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Measurement of serum immunoglobulin E (IgE) specific for house dust mite antigens in normal cats and cats with allergic skin disease

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Abstract

The purpose of this study was to determine whether cats with allergic skin disease have significant concentrations of serum Immunoglobulin E (IgE) specific for antigens derived from the house dust mites (HDM) *Dermatophagoides farinae* (DF) and *Dermatophagoides pteronyssinus* (DP). Enzyme-linked immunosorbent assays (ELISA) were developed for this purpose. Binding of serum allergen-specific IgE was detected via the use of biotinylated Fc-epsilon receptor alpha chain protein (FcεRIα). Following optimisation of the assay, serum samples from 59 cats with allergic skin disease and 54 clinically normal cats were screened. Results were expressed as ELISA units per ml (EU/ml) compared to a standard curve. Serological findings were correlated with the clinical presentation of affected cats. Cats with symptoms of feline allergic skin disease were grouped as follows: self-induced alopecia without lesions (group 1), papulocrusting dermatitis (group 2), eosinophilic granuloma complex (group 3), papular/ulcerative dermatitis of head and neck/facial dermatitis (group 4), and a combination of symptoms (group 5). Control normal cats comprised the final group (group 6). The Kruskal–Wallis test was used for statistical analysis. There was no significant difference between groups for DF- and DP-specific IgE concentrations with a *p*-value of 0.875 and 0.705, respectively. Although the FcεRIα-based ELISA was able to detect house dust mite-specific feline IgE, the presence of this allergen-specific IgE correlates poorly with the presence of clinical manifestations of allergic skin disease. The results of this study question the clinical relevance of house dust mite-specific IgE in feline allergic skin disease.
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Keywords: Cat; Allergic skin disease; Feline atopy; Immunoglobulin E (IgE); ELISA; Fcε-receptor

1. Introduction

Although feline atopy with cutaneous manifestations was first described 22 years ago (Reedy, 1982), the immunopathogenesis of this disease is still not entirely understood. Whilst atopic dogs show very

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characteristic clinical symptoms (Griffin and DeBoer, 2001), the dermatological lesions of feline atopy are variable and include cutaneous reaction patterns such as miliary dermatitis, self-induced alopecia, eosinophilic granuloma complex and pruritus of the face, neck and pinnae (Scott et al., 2001). Apart from these dermatological symptoms, according to some authors, feline atopy can also present as a respiratory disease (Corcoran et al., 1995) and in this aspect may be similar to human atopic asthma (Padrid et al., 1995).

In cats, as in humans and dogs, an immediate-type I hypersensitivity reaction appears to be involved in the immunopathogenesis of atopic skin disease and the role of aeroallergens and percutaneous allergens has been debated and investigated (Bevier, 1990). Immunoglobulin E (IgE) is the antibody involved in the initiation of immediate allergic responses (Gould et al., 2003). It is tightly bound to Fc-epsilon (Fcε)-receptor on mast cells, basophils and activated eosinophils. Binding of antigen to IgE cross-links these receptors, which triggers cell degranulation and the release of preformed chemical mediators (Janeway et al., 2001).

Intradermal testing is used to confirm a tentative diagnosis of feline atopy, but this test is often difficult to interpret because wheals (when they occur) are softer, non-erythematous, and often disappear more quickly than those observed in dogs. No studies have been performed on the production and regulation of feline IgE, and the clinical relevance of this antibody in allergic skin disease is unknown.

Powell et al. (1980) showed the passive transfer of reaginic antibodies in cats, which was later confirmed by Foster et al. (1995). DeBoer et al. (1993) reported that cats have a reaginic antibody showing cross-reactivity with canine IgE. However, antibodies produced against this molecule showed cross-reactivity with feline IgA and IgM. Gilbert and Halliwell (1998b) described the isolation and development of a polyclonal antibody reactive with putative feline IgE. Using these polyclonal antibodies, they found no significant difference between serum *Dermatophagoides farinae* (DF)-specific IgE concentrations of allergic cats and normal household cats (Gilbert and Halliwell, 1998a). Recently, Norris et al. (2003) also described the production of a polyclonal rabbit anti-feline IgE antiserum after purification of feline IgE from cats with experimentally induced asthma. Although antisera have been used for detection of allergen-specific feline IgE, their specificity is unclear. In some cases these anti-IgE reagents could show suboptimal performance (i.e., false-positive test results) due to significant cross-reactivity with IgG.

An in vitro test using the recombinant human Fc-epsilon receptor alpha (FcεRIα) chain protein as a reagent to detect allergen-specific IgE has been described (Wassom and Grieve, 1998; Stedman et al., 2001) and successfully applied to the detection of canine allergen-specific IgE (McCall et al., 2001; Foster et al., 2003). Although this reagent proved to be useful for the detection of feline IgE, only very limited reports have been published. Initially, the detection of flea allergen-specific IgE was shown (Bevier et al., 1997; McCall et al., 1997) and a later study reported that 75% of allergic cats screened had IgE to both house dust mite (HDM) antigens, DF and *Dermatophagoides pteronyssinus* (DP) (McCall, 2000).

The purpose of the current study was to evaluate the role of house dust mite-specific IgE in cats with allergic skin disease by enzyme-linked immunosorbent assay (ELISA) using the recombinant human FcεRIα-receptor as a detection reagent. An attempt was made to analyse results in relation to the clinical presentation, in order to gain more understanding of the immunopathogenesis of the different cutaneous reaction patterns in feline allergic skin disease.

2. Materials and methods

2.1. Cats with allergic skin disease

For this study excess serum was harvested from blood samples collected for diagnosis from 59 cats with allergic skin disease that had been referred to the School of Clinical Veterinary Science, University of Bristol. These cats ranged in age from 1 to 13 years (average age of 5.1 years). Two cats were of unknown age. This population consisted of 31 neutered female and 23 neutered male animals, 3 entire females and 2 cats where gender was unknown. Domestic short hair cats were the predominant breed ($n = 46$). Other breeds represented were Burmese (4), Persian (3), Bengal (1), Birman (1), British Blue (1), Cornish Rex (1), Maine coon (1), and Siamese (1).

2.2. Normal control cats

Control serum was collected from the excess of blood samples taken from 54 clinically normal cats that were presented for routine health examination at the University of Bristol ($n = 22$) and at a private veterinary practice in Austria ($n = 32$). The average age of these normal cats was 3.1 years with a range of 4 months to 15 years. The age of one cat was not known. There were 11 neutered female, 10 neutered male, 21 entire female and 12 entire male cats. Domestic short hair cats were again the predominant breed ($n = 49$); other breeds included Persian (4) and Burmese (1).

2.3. Clinical groups

Based on the clinical presentation, the cats were divided into the following six groups: self-induced alopecia without lesions (group 1, $n = 22$); papulo-crusting dermatitis (group 2, $n = 7$); eosinophilic granuloma complex (group 3, $n = 7$); papular/ulcerative dermatitis of head and neck/facial dermatitis (group 4, $n = 16$), a combination (group 5, $n = 7$) and a healthy control group (group 6, $n = 54$). Cats in groups 1–5 were investigated for allergic skin disease. Ectoparasites were ruled out by performing skin scrapings and coat brushings. A rigorous flea treatment programme was in place for most cats before referral (insecticidal treatment of the animal, other pets in the home and the environment for 3 months). The presence of microbial or dermatophyte infections was evaluated using standard diagnostic techniques in all cats (Scott et al., 2001). In 10 cats, food allergy as a cause of the skin condition was definitively ruled out by feeding an elimination diet for 8 weeks followed by rechallenge. Three further cats were undergoing a dietary trial at the time of blood sampling, whilst another three cats had commenced, but not finished, a dietary trial. For nine further patients, a dietary trial was suggested at the time of consultation but there was no further feedback on the outcome. No information about a food trial was available for the remaining 34 cats with allergic skin disease, although in these cats compliance with a diet trial, either on behalf of the owner or the cat, would have been poor and so a diet trial was not pursued.

2.4. Allergen-specific IgE ELISA

Preliminary assays using checkerboard titrations were performed to determine optimal dilutions of antigens, sera, and reagents. All incubations were performed within a humid chamber. Between incubation steps, plates were washed four times with an automatic plate-washer (Wellwash Ascent, Labsystems, Finland) with washing buffer (0.05% Tween 20 (polyethylene-sorbitan monolaureate; Sigma Chemical Company, Poole, Dorset, UK) in phosphate buffered saline (PBSTw), pH 7.2). Wells of 96-well polystyrene microtitre plates (Microtiter microplates, ThermoLabsystems) were coated overnight at 4 °C with 100 μl/well of 1 μg/ml DF- and DP-extract (mixed DF- and DP-extracts, 1:100 (w/v); purchased from Greer Laboratories Inc., Lenoir, NC, USA) in coating buffer (0.1 M sodium carbonate buffer, pH 9.6 (Sigma Chemical Company, Poole, Dorset, UK)). BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, USA) was used to determine protein contents of house dust mite (HDM)-extracts.

In the next step, 0.25% bovine serum albumin (BSA; Sigma Chemical Company, Poole, Dorset, UK) in PBSTw was used as blocking buffer (200 μl/well for 1 h at 37 °C). After blocking, 100 μl/well of cat serum was added and each serum sample was titrated using two-fold serial dilutions from 1/10 to 1/160.

Sera from two cats with a clinical diagnosis of allergic skin disease and a positive intradermal test result to both DF- and DP-antigens were pooled for use as a positive control in the assay. Both sera had been commercially tested previously using the FcεRIα receptor reagent (Heska™ Allercept™ Detection System, an ELISA performed at Axiom Veterinary Laboratories, UK) and high HDM-specific IgE titres had been reported. Serial doubling dilutions from 1/5 to 1/320 of this standard serum were included on each plate (Fig. 1). Sera were diluted in 0.25% BSA and 8% PEG (polyethylene glycol 6000; BDH Chemicals Ltd., Poole, England) in PBSTw. Plates were incubated for 2 h at 37 °C before 100 μl/well of biotinylated Fcε-receptor reagent (249 μg/ml; kindly provided by the Heska Corporation, Ft. Collins, CO, USA) was added at a dilution of 1/20,000. Following washing, 100 μl of Avidin linked to alkaline phosphatase (Avidin–AP; Sigma Chemical Company, Poole, Dorset, UK) was added to each well at a dilution of 1/70,000. Fcε-

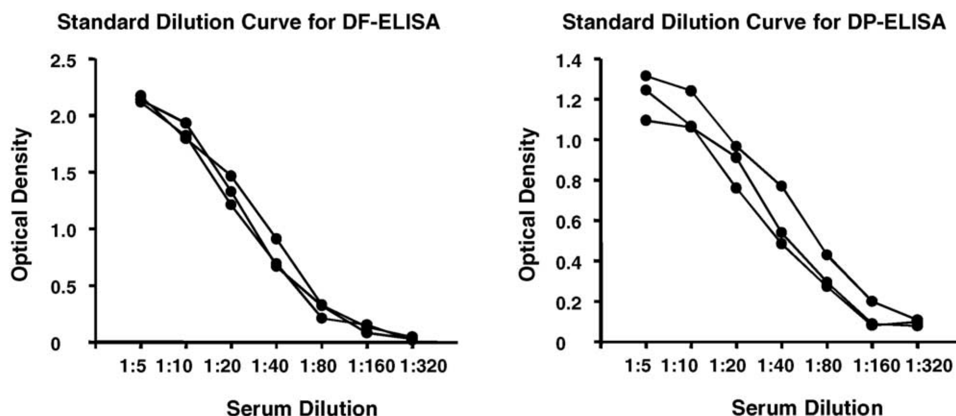

Fig. 1. Standard serial dilutions for DF- and DP-specific ELISA. The standards used in the quantification of IgE concentrations comprised pooled sera from two cats with a clinical diagnosis of allergic skin disease and a positive intradermal test result to both DF- and DP-antigens. The three parallel dilution curves represent standards run on three different plates; DF: *Dermatophagoides farinae*, DP: *Dermatophagoides pteronyssinus*.

receptor reagent and Avidin–AP were diluted in 0.25% BSA in PBSTw and plates incubated with each reagent for 1 h at 37 °C. Finally 100 μl of freshly prepared *p*-nitrophenylphosphate alkaline phosphatase substrate (*p*NPP; Sigma Fast™ *p*-nitrophenyl phosphate tablets in 0.2 M Tris buffer; Sigma Chemical Company, Poole, Dorset, UK) at a concentration of 1 mg/ml was added to all wells.

All plates included negative control wells where antigen, serum and Fcε-receptor reagent, respectively, were absent and highest concentrations of all other reagents or serum were used. Plates were read at 405 and 492 nm using an automated microtitre plate reader (Multiskan Biochromatic plate reader, Labsystems, Finland) after 1 and 2 h or when optical density (OD) values reached more than 1.0 for DF and a minimum of 0.4 for DP.

2.5. *ELISA to test specificity of the Fcε-receptor reagent*

In this direct ELISA, wells were coated overnight at 4 °C with 100 μl of 1 μg/ml purified feline IgG (Jackson Immuno Research Inc., PA, USA) in coating buffer. Blocking, incubation times and washing steps were performed as above. Fcε-receptor reagent and Avidin–AP were added to wells at a dilution of 1/20,000 and 1/10,000, respectively. As a positive control a 1/8000 dilution of alkaline phosphatase conjugated goat anti-cat IgG (Jackson Immuno Research Inc., PA, USA) was added instead. Assay development with *p*NPP–alkaline phosphatase substrate and OD readings were performed, as detailed previously. Plates included negative control wells where feline IgG, Fcε-receptor reagent or conjugated antibodies, respectively, was absent and highest concentrations of all other reagents had been used.

2.6. *Validation of ELISA technique*

To assess the reproducibility of the ELISA technique, three plates containing five repeats of a positive serum sample were run. The intra- and interplate coefficients of variation (CV) were determined.

2.7. *Statistical analysis*

Nonparametric Kruskal–Wallis and Mann–Whitney *U*-test were used to perform the statistical analysis of the data (SPSS 10.1 for Windows). Results were considered significantly different at $p < 0.05$.

2.8. *Data analysis*

Data were analysed in Excel. By plotting $\log_{10}$ OD values against $\log_{10}$ serum dilutions, curves for standard and test sera were generated after the mean value of the OD readings of the three negative controls (background) was subtracted from each sample OD

reading. Sample and standard curves were compared for parallelism and where this was not evident, dilution points on the sample curve were omitted. Results were recorded in ELISA units per ml (EU/ml) by reference to the standard curve. The standard curve was given a value of 100 EU when undiluted.

3. Results

In all six groups, the median concentration of both DF- and DP-specific IgE was 0 EU/ml. The mean concentration of DF-specific IgE in groups 1, 2 and 5 (14.39, 11.87, and 60.01 EU/ml) was higher than that of the control group (8.63 EU/ml), but this was not significant with $p = 0.875$. The mean concentration of DP-specific IgE in groups 1, 3 and 5 (7.74, 9.1 and 58.45 EU/ml) was higher than that of the control group (5.59 EU/ml) but this was not significant with $p = 0.705$ (Table 1 and Fig. 2). The 10 cats, which had undergone a full diet trial with a negative outcome were also assessed separately, but IgE measurements were not significantly different from the healthy control group with $p = 0.938$ for DF and $p = 0.639$ for DP.

Some individual sera (two samples in group 1, one sample in group 4 and six samples in group 6) showed positive OD readings with a titration effect, but because the sample curve was not parallel to the standard curve, these results were not included in the analysis. OD readings of wells coated with purified feline IgG were below 0.08 and were considered negative. The intraplate CV for the IgE-ELISA was 5.3%, while the interplate CV was 4.6%.

4. Discussion

The present study has quantified the concentrations of serum allergen-specific IgE in allergic and clinically normal cats. All of the cats in groups 1–5 fulfilled the majority of the clinical criteria for feline atopic skin disease (Prost, 1996; Scott et al., 2001). Unfortunately food hypersensitivity as a cause for the skin disease could only be totally ruled out in 10 of 59 cats. This reflects the practical difficulties and problems with owner compliance that are commonly encountered when cats are fed an elimination diet. To properly rule out food hypersensitivity, it is currently recommended to feed an exclusion diet (ideally home-cooked) for 9–13 weeks followed by rechallenge while cats are kept indoors only (Scott et al., 2001). Frequently food trials cannot be completed, however, because cats do not accept a change in their diet or find commercially prepared ‘‘hypoallergenic’’ foods unpalatable. As the vast majority of cats in the United Kingdom are kept as indoor/outdoor pets, for most owners (and cats) it is unacceptable that they be kept exclusively indoors for several weeks. The prevalence of feline food hypersensitivity is not clear, but has been variably described as either, uncommon or the third most common hypersensitivity in the cat (reviewed in Scott et al., 2001). On the other hand, atopic cats can have flea bite hypersensitivity and food

Table 1
Immunoglobulin E concentrations in serum from allergic and normal cats

Number of cats per group (n)	DF-specific IgE (EU/ml)		DP-specific IgE (EU/ml)		DF-negative cats per group	DP-negative cats per group
	Median	Mean	Median	Mean		
Group 1 ($n = 22$)	0	14.39	0	7.74	15	17
Group 2 ($n = 7$)	0	11.87	0	0	5	7
Group 3 ($n = 7$)	0	3.58	0	9.10	4	5
Group 4 ($n = 16$)	0	2.91	0	0.98	12	12
Group 5 ($n = 7$)	0	60.61	0	58.45	4	5
Controls ($n = 54$)	0	8.63	0	5.59	38	43

Fifty-nine clinical patients and 54 normal cats were screened by ELISA. Based on the clinical presentation, the cats with allergic skin disease were divided into the following groups: self-induced alopecia without lesions (group 1); papulocrusting dermatitis (group 2); eosinophilic granuloma complex (group 3); papular/ulcerative dermatitis of head and neck/facial dermatitis (group 4); a combination (group 5) and controls (group 6). Results were recorded in ELISA units per ml (EU/ml) by reference to the standard curve.

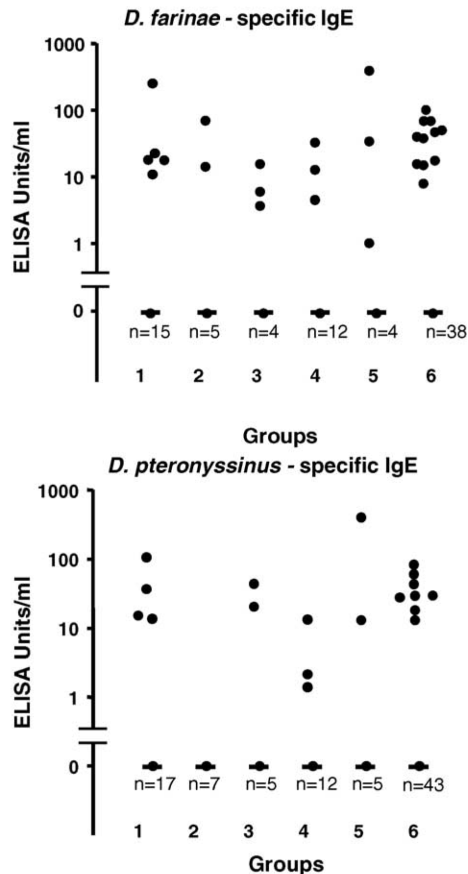

Fig. 2. *Dermatophagoides farinae* (DF) and *Dermatophagoides pteronyssinus* (DP)-specific IgE levels in ELISA units per ml vs. clinical groups (groups 1–5) and healthy control group (group 6). The horizontal black line corresponds to the median value of the group. Given that the majority of samples in all groups were negative, they were not plotted individually. They are represented by one black dot at the level of zero with the actual number of negative samples given below (*n*). No significant difference exists between groups with $p = 0.875$ for DF and $p = 0.705$ for DP.

hypersensitivity concurrently, but the incidence of these concurrent allergies is unknown and a wide variation has been reported (Scott et al., 2001). Taken together, the fact that not all cats underwent a dietary trial was deemed acceptable in the design of this study since it was unlikely to have a major influence on the results.

The majority of these cats had also been intradermally tested (data not shown) but we did not attempt to correlate the intradermal with serological test results. It is well established from canine studies that there is only partial correlation between serological and intradermal tests, but the significance of discrepant results is not completely understood (DeBoer and Hillier, 2001). This observation has proved to be very similar in cats. Foster and O'Dair (1993) evaluated 36 allergic cats with in vitro and in vivo tests and found a poor correlation between these tests. The antibody used in the in vitro test was a polyclonal rabbit anti-feline IgE reagent, which had been raised against a chimeric feline/murine IgE antibody. Gilbert and Halliwell (1998b) found a similar poor correlation with the use of a polyclonal antiserum assuming the intradermal test as the ‘‘gold standard’’. This poor correlation is not entirely surprising as the serological assay measures circulating allergen-specific IgE, while the skin test reflects IgE bound to dermal mast cells via the FcεRIα-receptor. Although shortcomings have been identified (for example, the need for determination of the major allergens, standardisation of allergen extracts and intradermal injection techniques, and criteria-selection for the interpretation of test results), the intradermal test is still used as the so-called ‘‘gold standard’’ to demonstrate allergen-specific hypersensitivity and to confirm the clinical diagnosis of canine atopic dermatitis (Hillier and DeBoer, 2001). In the cat, in addition to those weaknesses, the actual technique of skin testing is more difficult as the thin skin of this species makes accurate intradermal injection more challenging. The influence of a cortisol response due to restraint and anaesthesia, which is necessary for intradermal testing, has been considered a possible reason for the typically weak response observed in cats (Willemse et al., 1993). Nevertheless, some authors still consider intradermal testing as the optimal method for diagnosing atopic conditions in cats (Scott et al., 2001) although published evidence to support this is very limited. One abstract has been presented in which 90 cats with allergic skin disease were evaluated with intradermal allergens, and house dust mite proteins were identified as the dominant allergens (Prost, 1996). Taken together, serological testing could make the diagnosis of feline atopy much easier and less stressful for both the feline patient and the practicing veterinarian.

Published studies using a standardised in vitro test to aid the diagnosis of feline allergic skin disease have been very limited and not particularly promising. At

present, the most widely used in vitro test for canine allergy applies the recombinant truncated version of the human FcεRIα-receptor to detect allergen-specific IgE (Wassom and Grieve, 1998; McCall et al., 2001; Stedman et al., 2001). This assay has also shown the potential to reliably determine feline allergen-specific IgE (McCall et al., 1997; McCall, 2000) and formed the basis of the study reported here.

Previous studies of the serological response in feline allergic skin disease have used a range of different antibodies with serum from small numbers of cats. The present study reports the single largest group of serum samples analysed with a well-validated assay. One aim of this study was to determine whether an association existed between allergen-specific IgE concentrations and the different cutaneous reaction patterns that have been described in feline atopic dermatitis. Overall, most cats with skin disease in our study were negative for HDM-specific IgE and it was impossible to relate antibody titres to clinical presentation in the different groups. This could reflect the fact that IgE tends to be involved in the early stages of the allergic response (Gilbert and Halliwell, 1998a) and might no longer have been present at the time the cats were evaluated. At the point of presentation, animals could be in an advanced stage of the skin disease where the humoral immune response is mainly represented by allergen-specific IgG-levels. It was, however beyond the scope of this study to assess allergen-specific IgG-levels in these sera. In addition, Foster et al. (1997) have shown previously that HDM-specific IgG-levels were not significantly greater in allergic cats compared to normal cats. Although reactions to house dust mites are the most common of non-seasonal allergens, other indoor allergens such as moulds or outdoor allergens including grass, tree, and weed pollens have been associated with skin hypersensitivity in cats (Reedy, 1982; Prost, 1996). As the animals in this study were not serologically tested for prevalence of any other environmental allergens, these cannot be ruled out as a potential cause for the allergic skin reactions. Therefore, although the results of the present study suggest that house dust mite allergens may not be very important in feline allergic skin disease, other allergens might still have a role in the pathogenesis of these disorders.

Although the human FcεRIα-receptor reagent has been successfully utilised to detect allergen-specific IgE in atopic dogs (McCall et al., 2001; Foster et al., 2003), the fact that the median concentrations of serum allergen-specific IgE in all the groups of the present study equalled zero might suggest a lack in sensitivity of the assay. As we do not have an available recombinant feline FcεRα-chain, it is not possible to test whether such a reagent might have greater affinity for feline IgE than the recombinant human α-chain used here. In a recent report, Schleifer and Willemse (2003) suggested that skin test reactivity in cats should be evaluated after intravenous administration of 10% fluorescein solution by means of a Prausnitz–Kuestner test. Similarly, for further validation of the human FcεRIα-receptor reagent in cats, a passive cutaneous anaphylaxis (PCA) test could be performed on normal cats using Evans's Blue dye to visualise wheal development. This would ensure that cats, which showed a negative blood test, were truly negative and in due course could further prove the sensitivity of the reagent used.

It has previously been suggested that allergen-specific IgE might be produced without necessarily being of any clinical significance (Halliwell and DeBoer, 2001). This observation would be consistent with the observation that in our study, some of the 54 healthy control cats had detectable levels of DF- and DP-specific IgE-antibodies. These cats could have been exposed to house dust mite antigens in their environment and mounted an IgE response.

As IgE is only one element of the allergic immune response, further research into antigen-presentation and inflammatory cytokine profiles in feline allergic skin disease will be necessary. Several observations support a cellular mechanism in allergen-induced skin lesions in feline atopic dermatitis. Roosje et al. (1997) showed that lesional skin in allergic cats contained significantly increased numbers of CD1a+ and MHC II+ dendritic cells in the epidermis and dermis compared with the skin of healthy control animals. These authors also found a significant total increase in T-cell numbers in lesional skin of cats with miliary dermatitis (Roosje et al., 1998). In non-lesional skin a significant increase in CD4+ T-cells was found, while in the skin of healthy control cats only one or two CD4+ T-cells and no CD8+ T-cells were found. In another study, significantly more IL-4 positive cells were found in lesional and non-lesional skin in cats with allergic skin disease than in healthy control cats

(Roosje et al., 2002). To further investigate the concept of Th_2 lymphocyte expansion in allergic cat skin, it will be necessary to demonstrate a specific cytokine pattern. The determination of whether cells express a type-2 cytokine pattern (high IL-4, IL-5, IL-6, IL-13; low IL-2, IL-12, IFNγ) may be a better way to understand the pathogenesis of allergic skin disease in cats and its possible similarities with that in humans and dogs.

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