked immunosorbent assays (ELISAs) are marketed for the detection of allergen-specific feline IgE, details supporting the specificity of these antisera have not yet been published in the scientific literature. In a study of one of these assays, Foster and O'Dair (1993) found that the correlation with intradermal skin tests was very poor. Both the specificity and the sensitivity of the ELISA as compared with the intradermal skin test results were low with a positive or negative predictive value of 0.1 and 0.4, respectively.

The previous paper has documented the production of polyclonal antisera that were adjudged specific for feline IgE (Gilbert and Halliwell, 1997). It was important therefore to ensure that the antisera could detect IgE antibodies in the sera of cats with symptoms consistent with atopy and in the sera of normal cats in which allergen specific IgE was induced experimentally under controlled conditions. As house dust mites are the most common allergens involved in atopic disease in dogs and cats in Europe (Foster and O'Dair, 1993; Carlotti and Costargent, 1994; Sture et al., 1995) *Dermatophagoides farinae* (DF) was chosen as the antigen. The kinetics of IgE and IgG responses specific for DF in cats experimentally immunised were followed during the 8 weeks of the study.

2. Materials and methods

2.1. Animals

2.1.1. Clinical study

Serum was collected from 10 cats seen at the Royal (Dick) School of Veterinary Studies (R(D)SVS) at the University of Edinburgh and at the Faculté de Médecine Vétérinaire, Université de Liège in Belgium, in which a diagnosis of atopic disease was made by a combination of compatible clinical signs and positive intradermal skin tests (IDSTs) to one or more environmental allergens. The mean age of the cats was 6.5 years (range 3 to 12 years). Six of the cases were spayed females and the 4 males were castrated. In addition serum was collected from 15 healthy pet cats with no history of skin disease, presented to the Université de Liège for routine procedures (negative control group 1) and from 11 healthy laboratory reared cats (Hill Grove Family Farm, Oxford, UK) which had presumably never been exposed to significant amounts of DF allergens (negative control group 2). The mean ages of control group 1 and group 2 were 4.5 years (range 9 months to 13 years) and 9 years (range 4 to 12 years), respectively.

While all cats from control group 2 were entire females, control group 1 was composed of 7 females (2 spayed) and 8 males of whom 6 were castrated.

2.1.2. Experimental study

Ten healthy adult cats were obtained from Hill Grove Family Farm (Oxford, UK) and were used for immunisation with DF.

Five additional healthy adult cats, also from Hill Grove Family Farm, were used as recipients for Prausnitz–Küstner (PK) tests.

2.1.3. Antiserum production

New Zealand white rabbits, for antibody production, were obtained from Interfauna UK (Cambridge, UK).

All experimental protocols were in accordance with the requirements of the UK Home Office.

2.2. Allergen extracts

Dermatophagoides farinae (DF) extract (1/100 w/v) was purchased from Greer Laboratories (Lenoir, NC, USA).

Atopic cats were skin tested with a range of 52 additional allergens as described in a previous study on canine atopic disease (Sture et al., 1995).

2.3. Induction of DF-specific antibodies in normal cats

Ten parasite free cats were given 3 injections of 50 μ g of DF in aluminium hydroxide (Imject Alum[®]; Pierce, Rockford, USA) at weeks 0, 2 and 5. Five of the 10 cats were also given, at the same time, 50 μ g of *Ascaris* antigen (Asc-1; Greer Laboratories) mixed with the DF antigen in the same adjuvant. All cats were bled prior to immunisation (week 0) and at weekly intervals thereafter for 7 weeks (week 1 to week 7).

2.4. Performance of IDST

Cats were anaesthetised with 1 mg/kg xylazine (Virbaxyl[®], Virbac, Cambridge, UK) and 10 mg/kg ketamine (Vetalar[®], Parke-Davis, Pontypool, Gwent, UK). The lateral chest was clipped and 0.05 ml of 2 fold dilutions of a 1/1000 w/v dilution of DF (experimental cats) or of a 1/10 000 w/v dilution of DF (clinical cases) and of appropriate dilutions of the other allergens were injected intradermally. Phosphate buffered saline (PBS) and a 1/100 000 w/v solution of histamine phosphate (ARTU Biologicals, Lelystad, Netherlands) were used as negative and positive controls, respectively. Reactions were scored after 15 to 20 min on a +1 to +4 scale, incorporating both diameter and elevation above the surface. A +1 reaction was just discernibly greater than the PBS wheal, and a +4 reaction approximated the size of the histamine wheal.

2.5. PK tests

Healthy parasite-free cats were anaesthetised with 10 mg/kg ketamine hydrochloride intramuscularly and the hair of the lateral chest area was clipped. One-tenth ml of doubling dilutions from 1/10 of sera from the experimental cats were injected intradermally. Injection sites were challenged 48 h later by intradermal injection of 0.05 ml of a 1/1000 w/v dilution of DF. The antigen and also PBS were injected into unsensitised sites as negative controls, with a 1/100 000 w/v injection of histamine phosphate as a positive control. Skin reactions were recorded 20 min later using a +1 to +4 scale as in the IDST. For standardisation of responses between PK tests in different recipient cats, a reference serum from a cat which had a strong positive skin test to a high dilution of DF was used in each test.

2.6. Antisera employed

Polyclonal rabbit anti-feline IgE was prepared as described in the previous paper (Gilbert and Halliwell, 1997). The antiserum was passed through an affinity chromatography column containing DF coupled to cyanogen bromide activated Sepharose 4B (Pharmacia, Uppsala, Sweden) to remove possible naturally occurring antibodies with activity against DF.

Goat anti-feline IgG, Fc specific, alkaline phosphatase-conjugated, was obtained from Bethyl Laboratories (Montgomery, AL, US) for the measurement of DF-specific IgG in sera from clinical cases.

Rabbit anti-feline IgG, Fc specific, was purchased from Nordic Immunology (Tilburg, Netherlands) for the detection of DF-specific IgG in sera from experimental cats.

Monoclonal alkaline phosphatase-conjugated anti-rabbit immunoglobulin was purchased from Sigma (Poole, UK).

2.7. Enzyme-linked immunosorbent assay (ELISA)

2.7.1. DF-specific IgE

DF-specific IgE in feline sera was assessed by indirect ELISA. Polystyrene microtitre plates (Immulon 4®, Dynatech Laboratories, Billingshurst, UK) were coated overnight at 4°C with 100 µl/well of 1/5000 W/V dilution of DF extract in 0.1 M sodium carbonate buffer, pH 9.6 (coating buffer). Plates were washed three times with washing buffer which was 0.05% Tween 20 (Sigma) in 0.015 M PBS, pH 7.2 and blocked overnight at 4°C with 0.05% human serum albumin (HSA) (Sigma) in washing buffer (150 µl/well). After further washing, 50 µl of each sample diluted in ELISA buffer (0.05% Tween 20, 0.1% of HSA in 0.015 M PBS, pH 7.2) was added to duplicate wells. After incubation at 37°C for 2 h and further washing, 50 µl of the polyclonal anti-feline IgE diluted in ELISA buffer (1/50) was added in each well. After incubation for 2 h at 37°C, the plates were washed and 50 µl of the alkaline phosphatase-conjugated monoclonal anti-rabbit immunoglobulin diluted in ELISA buffer (1/2000) was added to each well and plates were incubated for a further hour at 37°C. After a last wash, 50 µl

of alkaline phosphatase substrate, namely 5 mg of PNP substrate tablets (Sigma) in 5.5 ml of MgCl_2 in diethanolamine buffer, pH 9.8, was added to each well. Plates were incubated at 37°C for 30 min and the optical density (OD) at 405 nm read using an automated microtitre plate reader after subtraction of the mean blank well values.

2.7.2. DF-specific IgG

In the case of the clinical study, a direct ELISA was used for the detection of DF-specific IgG, whereas in the case of the experimental study, DF-specific IgG was assayed by indirect ELISA as performed for allergen-specific IgE.

Incubation times were reduced from 2 h to 1 h 30 for the serum samples and from 2 h to 1 h for both the polyclonal rabbit anti-feline IgG and the alkaline phosphatase-conjugated goat anti-feline IgG. Incubation time for the monoclonal mouse anti-rabbit immunoglobulin was 1 h. Rabbit and goat anti-feline IgG were used at 1/5000 and 1/100, respectively. Monoclonal mouse anti-rabbit immunoglobulin was used at 1/2000.

2.8. Standardisation of results

A serum containing a high level of allergen-specific IgE or IgG antibody was selected as the standard and assigned a value of 1000 relative antibody units (RAU). Dilutions of the standard were included in each plate, and the results were recorded in RAU by reference to the standard curve.

2.9. Statistical analyses

Non-parametric Mann–Whitney test was used in the clinical study to perform the statistical analysis and to compare the IgE and IgG levels in the atopic cats and the 2 groups of negative control cats. Differences were considered significant at $P < 0.05$.

Correlation between the IgE and IgG antibody levels were calculated using a Pearson's rank coefficient correlation. The results were considered statistically significantly different if the probability (P) was less than 0.05.

3. Results

3.1. Validation of the assay

Allergen-specific IgE and IgG were measured in sera before and after heating to 56°C for 4 h. DF-specific IgE was no longer detected after heating, whereas IgG levels were substantially unaffected (Fig. 1a,b). This supports the isotype specificity of the anti-IgE antiserum.

3.2. Clinical study

3.2.1. IDST results

All the 10 cats showed a skin reactivity to at least one of the house dust mite (HDM) and/or house dust (HD) antigens (Table 1). The most common allergens implicated by

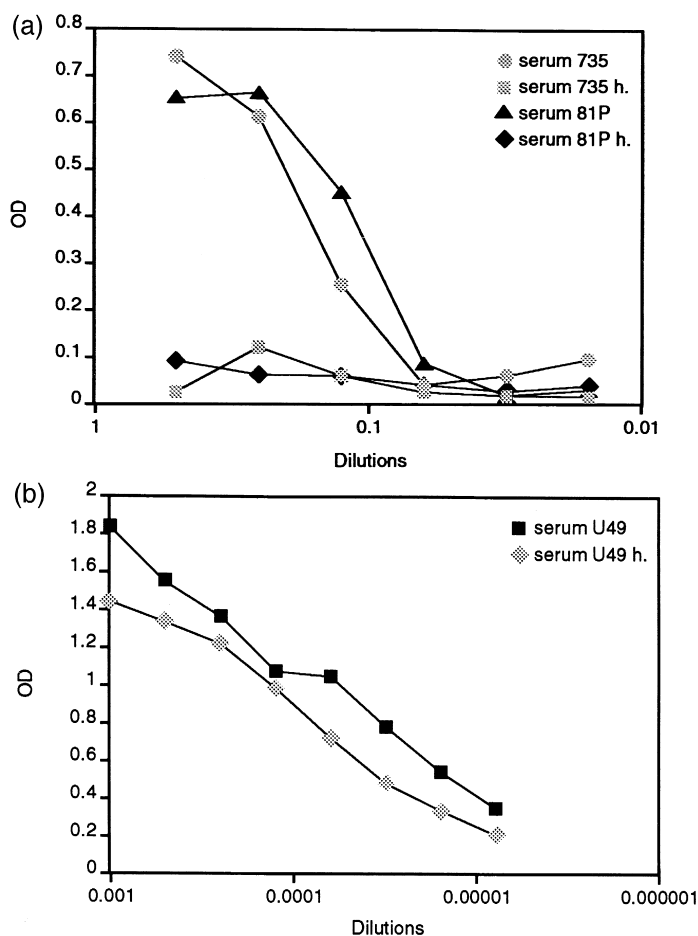


Fig. 1. DF-specific IgE (a, $n = 2$) and IgG (b) in cat sera before and after heating at 56°C for 4 h (h = sera after heating). IgE and IgG levels reported as absorbance at 405 nm.

the IDST were DF and HD which both gave positive reactions in 8 cats (Table 1). Six cats were positive to *Dermatophagoides pteronyssinus* (DP) and four cats were also positive to the flea antigen (Table 1). Other allergens to which positive reactions were seen were *Tyrophagus putrescentiae* ($n = 2$), mixed moth ($n = 1$), cockroach ($n = 1$), white ash ($n = 1$), birch ($n = 1$), american elm ($n = 1$), *Brassica* spp. ($n = 1$), *Chrysanthemum* spp. ($n = 2$), red clover ($n = 2$), Heather ($n = 1$), Velvet/Yorkshire fog ($n = 1$), red top/bent ($n = 1$), and couch ($n = 1$).

3.2.2. ELISA results

Although the median level in the atopic cats of both isotypes was higher than that of control group 1 (457.5 vs. 330 RAU for IgE and 16451 vs. 9860 RAU for IgG) in neither case was the difference significant (Table 2, Fig. 2a and b). However, in the

Table 1

Levels of DF-specific IgE and IgG, and IDST results for DF, DP and HD antigens in the 10 atopic cats

Atopic cats	IDST			DF-specific IgE (RAU)	DF-specific IgG (RAU)
	DF	DP	HD		
166324	+3	+4	+3	953	295793
165767	neg.	neg.	+3	576	15259
166349	+3	neg.	+2	282	1722
166064	neg.	+1	+1	657	31170
161640	+3	neg.	neg.	97	14456
165841	+2	+1	+2	447	1317
166735	+3	neg.	+2	680	5543
8317	+4	neg.	+3	421	26031
640	+2	+2	neg.	327	17644
Noirfa	+3	+3	+2	468	21177

control group 2, the median levels (37 RAU for IgE and 343 RAU for IgG) were significantly lower than in both the atopic cats and control group 1 ($P < 0.05$). The levels of DF-specific IgE and IgG were significantly correlated ($r = 0.629$, $P < 0.01$) (Fig. 3).

3.3. Experimental study

3.3.1. ELISA results

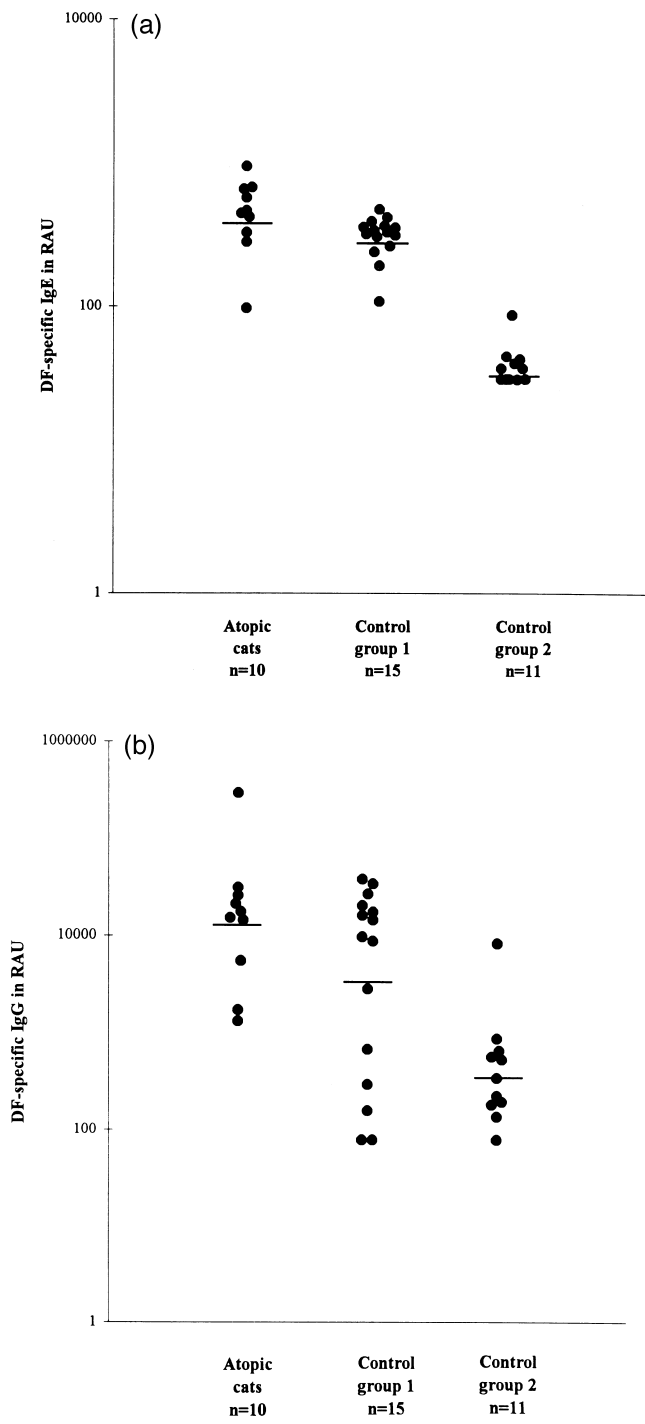
OD values slightly greater than the blanks were detected in some of the preimmunisation sera. These values were unchanged after the sera were passed through an immunoabsorbant column of DF coupled to Sepharose 4B (data not shown) which implied non-specific binding. These OD values were therefore subtracted from the results in each case before expression in RAU following reference to the standard curve.

All cats developed both an IgG and an IgE response to DF following immunisation (Fig. 4a,b). The kinetics appeared different, in that a detectable IgE response appeared earlier, and had fallen by week 5 prior to the third immunisation. In contrast, a detectable IgG antibody response commenced later and rose gradually through the 8

Table 2

Median and range of level of DF-specific IgE and DF-specific IgG in RAU in the atopic, normal household (control group 1) and laboratory maintained cats (control group 2).

	Atopic cats $n = 10$	Control group 1 $n = 15$	Control group 2 $n = 11$
<i>DF-specific IgE</i>			
Median	457.5	330	37
Range	97–953	108–478	31–87
<i>DF-specific IgG</i>			
Median	16451	9860	343
Range	295493–1317	78–38539	78–8367



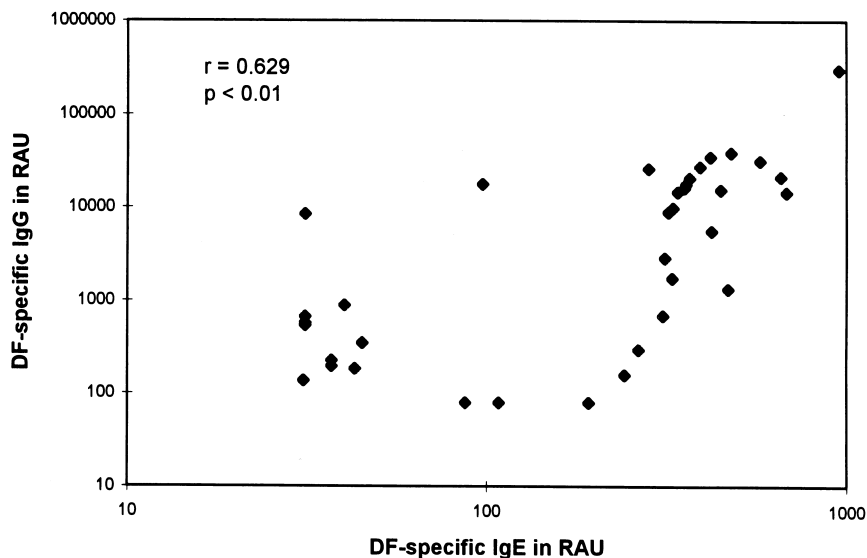


Fig. 3. DF-specific IgE and IgG in RAU in sera from atopic and normal cats. The levels of the two isotypes are significantly correlated ($r = 0.617$, $P < 0.01$).

weeks (Fig. 4a,b). Assessment of both isotypes in all serum samples in each cat did not reveal a significant correlation between them. However, when all the ELISA values for DF were considered together, there was a significant correlation between log DF-specific IgE and IgG antibodies ($r = 0.790$, $P < 0.01$), although some samples had very high IgG and low IgE levels and vice versa (Fig. 5). The 5 cats immunised with DF alone in alum produced responses that did not differ noticeably from those immunised with both DF and Asc-1 in alum.

3.3.2. IDST results

The 10 cats immunised with DF were skin tested weekly commencing immediately prior to the first injection. Assessment of the level of serum DF-specific IgE by ELISA and of the highest dilution of DF antigen to induce a positive skin test following intradermal injection revealed some striking anomalies and no correlation. Firstly, DF-specific IgE was, in general, detectable in sera prior to the time at which the cat showed positive skin tests. Indeed, 4 cats failed to develop positive skin tests during the 8 weeks of the experiment despite developing some of the highest levels of serum allergen-specific IgE. In general, strongly positive skin tests to high dilutions of antigen were always accompanied by high levels of detectable allergen-specific IgE. However the reverse was not the case. Representative examples of the varying responses are shown in Fig. 6a – d).

Fig. 2. The level of DF-specific IgE (a) and DF-specific IgG (b) in RAU in serum from atopic cats, normal pet cats (control group 1), and laboratory reared cats (control group 2). Bar represents the geometric mean for each group.

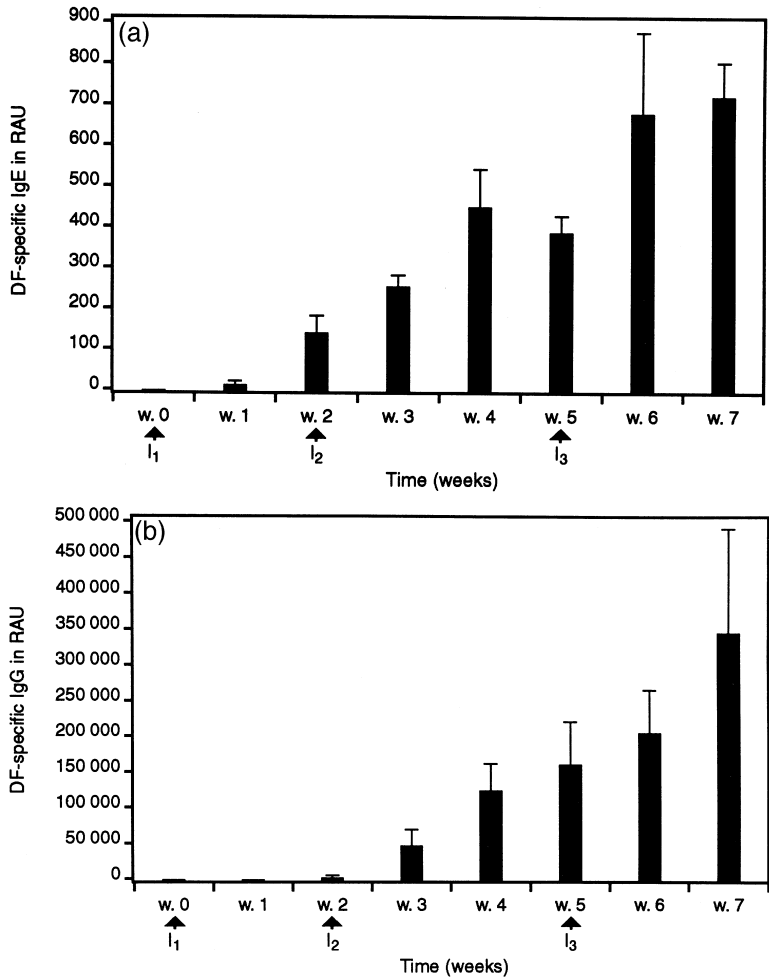


Fig. 4. Mean and standard error of the DF-specific IgE (a) and DF-specific IgG (b) responses in RAU in 10 cats (I₁, I₂, I₃ = immunisation dates).

3.3.3. PK test results

Positive PK tests were only obtained from cats with positive IDSTs and the IDST reactivity tended to parallel the PK titre. Thus the strongest PK test results were in general obtained with postimmunisation sera from cats which had strong positive skin tests to high dilutions of DF (up to 1/320). On the other hand sera from cats which had positive IDSTs only to low dilutions of DF, gave either negative or only weakly positive PK reactions. Furthermore postimmunisation sera from the 4 cats which failed to develop positive skin tests but had high levels of serum allergen-specific IgE as assessed by ELISA showed no PK reactivity. On the other hand, allergen-specific IgE was detected by ELISA in cats which had PK reactivity as well as in cats which had no such

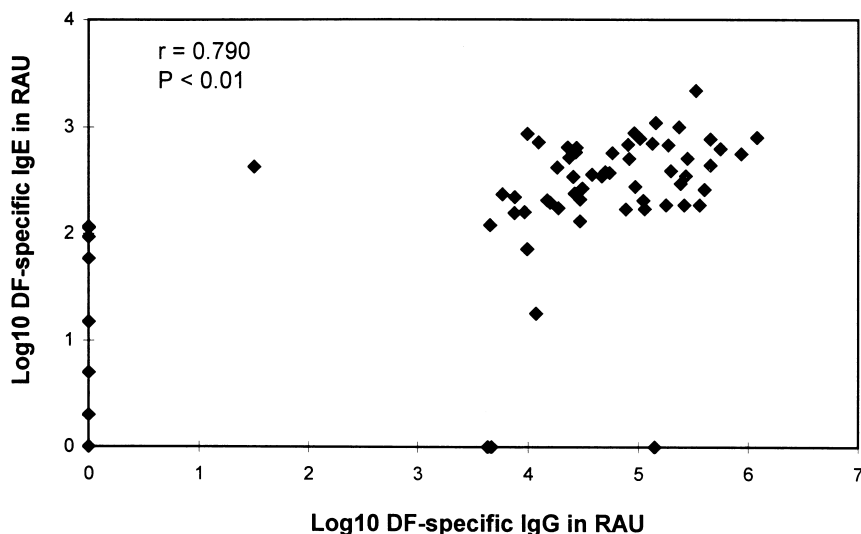


Fig. 5. DF-specific IgE and IgG in RAU in 78 blood samples from cats immunised with DF. The levels of the two isotypes are significantly correlated ($r = 0.790$, $P < 0.01$).

reactivity. In both situations the IgE was heat labile and was not detected by the ELISA after the serum was heated at 56°C for 4 h. Heating the sera also completely abolished the PK reactivity.

4. Discussion

One of the classical characteristic properties of reagenic antibody is its sensitivity to heating (Coca and Cooke, 1923). Ishizaka et al. (1967) observed that reagenic antibody to ragweed failed to precipitate with monospecific antiserum to IgE after exposure to 56°C for 2 to 4 h. Schwartzman and Rockey (1967) demonstrated that canine reagenic antibody is comparable to human reagenic antibody in many characteristics including heat sensitivity. Powell et al. (1980) demonstrated the heat sensitivity of reagenic antibody in cats. Such treatment does not inactivate other classes of antibody and thus allows the differentiation between IgE and other isotypes. In the present study, the complete abolition of detectable allergen-specific IgE in postimmunisation sera heated to 56°C for 4 h demonstrated the specificity of the rabbit anti-feline IgE.

Dermatophagoides farinae is believed to be the most common allergen implicated in feline atopy. Positive reactions to DF have been reported in 77 to 100% of the atopic cats (Carlotti and Prost, 1988; Foster and O'Dair, 1993; Prost, 1996). The results of the IDST in the present study were in agreement with other reports, with 80% of the cats showing positive reactions to DF.

The ELISA has only been introduced recently in the establishment of the diagnosis of atopic disease in cats (Foster and O'Dair, 1993). The antiserum used in the latter assay was raised against a chimaeric feline/murine IgE antibody and there is no published

data supporting its specificity. A comparison of this test with results of IDST, assuming the latter as the gold standard, showed a poor correlation between the two and it was concluded that ELISA was unreliable in establishing a diagnosis of feline atopy. In the present study the IDST also did not always correlate well with the in vitro test. Of the 10 atopic cats, 2 cats were IDST negative to DF but had a high level of DF-specific IgE as assessed by ELISA whereas one had a very low level of DF-specific IgE but showed a strong skin reactivity to DF. Although DF antigen at 1/10 000 dilution is believed to be non-irritant, it is possible that it was close to an irritant concentration in one cat and therefore could have represented a true false positive reaction in this cat. All the normal

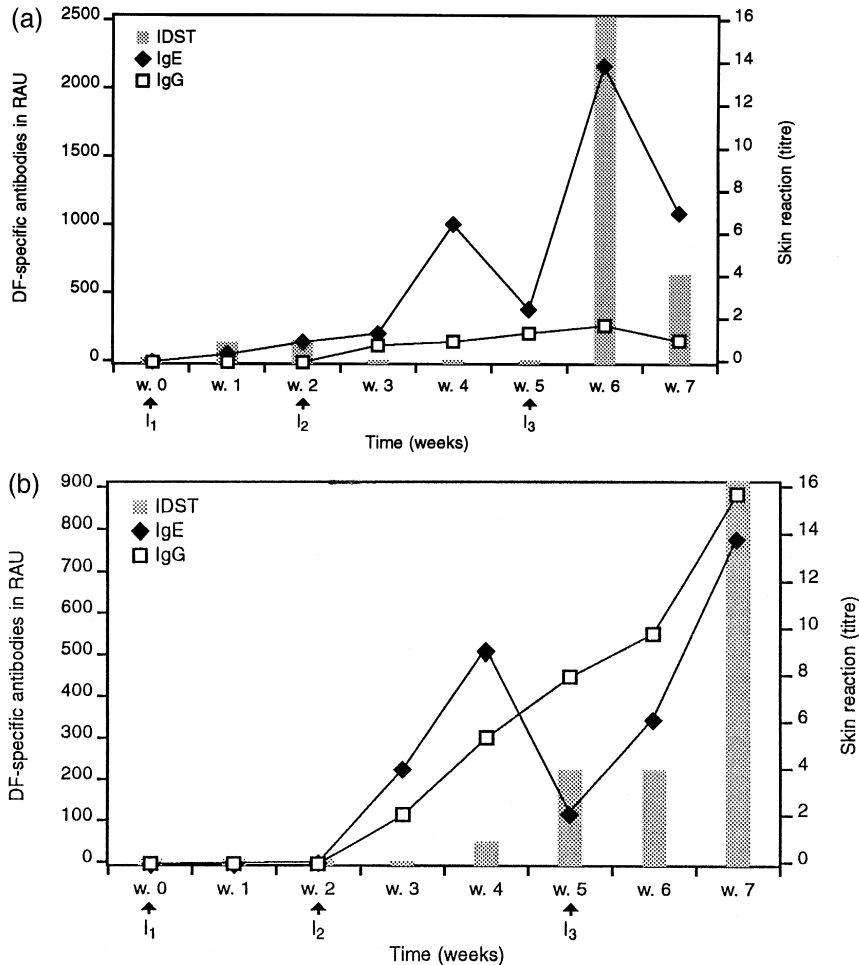


Fig. 6. The relationship between the DF-specific IgE and IgG (in RAU) and the highest dilution of antigen to give positive IDST (skin reaction titre) in 4 representative cats immunised with DF: cats U49 (a), B85 (b), A71 (c) and 735 (d) (I₁, I₂, I₃ = immunisation dates). IgG results are divided by 1000.

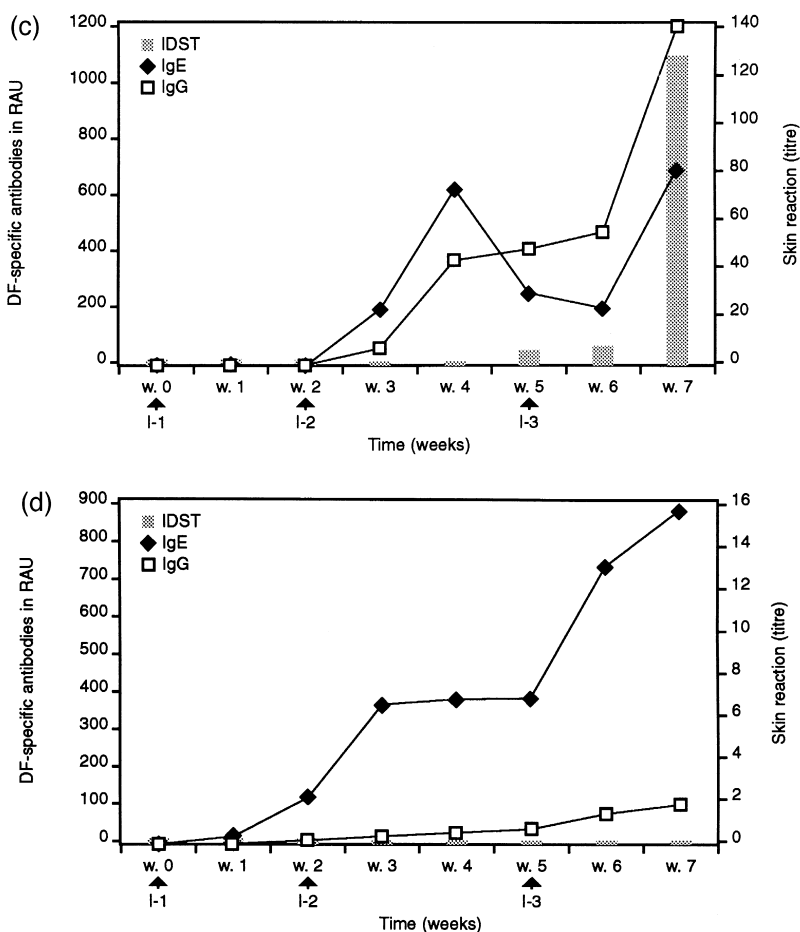


Fig. 6 (continued).

pet cats had detectable levels of DF-specific IgE, and high levels were found in 2 of them. However the median level of DF-specific IgE in sera from these cats was not significantly different from the median of that in the atopic cats. But it should also be emphasised that the normal pet cats were not skin tested and some of the cats may have had a positive IDST to this antigen, as was recently reported in normal dogs (Lian, 1997). On the other hand, it was also shown in the present study that normal cats may have allergen-specific IgE without developing skin test reactivity to this antigen. It was noteworthy that levels of both DF-specific IgE and IgG in the laboratory reared cats were significantly lower than that in both the other groups.

The fact that not only is DF-specific IgE the most frequently implicated allergen in feline atopy, but also is found in normal cats is of great interest. It has been suggested in man that the powerful allergic immune response commonly provoked by HDM could

result from its ability to cleave the low affinity IgE Fc receptor (CD23) from the surface of IgE-secreting B cells (Hewitt et al., 1995). This could ablate an important feedback inhibitory mechanism that normally limits IgE synthesis. Furthermore, fragments of CD23 released by HDM have been reported to promote IgE production (Sutton and Gould, 1993).

Injections of Asc-1 together with DF in 5 parasite free cats failed to stimulate a higher DF-specific IgE than in cats given DF alone. It is well-known that helminths are particularly adept at stimulating IgE synthesis. Helminth infection has the ability to produce an increase in non-parasite-specific IgE (Ogilvie, 1964; Zakroff et al., 1989) and furthermore to potentiate IgE responses to unrelated antigens. They also may disproportionately elevate serum levels of total non-specific IgE (Jarrett and Bazin, 1974; Turner et al., 1979). Halliwell (1972) showed that subcutaneous injections in dogs of *Ascaris suum* antigen alone or coupled to haptens was able not only to stimulate the production of reagin to haptens to which the antigen was coupled but also to any protein with which it was simultaneously administered. However the parasite status of the dogs was not known, although in those days *Toxocara canis* infection in the dogs was almost universal. The apparent disparity in the ability of ascaris antigens to stimulate allergen-specific IgE production in the 2 experiments may thus merely relate to the parasite status.

The DF-specific IgE response commenced early, being detectable 2 weeks after immunisation whereas IgG was essentially undetectable until week 3. Furthermore, the IgE level tended to fall off after the second immunisation whereas the IgG antibody continued to rise. IgE thus appears to be an early antibody in the cat, as has been shown in the rat (Tada, 1975). The decrease in DF-specific IgE after 5 weeks, as contrasted with the IgG response, could, of course merely be reflective of the shorter half-life of the former isotype, rather than an actual lowering of the production relative to IgG. The IgE antibody level depends not only on the dose of the antigen and the adjuvant used but also on the interaction of genetic background and environmental factors (Levine and Vaz, 1970; Vaz et al., 1971). It has been demonstrated that the IgE response to an allergen in mice depends on the strain immunised (Levine and Vaz, 1970). The marked variations of the antibody response seen in these cats are most probably the result of the genetic differences between them as it was suggested for the outbred dogs in the study of Schultz and Halliwell (1985).

The level of specific IgG to some aeroallergens has been shown to be higher in cats with either atopy, food and/or flea allergy than in normal cats or cats with pruritic skin disease but negative IDSTs (Foster et al., 1997). However this was not so in the case of HDM-specific IgG levels which were not significantly greater in allergic cats as compared to normal cats (Foster et al., 1997). In both the clinical and the experimental studies the levels of DF-specific IgE and IgG were significantly correlated. This is not surprising since both isotypes are under the control of a Th-2 type response.

The cats immunised with DF developed no clinical signs of allergy in spite of the strongly positive IDST developed by some of them. It is possible that this resulted from the limited exposure to DF in the laboratory conditions. However, the subcutaneous route used for antigen administration, both for the induction of IgE and for antigen challenge, is different to the natural disease in which the percutaneous route is believed

to be responsible for clinical signs (Halliwell, 1990; Olivry et al., 1996). However it is noteworthy that no species in which IgE was experimentally induced, including man, have shown any clinical signs of allergic disease (Marsh et al., 1972). This emphasises the fact that it is not merely the presence of IgE that distinguishes atopic individuals from normals. It has been suggested that this might be because the reaginic response can be accompanied by high levels of blocking antibodies (Marsh et al., 1972) but another reason could be the heterogeneity of the reaginic antibody response as discussed below. It was also noteworthy that relatively high levels of DF-specific IgE were also found in sera from some normal cats kept in normal household conditions, and presumably exposed to HDM antigen.

In the present study, circulating levels of DF-specific IgE did not correlate with tissue-bound IgE as assessed by IDSTs. In particular, 4 cats with high levels of IgE never developed a positive IDST. Moreover, it was noteworthy that there was a marked lack of correlation between the level of IgE as assessed by ELISA and by PK tests. Also, positive PK tests were never obtained using sera from cats which were IDST negative, although such sera had high levels of IgE as assessed by ELISA. On the other hand, sera from the cats which were only IDST positive to a low dilution of DF showed only a weak PK reactivity whereas sera from cats which had a positive IDST to high dilutions of DF showed much greater reactivity. It is, of course, possible that the cats which developed a high level of DF-specific IgE without a positive IDST may have developed a high level of blocking IgG. However, the level of IgG measured in these cats was not higher than in the cats with a positive IDST to a high dilution of DF who showed strong PK reactivity.

It could also be hypothesised that the negative IDST in the face of the positive ELISAs could be due to a delay in circulating IgE fixing to mast cells. Such a phenomenon is believed to occur clinically in some cases of canine atopic disease in which the positive *in vitro* tests develop weeks or months before a positive IDST. However this possibility could be excluded as the sera from these cats failed to give positive PK tests.

The most rational explanation for this finding would be the existence of heterogeneity of IgE in cats. Theoretically, a number of possibilities exist by which subclasses of IgE could differ. Firstly, the IgE antibody could be heterogeneous in terms of its ability to respond to histamine releasing factors (HRF), which are cytokines derived from macrophages, lymphocytes, mononuclear cells, platelets and vascular endothelial cells. It has been hypothesised that it is not the production of IgE but, rather the ability of the IgE to interact with the HRF which differentiates atopics from non atopic individuals (Lichtenstein, 1988). Thus the type of IgE produced by the cats experimentally immunised with DF and also that found in normal cats, in contrast to that produced by atopic cats, may not be reactive with HRF. In cats which become positive to the IDST, this first IgE antibody response (IgE –) could have been followed by the production (switching) to the second type of IgE antibody (IgE +). The cats which stayed negative to the IDST would never have developed the second IgE isotype and such animals would have positive ELISA results without positive IDST or PK reactivity. However HRF's appear to be involved only in the late phase reaction resulting from the activation of basophils. This reaction is seen in only a subpopulation of allergic individuals

(MacDonald, 1996). Therefore it is unlikely that, in the present study, the interaction of IgE with HRF would explain why some of the cats in which DF-specific IgE was experimentally induced did not develop an immediate response to the IDST.

Secondly, it is possible that the IgE antibody experimentally induced in the normal cats may be less effective at inducing degranulation, as is the case with IgG homocytotropic antibody (Valent and Bettelheim, 1992). It could also be that the IgE experimentally induced in the normal cats may have a low affinity for mast cell receptors and therefore not be able to interact with them in order to sensitise mast cells. In a recent study, Lyczak et al. (1996) demonstrated the existence of 4 human IgE isoforms. They suggested that this could be an additional possible explanation for the functional heterogeneity of IgE and that IgE may differ in its primary protein structure as well as glycosylation. Each isotope may have some unique properties as circulating, cytophilic immunoglobulins, and may differ in their ability to carry out IgE-mediated functions through binding to their different receptors (Lyczak et al., 1996). Interestingly, it has also been shown that the relative levels of mRNAs encoding various isoforms appears to be altered in allergic and parasitic diseases (Diaz-Sanchez et al., 1995). These findings suggest that the existence of more than one type of IgE in cats is not unexpected, and that the IgE antibody experimentally induced in normal cats may be different from the IgE produced in allergic cats. Moreover, it is not impossible that the cats which developed a positive IDST were genetically predetermined to be atopic.

Jackson et al. (1996) studied the leucocyte histamine release in atopic dogs, normal dogs and artificially sensitised dogs and found comparable results to those found in the present study, in that histamine release induced by antigen or anti-IgE was generally found only in atopic dogs. The same concepts could also account for the presence of IgE in normal cats without clinical disease, although, of course, the latter were not skin tested.

In conclusion, the present studies showed that normal cats developed a reagenic antibody response to an environmental allergen (DF) which was readily detected by use of the polyclonal anti-IgE in an ELISA. This antiserum also detected allergen-specific IgE in atopic cats and in normal pet cats kept under normal household conditions, but significantly lower levels were found in laboratory reared cats. In both the experimental and clinical studies there was a significant overall correlation between allergen-specific IgE and IgG responses. However, the level of allergen-specific IgE was not correlated with either skin test or PK reactivity, whereas the positive PK reactivity was generally correlated with positive skin test reactivity. These results are consistent with the existence of a heterogeneity of IgE antibodies in cats.

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