



Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm



Research paper

Comparison of intradermal skin testing (IDST) and serum allergen-specific IgE determination in an experimental model of feline asthma

Tekla M. Lee-Fowler^a, Leah A. Cohn^a, Amy E. DeClue^a, Christine M. Spinka^b,
Ryan D. Ellebracht^b, Carol R. Reinero^{a,*}

^a Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, 900 East Campus Drive, Columbia, MO 65211, USA

^b Department of Statistics, College of Arts and Sciences, University of Missouri, 146 Middlebush Hall, Columbia, MO 65211, USA

ARTICLE INFO

Keywords:

Allergy
Cat
Antibody
Animal model

ABSTRACT

Intradermal skin testing (IDST) and allergen-specific IgE determination are used to determine allergen sensitization. In cats, studies have found poor correlation between the two tests. However, these studies were mainly conducted in pet cats sensitized to unknown allergens with unknown dose and duration of exposure. We hypothesized that in an experimental model of allergic sensitization where these variables are controlled, IDST would demonstrate greater sensitivity and specificity than would serum allergen-specific IgE determination.

A model of feline asthma employing Bermuda grass allergen (BGA) or house dust mite allergen (HDMA) was used to test the hypothesis. Thirteen cats were assigned to undergo sensitization to BGA, HDMA or saline (placebo). Bronchoalveolar lavage fluid confirmed development of an asthmatic phenotype. Serum collection and IDST were performed on D0, D28 and D50. A portion of serum was pooled, and an aliquot heat inactivated (HI) to destroy IgE. Individual, pooled, and pooled HI samples were used for allergen-specific IgE determination using an FcεR1α-based ELISA; pooled samples were also analyzed using an enzymeimmunoassay. Sensitivity (SE), specificity (SP), and positive and negative predictive values (PPV and NPV) were calculated for IDST and for BGA- and HDMA-specific IgE.

Combined results for IDST found SE = 90.9%, SP = 86.7%, PPV = 83.3%, and NPV = 92.9%. For ELISA-based serum IgE testing, the SE = 22.7%, SP = 100%, PPV = 100% and NPV = 63.8%. The enzymeimmunoassay did not detect sensitizing IgE, but did detect IgE reactivity to a variety of irrelevant allergens (even in HI samples).

Sensitivity of IDST was greater than sensitivity of serum IgE measurement supporting use as a screening test for aeroallergens. Both IDST and allergen-specific IgE determination via ELISA were specific; either test can be used to guide selection of allergens for immunotherapy. The enzymeimmunoassay was unreliable and cannot be recommended.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Allergy is an immune-mediated inflammatory response to what should be harmless environmental allergens and is driven by activation of T helper 2 (Th2)

* Corresponding author. Tel.: +1 573 882 7821; fax: +1 573 884 7563.
E-mail address: reinero@missouri.edu (C.R. Reinero).

lymphocytes which produce IgE antibodies. Allergen-specific immunotherapy is the only treatment currently available with the potential to cure allergic disease and is a viable treatment for allergic skin disease in dogs and cats (Olivry and Sousa, 2001; Trimmer et al., 2005, 2006). In human medicine, immunotherapy has also been utilized as a clinical treatment for allergic asthma (Holgate and Polosa, 2008; Leatherman, 2008). Our laboratory has demonstrated that the use of an abbreviated (rush) allergen-specific immunotherapy protocol is effective in blunting eosinophilic airway inflammation in experimentally induced feline allergic asthma (Reinero et al., 2006, 2008; Lee-Fowler et al., 2009). As these studies have demonstrated promising results, it would be ideal to translate this therapy to pet cats with naturally occurring disease. It is first essential to determine to which aeroallergens the patient has been sensitized. Two tests, intradermal skin test (IDST) and allergen-specific serum IgE concentration measurements are routinely used to determine allergen sensitization in veterinary patients. These tests have been previously evaluated in multiple studies, and poor correlation between results of these tests has been demonstrated (Foster, 1993; Halliwell et al., 1998; Wassom and Grieve, 1998; Moriello et al., 2007). However, almost all studies have been performed in pet cats with unknown allergen sensitization, duration of exposure, and dose of exposure (Gilbert and Halliwell, 1998a,b). The studies in which research cats were injected with allergen in alum to induce allergen-specific IgE were not the models of any naturally occurring disease (Gilbert and Halliwell, 1998a,b), but they did confirm discordant results between skin and serum testing for allergens.

In allergic asthma, allergens are inhaled through the respiratory tract and induce local Th2 immunity driving IgE production. Using an experimental model of feline asthma in which the number and type of allergens as well as exposure (challenge) protocols are carefully controlled will be useful in determining the sensitivity and specificity of IDST and serum IgE concentrations for this disease. We hypothesized that in an experimental model of allergic sensitization where these variables were controlled, IDST would demonstrate greater sensitivity and specificity than would serum allergen-specific IgE determination.

2. Materials and methods

2.1. Animals

Thirteen purpose bred cats (nine intact females and four intact males) from a high responder asthmatic cat colony at the University of Missouri were used in the study. At the time of enrollment into the study, cats were 3 months of age. One additional non-sensitized cat was obtained from a commercial vendor (Liberty Research, Waverly, New York) for Prausnitz–Kustner testing. The study was conducted with the approval of the University of Missouri Animal Care and Use Committee and cats were cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Allergen sensitization

Cats were randomly assigned to one of three groups prior to the start of the study for allergen sensitization (Greer laboratories, Lenoir, NC): Bermuda grass allergen (BGA; $n = 7$), house dust mite allergen (HDMA; $n = 4$), or placebo (phosphate buffered saline; $n = 2$). Allergen sensitization was performed as previously described (Reinero et al., 2006). Cats were evaluated by intradermal skin test (IDST) on D0 to ensure that they were naive to the allergens of interest; they also had collection of bronchoalveolar lavage fluid (BALF) to confirm absence of preexisting eosinophilic airway inflammation. Cats then received subcutaneous injections of either BGA (12 μ g of BGA in 10 mg of alum), HDMA (12 μ g of HDMA in 10 mg of alum), or placebo (PBS in 10 mg alum) and 10^7 killed *Bordetella pertussis* organisms intramuscularly according to group assignment. On D14, the cats were administered an intranasal solution containing 75 μ g of each respective allergen in 0.2 ml PBS or placebo (0.2 ml PBS).

After D28, local delivery of allergen to the airway was performed using a series of seven aerosol challenges with BGA, HDMA or PBS (according to group assignment) with the last aerosol challenge on D48. Challenges occurred approximately every 3 days with the last aerosol challenge on D48. Allergen was delivered via a nebulizer to conscious, spontaneously breathing cats kept in chamber for the duration of aerosol delivery (Norris Reinero et al., 2004). Different chambers and tubing were used for each allergen or placebo solution. Cats in the BGA or HDMA groups received 125 μ g of allergen in 5 ml PBS, and cats receiving placebo were administered 5 ml of PBS.

2.3. Bronchoalveolar lavage (BAL)

Bronchoalveolar lavage fluid (BALF) was collected on D0 prior to allergen sensitization, and D50 after allergen sensitization using a blind technique. Cats were anesthetized using ketamine HCl (5–10 mg/kg IV; Fort Dodge Animal Health, Fort Dodge, IA) and intubated. An 8-french red rubber catheter was introduced and advanced through the endotracheal tube lumen until the catheter became wedged. An aliquot of 20 ml of sterile saline was instilled through the catheter, and the sample was immediately aspirated using gentle hand suction. Samples were placed on ice until the time of processing (within 2 h). Cytospin slides were prepared via cytocentrifugation (Shandon Cytospin 4, ThermoElectron Corporation, Waltham, MA), and 200 cell count differentials were performed.

2.4. Intradermal skin testing (IDST)

Intradermal skin tests were performed on D0, D28, and D50. Reconstituted lyophilized BGA (2.78 mg/ml) and HDMA (1.35 mg/ml) were used for skin testing. The allergens of interest (1:20 dilution of BGA and HDMA), a positive control (histamine, 1:10 dilution), and negative control (saline) were included in each skin test. A volume of 0.1 ml was injected intradermally for each agent. Wheals were measured in two dimensions after 20 min. A positive test result was recorded when a measurement was equal to

or greater than half the difference of the positive and negative control.

2.5. Serum allergen-specific IgE determination

Blood was collected into red top tubes without additives on D0, D28 and D50. The tubes were kept at room temperature for at least 1 h to allow for clotting. The samples were centrifuged at $1720 \times g$ for 20 min and serum harvested and banked in multiple aliquots at -20°C ; labeling of all samples was double checked. Coded samples were sent to two commercial laboratories unaware of the animal's sensitization for the determination of allergen-specific IgE. In the first commercial laboratory, ELISA technology using an alpha chain of the high-affinity mast cell receptor for IgE (Fc ϵ R1 α) was used to test reactivity against 48 different regional allergens (ALLERCEPTTM, Heska Laboratories, Loveland, CO) (Wasom and Grieve, 1998; Stedman et al., 2001; Taglinger et al., 2005). Minimum sample volume required was 2 ml of serum. Results were reported in EA units where a value >150 is considered significant provided it correlates with clinical signs. The second commercial laboratory used a liquid phase enzymeimmunoassay testing (VARL Liquid Gold, Veterinary Allergy Reference Laboratories (VARL), Pasadena, CA) (Alaba, 1997) to determine allergen-specific IgE against 40 regional allergens. Minimum sample volume required was 3 ml of serum. Results of this test were reported as classes 0–6; results in classes 3–6 are considered to have “high, very high or ultra high levels” of IgE.

Both individual aliquots of serum and samples pooled from all cats in each sensitization group were stored for IgE assays. Serum from individual cats within the same allergen sensitization group was pooled (i.e., PBGA and PHDMA). The pooled samples were split into two aliquots and one aliquot was heat inactivated to selectively destroy IgE (PBGA HI and PHDMA HI). Heat inactivation was performed by placing serum in a water bath (Isotemp 210, Fischer Scientific, Waltham, MA) at 56°C for 1 h. Pooled serum samples were further divided into 3 aliquots each for a total of 12 aliquots. One of the aliquots from each pooled sample (PBGA, PHDMA, PBGA HI and PHDMA HI) was submitted for enzymeimmunoassay. Another of the aliquots from each pooled sample was submitted for ELISA determination of IgE. The final aliquot of the pooled serum was used to conduct the Prausnitz–Kustner testing as described below. Additionally, individual aliquots of serum from each cat were submitted for ELISA measurement of IgE.

2.6. Prausnitz–Kustner (PK) testing

Prausnitz–Kustner testing was performed as previously described (Gilbert and Halliwell, 1998b,a; Norris et al., 2003). A naive, adult cat not sensitized to either allergen was sedated with ketamine HCl (5–10 mg/kg IV; Fort Dodge Animal Health, Fort Dodge, IA). The right lateral thoracic wall was clipped, and a grid (4 squares $\times$ 2 squares) was outlined on the skin using indelible ink. In the center of each square on the top row of the grid, undiluted,

pooled serum from the allergen sensitized cats was injected intradermally (0.1 ml of serum) so that each square contained PBGA, PHDMA, PBGA HI, or PHDMA HI. After 48 h, the cat was again sedated, and 0.1 ml of the respective allergen (BGA for PBGA and PBGA HI squares; HDMA for PHDMA and PHDMA HI squares) was injected intradermally directly over the area where the serum had been injected 48 h prior. Concurrently, the bottom of the grid was used for control samples with histamine (positive control; 1:10), saline (negative control), BGA (1:20), and HDMA (1:20) injected intradermally at a volume of 0.1 ml into the center of one square each. The allergens injected on the bottom of the grid were used to confirm that the cat was not naturally sensitized to either allergen. Results were interpreted in the same fashion as for IDST described above.

2.7. Statistical analysis

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for IDST and allergen-specific serum IgE testing at both D28 and D50 time points. These values were determined for each allergen individually and the allergens combined. Sensitivity was calculated as the number of cats sensitized to a particular allergen that were positive for that allergen divided by the total number of cats that were sensitized to that allergen. Specificity was calculated as the number of cats not sensitized to the allergen of interest (cats sensitized to the other allergen or placebo sensitized) that had a negative test result divided by the total number of cats that were not sensitized to the allergen of interest. Positive predictive value was calculated as the number of cats sensitized to the allergen of interest that had positive test results divided by the total number of cats with positive test results. Negative predictive value was calculated as the number of cats not sensitized to the allergen of interest that had negative test results divided by the total number of cats with negative test results. The larger serum volume required for enzymeimmunoassay precluded measurement of allergen-specific IgE in individual cats; for this reason sensitivity, specificity, positive predictive value, and negative predictive value could not be calculated. Instead, results of both enzymeimmunoassay and Prausnitz–Kustner testing on pooled sera are presented in a descriptive fashion. Similarly, pooled samples subjected to ELISA measurement of IgE are described.

3. Results

3.1. Bronchoalveolar lavage (BAL)

Evaluation of BALF at D0 revealed that none of the 13 animals in this study had $>10\%$ eosinophils (group mean eosinophil $\pm$ SEM%; BGA, $3.3 \pm 1.2\%$; HDMA, $3.0 \pm 0.7\%$; placebo, $1.5 \pm 1.5\%$). At D50 after the series of allergen challenges by aerosol, cats sensitized to BGA or HDMA had marked increases in the BALF eosinophil percentage compatible with the induction of experimental asthma (mean eosinophil $\pm$ SEM%; BGA, $36.5 \pm 7.3\%$; HDMA, $46.5 \pm 14.6\%$).

Table 1

Results of IDST on D28 and D50.

	BGA positive		BGA negative		HDMA positive		HDMA negative	
	D28	D50	D28	D50	D28	D50	D28	D50
BGA sensitized (<i>n</i> = 7)	7	7	0	0	0	1	7	6
HDMA sensitized (<i>n</i> = 4)	0	3	4	1	4	3	0	1
Placebo control (<i>n</i> = 2)	0	0	2	2	0	0	2	2

Table 2

Sensitivity, specificity, positive predictive value and negative predictive value of IDST in sensitized cats.

	Sensitivity	Specificity	PPV	NPV
BGA				
D28	100%	100%	100%	100%
D50	100%	50%	70%	100%
HDMA				
D28	100%	100%	100%	100%
D50	50%	88.9%	66.7%	80%
D28 + D50				
BGA	100%	75%	82.4%	100%
HDMA	75%	94.4%	85.7%	89.5%
Average (both times)	90.9%	86.7%	83.3%	92.9%

Experimental asthma was not induced in the group exposed to placebo in place of allergen (mean eosinophil $\pm$ SEM%; placebo, $0.5 \pm 0.5\%$).

3.2. Intradermal skin testing (IDST)

Prior to allergen sensitization (D0), all cats had negative IDST for both BGA and HDMA. Results of IDST were uniformly positive for sensitizing antigen and negative for the non-sensitizing antigen on D28 but not on D50 (Table 1). Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each allergen at each time point (D28 and D50) and for combined results for both allergens (Table 2). Additionally, these same parameters were calculated when both time points were combined, and when both allergens were combined at both time points (Table 2). The IDST was both sensitive and specific.

3.3. BGA- and HDMA-specific serum IgE

Prior to allergen sensitization (D0), all cats were negative by Fc ϵ R1 α -based ELISA technology for both BGA- and HDMA-specific IgE. Table 3 displays results of Fc ϵ R1 α -based ELISA testing for D28 and D50. The sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each allergen

Table 3

Allergen-specific Serum IgE (ELISA) results (HESKA).

	BGA positive		BGA negative		HDMA positive		HDMA negative	
	D28	D50	D28	D50	D28	D50	D28	D50
BGA sensitized (<i>n</i> = 7)	1	1	6	6	0	0	7	7
HDMA sensitized (<i>n</i> = 4)	0	0	4	4	0	3	4	1
Placebo control (<i>n</i> = 2)	0	0	2	2	0	0	2	2

Table 4

Sensitivity, specificity, positive predictive value and negative predictive value of allergen-specific IgE (HESKA) in sensitized cats.

	Sensitivity	Specificity	PPV	NPV
BGA				
D28	14.3%	100%	100%	50%
D50	14.3%	100%	100%	50%
HDMA				
D28	0%	100%	NA	69.2%
D50	75%	100%	100%	90%
BGA + HDMA				
D28	9.1%	100%	100%	60%
D50	36.4%	100%	100%	68.2%
Average(both times)	22.7%	100%	100%	63.8%

NA on D28 = no positive results at that time and should be dividing true positive/all positives.

Table 5

Allergen-specific IgE from pooled and heat inactivated pooled serum samples.

Test	PBGA	PBGA HI	PHDMA	PHDMA HI
PK test	+	–	++ ^a	+
Fc ϵ R1 α -based ELISA ^b	–	–	+	+
Liquid phase ^c	N/A ^d	–	–	–

^a ++ = strong positive (greater than the dimensions of the histamine positive control); + = positive ($\geq 50\%$ of the difference between the positive and negative controls); – = negative ($< 50\%$ of the difference between the positive and negative controls).

^b ALLERCEPT™, Heska Corporation, Loveland, CO.

^c Liquid Gold, VARL, Pasadena, CA.

^d Damaged during shipping and not available for analysis.

and for both allergens combined at each time point (D28 and D50) (Table 4). This assay showed excellent specificity and positive predictive values (100%) for both allergens tested, but did not have high sensitivity or negative predictive values.

Results of BGA- and HDMA-specific IgE determination from pooled samples are presented in Table 5. Prausnitz–Kustner testing documented the presence of both BGA- and HDMA-specific IgE in the pooled samples (i.e., PBGA and PHDMA). Additionally, a positive reaction was seen for PHDMA HI but the size of the wheal in response to the heat inactivated serum was smaller than that for non-heat

inactivated PHDMA suggesting the presence of homocytotropic IgG. This is supported by results of the FcεR1α-based ELISA which can also detect homocytotropic IgG. Although both PHDMA and PHDMA HI were considered positive by ELISA, quantitatively more immunoglobulin was detected in the non-heat inactivated sample than in the heat inactivated sample: PHDMA EA units = 739, PHDMA HI EA units = 241. The PBGA sample sent for enzymeimmuno-metric assay was damaged during shipping. The enzymeimmuno-metric assay did not identify HDMA-specific IgE in the PHDMA sample (class 0 = undetectable). More IgE was detected in the heat inactivated sample (PHDMA HI, class 2, low level IgE) than the non-inactivated sample, although both were interpreted as negative results.

3.4. Other allergen-specific IgE

Full regional allergen panels from the laboratory using FcεR1α-based ELISA technology were available for evaluation in all cats at each time point. Because study cats were born and raised in a closed colony, sensitization to other allergens was not expected. The two cats who received PBS in place of allergen throughout the sensitization and aerosol challenge phases of this study and who were housed in the same room as the allergen sensitized cats did not develop significant IgE (i.e., EA units < 150) against any of the 48 allergens tested in vitro at any time point (D0, D28 or D50). Ten of 11 cats in the BGA or HDMA sensitized groups did not develop significant IgE against any of the 48 allergens tested in vitro at D0 or D28. A single cat had IgE specific for cat epithelium (EA units = 338), white ash (EA units = 301), and yellow dock (EA units = 250) at D28. At D50, this cat had IgE specific for yellow dock (EA units = 492) and Russian thistle (EA units = 356). By D50, IgE cross-reactivity (EA units > 150) to sensitizing allergens was detected in several cats. Four of seven cats sensitized to BGA demonstrated IgE response to other grasses, while three of four cats sensitized to *Dematophagoides farinae* (i.e., HDMA) demonstrated IgE response to *D. pteronyssinus*. Aside from the aforementioned cat with IgE antibodies against yellow dock and Russian thistle, only one additional cat had a positive allergen-specific IgE unrelated at D50 unrelated to the sensitizing allergen (*Cladosporium sphaerospermum*, EA units = 151).

Full regional allergen panels were also available for all analyzed pooled samples from both commercial laboratories. From the laboratory using FcεR1α-based ELISA technology, no other allergen-specific IgE was identified in pooled or heat inactivated samples with the exception of cross-reactivity of *D. farinae* (EA units = 739) with *D. pteronyssinus* (EA units = 321) in the PHDMA sample. From the laboratory using liquid phase enzymeimmuno-metric assay testing, allergen-specific IgE was still detected even in pooled heat inactivated serum samples in which IgE was selectively destroyed. From the PBGA HI and PHDMA HI samples some IgE (class 1 or 2, very low level or low level of IgE) was noted to 13/40 and 15/40 allergens tested, respectively. The PBGA HI group had positive results for maple (class 3), mulberry (class 3) and fusarium (class 3). The PHDMA HI group had positive results for short ragweed (class 3), mulberry (class 4) and cockroach (class

4). Serum from the PHDMA group which had not been heat inactivated was positive for short ragweed (class 3), but negative for mulberry (class 2) and cockroach (class 0); it was also positive for Bahia grass (class 4). As mentioned earlier, serum from the PBGA group was not available for analysis from this laboratory.

3.5. Prausnitz–Kustner (PK) testing

The control IDST verified negative reactions for both allergens confirming that the cat was truly naive to BGA and HDMA. A positive reaction was determined for both pooled BGA and HDMA, and a mild positive reaction was present for pooled HDMA HI. The BGA HI sample elicited a negative IDST response. Measurements of the wheals are provided in Table 4.

4. Discussion

Results of this study demonstrated that IDST had a greater sensitivity and serum IgE determination (using the FcεR1α-based ELISA) had a greater specificity in cats in which BGA or HDMA were used as known sensitizing allergens in an experimental model of asthma. This is critical information if allergen-specific immunotherapy is to be evaluated in pet cats with naturally developing feline asthma. Failure to identify allergens to which a patient has been sensitized or selecting allergens not driving the allergic airway response would each diminish the efficacy of allergen-specific immunotherapy. While allergen-specific immunotherapy has shown promise as a curative treatment for feline asthma in an experimental model, in this model the same allergen (BGA) was used to sensitize and treat the cats (Reinero et al., 2006, 2008; Lee-Fowler et al., 2009). Identification of clinically relevant allergens poses a challenge in pet cats. As has been documented in other studies, the sensitivity of IDST makes it a good screening test, although false positives have been reported as common (Wassom and Grieve, 1998) and could lead to false positive identification of antigens for immunotherapy. On the other hand, FcεR1α-based ELISA tests were less sensitive but more specific. When serum was used for the evaluation of full allergen panels (i.e., not just screening for BGA- and HDMA-specific IgE), identification of IgE specific for allergens other than BGA or HDMA was almost always cross-reactive to the sensitizing antigen (i.e., grasses or mites, respectively). Thus, serum IgE determination by the FcεR1α-based ELISA for the selection of allergen-specific immunotherapy would likely be effective.

Intradermal skin testing has previously been regarded as the “gold standard” of allergy testing, but there has been little data to substantiate this claim in feline patients specifically in regards to aeroallergens. One study evaluated IDST and allergen-specific IgE determination in pet cats with small airway disease, but considering that these cats had an unknown sensitization history, it is impossible to determine test accuracy (Moriello et al., 2007). We proposed to address this issue by sensitizing cats to known allergens and evaluating the tests based on the known sensitization as the gold standard. Bermuda grass allergen and house dust mite allergen were used in this study

because these are common aeroallergens of pet cats and we have previously used these allergens successfully in sensitization protocols (Norris Reinero et al., 2004; Reinero et al., 2006, 2008). One limitation of the current study is that we only performed IDST using BGA and HDMA and not a full panel of allergens. We can thus not draw conclusions about IDST detecting IgE reactivity to other closely related or unrelated allergens.

This report showed that determination of serum allergen-specific IgE is not highly sensitive. This is not surprising in light of the fact that IgE is pulled out of circulation by high-affinity FcεR on the surface of mast cells and basophils. Importantly, the accuracy of serum allergen-specific IgE determination is laboratory and methodology dependent, with one laboratory (using liquid phase enzymeimmunoassay testing) identifying allergen-specific IgE in samples in which IgE had been destroyed by heat inactivation. Heat inactivation spares other immunoglobulin classes besides IgE. The less robust HDMA-specific IgE reactivity in both PK testing and the FcεR1α-based ELISA in heat inactivated samples suggests the presence of homocytotropic IgG, a heat stable form of IgG capable of binding mast cell FcεR1 (Wassom, 1997; Stedman et al., 2001). The laboratory using liquid phase enzymeimmunoassay testing uses a monoclonal antibody specific for feline IgE (O. Alaba, personal communication) which should not detect homocytotropic IgG. It is unclear why so many allergens from this laboratory recognized a low or very low amount of IgE (13/40 PBGA HI and 15/40 PHDMA HI) or high amounts of IgE (3/40 PBGA HI and 3/40 PHDMA HI) in serum in which IgE had selectively been destroyed. While it is possible that very high concentrations of allergen-specific IgE might result in a small amount of IgE remaining even after heat inactivation (Stedman et al., 2001), none of the allergen-specific IgE reported in the pooled samples was directed against BGA or HDMA, the two allergens used in sensitization and repeated aerosol challenge of the cats.

Both IDST and allergen-specific IgE determination have advantages and disadvantages. Intradermal skin testing has the benefit of relatively immediate results and is thought to be more biologically relevant. Drawbacks of IDST include the requirement for sedation, shaving a large portion of hair, and numerous allergen injections which must be kept in stock by the veterinarian. Additionally, certain medications such as glucocorticoids and antihistamines may blunt formation of wheals resulting false negative responses, while contents of some allergen extracts may locally trigger mast cell degranulation and induce wheal formation as a false positive response (Wassom and Grieve, 1998). Allergen-specific serum IgE determination has the advantage of being minimally invasive with only a single blood sample required. Medications such as glucocorticoids and antihistamines are not believed to interfere with this test; however this has not been adequately evaluated in cats. In traditional ELISAs, there is also the potential for other physiologically irrelevant antibodies, such as allergen-specific IgG, to interfere and cause false positive results since IgG is present in serum concentrations that are 10^3 – 10^5 times higher than IgE (Wassom and Grieve, 1998). Use of an

FcεR1α in place of a monoclonal or polyclonal antibody directed against feline IgE has been reported to improve the specificity of this assay as other classes of antibody in the serum (allergen-specific or not) will not bind to this receptor (Wassom and Grieve, 1998).

In conclusion, using an experimental model of feline asthma with known allergen exposure to BGA or HDMA, IDST had a higher sensitivity than serum IgE measurement and could be considered an initial screening test. However, serum IgE determination using an FcεR1α-based ELISA had 100% specificity for BGA and HDMA and would be an excellent test to guide selection of allergens for allergen-specific immunotherapy. Serum IgE determination using liquid phase enzymeimmunoassay testing was unreliable, both failing to identify sensitizing allergens and demonstrating allergen-specific IgE to irrelevant allergens, even in the face of selective IgE destruction by heat inactivation.

Conflict of interest statement

None.

Acknowledgements

This project was funded by a grant from the Morris Animal Foundation with sponsorship by the George Sydney and Phyllis Redmond Miller Trust at the San Francisco Foundation and the University of Missouri Phi Zeta chapter. We would like to thank the HESKA Corporation for providing the FcεR1α serum allergen testing.

References

- Alaba, O., 1997. Allergies in dogs and cats: allergen-specific IgE determination by VARL liquid gold compared with ELISA/RAST. *Veterinary Allergy and Clinical Immunology* 5, 93–101.
- Foster, A.P., O'Dair, H., 1993. Allergy testing for skin disease in the cat in vivo vs in vitro tests. *Veterinary Dermatology* 4, 111–115.
- Gilbert, S., Halliwell, R.E., 1998a. Feline immunoglobulin E: induction of antigen-specific antibody in normal cats and levels in spontaneously allergic cats. *Veterinary Immunology and Immunopathology* 63, 235–252.
- Gilbert, S., Halliwell, R.E., 1998b. Production and characterisation of polyclonal antisera against feline IgE. *Veterinary Immunology and Immunopathology* 63, 223–233.
- Halliwell, R.E.W., Gilbert, S.M., Mei Lian, T., 1998. Induced and spontaneous IgE antibodies to *Dermatophagoides farinae* in dogs and cats: evidence of functional heterogeneity of IgE. *Veterinary Dermatology* 9, 179–184.
- Holgate, S.T., Polosa, R., 2008. Treatment strategies for allergy and asthma. *Nature reviews* 8, 218–230.
- Leatherman, B., 2008. Injection and sublingual immunotherapy in the management of allergies affecting the unified airway. *Otolaryngologic clinics of North America* 41, 359–374 vii.
- Lee-Fowler, T.M., Cohn, L.A., DeClue, A.E., Spinka, C.M., Reinero, C.R., 2009. Evaluation of subcutaneous versus mucosal (intranasal) allergen-specific rush immunotherapy in experimental feline asthma. *Veterinary Immunology and Immunopathology* 129, 49–56.
- Moriello, K.A., Stepien, R.L., Henik, R.A., Wenzholz, L.J., 2007. Pilot study: prevalence of positive aeroallergen reactions in 10 cats with small-airway disease without concurrent skin disease. *Veterinary Dermatology* 18, 94–100.
- Norris, C.R., Decile, K.C., Byerly, J.R., Berghaus, R.D., Schelegle, E.S., Hyde, D.M., Gershwin, L.J., 2003. Production of polyclonal antisera against feline immunoglobulin E and its use in an ELISA in cats with experimentally induced asthma. *Veterinary Immunology and Immunopathology* 96, 149–157.

- Norris Reinero, C.R., Decile, K.C., Berghaus, R.D., Williams, K.J., Leutenegger, C.M., Walby, W.F., Schelegle, E.S., Hyde, D.M., Gershwin, L.J., 2004. An experimental model of allergic asthma in cats sensitized to house dust mite or Bermuda grass allergen. *International Archives of Allergy and Immunology* 135, 117–131.
- Olivry, T., Sousa, C.A., 2001. The ACVD task force on canine atopic dermatitis (XIX): general principles of therapy. *Veterinary Immunology and Immunopathology* 81, 311–316.
- Reinero, C.R., Byerly, J.R., Berghaus, R.D., Berghaus, L.J., Schelegle, E.S., Hyde, D.M., Gershwin, L.J., 2006. Rush immunotherapy in an experimental model of feline allergic asthma. *Veterinary Immunology and Immunopathology* 110, 141–153.
- Reinero, C.R., Cohn, L.A., Delgado, C., Spinka, C.M., Schooley, E.K., Declue, A.E., 2008. Adjuvanted rush immunotherapy using CpG oligodeoxynucleotides in experimental feline allergic asthma. *Veterinary Immunology and Immunopathology* 121, 241–250.
- Stedman, K., Lee, K., Hunter, S., Rivoire, B., McCall, C., Wassom, D., 2001. Measurement of canine IgE using the alpha chain of the human high affinity IgE receptor. *Veterinary Immunology and Immunopathology* 78, 349–355.
- Taglinger, K., Helps, C.R., Day, M.J., Foster, A.P., 2005. Measurement of serum immunoglobulin E (IgE) specific for house dust mite antigens in normal cats and cats with allergic skin disease. *Veterinary Immunology and Immunopathology* 105, 85–93.
- Trimmer, A.M., Griffin, C.E., Boord, M.J., Rosenkrantz, W.S., 2005. Rush allergen specific immunotherapy protocol in feline atopic dermatitis: a pilot study of four cats. *Veterinary Dermatology* 16, 324–329.
- Trimmer, A.M., Griffin, C.E., Rosenkrantz, W.S., 2006. Feline immunotherapy. *Clinical Techniques in Small Animal Practice* 21, 157–161.
- Wassom, D.L., 1997. Principles and history of the Fc epsilon receptor (FcεR1) for IgE detection. *Supplement to Compendium on Continuing Education for the Practicing Veterinarian* 19, 6–7.
- Wassom, D.L., Grieve, R., 1998. In vitro measurement of canine and feline IgE: a review of FcεR1α-based assays for detection of allergen-reactive IgE. *Veterinary Dermatology* 9, 173–178.