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Research paper

A study for characterization of IgE-mediated cutaneous immediate and late-phase reactions in non-allergic domestic cats

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ABSTRACT

Immunoglobulin-E (IgE) mediated reactions can be induced by intradermal injection of anti-IgE antibodies in both humans and dogs. These reactions grossly and histologically mimic changes seen in naturally occurring allergic dermatitis in these species. Similar studies have not been conducted in the cat. Purified polyclonal rabbit-origin IgG specific for canine IgE (anti-IgE) and rabbit immunoglobulin G (IgG) were injected intradermally in 7 non-allergic laboratory colony cats. Wheal measurements were obtained and biopsies collected before injection and at injection sites after 20 min, 6, 24, and 48 h. Injection of anti-IgE induced an immediate wheal response which was significantly larger than that seen after injection of rabbit IgG. Anti-IgE injected skin was also significantly thicker than IgG-injected skin. This corresponded with a significant increase in number of visibly degranulated mast cells in anti-IgE samples when compared to IgG samples. Injection of anti-IgE was associated with the rapid recruitment of inflammatory cells to the injected dermis. The number of inflammatory cells and mononuclear cells were significantly elevated after the injection of anti-IgE when compared to IgG-injected skin. Both eosinophils and neutrophils were significantly increased in anti-IgE samples relative to IgG, although neutrophils were only transiently increased. The high eosinophil and relatively low neutrophil cell counts in these samples were consistent with previously documented histologic features of naturally occurring feline allergic skin disease. Immunohistochemistry identified a significantly overall increased CD1a⁺ cells after the intradermal injection of anti-IgE when compared to IgG and non-injected skin. CD3⁺, CD8⁺ and CD4⁺ were also significantly increased overall in anti-IgE injected skin relative to IgG injected skin. These data document the gross and cellular response to injection of anti-IgE in the skin of healthy, non-allergic cats and support a possible role for IgE in the development of feline allergic dermatitis.

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1. Introduction

Atopic dermatitis is a common inflammatory disease of the skin, which appears to be increasing in prevalence (Leung et al., 2003). The condition may be seen in several species, including humans, dogs and horses (Olivry et al.,

Abbreviations: Anti-IgE, polyclonal rabbit anti-canine IgE; IgG, rabbit immunoglobulin G; LPR, late-phase reaction.

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2001; Leung et al., 2003; Stepnik et al., 2012). Humans and dogs with atopic dermatitis demonstrate a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features, including a young age of onset, flexural dermatitis and predisposition to secondary infections with bacteria or fungi (Olivry et al., 2001; Leung and Bieber, 2003).

In many species, the development of atopic skin and respiratory disease has been associated with the development of IgE antibodies to environmental antigens (Hill and DeBoer, 2001; Olivry et al., 2001). Although many environmental allergens have been associated with AD (including pollens, danders, and molds), the most common allergens appear to be those associated with house-dust mites (HDMs), including *Dermatophagoides pteronyssinus* and *D. farinae* (Hill and DeBoer, 2001; Bexley et al., 2009). A similar range of allergens has been described in association with the development of allergic respiratory disease, including allergic asthma (AA) and allergic rhinitis (Masuda et al., 2001).

Cats with allergic dermatitis have a pleomorphic clinical appearance, and affected animals may display self-inflicted alopecia, miliary dermatitis (crusted papular dermatitis), head and neck pruritus or the development of eosinophilic dermatitis (eosinophilic granulomas) (Taglinger et al., 2007). In addition, both cats and humans are frequently affected with varying degrees of allergic respiratory disease, including allergic asthma (AA) or allergic rhinitis (Masuda et al., 2001; Delgado et al., 2010). In humans, the development of atopic dermatitis in childhood frequently heralds the development of asthma later in life—the so-called “atopic march” (Leung and Bieber, 2003).

Cutaneous immunoglobulin-E (IgE) mediated inflammation can be modelled by the intradermal injection of anti-IgE antibodies in both humans and dogs (Olivry et al., 2001; Pucheu-Haston et al., 2006). In the dog, this injection induces degranulation of cutaneous mast cells, and has been demonstrated to produce immediate wheal and flare reactions as well as late phase reactions (LPR) in the skin of both normal and atopic dogs (Olivry et al., 2001; Pucheu-Haston et al., 2006). These reactions grossly and microscopically resemble those generated by the intradermal injection of allergen or anti-IgE in humans with atopic disorders (Olivry et al., 2001).

Similar studies have not yet been performed in the cat. Indeed, some controversy exists as to the role of IgE in the development of feline allergic skin disease, as feline allergen-specific IgE serum levels do not always correlate with clinical disease (Gilbert and Halliwell, 1998a; Halliwell et al., 1998; Taglinger et al., 2005). However, allergen specific IgE levels also do not always correlate with clinical disease in the dog, a species in which the role of IgE in allergic dermatitis is well accepted (Egli et al., 2002; Zemmann et al., 2002; Lauber et al., 2012). In contrast, the role of IgE in the mediation of other feline allergic diseases (such as asthma) is better documented (Norris Reinero et al., 2004; Lee-Fowler et al., 2009). Given the frequent co-association of the feline asthmatic phenotype with (presumably allergic) skin disease (Moriello et al., 2007), it is tempting to speculate that IgE may in fact play a role in both disorders.

The specific aims for this study were two-fold. The first objective was to characterize feline IgE-mediated cutaneous immune responses grossly and microscopically. The evaluation of anti-IgE injected skin would allow us to identify important events and cellular participants during the development of IgE-mediated inflammation in non-allergic cats. The second objective was to compare these results to those previously reported in cats with spontaneous cutaneous allergic dermatitis. Ultimately, it is expected that this model will enable the pre-clinical evaluation of the potential efficacy of anti-inflammatory medications for the control of feline hypersensitivity, and facilitate improved diagnostic and therapeutic methods to improve feline quality of life.

2. Materials and methods

2.1. Tested subjects

Seven healthy, sexually intact, laboratory colony female domestic short haired cats were used for the study. Their age ranged from 5 to 7 years of age with a mean age of 5.5 years. The cats were selected from the colony kept at the Audubon Center for Research of Endangered Species. The cats were selected based upon the lack of clinical or historical evidence of allergic skin or respiratory disease, cutaneous bacterial infections, or systemic disease. Cats that had received medications 14 days prior to the study were excluded. Physical examinations were performed on all cats 1 day prior to the start of the study. Housing and experimental samplings were in accordance with the National Research Council's 1996 *Guide for Care and Use of Laboratory Animals*. All experimental protocols were approved by an Institutional Animal Care and Use Committee.

2.2. Generation of IgE-mediated cutaneous immediate and late-phase reactions

On study Day 1, each cat was anesthetized by administration of isoflurane. The cats were then shaved on their lateral thorax. Each cat was injected intradermally (0.05 ml each injection site) at one site with phosphate buffered saline (PBS), at four sites with protein G affinity purified rabbit polyclonal IgG specific for canine IgE (anti-canine IgE) (Bruce Hammerberg, North Carolina State University) diluted to 0.1 mg/ml in PBS, and at four sites with normal rabbit IgG (Jackson ImmunoResearch Laboratories, West-grove, PA) diluted to 0.1 mg/ml in PBS. Table 1 defines the injection sites. Individual injection sites were marked with an indelible marker to facilitate identification and localization at later time points. The optimal injection volume and concentration had been previously determined during a 3 cat pilot study (data not presented).

2.3. Grading the macroscopic reactions

Injected skin sites were examined at 20 min and at 6, 24 and 48 h after injection. The diameter of the cutaneous reactions was measured with calipers in two perpendicular directions to determine the reaction area. Skin thickness

Table 1
Sample injection site and collection.

Time	Time zero	20 min	6 h	24 h	48 h
Samples collected	PBS Non-injected skin	Anti-IgE IgG	Anti-IgE IgG	Anti-IgE IgG	Anti-IgE IgG

Intradermal PBS was assessed for control of wheal diameter and skin thickness. All solutions were injected intradermally at time zero. Biopsies were collected from non injected skin and from anti-IgE, and IgG injected areas at the specified time points.

was also measured with calipers at these times. The presence or absence of erythema or palpable induration was also recorded.

2.4. Specimen collection and processing

The cats were placed under isoflurane anesthesia before each biopsy collection. Each biopsy site received 0.5 ml of 2% lidocaine (Sparhawk Laboratories, Lenexa, KS) buffered with sodium bicarbonate s.c. One 8 mm punch biopsy of non-injected skin was collected on the lateral thorax, and then similar samples were collected at injection sites at 20 min and 6, 24 and 48 h after injection with anti-IgE and normal rabbit IgG. Each sample was bisected immediately after collection. One half was placed in 10% neutral buffered formalin for routine processing in paraffin. The other half was placed in Optimal Cutting Temperature medium (OCT Tissue Tek, Baxter Diagnostics Inc.), immersed in isopentane cooled in liquid nitrogen and stored at -80°C until cryosectioning. The skin was closed using a single cruciate stitch using 3-0 poliglecaprone 25 monofilament absorbable suture.

2.5. Histological staining

Five-micrometer sections were cut from paraffin blocks and stained with haematoxylin and eosin for examination of inflammatory cells. Eosinophils were visualized and counted using Luna's stain for eosinophils (Luna, 1968). A low-pH toluidine blue stain (Preece, 1972; Sheehan and Hrapchak, 1980) was used to identify dermal mast cells.

2.6. Immunohistochemistry of cutaneous mononuclear cells

Frozen samples were sectioned at -20°C in a cryotome. The slides were air dried for 10 min, then fixed by a 10 min immersion in acetone. Endogenous peroxidase activity was neutralized by a 10 min immersion in 0.3% hydrogen peroxide/0.1% sodium azide. Nonspecific binding was inhibited by blocking for 20 min in 1% fetal calf serum. After thoroughly washing with PBS, the slides were incubated with mouse-origin monoclonal antibodies specific for feline CD1a⁺, CD4⁺ or CD8⁺ (P.F. Moore, Leukocyte Antigen Biology Laboratory, UC Davis) for 1 h. This was followed by a 30 min incubation using biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA), then a 30 min incubation with horseradish peroxidase-conjugated streptavidin (Zymed-Invitrogen, Carlsbad, CA). Finally, the slides were developed using AEC chromogen (BioGenex, Fremont, CA) and counterstained with hematoxylin (Dako, Carpinteria, CA).

For CD3⁺ immunohistochemistry, samples were prepared from 5-micrometer sections cut from paraffin blocks. Each slide was deparaffinized in 3 changes of xylene for 5 min. Slides were rehydrated with 3 changes of 100% alcohol and 1 change with 95% alcohol for 5 min each. The slides were rinsed 3 times with distilled water. This was followed by antigen retrieval using 10mMCitrate buffer, 6.0 pH, for 10 min at $125-127^{\circ}\text{C}$ (Biocare Medical (Concord, CA) Decloaker). The slides were allowed to cool for 20 min, followed by a rinse with deionized water, and then with a Tris-buffer, pH 7.6. The slides were loaded into a DAKO Autostainer LINK 48. Endogenous peroxidase activity was blocked by application of a 3% hydrogen peroxide solution for 3 min, and then the slides were rinsed with buffer solution. Normal goat serum was then applied to each slide for 30 min to inhibit non-specific antibody binding. Slides were not rinsed after blocking. The rabbit anti-human CD3⁺ primary antibody (Dako A 0452) at a 1:400 dilution was applied to each slide for 30 min, and then rinsed with buffer solution. Biotinylated anti-rabbit secondary antibody, was then applied to each slide for 30 min, and then rinsed with buffer solution. The detection reagent ABC (Vector Laboratories, INC.; Burlingame, CA) was applied for 30 min, and then rinsed with buffer solution. The substrate Nova Red (SK-4800, Vector Laboratories, INC.; Burlingame, CA) was applied to each slide, and then rinsed with buffer solution and deionized water. The counter stain Mayers Hematoxylin was applied to each slide for 5 min, and then rinsed with buffer solution followed by a deionized water rinse. The slides were removed from the stainer, and allowed to dry in an oven at 60°C for 30 min. The slides were removed from the oven and allowed to cool for 10 min. The slides were rinsed in 3 changes of xylene for 3 min, and a cover slip was applied.

2.7. Enumeration of dermal cells

The stained slides were blinded to the investigator, and the total nucleated cells/mm² were counted in the superficial dermis. Cells were counted in 16 consecutive 0.254 mm × 0.254 mm fields with a 40× objective lens (Olympus BX53 microscope, Olympus America Inc, Center Valley, PA), excluding endothelial cells and adnexa. Eosinophils, neutrophils, mast cells, as well as CD1a⁺, CD3⁺, CD4⁺, and CD8⁺ mononuclear cells/mm² were counted in the same manner.

2.8. Statistical analysis

Statistical analyses were performed using SAS Software, Version 9.3 for Windows (SAS institute, Cary, NC, USA). Statistical analysis of gross measurements and dermal cell

counts was performed by using the MIXED procedure. The evaluation of repeated measures analysis of variance in a factorial arrangement of treatments was conducted. The fixed effects were time and the injected substance type (PBS, IgG, and anti-IgE). The random effect was the tested animals (7 cats). When overall significance was detected, pairwise comparisons of least-squares means were conducted with *t* tests. All tests were considered significant at $p \leq 0.05$.

3. Results

3.1. Intradermal injection of anti-IgE produced macroscopic immediate reactions

Intradermal injection of anti-IgE produced a significant ($p < 0.001$) immediate wheal response at 20 min when compared to PBS and IgG injected skin (Fig. 1A). Skin thickening was also significantly greater overall after the injection of anti-IgE when compared to IgG-injected skin ($p < 0.0001$), although a treatment $\times$ time effect could not be demonstrated (Fig. 1B). Erythema was not noted after injecting the skin with testing agent or controls.

3.2. Intradermal injection of anti-canine IgE induced degranulation of dermal mast cells

Toluidine blue staining improved the contrast of granules and permitted visualization of intact and degranulated mast cells. The cells were predominately located in a perivascular and perifollicular location. The intradermal injection of anti-IgE was associated with the dispersal of dark staining granules into the surrounding dermis and overall diminution of cell-associated granule staining density (Fig. 2A and B). This finding was interpreted as mast cell degranulation (Olivry et al., 2001). Overall, a significant increase in degranulated superficial dermal mast cells was seen after the injection of anti-IgE when compared to both non-injected and IgG injected skin ($p < 0.0001$; Fig. 2A). However, no significant treatment $\times$ time effect was found. These changes correlated with the observed wheal and dermal thickening changes seen in anti-IgE injected skin.

3.3. Intradermal injection of anti-canine IgE induced an increase of inflammatory cells

Injection of anti-IgE was associated with the rapid recruitment of inflammatory cells. The majority of these cells were distributed in a perivascular to perifollicular pattern, similar to that documented in spontaneous disease (Taglinger et al., 2007). In addition, mild to marked superficial dermal edema was noted in the anti-IgE injected samples when compared to IgG-injected samples (Fig. 3A–D). The total number of inflammatory cells was highest at 20 min after injection of anti-IgE, and then decreased only slightly thereafter (Fig. 4A). In contrast, the eosinophil numbers peaked at 6 and 24 h then decreased (Fig. 4B). Neutrophils demonstrated only a slight increase at 20 min and 6 h (Fig. 4C). A group statistical effect was demonstrated, with total inflammatory cells and eosinophils increased overall in anti-IgE injected skin

relative to both non-injected and IgG-injected skin. For neutrophils, a group effect could also be demonstrated, with increased numbers in anti-IgE relative to IgG-injected skin. However, type $\times$ time interactions were not identified for any of these parameters.

3.4. Intradermal injection of anti-canine IgE was associated with an increase in dermal CD1a⁺ dendritic cells but had minimal effect upon dermal lymphocytes

A group statistical effect was demonstrated for CD1a⁺ dendritic cells, which were increased overall when compared to non-injected and IgG-injected skin (Table 2). In contrast, the effect of anti-IgE injection on lymphocyte numbers was less remarkable. The numbers of CD3⁺, CD4⁺ and CD8⁺ T cells were increased overall after the injection of anti-IgE when compared to the control IgG, but a significant group effect could not be demonstrated between anti-IgE and non-injected skin for these parameters (Table 2). Although a significant group difference could be demonstrated between anti-IgE and IgG, significant type $\times$ time interactions were not identified.

4. Discussion

This study documented the immediate and delayed inflammatory responses of normal cat skin to the intradermal injection of anti-IgE. The macroscopic and microscopic changes seen after the intradermal injection of anti-IgE shared many features with those previously reported in cats with naturally occurring allergic skin disease (Moriello, 2001; Taglinger et al., 2005). In contrast, the intradermal injection of normal rabbit IgG did not cause significant macroscopic or microscopic changes. These results suggest that the intradermal-injection of anti-IgE may be a useful tool for modelling IgE-dependent inflammatory responses in normal cat skin.

Few studies have investigated the role and importance of IgE in allergic skin conditions of cats (Hobi et al., 2011). Some authors question the importance of IgE in feline allergic disease (Hobi et al., 2011), as feline allergen-specific IgE serum levels do not always correlate with clinical disease (Gilbert and Halliwell, 1998a; Halliwell et al., 1998; Taglinger et al., 2005). However, allergen specific IgE levels also do not always correlate with clinical disease in the dog, a species in which the role of IgE in allergic dermatitis is well accepted (Egli et al., 2002; Zemmann et al., 2002; Lauber et al., 2012). In addition, some studies in peer reviewed literature have drawn conclusions about feline serum IgE levels based upon the results of non-validated assays (Reinero, 2009). Nonetheless, considerable evidence supports the assertion that IgE may play a pivotal role in the development and perpetuation of feline hypersensitivity, as it does in the dog and in humans. For example, allergen-specific cutaneous reactivity can be transferred between individuals by the injection of serum from a sensitized cat (passive cutaneous anaphylaxis testing; PCA) (Gilbert and Halliwell, 1998a; Reinero, 2009). This reactivity is abrogated if the serum is heated before transfer, which inactivates IgE, but not other antibody isotypes (Gilbert and Halliwell, 1998a,b; Lee-Fowler et al., 2009). In

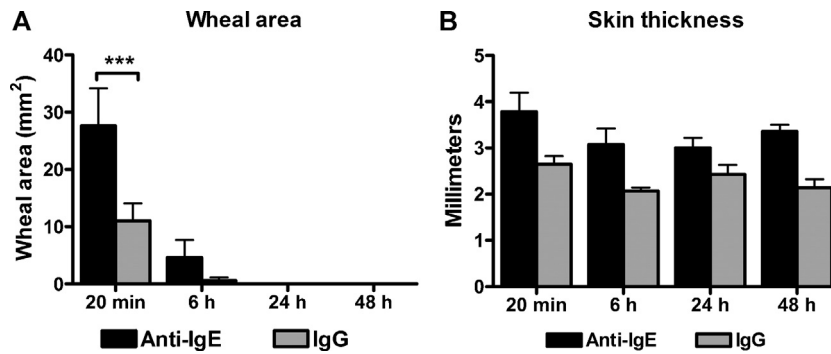


Fig. 1. Injection of cross-linking anti-IgE is associated with the development of an immediate wheal response and dermal thickening. Intradermal injection of anti-IgE produced a significant ($p < 0.001$) immediate wheal response at 20 min when compared to IgG injected skin (A); Skin thickness was significantly greater overall after the injection of anti-IgE when compared to IgG-injected skin ($p < 0.0001$), although a treatment $\times$ time effect could not be demonstrated.

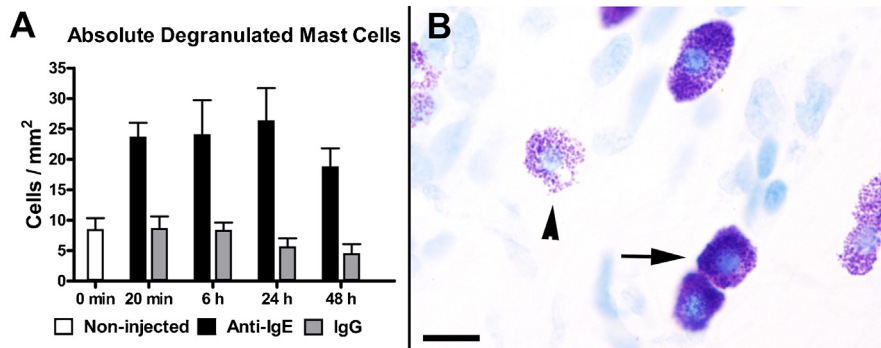


Fig. 2. Intradermal injection of anti-IgE is associated with cutaneous mast cell degranulation. A significant increase overall in degranulated dermal mast cells seen after the injection of anti-IgE when compared to both non-injected and IgG injected skin ($p < 0.0001$) although a treatment $\times$ time effect could not be demonstrated (A); The arrowhead points to a degranulating mast cell and the arrow points to an intact mast cell (B). Toluidine blue stain, 100 $\times$, bar = 10 μ m.

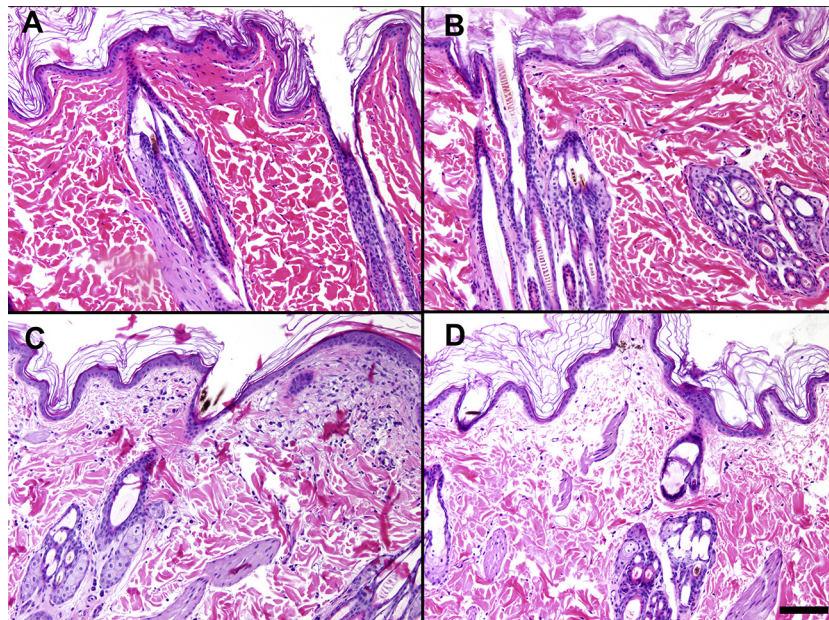


Fig. 3. Injection of cross-linking anti-IgE is associated with the development of superficial dermal edema and the recruitment of inflammatory cells. Non-injected skin (A); IgG injected skin (B); Anti-IgE injected skin demonstrating superficial dermal edema and a superficial perivascular infiltrate (C); anti-IgE injected skin from a different cat demonstrating less cellularity, but more obvious superficial edema (D). Hematoxylin and eosin stain, 20 $\times$, bar = 100 μ m.

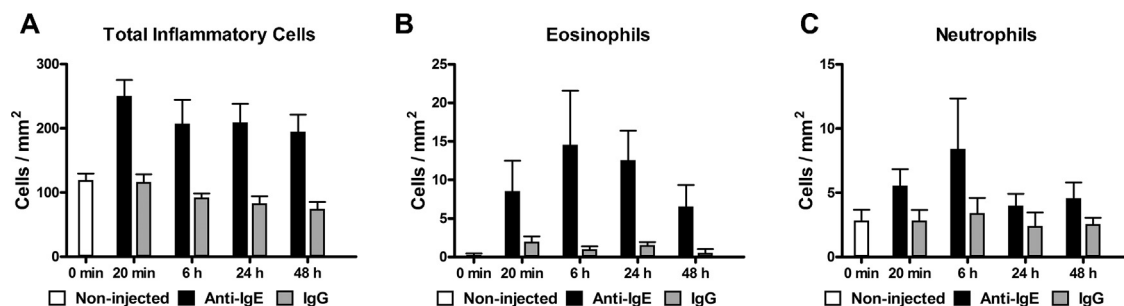


Fig. 4. Intradermal injection of anti-IgE was associated with the rapid recruitment of inflammatory cells to the dermis. Total number of inflammatory cells in anti-IgE injected skin were significantly higher overall than both IgG and non-injected skin ($p < 0.001$) (A). Eosinophils were increased overall in anti-IgE injected skin relative to IgG-injected ($p < 0.0001$) and non-injected skin ($p < 0.01$) (B). Neutrophils demonstrated only a transient increase but were still increased overall relative to IgG-injected skin ($p < 0.05$) (C).

addition, intradermal injection of polyclonal, cross-linking anti-feline IgE will induce the formation of a wheal and flare in normal feline skin (Gilbert and Halliwell, 1998b; Norris et al., 2003). Increased levels of allergen specific IgE have been demonstrated in association with spontaneous allergic asthma, rhinitis and dermatitis in the cat (Gilbert and Halliwell, 1998a; Masuda et al., 2001; Moriello et al., 2007). Furthermore, experimental sensitization of cats by injectable or aerosol exposure has also been demonstrated to induce increases in serum allergen-specific IgE (DeBoer, 1993; Lee-Fowler et al., 2009).

Several published studies have documented the importance of IgE in mediating immediate- hypersensitivity responses and LPR in humans and dogs (Leung and Bieber, 2003; Pucheu-Haston et al., 2006; Stone et al., 2010). This antibody is bound by high affinity IgE receptors (FcεRI) that are located on mast cells, basophils and activated eosinophils (Stone et al., 2010). When an antigen binds to IgE and cross links the FcεRI it triggers cell degranulation (Leung and Bieber, 2003; Stone et al., 2010). This

degranulation is associated with the release of pre-formed chemical mediators such as: biogenic amines, granule enzymes, proteoglycans, and lipid mediators. These mediators are responsible for the classic wheal and flare reaction (Leung and Bieber, 2003; Stone et al., 2010). In addition, pre-formed and *de novo* synthesized cytokines such as TNF, IL-1, IL-4, IL-6, IL-13, CCL3, CCL4, IL-3 and GM-CSF are also released from the cytoplasm of mast cells and are largely responsible for promoting LPR (Leung and Bieber, 2003; Stone et al., 2010). To the authors' knowledge, this study is the first to characterize late phase reactions in the cat, and our results are similar (if not as dramatic) as those demonstrated in humans and dogs (Leung and Bieber, 2003; Pucheu-Haston et al., 2006; Stone et al., 2010).

In the current study, the intradermal injection of anti-IgE in cats induced an immediate wheal response at 20 min, which was greatly decreased by 6 h and undetectable afterwards. This wheal response correlated with the development of mild to marked superficial edema in anti-IgE injected skin. The rapidly fading response is

Table 2
Immunohistochemistry Results.

	Non injected (n = 7)		Normal rabbit IgG (n = 7)				Anti-IgE (n = 7)			
	Time 0		20 min	6 h	24 h	48 h	20 min	6 h	24 h	48 h
Paraffin samples										
CD3 ⁺										
Mean	21.71		15.00	13.00	13.14	12.50	30.00	22.14	24.14	31.14
(SE)	(2.44)		(3.67)	(4.31)	(3.99)	(4.07)	(6.33)	(4.38)	(4.88)	(7.23)
Median	20		15	9	7	11	22	25	27	24
Frozen samples										
CD1a ⁺										
Mean	25.71		23.14	30.00	22.85	23.28	38.14	46.57	40.28	40.42
(SE)	(4.18)		(3.92)	(6.19)	(1.64)	(3.39)	(5.43)	(6.55)	(4.53)	(5.15)
Median	24		25	26	23	23	40	40	44	45
CD4 ⁺										
Mean	40.85		21.57	9.14	14.57	17.14	40.42	33.29	34.71	37.42
(SE)	(15.06)		(6.62)	(3.49)	(4.67)	(5.15)	(6.03)	(11.35)	(10.42)	(7.11)
Median	22		19	4	13	15	43	21	34	50
CD8 ⁺										
Mean	7.71		10.42	7.14	5.14	2.14	12.71	16.85	11.42	9.42
(SE)	(4.16)		(5.15)	(1.88)	(1.65)	(0.40)	(6.25)	(8.51)	(4.36)	(2.59)
Median	3		3	5	4	2	5	7	6	7

CD3⁺ was significantly increased overall in anti-IgE injected skin relative to IgG-injected skin ($p < 0.001$). CD1a⁺ was significantly increased overall in anti-IgE injected skin relative to IgG- and non-injected skin ($p \leq 0.001$). CD4⁺ was significantly increased overall in anti-IgE injected skin relative to IgG- ($p < 0.001$) and non-injected skin ($p < 0.01$). CD8⁺ was significantly elevated overall in anti-IgE injected skin relative to IgG-injected skin ($p < 0.05$).

similar to the results often seen during intradermal allergen challenge in cats with spontaneous allergic skin disease (Taglinger et al., 2005). Despite the evanescent quality of the wheal response, injection of anti-IgE was associated with a significant overall increase in skin thickness compared to IgG-injected skin, although no type $\times$ time interaction could be demonstrated.

Anti-IgE injected skin exhibited an overall increase in the number of visibly degranulated mast cells when compared to IgG-injected skin. This increase in degranulated cells would be consistent with the increases in wheal area and dermal thickness following injection of anti-IgE. The degranulated cells were identified with toluidine blue staining and had a pale blue cytoplasmic appearance with dispersion of granules into the surrounding dermis (Olivry et al., 2001) (Fig. 2B). Similar appearing degranulated mast cells have been documented in dogs after the injection of anti-IgE (Olivry et al., 2001).

The inflammatory cellular infiltrate in cutaneous late phase responses in humans and dogs is well documented and is typically comprised of eosinophils, neutrophils, dendritic cells and lymphocytes (Olivry et al., 2001; Leung and Bieber, 2003; Stone et al., 2010). In contrast to the extensive research that has been performed in these species, relatively little work has been performed to characterize the inflammatory response associated with feline allergic skin disease. Eosinophilia appears to be a relatively consistent feature of spontaneous allergic skin disease in the cat. One case report identified a mild perivascular dermatitis made up of mast cells, lymphocytes and eosinophils (Moriello, 2001). A second peer reviewed study reported demonstration of prominent numbers of eosinophils, mast cells, neutrophils, and lymphocytes in the superficial dermis (Roosje et al., 1998). A third study identified a similar histological dermal inflammatory pattern (Roosje et al., 2002). Finally, Taglinger et al. (2007) reported that feline allergic skin was infiltrated by activated antigen-presenting cells and T lymphocytes in addition to dermal mast cells.

The present study identified an overall increase in the number of eosinophils in anti-IgE injected skin compared to non-injected and IgG injected skin. Although the lack of a significant treatment $\times$ time interaction precludes a definitive statement on the kinetics of the eosinophil response, there was a trend for dermal eosinophils to peak at 6 and 24 h then decline. This finding was similar with previous studies in the dog, which identified migrating eosinophils within 12 h post challenge of allergen (Becker et al., 1988) or anti-IgE antibodies (Olivry et al., 2001). In humans, the peak eosinophil influx takes place about 6 h after induction of LPR (Frew and Kay, 1988; Charlesworth et al., 1989; Gaga et al., 1991; Tsiocopoulos et al., 1994). The exact cause for this eosinophil influx in cats remains unknown; however, it may be similar to that reported in humans, in which release of Th2-type cytokines such as IL-3, IL-4, IL-5, GM-CSF, and CCL11 have been found to contribute to eosinophil development, proliferation, differentiation, and recruitment to LPR inflammatory sites (Gilfillan and Tkaczyk, 2006; Rothenberg and Hogan, 2006; Abraham and St John, 2010; Stone et al., 2010). Other chemoattractants such as C5a, PAF, and LTB₄ may also play a role in recruiting eosinophils to these areas in humans (Rothenberg and

Hogan, 2006; Stone et al., 2010). Unfortunately, very little work has been performed to investigate the cytokine profile in the skin or blood of cats with spontaneous allergic skin disease. In one study, no clear difference could be demonstrated in the expression of IL-4 and IL-5 in the skin of cats with allergic skin disease compared to healthy cats (Taglinger et al., 2008). Similarly, serum levels of IL-5 were not found to correlate with circulating eosinophil numbers in cats (Nakazato et al., 2007). In contrast, CD4⁺ T cells in lesional and nonlesional skin of cats with naturally occurring allergic skin disease have been demonstrated to produce IL-4 (Roosje et al., 2002).

Neutrophil infiltration has been documented in human and dog LPR. However, the relative numbers of these cells are somewhat variable between studies (Solley et al., 1976; Olivry et al., 2001; Pucheu-Haston et al., 2006). In general, neutrophil infiltrate in LPR increases between 6 and 12 h and is transient in nature. In the present study, intradermal injection of anti-IgE was associated with a minimal increase of neutrophils at 20 min and 6 h. This relative paucity of neutrophils was consistent with previously reported histological changes in spontaneous feline allergic disease. Neutrophils appear to be relatively scarce in non-infected allergic cat skin. For example, biopsy results of three littermates diagnosed with atopy did not contain any neutrophils, but rather mast cells, lymphocytes, and eosinophils (Moriello, 2001). Another study reported mixed perivascular inflammation with few neutrophils in lesional allergic skin; however neutrophils were absent in non-lesional allergic cat skin (Roosje et al., 2002).

In dogs, the number of CD1c⁺ dendritic cells have been shown to increase significantly in LPR after the intradermal injection of anti-IgE (Olivry et al., 2001; Pucheu-Haston et al., 2006). This experimental finding echoes the increased numbers of dermal and epidermal dendritic cells associated with canine spontaneous atopic dermatitis. In the current study, there was a significant overall increase in the number of CD1a⁺ dermal dendritic cells in skin injected with anti-IgE relative to non-injected and IgG injected skin. This increase is similar to that reported in feline spontaneous allergic dermatitis. A previous study demonstrated significantly increased numbers of CD1a⁺ and MHCII⁺ dendritic cells in the epidermis and dermis of lesional skin in allergic cats compared to healthy control cats (Roosje et al., 1997). In another study, increased numbers of cells expressing MHC class II⁺ (morphologically resembling dermal dendritic cells, macrophages and epidermal Langerhans cells) were found in lesional and non-lesional skin in cats with spontaneous allergic skin disease relative to healthy control cats (Taglinger et al., 2007). Together, these results suggest that Langerhans cells, dendritic cells and other MHC class II⁺ cells play a significant role in the pathogenesis of feline allergic skin disease.

Intradermal injection of anti-IgE was associated with an overall increase in dermal CD3⁺ T-lymphocytes relative to that seen following injection of IgG, although this increase was not significant compared to non-injected skin. There was a tendency for the lymphocyte numbers to increase with time, but this increase was not statistically significant. The lymphocytes in the present study were distributed in a superficial perivascular and perifollicular pattern. A recent

report documented an increase in the number of CD3⁺ lymphocytes in the skin of cats with spontaneous allergic disease, frequently in association with dendritic cells expressing MHC class II⁺ (Taglinger et al., 2007). This report suggests that dendritic cells may participate in the maintenance of the local immune response by presenting antigen to T lymphocytes, as has been reported in other species (Taglinger et al., 2007).

The CD4⁺ and CD8⁺ cell counts were increased overall after the injection of anti-IgE when compared to IgG injected skin. Evaluation of the CD4⁺ to CD8⁺ ratio is an alternate method of evaluating the dermal lymphocyte response, and has been used in previous studies (Bos et al., 1987; Roosje et al., 1998). In the present study, the ratio of CD4⁺/CD8⁺ T cells in anti-IgE injected skin was calculated as 3.97:1. In contrast, the CD4⁺/CD8⁺ ratios in non-injected and IgG-injected skin were somewhat lower (3.5:1 and 3.2:1, respectively). One study documented a relative increase of CD4⁺ T cells and a CD4⁺/CD8⁺ ratio of 3.9:1 in lesional skin of cats diagnosed with allergic dermatitis (Roosje et al., 1998). The same study documented that the dermis of healthy control cats had only 1 or 2 CD4⁺ cells and no CD8⁺ cells noted (Roosje et al., 1998).

5. Conclusion

In conclusion, intradermal injection of cross-linking anti-IgE was associated with a transient immediate wheal response and the development dermal thickening and superficial dermal edema. Histologic examination of injected skin demonstrated an increase in the number of degranulated mast cells, which was associated with the rapid recruitment of inflammatory cells, especially eosinophils and dermal dendritic cells. These cells were typically distributed in a perivascular to perifollicular fashion. Overall, these results are very similar to those reported in experimentally induced IgE-mediated cutaneous inflammation in both dogs and humans. Furthermore, the responses seen in the current study shares many features with those previously described in spontaneous feline allergic skin disease. Although it would be premature to state that the results of the current study prove that IgE plays a relevant role in feline allergic skin disease, the model does provide evidence to support such a hypothesis.

In summary, our laboratory has developed an experimental *in vivo* model of feline cutaneous IgE-mediated inflammation. The model shares many similarities with both experimentally induced IgE-mediated inflammation in other species as well as with spontaneous allergic skin disease in the cat. This model shows promise for further evaluation of the events involved in the development of feline LPR, and may prove useful in the pre-clinical evaluation of drugs designed to target the LPR in this species.

Conflict of interest

None declared.

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