# Effects of cyproheptadine and cetirizine on eosinophilic airway inflammation in cats with experimentally induced asthma

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**Objective**—To determine whether oral administration of cyproheptadine or cetirizine blocks the action of serotonin and histamine, respectively, and results in diminished eosinophilic airway inflammation in cats with experimentally induced asthma.

**Animals**—9 cats in which asthma was experimentally induced through exposure to Bermuda grass allergen (BGA) during a 3-month period.

**Procedures**—Cats were randomized to receive monotherapy with each of 3 treatments for 1 week: placebo (flour in a gelatin capsule, PO, q 12 h), cyproheptadine (8 mg, PO, q 12 h), or cetirizine (5 mg, PO, q 12 h). A 1-week washout period was allowed to elapse between treatments. Prior to and following each 1-week treatment period, blood and bronchoalveolar lavage fluid (BALF) samples were collected. The percentage of eosinophils in BALF was evaluated to determine treatment efficacy. Serum and BALF BGA-specific immunoglobulin contents and plasma and BALF histamine concentrations were determined via ELISAs. Plasma and BALF serotonin concentrations were measured by use of a fluorometric method.

**Results**—The mean  $\pm$  SD percentage of eosinophils in BALF did not differ significantly among treatment groups (placebo, 40  $\pm$  22%; cyproheptadine, 27  $\pm$  16%; and cetirizine, 31  $\pm$  20%). Among the treatment groups, BGA-specific immunoglobulin content and histamine and serotonin concentrations were not significantly different.

**Conclusions and Clinical Relevance**—In cats with experimentally induced asthma, cyproheptadine and cetirizine were not effective in decreasing airway eosinophilic inflammation or in altering several other measured immunologic variables. Neither cyproheptadine nor cetirizine can be advocated as monotherapy for cats with allergen-induced asthma. (*Am J Vet Res* 2007;68:1265–1271)

A sthma in cats is believed to be allergic in origin, caused by a Th2-lymphocyte–driven response to inhaled environmental aeroallergens.<sup>1,2</sup> The Th2 cells elaborate cytokines that orchestrate synthesis of allergen-specific IgE antibody by plasma cells. The IgE antibody avidly binds mast cells on the mucosal surface of the airways. On reexposure to the specific allergen, 2 IgE antibodies on the surface of mast cells are cross-linked, which leads to an intracellular signal that triggers mast cell degranulation. As part of the acutephase response, preformed mediators within mast cells

AJVR, Vol 68, No. 11, November 2007

	ABBREVIATIONS
Th	T-helper
BGA	Bermuda grass allergen
IL	Interleukin
BALF	Bronchoalveolar lavage fluid
OD	Optical density

(eg, serotonin, histamine, and chemotactic factors) are released. Within seconds to minutes, these mediators result in vasodilation, an increase in vascular permeability, an inflammatory cell influx, and smooth muscle constriction.<sup>3,4</sup>

The hallmark features of allergic asthma include eosinophilic airway inflammation and airway hyperreactivity and remodeling (permanent architectural changes in the lungs). Airway inflammation is a cardinal feature of asthma because it can contribute to both airway hyperreactivity and remodeling.<sup>5</sup> Treatments that can blunt the inflammatory cascade may be of maximal benefit to patients if those treatments can also diminish airway hyperreactivity and remodeling.

Currently, there is no cure for allergic asthma, and the best available treatments focus on antagonizing or

Received January 25, 2007.

Accepted April 25, 2007.

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This manuscript represents a portion of a thesis submitted by the first author to the University of Missouri Graduate School as partial fulfillment of the requirements for a Master of Science degree.

Supported by a grant from the Committee on Research, College of Veterinary Medicine, University of Missouri.

Presented in part at the 24th Annual Forum of the American College of Veterinary Internal Medicine, Louisville, May–June 2006. Address correspondence to Dr. Reinero.

suppressing the inflammatory cascade once it has become well established. Glucocorticoids are the mainstay of treatment; they are highly effective anti-inflammatory drugs because the glucocorticoid receptor has pleiotropic effects on multiple cell-signaling pathways involved in inflammation.<sup>6</sup> In addition to glucocorticoids, bronchodilators are prescribed for cats that develop life-threatening respiratory distress as a result of bronchoconstriction.<sup>7,8</sup>

Long-term use or high doses of glucocorticoids may be contraindicated in cats with asthma that also have diabetes mellitus, heart disease, or concurrent infections. In these situations, other treatment options would be desirable. Because serotonin and histamine have been implicated in the acute-phase response, blocking these mediators should have a beneficial effect against smooth muscle constriction, vasodilation, increased vascular permeability, and inflammatory cell influx.

Serotonin is a common mediator in mast cells in many species including cats.9,10 Intravenous infusion of serotonin causes airway constriction in cats in vivo.<sup>11</sup> There is also evidence that endogenous serotonin constricts feline smooth muscle.10 Tracheal and bronchial smooth muscle strips obtained from cats with experimentally induced asthma released serotonin into a perfusate in vitro following (but not before) addition of allergen.<sup>10</sup> Furthermore, when those airway smooth muscle strips were incubated with cyproheptadine (a serotonin antagonist) prior to addition of allergen, the degree of constriction in response to allergen was attenuated.<sup>10</sup> Although it is well accepted that serotonin can lead to bronchoconstriction, its effects on inflammation have not been thoroughly evaluated in cats. In other species, there is evidence that serotonin contributes, albeit indirectly, to airway inflammation. For example, in mice sensitized with ovalbumin (as a model of asthma), serotonin induced secretion of IL-16 from epithelial cells.<sup>12</sup> Interleukin-16 is a cytokine that recruits and activates Th cells, which ultimately drive the asthmatic response. As another example, in human lung fibroblasts, serotonin modulated the release of eotaxin (a chemokine that attracts eosinophils).<sup>13</sup>

Similar to serotonin, histamine is stored as a preformed mediator in mast cells and induces bronchoconstriction in humans with asthma.14 The effects of histamine on smooth muscle in cats are not as clear-cut. In a study<sup>15</sup> involving tracheal and bronchial smooth muscle strips from healthy cats, histamine induced tracheal and bronchial smooth muscle dilation. This is in contrast to results of an in vivo study16 in which histamine that was administered IV or via aerosol induced bronchoconstriction. Interestingly, inflammation may modulate the response to histamine; lung strips from cats with chemically induced airway inflammation have increased airway reactivity when treated with histamine.<sup>17</sup> In vivo, histamine is released in the lungs in response to allergenic stimulation in humans as well as cats with experimentally induced asthma.9,18 Histamine is a specific chemoattractant and activator of human eosinophils and mediates airway inflammation.<sup>19,20</sup> Although an increased content of histamine in BALF has been detected in cats with experimentally induced

asthma following allergen challenge, the exact role of histamine in mediating airway inflammation in cats is not well understood.<sup>9</sup>

Because of the roles that serotonin and histamine play in the acute-phase response of allergic asthma in other species, therapeutic blockade of these mediators in vivo deserves further evaluation in asthma-affected cats. Cyproheptadine, a serotonin antagonist, decreases airway reactivity to serotonin infusion in healthy cats.<sup>11</sup> In a study<sup>21</sup> of cats with experimentally induced asthma, cyproheptadine administered orally at a commonly used low dose (2 mg, q 12 h) decreased airway hyperreactivity in a subpopulation of cats, although it did not significantly decrease eosinophilic airway inflammation, compared with the effects of the placebo treatment. However, the pharmacokinetics of cyproheptadine suggest that some cats may require substantially higher doses (as high as 8 mg) to reach therapeutic concentrations.<sup>22</sup> Such a higher dose has not previously been evaluated in cats with asthma. Cetirizine, a second-generation histamine 1 receptor antagonist, causes dose-dependent protection against bronchoconstriction in humans affected with mild asthma during histamine challenge.<sup>23,24</sup> Cetirizine also inhibits expression of proinflammatory mediators such as tumor necrosis factor- $\alpha$ , intercellular adhesion molecule-1, and superoxide radicals and inhibits eosin-induced transendothelial migration of eosinophils in vitro.<sup>25,26</sup> Although these effects of cetirizine may diminish inflammatory cell infiltration in the airways, no studies to date have been performed to evaluate this drug in cats with naturally occurring or experimentally induced asthma to our knowledge.

We previously developed a means of inducing allergic asthma in cats through sensitization and challenge with BGA.<sup>2</sup> This procedure results in eosinophilic airway inflammation, increases in serum concentration of allergen-specific IgE, and bronchoconstriction after allergen exposure, which mimic naturally occurring asthma in cats.<sup>2</sup> The purpose of the study reported here was to test the hypothesis that cyproheptadine and cetirizine would result in diminished eosinophilic airway inflammation by blocking the actions of serotonin and histamine, respectively, in cats with experimentally induced asthma. An additional objective of the study was to determine whether serotonin or histamine blockade would affect blood and BALF concentrations of serotonin, histamine, and BGA-specific immunoglobulins.

### **Materials and Methods**

**Experimental animals**—Nine sexually intact male domestic shorthair cats (6 to 9 months of age) that weighed 4.4 to 5.0 kg were obtained from a specificpathogen—free colony maintained by a commercial research animal provider.<sup>a</sup> The cats were cared for according to the principles outlined in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and the University of Missouri Animal Care and Use Committee.

No abnormalities were detected in any cat during physical examination. The cats were housed as a group in an indoor facility. Water and a dry feline maintenance diet were fed ad libitum. Prior to enrollment in the study, all cats were confirmed to have negative results of an intradermal skin test for BGA<sup>b</sup> and a lack of eosinophilic airway inflammation (percentage of eosinophils in BALF was < 5% in all cats; reference range,<sup>27</sup> 16 ± 14%).

Allergen sensitization—Cats were sensitized to BGA by use of a previously described protocol.<sup>2</sup> Briefly, the cats were administered 12 µg of BGA in 10 mg of alum, SC, and 10<sup>7</sup> Bordetella pertussis organisms, IM, on day 0. On day 14, 0.2 mL of BGA (0.08 mg) was administered intranasally. On day 21, 12 µg of BGA in 10 mg of alum was administered SC. Intradermal skin testing was repeated on day 28; parenteral sensitization was confirmed by examination of wheal formation in response to BGA. Three cats were not adequately sensitized (ie, wheals that developed in response to BGA were < 50%of the difference between the diameters of the wheals at the positive and negative control sites) and were given another injection of 12  $\mu$ g of BGA in 10 mg of alum on day 28. To perform aerosol challenge, unsedated cats were placed in a sealed chamber to which an air compressor<sup>c</sup> with a nebulizer was fitted; the allergen solution was aerosolized so that each cat received 0.5 mg of BGA in 5 mL of PBS solution/exposure. Aerosol exposure was started on day 30 and repeated every other day until each cat had received 7 exposures. Each cat was exposed to allergen for 15 minutes during each of the first 3 exposures; the duration of each exposure was decreased to 10 minutes (0.3 mg of BGA/exposure) for the remainder of the study. Two days after allergen challenge (day 44), BALF was collected and the percentage of eosinophils was determined to confirm the asthmatic phenotype (mean  $\pm$  SD percentage of eosinophils, 56  $\pm$  14%). To maintain airway sensitivity to allergen, the cats were then delivered an aerosol challenge weekly for the remainder of the study; these challenges were administered 48 hours prior to collection of pretreatment or posttreatment samples of blood and BALF.

Study design—After confirmation of the asthmatic phenotype, cats were enrolled in the blinded, randomized, placebo-controlled, crossover study to evaluate the effects of cetirizine and cyproheptadine. By use of a table of random numbers, cats were assigned to receive monotherapy with each of 3 treatments for 1 week: cyproheptadine<sup>d</sup> (8 mg, PO, q 12 h), cetirizine<sup>e</sup> (5 mg, PO, q 12 h), and placebo (flour in a No. 4 gelatin capsule,<sup>f</sup> PO, q 12 h). Cats were monitored for adverse effects of these medications at least twice daily during the treatment periods. Cyproheptadine and cetirizine tablets were crushed and also placed in No. 4 gelatin capsules,<sup>f</sup> and the individual who administered the treatments to the cats was unaware of which substance was in the capsules. All treatments were administered for 1 week followed by a 1-week washout period; the protocol was repeated for each of the other treatments, so that each cat received all 3 treatments during the study. Samples of blood and BALF were collected on the same day every week (including washout periods). The cats were administered a BGA aerosol challenge 48 hours prior to weekly sample collection. Samples obtained during the washout period (prior to treatment) were considered baseline samples, and samples obtained at the end of

the 1-week treatment period were considered posttreatment samples.

Collection of BALF—For baseline and posttreatment BALF sample collections, cats were anesthetized with ketamine<sup>g</sup> (8 to 14 mg/kg, IV). Cats were intubated with cuffed 4.0- to 4.5-mm endotracheal tubes, and a 7-F polypropylene catheter was gently inserted through the endotracheal tube until the catheter was wedged into a small airway. A 12-mL aliquot of 0.9% PBS solution was flushed into the catheter and retrieved via gentle hand suction. During collection of all samples, at least 50% of this fluid was retrieved. The BALF samples were placed immediately on ice and processed within 2 hours after collection.

For each BALF sample, the total nucleated cell count was determined by use of a hemacytometer. A cytocentrifuge was used to prepare slides for cytologic evaluation; slides were stained with a modified Wright's stain. By evaluating 200 nucleated cells/slide, differential cell counts were determined by 1 investigator (EKS), who was unaware of the treatments administered. The number of eosinophils was determined by multiplying the percentage of eosinophils and the total number of nucleated cells. The remaining BALF was centrifuged at  $300 \times g$  for 10 minutes, and the supernatant was collected and stored at  $-20^{\circ}$ C until further analysis.

Collection of blood—Blood samples were collected in tubes containing EDTA and additive-free tubes via jugular venipuncture. The EDTA-containing tubes were placed directly on ice and then centrifuged at 400  $\times$  g for 10 minutes within 2 hours after collection. Plasma was collected and stored at –20°C until further analysis. Blood samples in the additive-free tubes were allowed to clot at 24°C and then centrifuged at 1,730  $\times$  g for 20 minutes. The serum was collected and stored at –20°C until time of analysis.

Plasma and BALF histamine concentrations—A commercially available histamine competitive ELISA kit<sup>h</sup> was used to quantitate plasma and BALF histamine concentrations, in accordance with the manufacturer's instructions. All standards and unknown samples were assayed in duplicate. Coefficients of variation for the plasma and BALF histamine assays were < 20%. The histamine concentration in each sample was calculated by use of values generated from a standard curve. The range of detection of histamine in the ELISA was 2.5 to 50 ng/mL.

Plasma and BALF serotonin concentrations—Quartz cuvettes were loaded with plasma samples in a solution of Tris buffer or undiluted BALF samples for assessment of absorbance and emission spectra. Absorbance spectra were collected by use of a scanning diode array spectrometer<sup>i</sup> with 1-nm resolution. Emission spectra were collected by use of a fluorometer<sup>j</sup> in excitation-emission-matrix mode with a 2.5-nm band-pass from 275 to 500 nm at 2nm intervals. These spectra were collected by use of excitation wavelengths from 250 to 400 nm at 5-nm intervals and a 2.5-nm excitation band-pass. To improve the signalto-noise ratio, the software was configured to average 8 individual intensity measurements/datum point. The assay was performed on a set of 9 standards with various

noncorrelated concentrations of tryptophan (410 ng/mL to 4.08 µg/mL) and serotonin (10 ng/mL to 1.59 µg/mL) prior to performing the assay on the study samples (plasma and BALF). Plasma samples were assessed at a concentration of 2 and 4  $\mu$ L/mL in Tris buffer, and the BALF samples were examined without solvent dilution. The minimum detectible concentrations of tryptophan and serotonin were 40 and 5 ng/mL, respectively. The sample data were collected and exported into a software program<sup>k</sup> for parallel factor analysis. Parallel factor analysis in combination with excitation-emission-matrix fluorescence spectroscopy has been widely applied to the resolution of highly overlapped fluorescence spectra.<sup>28,29</sup> A weighted parallel factor analysis algorithm was used to eliminate the contribution of Rayleigh and Raman scattering to the analysis.<sup>30</sup> Results for plasma and BALF serotonin concentrations were reported as micrograms per milliliter. Both the interassay and intra-assay coefficients of variation were < 7%. Because of the dilutional effects of BAL sample collection, BALF serotonin concentrations were referenced to BALF total protein concentrations (results reported as mg/mL) that were obtained by use of a protein assay.<sup>1</sup> Corrected BALF values were calculated by use of an equation as follows:

### Corrected BALF value =

(BALF concentration of unknown sample/ total protein concentration of unknown sample)

(BALF concentration of positive control sample/ total protein concentration of positive control sample)

Serum concentration of BGA-specific IgE—By use of an ELISA involving a polyclonal rabbit anti-feline IgE antibody previously developed by our group,<sup>31</sup> BGA-specific IgE concentration in serum samples was determined. Sera obtained from cats that had been sensitized to BGA and used in another study<sup>2</sup> were pooled as a positive control sample. Results were reported as OD values and expressed as a percentage of the OD value of the pooled positive control sample.

Serum and BALF concentrations of BGA-specific IgG and IgA—By use of an ELISA method that had been previously validated by our group,<sup>32</sup> BGA-specific IgG and IgA concentrations were measured in serum and BALF samples. Pooled samples of serum and BALF obtained from BGA-sensitized cats used in a previous study<sup>2</sup> were used as positive control samples. Serum and absolute BALF concentrations were reported as OD values expressed as a percentage of the OD value of the respective pooled positive control sample.

Because of the dilutional effects of the BAL procedure, BALF concentrations were referenced to BALF total protein concentrations (results reported as mg/mL) that were obtained by use of a protein assay.<sup>1</sup> Corrected BALF values were calculated by use of an equation as follows:

Corrected BALF concentration =

(OD of unknown sample/total protein concentration of unknown sample)

(OD of positive control sample/total protein concentration of positive control sample)

Statistical analysis—A commercially available software program<sup>m</sup> was used to perform the Shapiro-Wilk test for normality. Differences in percentage of BALF eosinophils, plasma and BALF histamine and serotonin concentrations, serum allergen–specific IgE, and serum and BALF allergen–specific IgG and IgA among treatments were assessed by use of a mixed-model AVOVA to adjust for the effects of individual cat variation. All variables met criteria suitable for normality assumptions. Data are presented as mean  $\pm$  SD. For all comparisons, a *P* value < 0.05 was considered significant.

# Results

Adverse effects—During each 1-week treatment period, cats were monitored at least twice daily by the same individual (EKS). No adverse effects attributable to drug administration were detected.

Percentage of eosinophils in BALF—The percentage of eosinophils in BALF did not differ significantly (P = 0.196) among treatment groups. Mean  $\pm$  SD percentage of eosinophils in BALF was  $40.4 \pm 22.4\%$  after placebo treatment,  $31.2 \pm 19.9\%$  after cetirizine treatment, and  $26.5 \pm 15.6\%$  after cyproheptadine treatment. Additionally, comparison of the posttreatment and baseline percentages of eosinophils in BALF revealed no significant differences among treatment groups (P = 0.080), time (P = 0.127), or treatment versus time (P = 0.130). Similarly, there was no significant (P = 0.871) difference in absolute number of BALF eosinophils among treatment groups (data not shown). This indicated that the degree of eosinophilic inflammation in response to BGA in the cats did not change during the study period and was not affected by treatment.

Plasma and BALF histamine concentrations— Plasma histamine concentration did not differ significantly (P = 0.094) among treatment groups. Mean  $\pm$  SD plasma histamine concentration was  $21.9 \pm 9.3$  ng/mL,  $15.8 \pm 5.7$  ng/mL, and  $25.5 \pm 10.8$  ng/mL for placebo, cetirizine, and cyproheptadine treatments, respectively. Histamine concentrations in BALF were below the limit of detection of the ELISA in 12 of 25 (48%) of the samples collected; therefore, meaningful statistical analysis could not be performed on these data.

Plasma and BALF serotonin concentrations-Plasma and BALF serotonin concentrations did not differ significantly (P = 0.772 and P = 0.459, respectively) among treatment groups. Mean ± SD serotonin concentration in plasma was  $4.03 \pm 4.96 \,\mu$ g/mL after placebo treatment,  $4.9 \pm 4.28 \,\mu$ g/mL after cetirizine treatment, and  $5.36 \pm 5.88 \,\mu$ g/mL after cyproheptadine treatment. Mean serotonin concentration in BALF was 0.015  $\pm$ 0.02  $\mu$ g/mL after placebo treatment, 0.009  $\pm$  0.08  $\mu$ g/ mL after cetirizine treatment, and  $0.017 \pm 0.018 \,\mu\text{g/mL}$ after cyproheptadine treatment. Mean BALF protein concentrations were 0.097  $\pm$  0.046 mg/mL, 0.069  $\pm$ 0.057 mg/mL, and 0.064  $\pm$  0.047 mg/mL for placebo, cetirizine, and cyproheptadine treatments, respectively. The BALF serotonin-to-total protein ratios were 10.19  $\pm$  11.2, 18.33  $\pm$  20.58, and 19.69  $\pm$  15.49 for placebo, cetirizine, and cyproheptadine treatments, respectively.

Serum concentration of allergen-specific IgE—No significant (P = 0.506) difference in serum BGA-specific IgE concentration was detected among treatment

Table 1—Mean  $\pm$  SD serum (expressed as a percentage of a positive control sample value) and BALF concentrations (expressed as the BALF immunoglobulin concentration–to–total protein concentration ratio) of BGA-specific IgG and IgA in 9 cats with experimentally induced asthma following treatment with placebo (flour in a gelatin capsule), cyproheptadine (8 mg, PO, q 12 h), and cetirizine (5 mg, PO, q 12 h) in a crossover study. No significant differences were detected among treatment groups.

Treatment	Serum IgG (%)	Serum IgA (%)	BALF IgG	BALF IgA
Placebo Cetirizine Cyproheptadine	$\begin{array}{c} 50\pm28\\ 42\pm23\\ 44\pm22 \end{array}$	$\begin{array}{c} 5.33 \pm 3.9 \\ 7.11 \pm 5.51 \\ 6.22 \pm 4.41 \end{array}$	$\begin{array}{c} 0.18 \pm 0.15 \\ 0.67 \pm 0.68 \\ 0.35 \pm 0.37 \end{array}$	$\begin{array}{c} 0.15 \pm 0.12 \\ 0.55 \pm 0.53 \\ 0.34 \pm 0.35 \end{array}$

groups. Mean  $\pm$  SD concentrations of IgE (expressed as a percentage of the pooled positive control value) were 143.1  $\pm$  84.7% after placebo treatment, 125.9  $\pm$  74.5% after cetirizine treatment, and 147.1  $\pm$  99.7% after cyproheptadine treatment.

Serum and BALF concentrations of allergenspecific IgG and IgA—The BGA-specific IgG and IgA concentrations in plasma and BALF did not differ significantly (P > 0.05) among treatment groups (Table 1). The BALF immunoglobulin concentration—to—total protein concentration ratios for IgG and IgA were also not significantly (P > 0.05) different among groups.

# Discussion

In the cats with experimentally induced asthma used in the present study, treatment with cyproheptadine or cetirizine did not significantly decrease the percentage of eosinophils in BALF, compared with pretreatment baseline values. Because serotonin has proinflammatory effects in other species,<sup>12,13</sup> we had postulated that blockade of serotonin might be beneficial for the treatment of cats with allergen-induced asthma. Similarly, because histamine is an attractant to human eosinophils and histamine-induced vasodilation has been implicated in airway inflammation, it seemed rational that blockade of this mediator might blunt the severity of eosinophilic airway inflammation in cats.<sup>14,19</sup> Because airway inflammation is thought to contribute to airway hyperreactivity and remodeling, drugs that fail to dampen airway inflammation cannot be recommended as monotherapy for asthma.<sup>5</sup> Thus, on the basis of the findings of the present study, we cannot advocate administration of cyproheptadine or cetirizine as the sole treatment for cats with asthma.

The inability of these drugs to attenuate eosinophilic airway inflammation could have several explanations. First, mediators other than serotonin and histamine may play more important roles in inducing eosinophilic inflammation in asthma-affected cats. For example, Th2 cells synthesize IL-5, which is responsible for maturation, differentiation, and survival of eosinophils and for the initial migration of those cells into the airways.<sup>5</sup> Second, the release of serotonin and histamine from mast cells is a relatively late event in the allergic inflammatory cascade, occurring a considerable time after allergen presentation, activation of Th2 cells with their subsequent cytokine elaboration, and plasma cell synthesis of allergen-specific IgE. Therefore, antagonism of serotonin and histamine receptors on mast cells may not make a substantial difference in the extent of airway eosinophilia because other inflammatory pathways have already been activated. Finally, because serotonin and histamine have been implicated in the acute-phase response, it is possible that their maximal beneficial effects were not detectable in BALF samples collected 48 hours after the cats were exposed to allergen. On the basis of the results of our study, it is not possible to determine whether these drugs blunted the extent of the acute-phase response or whether the latephase response (which is driven by release of cysteinyl leukotrienes and cytokines in other species) played a more dominant role in establishing eosinophilic airway inflammation. It is known that cysteinyl leukotrienes are not important mediators in cats with experimentally induced asthma or naturally occurring allergic bronchitis,<sup>21,33,n</sup> but the role of cytokines in the latephase response in cats has not yet been studied to our knowledge. However, even if cyproheptadine or cetirizine diminished the eosinophilic inflammation during the acute-phase response, eosinophilic inflammation of the airways at the time of sample collection was too severe to consider administration of these medications to be successful clinical treatments.

In the present study, serotonin concentrations in plasma and BALF samples were not significantly different among treatment groups. Similarly, histamine concentrations in plasma samples were not significantly different among treatment groups. The authors are unaware of previous studies in which serotonin concentrations in plasma and BALF samples collected from cats with experimentally induced asthma have been evaluated; however, it is known that incubation with allergen triggers the release of serotonin from airway smooth muscle strips obtained from sensitized cats.9 On the other hand, histamine concentrations in BALF obtained before and after allergen challenge from cats with experimentally induced asthma have been evaluated.<sup>9</sup> In that study,<sup>9</sup> mean  $\pm$  SD histamine concentration in BALF in sensitized cats prior to allergen challenge was 74  $\pm$  19 pg/mL and was significantly increased after allergen challenge (970  $\pm$  180 pg/mL). In the control group of nonsensitized cats in that study, mean ± SD baseline BALF histamine concentration was  $6 \pm 3$  pg/mL; after sham allergen challenge, BALF histamine concentration increased to  $55 \pm 26$  pg/mL.<sup>9</sup> The histamine concentrations in BALF reported in that study were less than the limit of detection of the ELISA used in our study; therefore, it is perhaps not surprising that the BALF histamine concentration in 48% of the samples in our study was less than the standard curve generated for the kit. In contrast to BALF histamine concentrations, plasma histamine concentrations in cats sensitized to Ascaris suum that were measured prior to and immediately after allergen challenge were 14.0  $\pm$  0.7 ng/mL and 36.3  $\pm$  9.1 ng/mL, respectively,<sup>34</sup> which are similar to the concentrations determined in the present study.

Because the action of cyproheptadine and cetirizine involves competitive inhibition of serotonin and histamine receptors, respectively,<sup>11,35</sup> we had postulated that serum and BALF histamine concentrations would be increased after cetirizine treatment and that serum and BALF serotonin concentrations would be increased after cyproheptadine treatment in the asthma-affected cats of the present study. It is possible that we did not detect the maximal effect of these medications because samples were collected 2 days after allergen exposure. We elected to collect BALF samples 2 days after allergen challenge on the basis of a large body of data that historically supported the presence of substantial eosinophilic inflammation after that interval and on Th2 cytokine profile data relating to that time point; collection of BALF 2 days after allergen challenge is now the standard protocol for experimental induction of asthma in cats by use of BGA.<sup>2</sup> Although evaluation of eosinophilic airway inflammation at that time point is apparently appropriate, in retrospect, it probably would have been preferable to collect blood samples for analysis of mediator concentrations shortly after allergen challenge. However, at least for the mediator histamine, pharmacologic blockade may not decrease plasma concentrations of histamine but may still have an impact on the asthmatic phenotype.<sup>34</sup> In another study<sup>34</sup> involving cats sensitized to A suum, researchers evaluated plasma histamine concentrations after allergen challenge in cats pretreated with the antihistamine pyrilamine and untreated control cats. Both groups had a significant increase in plasma histamine concentration 5 to 10 minutes after antigen challenge, compared with baseline values, but there was no significant difference in histamine concentration between groups (35  $\pm$ 9.7 ng/mL and 42.2  $\pm$  11.5 ng/mL in the antihistaminetreated and control group, respectively).<sup>34</sup> Interestingly, pretreatment with pyrilamine blunted the severity of the increase in airway resistance, compared with findings in control cats, suggesting that although plasma concentrations of histamine were not significantly reduced in the antihistamine-treated cats, blockade of histamine led to physiologic effects (ie, diminution of acute postantigen challenge bronchoconstriction).<sup>34</sup>

In addition to the finding that cyproheptadine and cetirizine were ineffective in dampening eosinophilic airway inflammation in cats with allergic asthma in the present study, those drugs also failed to significantly alter any of the other measured immune variables. Serotonin and histamine have been implicated in vasodilation, increases in vascular permeability, inflammatory cell influx, and airway smooth muscle constriction, and it would be reasonable to speculate that blockade of these mediators might have far-reaching effects on other components of the allergic inflammatory cascade.<sup>3,4</sup> For instance, both serotonin and histamine can lead to release of IL-16, which causes recruitment of activated T cells.12,19 In asthma, activated Th2 cells are responsible for elaboration of a variety of cytokines, some of which are involved in B-cell maturation, terminal differentiation of B cells to plasma cells, synthesis of antibodies, and ultimately isotype switching to the IgE class of antibody. Bermuda grass allergen-specific IgE, IgG, and IgA concentrations in serum and BGA-specific IgG and IgA concentrations in BALF were evaluated in the present study to determine whether blockade of serotonin and histamine would indirectly alter the formation of allergen-specific immunoglobulins. Significant differences in immunoglobulin synthesis among treatment

groups were not detected, suggesting that blockade of serotonin or histamine has negligible effects on allergen-specific immunoglobulin production. Because serotonin and histamine are released relatively late in the cascade of inflammatory events associated with allergic asthma and because of the overlapping, redundant, and additive or synergistic actions of various components of the inflammatory cascade, it is not surprising that these drugs failed to induce substantial effects on immunoglobulin concentrations in the cats of the present study.

Eosinophilic inflammation is not the only important pathologic change associated with allergic asthma. Airway hyperreactivity and remodeling are also important in the pathogenesis of this disease. Serotonin has been implicated as an important mediator of airway smooth muscle constriction.<sup>11</sup> Previous studies<sup>10,11</sup> to evaluate the effects of cyproheptadine in cats with asthma have focused on the drug's ability to alleviate bronchoconstriction. Another study<sup>21</sup> to investigate the effects of cyproheptadine on airway inflammation in cats with experimentally induced asthma revealed that a subpopulation of cats had decreased airway hyperreactivity following treatment despite the fact that airway inflammation was not reduced significantly. The pharmacokinetics of cyproheptadine in cats suggest that for maximal effect, the dose of cyproheptadine required by cats has to be substantially higher than that used in the aforementioned study.<sup>22</sup> We did not assess airway hyperreactivity in the present study, but investigation of the effects of a high dose of cyproheptadine on this variable in cats is warranted.

Adverse effects of these drugs in cats have not been widely reported. Cyproheptadine can increase appetite and cause hyperreactivity in some cats.<sup>22</sup> In humans, cetirizine can cause somnolence, fatigue, dry mouth, pharyngitis, and dizziness.<sup>36</sup> In the cats of the present study, no drug-associated adverse effects were detected.

The findings of the present study have indicated that cyproheptadine and cetirizine do not significantly diminish eosinophilic inflammation in cats with BGAinduced asthma. Additional studies are warranted to examine the effects of these medications on airway hyperreactivity or their use in combination treatments, particularly with regard to glucocorticoid-sparing effects. However, on the basis of the results of our study, these medications cannot be recommended for use as monotherapy for the treatment of cats with allergeninduced asthma.

- Liberty Research, Waverly, NY. a.
- Bermuda grass allergen, Greer Laboratories Inc, Lenoir, NC. b.
- c. Easy Air 15, Model PM15P, Precision Medical Inc, Northampton, Pa.
- Cyproheptadine HCl, IVAX Pharmaceuticals Inc, Miami, Fla. d.
- Zyrtec, Pfizer Inc, New York, NY. e.
- Gelatin capsule, Eli Lilly and Co, Indianapolis, Ind. f.
- Ketaset, Fort Dodge, Fort Dodge, Iowa. g.
- Histamine ELISA kit No. 409010, Neogen, Lexington, Ky. h.
- Cary Bio 50 scanning diode array spectrometer, Varian Inc, Palo i. Alto, Calif.
- Cary Eclipse L-format fluorometer, Varian Inc, Palo Alto, Calif.
- k. MATLAB, version 7.1 (R14), The MathWorks Inc, Natick, Mass.
- 1. Bio-Rad protein assay, Bio-Rad Laboratories, Hercules, Calif. m.
  - SAS, version 9.1, SAS Institute Inc, Cary, NC.

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