

Allergen-specific IgG and IgA in serum and bronchoalveolar lavage fluid in a model of experimental feline asthma

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Accepted 24 June 2003

Abstract

Allergic asthma, a Th2 cell driven response to inhaled allergens, has classically been thought of as predominantly mediated by IgE antibodies. To investigate the role of other immunoglobulin classes (e.g., IgG and IgA) in the immunopathogenesis of allergic asthma, levels of these allergen-specific immunoglobulins were measured in serum and mucosal fluids. Bermuda grass allergen (BGA)-specific IgG and IgA ELISAs in serum and bronchoalveolar lavage fluid (BALF) were developed and optimized in an experimental model of BGA-induced feline asthma. Levels of BGA-specific IgG and IgA significantly increased over time in serum and BALF after allergen sensitization. Additionally, these elevated levels of BGA-specific IgG and IgA were seen in conjunction with the development of an asthmatic phenotype indicated by positive intradermal skin tests, enhanced airways hyperreactivity, and increased eosinophil percentages in the BALF.

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Keywords: Immunoglobulin; Antibody; Pulmonary; Allergy; Cat; ELISA

1. Introduction

Allergic asthma is a Th2 cell driven immune response against what otherwise would be harmless environmental aeroallergens. Characterized by bronchoconstriction and airways inflammation, allergic asthma has classically been described as a type I hypersensitivity reaction mediated by allergen-specific

IgE antibodies. The balance between Th1 and Th2 lymphocyte subsets, with a Th2 cell dominant cytokine profile predominating, favors production of IgE antibodies. Because of the central role IgE plays in asthma pathogenesis, most studies evaluating the role of immunoglobulins in asthma have focused on IgE; however, other immunoglobulin classes are likely to influence the asthmatic phenotype. For example, allergen-specific IgG can neutralize allergen, or downregulate IgE production (Djurup, 1985). On the other hand, one subclass of IgG (e.g., the IgG4 subclass in humans) has actually been shown to exacerbate allergic

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reactions by binding to mast cells and triggering degranulation (Van der Zee and Aalberse, 1991). IgA, most of which is produced at mucosal surfaces, acts locally where it is hypothesized to prevent allergen absorption, thus diminishing the chance for allergic sensitization (Bottcher et al., 2002). The role of IgA in asthma is not well defined; however, in established allergic lung inflammation, IgA is speculated to induce eosinophil degranulation and exacerbate airways inflammation (Arnaboldi and Metzger, 2002). The interactions are complex between effector cells and IgE, IgG, and IgA in allergic asthma, and are likely to be affected by dose of allergen and chronicity of exposure (Djurup, 1985; Van der Zee and Aalberse, 1991).

Feline asthma is a naturally developing condition in pet cats (Dye et al., 1996) and closely mimics human asthma. A model of feline asthma is useful to study the immunopathogenesis of the disease in both species. An experimental model of feline asthma using Bermuda grass allergen (BGA) has been developed, and sensitized cats develop BGA-specific IgE, airways eosinophilia, airways hyperreactivity, and have a Th2 cytokine profile (Norris et al., 2001a,b). To date, no studies in naturally developing or experimentally induced feline asthma have been performed to evaluate either serum or bronchoalveolar lavage fluid (BALF) allergen-specific IgG or IgA levels and to determine if they correspond with the development of the asthmatic phenotype (i.e., as shown by results of intradermal skin tests, airways hyperreactivity measurements, and eosinophil percentages in the BALF). The objective of this study was to evaluate fluctuations in levels of serum and BALF allergen-specific IgG and IgA over the course of allergic sensitization and challenge in cats with experimentally induced asthma.

2. Materials and methods

2.1. Experimental animals

Seven mixed-breed cats aged 1 year were obtained from a University of California, Davis, research cat colony as part of a larger ongoing study on drug therapy for asthma. All cats had normal physical examinations and were FeLV- and FIV-negative.

Cats were housed together in an indoor research facility in a single open room. Prior to entry into the study, cats had intradermal skin testing (IDST) to ensure no previous sensitization to Bermuda grass allergen (BGA; Greer Laboratories, Inc., Lenoir, NC).

2.2. Intradermal skin testing

Cats were sedated with ketamine HCl (5 mg/kg, intramuscularly) for intradermal injections (0.1 ml) of: histamine phosphate (1:100,000), which served as a positive-control; sterile saline, which served as a negative control; and BGA (0.75 mg/ml). 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. Prausnitz-Kustner (PK) tests

A recipient, 8-month old non-sensitized cat was sedated as described for IDST to perform PK tests. A series of square grids measuring approximately 3 cm in diameter were delineated on the skin using a permanent marker and 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 serum, intradermal injection of BGA 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.

2.2.2. Evaluation of airway hyperresponsiveness

Prior to sensitization (day 0), and after BGA sensitization and allergen challenge (week 6), cats were anesthetized with propofol (6 mg/kg iv for induction; 0.2–0.6 mg/kg min constant rate infusion for maintenance anesthesia), incubated, placed in sternal recumbency, and the endotracheal tube was attached to a

blow-by air delivery system set to deliver 100% O₂ at 2 l/min. Cats were allowed to breathe spontaneously throughout the remainder of the testing. The change in outflow was recorded using a Hans–Rudolph pneumotachometer (Series 8300, Hans Rudolph, Inc., Kansas City, MO) attached to a Validyne pressure transducer (model MP45, Validyne Engineering, Northridge, CA). Transpulmonary pressure (PTP) was measured using a Validyne differential pressure transducer (model DP15-26, Validyne Engineering, Northridge, CA) with one port attached to a water-filled cannula placed in the esophagus at mid-chest level and the other port attached to a side port of the pneumotachometer. These analog signals were sent to a Po–Ne–Mah analog-to-digital data acquisition system (Gould Instrument Systems, Valley View, OH).

For bronchoprovocation studies, methacholine (MCh) was used. Aerosol was generated with a low-volume nebulizer (Miniheart[®], Westmed, Inc., Tucson, AZ) and delivered to the blow-by system at 2 l/min. Baseline pulmonary resistance measurements were collected for 4 min after nebulization of a 1 min aerosol of sterile saline. Methacholine challenge was performed by delivering increasing half-log doses of MCh for 1 min starting at a dose of 0.0625 mg/ml, which was followed by 4 min of data collection. The dose-response study was terminated when the airways resistance increased to 200% of the post-saline challenge value (EC200R_L) or the arterial oxygen blood saturation fell to 75% or less.

2.3. Sensitization protocol

Cats underwent parenteral sensitization and aerosol challenge according to a previously described protocol (Norris et al., 2001a). Briefly, cats were administered 12 µg of BGA in alum s.c. and *Bordetella pertussis* 10⁵ organisms i.m. on day 0; 0.2 ml BGA (0.75 mg/ml) intranasally on day 14; and 12 µg of BGA in alum s.c. on day 21. Intradermal skin testing was used to confirm sensitization to BGA on day 28. Aerosol challenge followed parenteral sensitization and consisted of twice weekly treatments for 2 weeks, then weekly thereafter. Allergen challenge was performed in awake and spontaneously breathing cats placed in a sealed plastic chamber. Compressed air at a pressure of 2.93 kg/cm² was supplied via an air compressor (Easy Air 15, Precision Medical, Inc., Northampton,

PA) to a nebulizer to aerosolize the allergen solution in an air flow rate of 9.3 l/min. The polydispersed aerosol from the nebulizer was conveyed to the exposure chamber through 22 mm inside diameter smooth interior flexible tubing (A-M Systems, Inc., Carlsburg, WA). Cats were nebulized with 0.07 mg/ml allergen (diluted in phosphate buffered saline) for 5 min per treatment.

2.3.1. Sample collection

Whole blood was drawn by jugular venipuncture on day 0 (baseline), and days 14, 21, 28, and 60. 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 assay.

Bronchoalveolar lavage fluid was collected on day 0, day 45, and day 60 by sequentially instilling three 10 ml aliquots of warmed 0.9% sterile saline through a gently wedged 7 French polypropylene catheter passed through an endotracheal tube. Lavage samples were placed on ice until processing, which took place within 2 h. Samples were centrifuged at 2000 rpm for 20 min, and the supernatant harvested and banked at –20 °C until the time of assay.

2.4. Optimization of the ELISA

Each ELISA was performed using a 96-well plate (Dynatech Immulon 4, Alexandria, VA) sensitized with Bermuda grass allergen (Greer Laboratories, Lenoir, NC) at 1 µg/well in 100 µl of 1 M 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. Samples of banked cat sera and BALF were run on the same plate as positive- and negative-controls. The positive-control consisted of pooled cat sera or BALF taken from cats in another asthma study at 6 months post-BGA sensitization, and the negative control consisted of pooled cat sera or BALF taken from those same cats prior to sensitization. Wash buffer (phosphate buffered saline (PBS)/Tween 20, 0.2%) was used as an additional negative control. For each type of ELISA developed, optimal concentrations of sera or BALF, and secondary antibody were determined using the aforementioned control sera and BALF. The sera were serially diluted, and the optimal dilution was chosen according to that

which appeared in the linear portion of the curve when plotting the negative log of serum dilution versus optical density (OD). Once the serum dilution had been established, the secondary antibody was serially diluted while keeping the serum dilution fixed. Dilution of secondary antibody was chosen according to that consistently yielding the highest positive-control to negative control ratio (P:N ratio). All 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 Corp., Menlo Park, CA) every 5 min for 30 min to determine the optimal time of substrate incubation.

Sensitized plates were incubated for 1 h at 37 °C with 100 µl per well of rabbit serum albumin (RSA) 1% diluted in wash buffer. Next, the plate was washed with wash buffer by first soaking 10 min, and followed by two washes by hand. Serum or BALF samples were appropriately diluted as described above for each immunoglobulin class with wash buffer and loaded at 100 µl per well in duplicate. The same was done for the pool of positive and negative cat serum. The samples were then incubated at 37 °C for 1–1.5 h, and washed as described above. The secondary antibody (goat anti-cat IgG Fc or goat anti-cat IgA) conjugated with horse-radish peroxidase (Bethyl Laboratories, Inc., Montgomery, TX) was diluted with wash buffer, and 100 µl per well was added to the plate. 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 15 mg/15 ml of 0.1 M citrate buffer pH 4.5 and 6 µl of 30% hydrogen peroxide, was added at 100 µl per well and incubated for 15 min. After substrate addition and incubation, the plates were read at dual wavelengths of 450 and 650 nm on an ELISA plate reader. The optical density (OD) for each well was recorded.

2.5. Serum BGA-specific IgG ELISA

The positive- and negative-control pooled cat sera were serially diluted from 1:10 to 1:3200, and the optimal dilution was chosen as 1:100. The secondary antibody was diluted at 1:50,000, 1:100,000 and 1:150,000 and the dilution of 1:150,000 was selected based on a P:N ratio of 8. The optimal time of substrate incubation was 15 min.

2.6. Bronchoalveolar lavage BGA-specific IgG ELISA

The positive- and negative-control pooled cat BALF was serially diluted from 1:2 to 1:512, and the optimal dilution was chosen as 1:4. The same secondary antibody and dilution as the serum IgG ELISA was used. The optimal time of substrate incubation was 15 min.

2.7. Serum BGA-specific IgA ELISA

The positive- and negative-control pooled cat sera were serially diluted from 1:4 to 1:512. The optimal dilution was chosen as 1:32. The secondary antibody was diluted to 1:50,000, 1:100,000, and 1:150,000 and was selected to be 1:100,000 based on a P:N ratio of 15.5. The optimal time of substrate incubation was 15 min.

2.8. Bronchoalveolar lavage BGA-specific IgA ELISA

The positive- and negative-control pooled cat BALF was serially diluted at 1:10, 1:20, 1:40, 1:60, 1:80, 1:100, 1:150, 1:200, 1:400, 1:800, 1:1600, and 1:3200, and the optimal dilution was chosen as 1:60. The same secondary antibody and dilution as the serum IgG ELISA was used. The optimal time of substrate incubation was 15 min.

2.9. Protein assay

A protein assay (Bio-Rad, Hercules, CA) was performed according to manufacturer's instructions on BALF samples to normalize for total protein content in BALF.

2.9.1. Inhibition assays

The specificity of the methods were evaluated using inhibition assays. Both the positive- and negative-controls (pooled cat sera and BALF) were incubated with BGA at a final concentration of 0, 1, 10, and 100 µg/ml at 37 °C for 1 h, followed by 4 °C overnight. Additional aliquots of the same control samples were also incubated with an irrelevant allergen (house dust mite allergen, HDMA), at a final concentration of 0, 1, 10, and 100 µg/ml in the same manner. The incubated samples were centrifuged at 10,000 × g for

15 min to pellet immune complexes, and the supernatants were analyzed in each of the aforementioned optimized ELISAs (serum BGA-specific IgG, serum BGA-specific IgA, BALF BGA-specific IgG, and BALF BGA-specific IgA).

2.9.2. Data analysis

Paired *t*-tests were used to compare differences in the group means for the ED200R_L and BALF eosinophil percentages at baseline and post-sensitization.

Results of the ELISA were reported by first subtracting the OD of the PBS/Tween 0.2% well (background). To normalize samples run on different plates and on different days, the following equation was used: $OD_{\text{normalized}} = OD_{\text{sample}} \times (OD_{\text{mean positive control}} / OD_{\text{positive control}})$. For BALF samples, the OD of the sample antibody being evaluated (once normalized) was divided by the total protein concentration as determined by the Bio-Rad assay.

For serum BGA-specific IgG and IgA, the group mean $OD_{\text{normalized}}$ was calculated at each time point (days 0, 28, 50, 87, and 180). Similarly, BALF BGA-specific IgG and IgA, had the group mean $OD_{\text{normalized}}$ calculated at each time point (days 0, 45, and 60). Data series were evaluated for normality of distribution and homogeneity of variance; if assumptions of normality were met, data were analyzed by performing a univariate repeated-measures ANOVA using SPSS

statistical software (version 10.0). If the data did not meet the assumptions of normality or equal variance required for parametric testing, they consequently were analyzed using Friedman's analysis of variance by ranks. Post-hoc comparisons were conducted to determine which means were different within each series while limiting the experiment-wise type I error rate to 5%. Pair-wise comparisons for parametrically analyzed data were performed using Tukey's procedure, while those for non-parametrically analyzed data were based on the differences between rank sums (Daniel, 1990).

3. Results

After sensitization with BGA (day 28), all cats demonstrated the presence of BGA-specific IgE antibodies fixed to mast cells in the dermis using intradermal skin testing. Results of the PK tests showed positive blue wheals for the donor serum of all sensitized cats. Intradermally injected heat inactivated serum from each donor cat showed no reactivity. With respect to airways hyperreactivity, the group mean $\pm$ S.E. EC200R_L at baseline (8.8 ± 3.0 mg/ml) was significantly ($P = 0.049$; $n = 7$) higher than after allergen sensitization and challenge (1.7 ± 0.5 mg/ml). The group mean eosinophil percentage at baseline

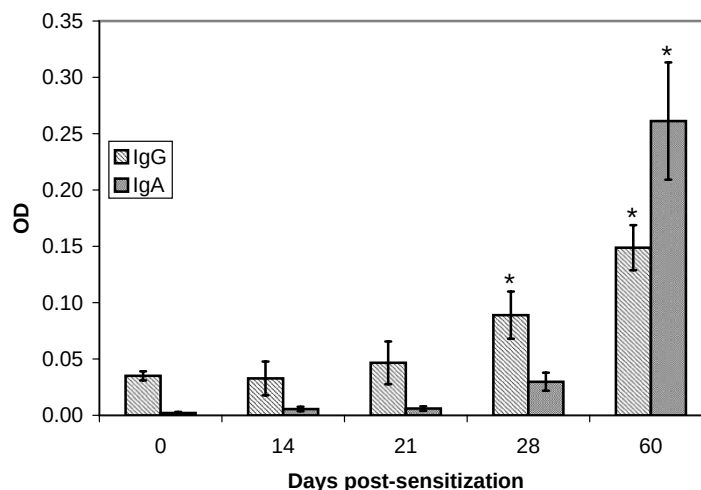


Fig. 1. Group mean $\pm$ S.E. optical densities for serum BGA-specific IgG and IgA over the course of allergic sensitization and challenge in cats with experimentally induced asthma. Means marked with an asterisk (*) are significantly higher than baseline values ($P < 0.05$ over all comparisons).

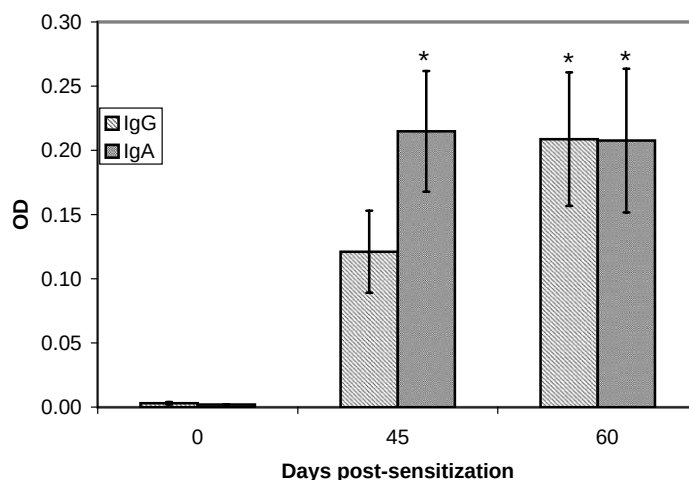


Fig. 2. Group mean $\pm$ S.E. optical densities for BALF BGA-specific IgG and IgA over the course of allergic sensitization and challenge cats with experimentally induced asthma. Means marked with an asterisk (*) are significantly higher than baseline values ($P < 0.05$ over all comparisons).

($4 \pm 1\%$) was significantly ($P = 0.013$; $n = 7$) lower than after sensitization ($32 \pm 10\%$).

Comparisons of group mean OD values for serum BGA-specific IgG and IgA (from the five cats that had measurements at every time point) revealed significant differences over time for both IgG ($P < 0.001$), and IgA ($P < 0.006$) (Fig. 1). Similarly, comparisons of group mean OD values for BGA-specific immunoglobulins in BALF revealed significant differences over time for both BGA-specific IgG ($P < 0.005$), and BGA-specific IgA ($P < 0.008$) (Fig. 2).

The inhibition assays in which control serum and BALF were incubated overnight with a relevant allergen (BGA) or irrelevant allergen (HDMA), prior to analysis using the optimized ELISA, demonstrated the specificity of IgG and IgA for BGA. For the serum BGA-specific IgG ELISA, incubating the pooled positive serum samples overnight with BGA at final concentrations of 1, 10, and 100 $\mu\text{g/ml}$ abolished 24, 59, and 90% of the activity, respectively. Comparatively, incubating with HDMA at final concentrations of 1, 10, and 100 $\mu\text{g/ml}$ for the same samples abolished 0, 0, and 7% of the activity, respectively. For the BALF BGA-specific IgG ELISA, incubating the positive pooled BALF samples overnight with BGA at final concentrations of 1, 10, and 100 $\mu\text{g/ml}$ abolished 34, 87, and 97% of the activity, respectively, whereas incubating with the same concentrations of HDMA abolished 10,

11, and 19% of the activity, respectively. The OD readings of the negative pooled serum and BALF samples at all concentrations were similar to the plate blank (PBS/Tween 0.2%). Results were similar for the serum and BALF BGA-specific IgA ELISA, with BGA final concentrations of 100 $\mu\text{g/ml}$ demonstrating the maximum inhibition at 87 and 100%, respectively.

4. Discussion

In this study, cats with experimentally induced asthma developed systemic and local immune responses to a single allergen, Bermuda grass. The immunopathologic events in allergic asthma are initiated by Th2 cytokines that promote the differentiation, proliferation, and survival of other key effector cells (Foster et al., 2002). The Th2 cytokines lead to IgE production; eosinophil, mast cell, and basophil infiltration; mucus hypersecretion; airway remodeling; and airway hyperreactivity in asthmatics (Foster et al., 2002; Robinson et al., 1993; Romagnani, 2000). The cats in this study developed BGA-specific IgE, which fixed to mast cells in the dermis (as indicated by positive intradermal skin tests and PK tests), airways hyperreactivity (as manifested by a decrease in the EC200R_L), and airways eosinophilia, all compatible with an asthmatic phenotype.

Most studies evaluating the role of immunoglobulins in asthma have focused only on IgE because of its central role in the acute-phase response, where allergen cross linking IgE on mast cells triggers degranulation. The immunologic function of allergen-specific IgG and IgA in the serum and mucosal secretions is less well defined than IgE in allergic asthma. An association between allergen-specific IgE and both IgG and IgA in atopic humans has been described previously (Aalberse, 2000). The cats in this study demonstrated an increase in BGA-specific IgG and IgA levels in both serum and BALF over time with sensitization. These increases in allergen-specific IgG and IgA levels were seen in conjunction with both development of allergen-specific IgE and an asthmatic phenotype, although the specific role they play in the expression of this phenotype is still unclear and will require further research.

Allergen-specific IgG antibodies generated in allergic disease are thought not to be innocent bystanders, but may play either protective or pathogenic roles (Van der Zee and Aalberse, 1991). Certain subclasses of IgG can neutralize allergens before they interact with IgE antibodies and trigger mast cell degranulation; these antibodies have been referred to as “blocking antibodies” (Djurup, 1985; Van der Zee and Aalberse, 1991). Allergen-specific IgG may also inhibit or downregulate IgE production (Djurup, 1985). In a pathogenic role, IgG antibodies may be capable of binding to mast cells and triggering anaphylaxis, and these antibodies are referred to as “homocytotropic” (Van der Zee and Aalberse, 1991). No evidence of homocytotropic antibodies were found in the cats of this study using Prusnitz–Kustner (PK) tests; all positive PK tests were attributed to IgE, as heat inactivation of the serum abolished reactivity.

It is well accepted that different subclasses of IgG have different biological activities such as the ability to fix complement or to bind to Fc receptors on various effector cells. It has been suggested that the production of the various IgG subclasses might be under different control mechanisms (Nahm et al., 1998). For example, IL-4 production may increase both IgE and IgG4 antibodies in humans (Ishizaka et al., 1990). Serial measurement of allergen-specific IgG4 in humans has been proposed to be an indicator of exposure to the particular allergen (Nahm et al., 1998). In contrast to IgG4, IgG1 production may be inversely associated

with synthesis of IgE (Djurup, 1985). In the cat, characterization of IgG subclasses in allergic disease has not been performed to date due to the lack of specific reagents.

IgG and IgA antibody classes may have different effector functions depending on the phase of asthma. Asthma has an early phase consisting of an initial period of bronchoconstriction within 15 min after allergen provocation, and a late-phase reaction that can occur 3–24 h later (Crosby et al., 2002). In a murine model of asthma, the early-phase reaction (EPR) has been associated with allergen-specific IgG via transfer experiments in knockout mice deficient in B or T cells, suggesting the EPR is mediated by allergen-specific IgG but may be IgE independent (Crosby et al., 2002). An association between elevated allergen-specific IgA in BALF and an enhanced pulmonary late-phase reaction (LPR) in asthmatic humans has been reported (Peebles et al., 1998, 2001). This LPR occurs as inflammatory cells migrate into airways after mediators have been released or synthesized during the APR. Eosinophils are important effector cells that migrate into inflamed airways and, in turn, release their own inflammatory mediators upon degranulation. They have surface receptors for IgE (FcεRI), IgA (FcαR), and IgG (FcγRII) (Kaneko et al., 1995; Monteiro et al., 1993; Peebles et al., 1998). While IgE is believed to be involved in eosinophil recruitment to inflamed airways, IgA has been shown to cause eosinophil degranulation and to exacerbate airways inflammation (Arnaboldi and Metzger, 2002; Peebles et al., 1998). Although analysis of the BALF from the cats of this study demonstrated increases in both BGA-specific IgA and eosinophil percentages after sensitization, no attempt was made to look at a marker for eosinophil degranulation (e.g., eosinophil cationic protein) (Peebles et al., 2001).

While IgG is important in systemic immune responses and is in highest concentrations in the serum, the majority of IgA is formed in mucosal tissues and exerts its effects locally. IgA is found in monomeric form in the blood and in inflammatory states can permeate the respiratory epithelial lining to gain access to the airways; it also occurs as a dimer and is produced by mucosal plasma cells. In sensitized patients, high levels of secretory IgA in the airway mucosa could interfere with the binding of IgE with

allergen, ultimately preventing symptoms of asthma (Bottcher et al., 2002). Sampling of total IgA, allergen-specific IgA, and secretory IgA in saliva from children up to 2 years of age, correlated development of allergic symptoms (atopic dermatitis, allergic rhinoconjunctivitis, or asthma) with high levels of total and allergen-specific IgA, but low levels of secretory IgA (Bottcher et al., 2002). High levels of BGA-specific IgA in BALF were seen in conjunction with the development of the asthmatic phenotype in this model of experimental feline asthma.

The measurement of allergen-specific IgG and IgA is likely to have important implications in both research and clinical settings. Trends in these immunoglobulins over time with allergen sensitization may help distinguish research animals that are high versus low responders in experimental models, which is common in outbred (non-rodent) models (Schelegle et al., 2001). Correlating levels of allergen-specific IgG and IgA with allergen-specific IgE may provide insight into differences in the asthmatic phenotype in these outbred species. In a clinical setting, monitoring allergen-specific IgG and IgA in serum or mucosal secretions may be useful in clinical patients undergoing therapy to modulate the allergic response. For example, immunotherapy, the administration of repeated small doses of allergens to sensitized patients, has been employed in patients with inhalant allergies (allergic rhinitis and asthma). Changes in allergen-specific IgG and IgE antibodies in the serum have been described, with the former rising and the latter falling (Djurup, 1985). Allergen-specific IgG and IgA have also been shown to increase in nasal secretions in these patients (Djurup, 1985). In humans, subclasses of allergen-specific IgG antibodies vary in response to seasonal changes of the allergen in the environment (Nahm et al., 1998). Much research remains to be done on the immunopathogenesis of asthma in the cat, and use of allergen-specific ELISAs for IgG and IgA in serum and BALF may help provide answers to etiologic and mechanistic questions in this and other related allergic diseases.

Acknowledgements

This project was supported in part by a grant from the Center for Companion Animal Health, School of

Veterinary Medicine, University of California, Davis; a Postdoctoral training grant in environmental pathology (NIEHS T32 ES 007055-26; Dr. Norris) and the Dr. Vicki Krade Memorial Feline Research Scholarship (Ms. Byerly). The authors would like to thank Londa Berghaus for technical assistance.

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