

# Production of polyclonal antisera against feline immunoglobulin E and its use in an ELISA in cats with experimentally induced asthma<sup>1</sup>

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## Abstract

Serum samples from six cats with experimentally induced asthma were used to purify feline IgE using gel filtration and affinity chromatography. The resultant IgE, evaluated for purity by immunoelectrophoresis (IEP) and reactivity by Prausnitz–Kustner (PK) testing, was used to develop polyclonal rabbit anti-feline IgE antisera. Using reverse cutaneous anaphylaxis (RCA), the antisera were determined to be specific for feline IgE. The polyclonal rabbit anti-feline IgE antiserum was then validated in an allergen-specific ELISA. Serum samples from an additional five asthmatic cats sensitized with Bermuda grass allergen (BGA) were evaluated prior to sensitization, after parenteral sensitization, and after aerosol sensitization and challenge. A significant increase in serum BGA-specific IgE was noted over time.

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## 1. Introduction

Naturally developing feline asthma is a clinically important respiratory disease affecting pet cats (Dye et al., 1996; Moise et al., 1989). Since the cat is the only animal species to commonly and spontaneously

develop asthma with the major features of the disease in humans, the cat has also been used as an experimental model of human asthma (Norris et al., 2001; Padrid, 1995; Padrid et al., 1995b). Markers of the allergic response are an important part of being able to characterize alterations in the asthmatic phenotype over time, or with drug intervention. Commercial sources of polyclonal or monoclonal antisera recognizing feline IgE are not currently available.

Allergic hypersensitivity responses in asthma are mediated by allergen-specific IgE (Holgate, 1999).

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Immunoglobulin E bound to receptors on mast cells, basophils, and activated eosinophils triggers degranulation when cross-linked by allergen; this in turn leads to release of mediators responsible for bronchoconstriction, inflammatory cell influx, and mucus hypersecretion. Evaluation of hypersensitivity can be performed by intradermal skin tests to specific allergens, Prausnitz–Kustner (PK) or passive cutaneous anaphylaxis (PCA) tests, allergen-specific ELISA, or by mucosal testing for cell-bound IgE (e.g., allergenic airway challenge with pulmonary function testing, or identification of IgE positive cells in bronchoalveolar lavage fluid) (De Boer and Hillier, 2001; Gilbert and Halliwell, 1998a,b; Kleinbeck et al., 1989; Zemmann et al., 2002). With the exception of the allergen-specific ELISA, the remaining tests are invasive, and determination of the allergic response requires live animals. Applications of an ELISA for measurement of allergen-specific feline IgE antibodies would include screening cats for preexisting sensitization, identifying high versus low responder cats, and correlating serum allergen-specific IgE levels with other immunologic, physiologic and histologic parameters. The purpose of this study was to purify feline IgE from cats with experimentally induced asthma for development of polyclonal rabbit anti-feline IgE antisera, and to use the antisera in an ELISA to detect changes in allergen-specific IgE over time in the asthmatic cat model.

## 2. Materials and methods

### 2.1. Experimental animals

Six mixed-breed cats obtained from a University of California, Davis research cat colony were used to harvest serum for IgE purification after induction of asthma according to a previously described protocol (Norris et al., 2001). Cats first underwent parenteral sensitization by receiving allergen with alum subcutaneously on days 0 and 21, *Bordetella pertussis* ( $10^5$  organisms) as an adjuvant intramuscularly on day 0, and intranasal allergen on day 14. Aerosol challenge followed parenteral sensitization starting on day 28, and consisted of twice a week treatments for 2 weeks, then weekly thereafter. Aerosolization took place in awake cats in a sealed chamber fitted for a DeVillbiss air compressor with a nebulizer for 5 min per treatment.

Cats were challenged with BGA aerosol 48 h prior to the blood draw on day 50 (post-aerosol sensitization and challenge time point).

Prior to entry into the study, cats had intradermal skin testing (IDST) to ensure no previous sensitization to house dust mite allergen (HDMA, *Dermatophagoides farinae*;  $n = 3$ ; Greer Laboratories, Lenoir, NC) or Bermuda grass allergen (BGA,  $n = 3$ ; Greer Laboratories, Lenoir, NC). The purified IgE was used to produce polyclonal rabbit anti-feline IgE antisera as described below.

An additional five mixed-breed cats (IDST negative for BGA) from the same source underwent sensitization to BGA and were used for evaluation of changes in their serum IgE levels before and after sensitization and challenge using the polyclonal IgE antisera that had been generated and optimized for use in an ELISA.

### 2.2. Intradermal skin testing

On day 0 (baseline), cats were sedated with ketamine HCl (5 mg/kg, intramuscularly) and hair was clipped from their lateral thorax. Intradermal injections of 0.1 ml of the following were administered: histamine phosphate (1:100,000) and serotonin HCl (1:100), which served as positive controls; sterile saline, which served as a negative control; and HDMA or BGA (20,000 PNU/ml, both diluted 1:10). Cats were considered to have a positive skin reaction if the diameter of the wheal formed in response to BGA was greater than or equal to that halfway between the diameter of the wheals produced by the positive and negative controls (Scott et al., 1995). The width and length of the wheals were measured in centimeters after 20 min.

#### 2.2.1. PK tests

On day 28 of the parenteral sensitization protocol, evaluation of serum allergen-specific IgE from the six cats donating serum for IgE purification was performed using PK testing. Two recipient, 6-month-old non-sensitized cats were sedated as described for IDST. After confirming a negative skin reaction for each allergen (HDMA or BGA), one cat was used to test for the presence of HDMA-specific IgE in the donor serum, and the other for BGA-specific IgE in the donor serum. A series of square grids measuring

approximately 3 cm in diameter were delineated on the skin of each cat using a permanent marker. Intradermal injections of 0.1 ml of undiluted sensitized cat serum were given in the center of these squares. Heat inactivated serum (undiluted; at 56 °C for 30 min) was also administered intradermally. After 48 h, cats were again sedated and given intradermal injections of histamine and sterile saline as positive and negative controls. In the marked sites of previous intradermal injection of sensitized cat sera, intradermal injection of respective allergen was administered. An intravenous injection of Evans Blue dye (20 mg diluted in 1 ml sterile saline) was given, and the sites of intradermal injection were observed and the size of the blue wheals was measured after 20 min.

### 2.3. Sample collection

Whole blood was drawn by jugular venipuncture from the six cats donating serum for IgE purification on day 0 (baseline), and on days 14, 21, 28, 35, 42, 50, 56, 70, 80, 110, 140 and 180 after sensitization with HDMA or BGA. The blood was allowed to clot at room temperature and was then centrifuged at 2000 rpm for 30 min. Serum was harvested and banked at –20 °C until the time of IgE purification.

### 2.4. Serum IgE purification

A total volume of 22.5 ml of pooled serum from the aforementioned days post-allergen sensitization was used for feline IgE purification. The globulin fraction of the serum was obtained by precipitating the serum with an equal volume of 50% saturated ammonium sulfate (SAS). The ammonium sulfate fractionation of serum was performed three times, followed by dialysis in borate buffered saline (pH 8.0) to remove the sulfate. Further purification of the globulin fraction was attained by gel-filtration chromatography on Sephacryl S-300 (Pharmacia Fine Chemicals, Uppsala, Sweden) using 0.1 M Tris/0.5 M HCl, pH 8.0 buffer. The protein profile read at an optical density (OD) of 254 nm had two peaks: the first being a narrow peak consistent with IgM, and the second a wide peak having an early shoulder consistent with IgE and the remainder consistent with IgG. The fractions comprising the first half of the second peak were pooled and passed through an affinity chromatography

column using an EDC/diaminodipropylamine immobilization kit (#44899, Pierce Chemical Company, Rockford, IL) to couple affinity purified goat anti-cat IgG Fc (#602-1103, Rockland, Gilbertsville, PA) to the solid support. This was done to further enrich for IgE by removing contaminating IgG.

### 2.5. Immuno-electrophoresis (IEP) and PK testing on purified feline IgE

IEP was performed using slides coated with an adhesive 1% agar undercoat and a 1% agar electrophoresis gel layer in 0.03 M barbital buffer (pH 8.9). Gels were run for 90 min at 3–5 mA/frame and incubated in a humidity chamber for 48 h at room temperature. Normal cat sera and purified cat IgE were tested for reactivity against goat anti-feline IgG (AA126, Serotec, Raleigh, NC), goat anti-feline IgM (AA124, Serotec, Raleigh, NC), goat anti-feline IgA (AA125, Serotec, Raleigh, NC), and mouse anti-feline IgM, A, G (MCA623, Serotec, Raleigh, NC).

### 2.6. Immunization of rabbits

To make polyclonal antisera against feline IgE, two rabbits were immunized with the purified IgE fraction. A protein concentration was performed on the purified IgE using a protein assay (Bio-Rad, Hercules, CA) performed according to manufacturer's instructions and demonstrated a concentration of 0.137 mg/ml (total volume 11 ml).

In each rabbit, on day 0, 0.2 mg of purified feline IgE emulsified 2:1 in incomplete Freund's adjuvant (Life Technologies, Grand Island, NY) was given by the im route divided in four sites. Second and third immunizations (identical protocol) were administered on days 14 and 21. Blood was obtained from the rabbits at baseline and on days 14, 21, 28, 37, and 60 from the central ear artery. The blood was allowed to clot at room temperature and was then centrifuged at 2000 rpm for 30 min. Serum was harvested and banked at –20 °C until further prepared and characterized.

### 2.7. Preparation of antiserum

Ouchterlony double immunodiffusion tests were performed to demonstrate rabbit antibody precipitation

with components of normal cat serum. The antiserum from the rabbits underwent a 50% SAS cut three times prior to being run through an affinity chromatography column multiple times using an EDC/diaminodipropylamine immobilization kit (#44899, Pierce Chemical Company, Rockford, IL) which coupled the solid support to affinity purified cat IgG H + L (JOC-000003, Accurate Chemical and Scientific Corporation, Westbury, NY). IEP was used to monitor the disappearance of rabbit anti-cat IgG from the antiserum (data not shown).

## 2.8. Reverse cutaneous anaphylaxis (RCA)

Polyclonal rabbit anti-feline IgE antisera was diluted at 1:2 in PBS and injected intradermally at a volume of 0.1 ml into three recipient cats along with the aforementioned positive and negative controls. Serum from one of the rabbits prior to immunization with purified feline IgE was used as an additional negative control. The tests were read for wheal development at 20 min.

## 2.9. Optimization of the ELISA

Each ELISA was performed using a 96 well plate (Dynatech Immulon, Alexandria, VA) sensitized with BGA (Greer Laboratories, Lenoir, NC) at 1 µg/well in 100 µl of carbonate/bicarbonate buffer at pH 9.6. Each plate was incubated overnight at 4 °C; plates were stored at 4 °C for up to 2 months. Sensitized plates were incubated for 1 h at 37 °C with 100 µl/well of rabbit serum albumin (RSA) 1% diluted in wash buffer (PBS/Tween 0.2%). The plate was washed with wash buffer by first soaking 10 min, and following with two washes by hand. One-hundred microliters of the positive and negative controls were added to each well in duplicate. Two positive controls were run during the optimization: one consisted of pooled cat sera that was PK test positive on day 28 post-sensitization, and the other consisted of pooled cat sera taken from cats in another experimental asthma study at 6 months post-BGA sensitization. The negative controls consisted of pooled cat sera taken from the latter cats prior to sensitization, as well as wash buffer. All sera were diluted 1:2 in wash buffer. Samples were incubated for 1.5 h at 37 °C, followed by washing as described above. The affinity purified rabbit anti-feline IgE

was diluted at 1:50, 1:100, and 1:200 in wash buffer, and 100 µl of each dilution was added to a set of positive and negative control samples for 1 h at 37 °C. The plate was washed, and a 1:10,000 dilution was made of goat anti-rabbit horse-radish peroxidase H/L chain (Bethyl Laboratories, Montgomery, TX), and 100 µl was added to each well. After a 1 h incubation at 37 °C, the plates were washed as previously. Substrate, which consisted of *o*-phenylenediamine (Sigma, St. Louis, MO) at 10 mg/10 ml of citrate buffer pH 4.5 and 4 µl of hydrogen peroxide, was added at 100 µl/well and incubated for 15 min. After substrate addition and incubation, the plates were read at dual wavelengths of 450–650 nm on an ELISA plate reader (UV max kinetic microplate reader model 0200-0340, Molecular Devices, Menlo Park, CA) every 5 min for 30 min to determine the optimal time of substrate incubation. The OD for each well was recorded.

Dilution of rabbit anti-feline IgE was chosen according to that consistently yielding the highest positive control to negative control ratio (P:N ratio). The dilution was selected as 1:100 which demonstrated a P:N ratio of 24.6 for the PK+ pooled cat sera and 16 for the other positive control pooled cat sera. The optimal time of substrate incubation was 20 min. Results of the ELISA were reported by first subtracting the OD of the PBS/Tween 0.2% well (background). The intra-assay coefficient of variance was 4.1%. Samples were then expressed as a percentage of the positive control run on the same plate.

Serum samples from an additional five mixed breed cats taken prior to sensitization (day 0), after parental sensitization (day 28), and after aerosol challenge (day 50) were diluted 1:2 in wash buffer and used in the optimized ELISA. The positive control consisted of pooled cat sera taken from cats in another experimental asthma study at 6 months post-BGA sensitization, and the negative controls consisted of pooled cat sera taken from those same cats prior to sensitization, as well as wash buffer.

## 2.10. Preferential precipitation of serum samples with SAS

To determine if feline BGA specific non-IgE antibodies might be causing interference in the ELISA,

positive and negative control pooled samples at a dilution of 1:2 underwent precipitation with SAS. Feline IgG can be purified from 33.3% ammonium precipitates (Gilbert and Halliwell, 1998b). Into labeled microcentrifuge tubes, 300  $\mu$ l of sera was added to appropriate amounts of 0.85% saline and SAS, so that final ammonium sulfate concentrations of 25, 27.5, 30, 32.5 and 35% were reached. One drop of 1.0 N NaOH was added to each tube, and each tube was vortexed for 15–20 s. Tubes were allowed to incubate at room temperature, and were vortexed every 15 min. Samples were centrifuged at  $10,000 \times g$  for 10 min, and the supernatants were transferred to new tubes and stored at 4 °C overnight. The pellets, which contained various amounts of precipitated immunoglobulins, were discarded.

### 2.11. Data analysis

Repeated measurements of serum IgE were analyzed by using commercially available software (SAS Analyst Repeated Measures ANOVA, SAS version 8.02, SAS Institute, Cary, NC) to fit a mixed linear model. Tukey's procedure was used to make post-hoc comparisons of the mean IgE concentrations obtained at the different time points while controlling the overall error rate at 5%.

## 3. Results

### 3.1. IDST and PK tests in six cats donating serum for IgE purification

Prior to sensitization with HDMA or BGA, all cats had negative skin reactions to intradermally injected allergen (Table 1a). After parenteral sensitization, all cats showed reactivity to respective allergen (Table 1b). Formation of wheals in response to histamine and serotonin was variable prior to and after sensitization with allergen, although all cats had a positive skin reaction to either histamine or serotonin. Results of the PK tests showed positive blue wheals in the recipient cats at 48 h after intradermal injection of the serum collected on day 28 from all six sensitized cats (Table 2a and b). Intradermal injection of heat inactivated serum from each donor cat abolished the skin reactivity.

Table 1

Size of wheals (measured in cm) from six cats having IDST (a) prior to parenteral sensitization; (b) on day 28 of parenteral sensitization

| Cat number                                | Histamine        | Serotonin        | Saline           | Allergen         |
|-------------------------------------------|------------------|------------------|------------------|------------------|
| (a) Prior to parenteral sensitization     |                  |                  |                  |                  |
| 1 <sup>a</sup>                            | 0                | 1.3 $\times$ 1.4 | 0                | 0                |
| 2 <sup>a</sup>                            | 0                | 1.0 $\times$ 1.3 | 0                | 0                |
| 3 <sup>a</sup>                            | 1.0 $\times$ 1.0 | 0                | 0                | 0                |
| 4 <sup>b</sup>                            | 1.0 $\times$ 1.0 | 0                | 0                | 0                |
| 5 <sup>b</sup>                            | 1.0 $\times$ 1.0 | 0.9 $\times$ 1.2 | 0                | 0                |
| 6 <sup>b</sup>                            | 1.1 $\times$ 1.2 | 0                | 0                | 0                |
| (b) On day 28 of parenteral sensitization |                  |                  |                  |                  |
| 1 <sup>a</sup>                            | 1.2 $\times$ 1.5 | 0                | 0                | 1.0 $\times$ 0.9 |
| 2 <sup>a</sup>                            | 1.5 $\times$ 1.5 | 1.7 $\times$ 1.9 | 0                | 1.5 $\times$ 1.6 |
| 3 <sup>a</sup>                            | 2.0 $\times$ 1.8 | 1.6 $\times$ 1.6 | 0                | 1.2 $\times$ 1.4 |
| 4 <sup>b</sup>                            | 1.6 $\times$ 1.7 | 1.9 $\times$ 1.6 | 1.0 $\times$ 0.9 | 1.7 $\times$ 1.6 |
| 5 <sup>b</sup>                            | 1.5 $\times$ 1.2 | 1.8 $\times$ 2.0 | 0                | 1.0 $\times$ 1.0 |
| 6 <sup>b</sup>                            | 1.5 $\times$ 1.8 | 1.6 $\times$ 1.8 | 0                | 1.3 $\times$ 1.2 |

<sup>a</sup> Sensitized to HDMA.

<sup>b</sup> Sensitized to BGA.

### 3.2. IEP and PK testing on purified feline IgE

Normal cat sera reacted with arcs in the expected regions for IgG, IgM and IgA. Purified cat IgE reacted only with mouse anti-feline IgG, IgM and IgA with a small arc in the region consistent with IgE (likely a result of reaction to light chains), and failed to react with the isotype specific reagents (data not shown).

Purified IgE was used in a PK test on a non-sensitized recipient cat, and the size of the wheals

Table 2

Results of PK testing in cats sensitized to (a) HDMA and (b) BGA. Size of wheals on the recipient (non-sensitized cat) are measured in cm

| Cat number            | Undiluted serum  | 1:4 Diluted serum |
|-----------------------|------------------|-------------------|
| (a) HDMA <sup>a</sup> |                  |                   |
| 1                     | 1.0 $\times$ 1.0 | 0.8 $\times$ 0.8  |
| 2                     | 1.2 $\times$ 0.9 | 1.0 $\times$ 0.8  |
| 3                     | 2.0 $\times$ 1.8 | 0.9 $\times$ 0.8  |
| (b) BGA <sup>b</sup>  |                  |                   |
| 4                     | 1.2 $\times$ 1.2 | 0.7 $\times$ 1.0  |
| 5                     | 1.9 $\times$ 1.8 | 1.5 $\times$ 1.4  |
| 6                     | 1.6 $\times$ 1.5 | 1.2 $\times$ 1.2  |

<sup>a</sup> Histamine 1.9 cm  $\times$  1.0 cm; serotonin 1.2 cm  $\times$  0.9 cm; PBS 0 cm  $\times$  0 cm. Heat inactivated serum showed no reactivity.

<sup>b</sup> Histamine 1.6 cm  $\times$  1.6 cm; serotonin 1.6 cm  $\times$  0.6 cm; PBS 0 cm  $\times$  0 cm. Heat inactivated serum showed no reactivity.

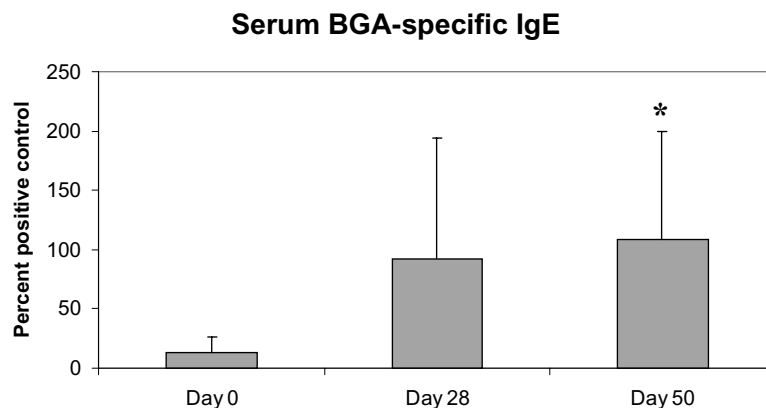


Fig. 1. The group mean  $\pm$  S.E.M. for BGA-specific IgE expressed as the percentage of the positive control in five cats with experimentally induced asthma at baseline (day 0), after parenteral sensitization (day 28), and after aerosol sensitization and challenge (day 50) using polyclonal rabbit anti-feline IgE antisera in an ELISA.

in centimeters were: purified IgE,  $1.7 \times 1.7$ ; heat-inactivated purified IgE,  $0 \times 0$ ; histamine,  $1.5 \times 1.3$ ; and saline  $0 \times 0$ .

### 3.3. RCA using rabbit anti-feline IgE

All three recipient cats used for RCA testing had intradermal wheals form in response to injections of rabbit anti-feline IgE antisera. None of the cats had a wheal form in response to saline or the rabbit serum obtained prior to immunization with purified feline IgE. The size of the wheals formed in response to histamine were  $1.0 \text{ cm} \times 1.8 \text{ cm}$ ,  $1.0 \text{ cm} \times 1.0 \text{ cm}$ , and  $1.1 \text{ cm} \times 1.0 \text{ cm}$ , and the size of the wheals formed in response to the 1:2 diluted polyclonal rabbit anti-cat IgE antisera were  $0.7 \text{ cm} \times 0.8 \text{ cm}$ ,  $1.0 \text{ cm} \times 1.0 \text{ cm}$ , and  $0.8 \text{ cm} \times 1.0 \text{ cm}$ , respectively.

### 3.4. Preferential precipitation of serum samples with SAS

Samples were assayed in duplicate for BGA-specific IgE by the ELISA technique described above. The mean ODs for final SAS concentrations from 25 to 35% from positive pooled samples were 0.241, 0.215, 0.232, 0.201, and 0.218; the mean OD from the positive pooled sample (uncut with SAS) was 0.227. Similarly, the mean ODs for final SAS concentrations from 25 to 35% from negative pooled samples were 0.065, 0.078, 0.071, 0.076, and 0.072;

the mean OD from the negative pooled sample (uncut with SAS) was 0.062.

### 3.5. BGA-specific IgE levels in sensitized and challenged cats

The BGA-specific IgE ELISA was used to evaluate serum samples from five cats prior to sensitization with BGA (day 0), after parenteral sensitization (day 28), and after aerosol challenge (day 50). The group mean percentage of the positive control  $\pm$  S.E.M. was  $12.9 \pm 13.6$  for day 0,  $92.5 \pm 101.6$  for day 28, and  $108.8 \pm 91.1$  for day 50. There was a statistically significant increase in the group mean OD on day 28 ( $P = 0.04$ ) and day 50 ( $P = 0.01$ ) compared with day 0 (Fig. 1).

## 4. Discussion

Allergic asthma results from polarization of T-lymphocyte responses towards Th2-cells, which subsequently produce an array of cytokines that regulate IgE production (Holgate, 1999). Immunoglobulin E levels in the serum are usually present in very small quantities, as these antibodies are effectively captured and removed from circulation by high affinity Fc $\epsilon$  receptors (Fc $\epsilon$ RI) bound to mast cells, basophils and activated eosinophils (Janeway et al., 2001). Immunoglobulin E, when cross-linked by allergen, leads to

release of mediators responsible for bronchoconstriction, inflammatory cell influx, and mucus hypersecretion in sensitized patients. In the current study, cats sensitized with house dust mite or BGA developed ample IgE in the serum for purification of IgE by gel filtration and affinity chromatography. The resultant IgE, evaluated for purity by IEP, was used to develop polyclonal rabbit anti-feline IgE antisera and was subsequently validated for use in an allergen-specific ELISA. The ability to detect and monitor changes in allergen-specific IgE is helpful for identifying cats with preexisting sensitization, discriminating high versus low responder cats (as genetic differences are hypothesized to contribute to variation in individual cat IgE responses (Gilbert and Halliwell, 1998a)), and correlating serum allergen-specific IgE levels with other immunologic parameters.

Atopy is the inherited predisposition to form IgE in response to allergen (Wassom, 1997). The atopic state is commonly recognized by employing IDST to evaluate for the presence of allergen-specific IgE fixed to mast cells in the dermis (Cookson, 1999). Prior to entry in the study, all cats had negative skin tests to their respective allergen, indicating they had not been previously sensitized to HDMA or BGA. Both allergens have been implicated in the pathogenesis of asthma in humans (Holgate, 1999; Cookson, 1999; Tournoy et al., 2000). After undergoing parenteral sensitization, the six cats used to purify IgE developed positive skin reactions to allergen (Table 1b). Since IDST do not provide information about allergen-specific IgE antibodies in the serum, PK tests were employed. PK tests were chosen since there currently are no commercially available polyclonal or monoclonal antibodies specific for feline IgE. The commercial assay that can detect feline IgE uses an Fcε receptor to bind to the feline IgE antibody, but does not differentiate between IgE and homocytotropic IgG (Wassom, 1997). Immunoglobulin E in the cat, like in other species, is heat sensitive (Powell et al., 1980). Heating sera does not inactivate other immunoglobulin classes, allowing for differentiation between IgE and homocytotropic IgG (Gilbert and Halliwell, 1998a). Heating the sera of the sensitized cats in this study abolished the PK reactivity (Table 2a and b), proving the cats sensitized to HDMA or BGA produced allergen-specific IgE in their serum. PK tests are impractical to perform on large number of serum

samples because of the requirement for non-sensitized recipient cats, which made validation of an allergen-specific ELISA appealing. Additionally, PK tests have not always resulted in development of a detectable IgE response in sensitized cats, perhaps because the IgE isoform produced either does not effectively bind mast cells, or trigger their degranulation (Gilbert and Halliwell, 1998b).

In cats with allergic disease, correlation of allergen-specific ELISA with IDST has been often been poor (Foster and O'Dair, 1993; Gilbert and Halliwell, 1998a). This disparity is not unexpected, given that the two tests are measuring different forms of IgE. Allergen-specific ELISAs detect circulating IgE in the serum, and these serum antibodies have a short half-life (2–3 days). The half-life of IgE is substantially longer (months) when bound to FcεRI on mast cells in the dermis; cell bound IgE serves as the basis for the IDST (Janeway et al., 2001). Additionally, it has been speculated that there is heterogeneity in feline IgE in terms of its ability to bind mast cell receptors and carry out effector functions (i.e., degranulation) (Gilbert and Halliwell, 1998a). Serum-based “allergy” tests are not completely sensitive or specific for the diagnosis of hypersensitivities in atopic animals see De Boer and Hillier (2001). Correlation of immunologic, physiologic and histologic parameters with both allergen-specific ELISA and IDST in the feline asthma model deserves further study.

To our knowledge, the use of serotonin for IDST in cats has not been previously reported. In humans and non-human primates, histamine is the primary mediator of type I hypersensitivity, but in rats and mice, the primary mediator is serotonin (Dvorak et al., 1983; Schelegle et al., 2001). We chose to evaluate serotonin as a positive control based on a previous study implicating serotonin as an important mediator of type I hypersensitivity in *Ascaris suum*-sensitized airways of cats (Padrid et al., 1995a). It was interesting to note that prior to allergen sensitization, two cats that failed to develop reactivity to histamine did develop positive skin reactions to serotonin (Table 1a). It is unknown why after sensitization the size of the wheals in response to histamine and serotonin increased, and why five cats developed wheals to both positive controls (compared with one cat prior to sensitization). It is possible that sensitization induces an upregulation of receptors for these mediators on blood vessels.

Diagnostic tests using polyclonal antisera for detection of IgE have been criticized for their lack of specificity because of recognition of allergen-specific IgG antibodies along with allergen-specific IgE antibodies (Hammerling and deWeck, 1998). In this study, the polyclonal rabbit antisera were made more specific for feline IgE by affinity purification using a column bound with feline IgG H + L chains, and subsequently checked for disappearance of reactivity against feline IgG using IEP. The possibility that the rabbit antisera partially recognized allergen-specific IgG antibodies causing interference in the ELISA was tested by preferential precipitation of serum samples with SAS. A previous study has shown feline IgG can be purified from 33.3% ammonium precipitates (Gilbert and Halliwell, 1998b). Final ammonium sulfate concentrations of 25, 27.5, 30, 32.5 and 35% were reached prior to running the serum samples in the ELISA. No significant differences were noted in the final OD readings for any of the serum samples with precipitation of the IgG component. As further evidence that the polyclonal rabbit anti-feline IgE antisera were capable of recognizing feline IgE, the functional activity was evaluated in RCA tests performed on three different cats. In all cats, the polyclonal antisera caused skin reactivity as the anti-feline IgE antibodies cross-linked IgE bound to mast cells in the dermis.

The utility of the allergen-specific ELISA was demonstrated in the five cats undergoing sensitization to BGA. As predicted, cats had negligible amounts of IgE at baseline, and showed an increase in serum BGA-specific IgE over time with BGA sensitization. Future applications for this ELISA in the asthmatic cat model include assessment of the dynamics of serum IgE production, as well as the efficacy of drug therapy and immunomodulation on serum IgE levels.

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