Effects of drug treatment on inflammation and hyperreactivity of airways and on immune variables in cats with experimentally induced asthma

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Objective—To compare the effects of an orally administered corticosteroid (prednisone), an inhaled corticosteroid (flunisolide), a leukotriene-receptor antagonist (zafirlukast), an antiserotonergic drug (cyproheptadine), and a control substance on the asthmatic phenotype in cats with experimentally induced asthma.

Animals—6 cats with asthma experimentally induced by the use of Bermuda grass allergen (BGA).

Procedures—A randomized, crossover design was used to assess changes in the percentage of eosinophils in bronchoalveolar lavage fluid (BALF); airway hyperresponsiveness; blood lymphocyte phenotype determined by use of flow cytometry; and serum and BALF content of BGA-specific IgE, IgG, and IgA determined by use of ELISAs.

Results—Mean ± SE eosinophil percentages in BALF when cats were administered prednisone (5.0 ± 2.3%) and flunisolide (2.5 ± 1.7%) were significantly lower than for the control treatment (33.7 ± 11.1%). We did not detect significant differences in airway hyperresponsiveness or lymphocyte surface markers among treatments. Content of BGA-specific IgE in serum was significantly lower when cats were treated with prednisone (25.5 ± 5.4%), compared with values for the control treatment (63.6 ± 12.9%); no other significant differences were observed in content of BGA-specific immunoglobulins among treatments.

Conclusions and Clinical Relevance—Orally administered and inhaled corticosteroids decreased eosinophilic inflammation in airways of cats with experimentally induced asthma. Only oral administration of prednisone decreased the content of BGA-specific IgE in serum; no other significant local or systemic immunologic effects were detected among treatments. Inhaled corticosteroids can be considered as an alternate method for decreasing airway inflammation in cats with asthma. (*Am J Vet Res* 2005;66:1121–1127)

Allergic asthma is a **T-helper 2 (Th2)**–lymphocyte-driven hypersensitivity reaction to aeroallergens. Activation of allergen-specific Th2 cells triggers production of cytokines that orchestrate the asthmatic response, which includes recruitment and activation of inflammatory cells, induction of hyperreactivity in airways, synthesis of allergen-specific antibodies (especially the IgE class), and remodeling of tissues in humans and cats.[1–4] Considerable emphasis has been placed on developing novel treatments that block portions of the inflammatory cascade in human asthmatics. Although asthma is a common bronchopulmonary disorder in cats and is strikingly similar to the disease in humans, randomized, controlled studies evaluating novel treatments have not been performed in cats, despite parallel advances in humans.

Treatments for cats with asthma have traditionally relied on bronchodilators and corticosteroids.[5,6] Few studies in cats have explored other therapeutic options, and some of these studies[7–9] have evaluated in vitro rather than in vivo responses. Orally administered or injectable corticosteroids have been successfully used therapeutically because of their potent effects on inflammation. Corticosteroids bind a cytosolic glucocorticoid receptor and are translocated into the nucleus where they suppress transcription of multiple proinflammatory genes, including those encoding for cytokines, inflammatory enzymes, receptors for inflammatory mediators, and cell adhesion molecules.[10] Inhaled corticosteroids are used in preference to orally administered corticosteroids in humans with asthma because of fewer systemic adverse effects and the fact that high drug concentrations are delivered directly to the lungs[10]; however, to our knowledge, the efficacy of inhaled corticosteroids in cats with asthma has not been evaluated.

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Leukotriene (LT)-receptor antagonists (eg, zafirlukast) are also used in humans with asthma because they block the function of LTs produced after allergenic exposure.[11] Cysteinyl LTs (ie, LTC4, LTD4, and LTE4), which are derived from lipoxygenation of arachidonic acid in cell membranes, are potent constrictors of bronchial smooth muscle that impair mucociliary transport, enhance mucus release, and potentiate influx of inflammatory cells in humans.[12] Although LT-receptor antagonists are empirically used in feline asthmatic patients, their clinical use has yet to be justified, especially in light of studies[9,13,a] that have questioned the role of LTs in the pathogenesis of asthma in cats.

In contrast to LTs, serotonin has been described in vitro as an important mediator in the acute-phase response of cats with experimentally induced asthma.[9] Cyproheptadine blocks serotonin receptors on smooth muscle cells, and cyproheptadine in nonasthmatic research cats reverses bronchoconstriction induced by an infusion of serotonin.[14] However, its effects on the asthmatic responses in vivo are unknown.

Methods for evaluating the response to treatment in cats with asthma include improvement or resolution of clinical signs (eg, coughing, wheezing, or episodes of dyspnea), changes in radiographic appearance of the lungs, diminished inflammatory cells (especially eosinophils) in **bronchoalveolar lavage fluid (BALF)**, and, in a few studies,[5,6] improvement in hyperresponsiveness of airways as detected by pulmonary function testing.

We have developed a method of experimentally inducing asthma in cats by use of the clinically relevant aeroallergen Bermuda grass.[1] This method replicates the major immunopathophysiologic features that characterize this disease (ie, an increase in allergen-specific IgE concentrations, eosinophilic inflammation of the airways, patterns of Th2 cytokines, bronchoconstriction in response to inhalation of specific allergens, and histologic evidence of remodeling of the airways [notably, hypertrophy of smooth muscles and metaplasia of mucus cells]). The purpose of the study reported here was to compare the effects of treatment with an orally administered corticosteroid (prednisone), an inhaled corticosteroid (flunisolide), an LT-receptor antagonist (zafirlukast), an antiserotonergic drug (cyproheptadine), and a control substance on the asthmatic phenotype in cats with asthma experimentally induced by use of **Bermuda grass allergen (BGA)**. We hypothesized that the orally administered and inhaled corticosteroids would diminish eosinophilia and hyperresponsiveness of airways. Assuming that cysteinyl LTs and serotonin, respectively, are important mediators in cats with asthma, zafirlukast and cyproheptadine would ameliorate these effects. Additionally, because it is postulated that inhaled corticosteroids have minimal systemic absorption and the anti-LT and antiserotonergic drugs target specific and narrow sites in the inflammatory cascade, we hypothesized that only the orally administered corticosteroids would have a global suppressive effect on the immune system.

Materials and Methods

Animals—Six neutered mixed-breed cats (3 males and 3 females) were obtained from a research cat colony maintained at the University of California at Davis for use in the study. Cats were 12 to 14 months old and weighed between 4.5 and 5.4 kg. Cats were cared for in accordance with principles outlined in National Institutes of Health guidelines.[15]

All cats had results within expected limits for physical examinations and negative results when tested for FeLV and FIV. Cats were housed together in an indoor facility in a single open room. Water and a dry maintenance diet formulated for cats were available ad libitum. Cats had negative results for an ID skin test with BGA and a lack of eosinophilic airway inflammation at baseline (percentage of eosinophils in BALF was $< 7\%$ in all cats).

Induction of asthma—Cats were sensitized to BGA[b] in accordance with a protocol described elsewhere.[1] Briefly, cats were administered 12 µg of BGA in 10 mg of alum, SC, and 10^5 *Bordetella pertussis* organisms, IM, on day 0; 0.2 mL of BGA (0.75 mg/mL; intranasal) on day 14; and 12 µg of BGA in 10 mg of alum, SC, on day 21. Detection of wheals (positive result) during ID skin testing was used to confirm sensitization to BGA on day 28. After parenteral sensitization, aerosol challenge exposure was conducted on awake, spontaneously breathing cats in a sealed chamber. An air compressor[c] attached to a nebulizer was used to aerosolize the allergen solution. Aerosol challenge exposure was performed 3 times/wk for 2 weeks, then at weekly intervals thereafter for the duration of the study. Cats were exposed to 0.5 mg of BGA dissolved in PBS solution followed by nebulization for 5 min/treatment.

Study design—After it was confirmed that cats had an asthmatic phenotype, they were enrolled in the randomized crossover study. Cats were randomly assigned to initially receive 1 of 5 drugs. Each drug was administered for 2 weeks, which was then followed by a washout period of 4 weeks. Each cat received all 5 drugs during the course of the study. The 5 treatments were prednisone[d] (5 mg, PO, q 12 h), flunisolide[e] (250 µg, inhaled, q 12 h; administered by use of a metered dose inhaler and pediatric holding chamber[f]), an LT-receptor antagonist (zafirlukast[g]; 10 mg, PO, q 12 h), an antiserotonergic drug (cyproheptadine[h]; 2 mg, PO, q 12 h), and a control substance (flour placed in a No. 4 gelatin capsule, PO, q 12 h). When cats received the flunisolide by use of the metered dose inhaler, 1 puff of the inhaler (250 µg/puff) was discharged into the pediatric holding chamber, which was held over the cat's nose and mouth for 10 breaths. The first day of each treatment was designated as day 0.

Data collection—On day 14 of each treatment period, cats were anesthetized with propofol[i] (6 mg/kg, IV, for induction, which was followed by 0.2 to 0.6 mg/kg/min as a constant rate infusion for maintenance of anesthesia). Cats were then intubated with cuffed 4.5-mm endotracheal tubes.

Evaluation of airway hyperresponsiveness—Anesthetized cats were evaluated for airway hyperresponsiveness by challenge exposure to methacholine, as described elsewhere.[13] Briefly, cats were allowed to breathe spontaneously, and the endotracheal tube was attached to a blow-by delivery system set to deliver 100% oxygen at a rate of 2 L/min; change in outflow exhausted from this system (indicating inspiratory and expiratory flow) was recorded by use of a pneumotachometer[j] attached to a pressure transducer.[k] Transpulmonary pressure was measured by use of a differential pressure transducer,[l] with 1 port attached to a water-filled cannula placed in the esophagus in the midthoracic region

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and the other port attached to a side port of the pneumotachometer. These signals were sent to an analogue-to-digital data acquisition system.[m] Sterile saline (0.9% NaCl) solution was administered as a nebulized aerosol for 1 minute, after which baseline measurements of pulmonary resistance were continuously collected for 4 minutes. Methacholine, a short-acting analogue of acetylcholine that is resistant to acetylcholinesterase, was delivered in increasing half-log doses for 1 minute starting at a dosage of 0.0625 mg/mL (maximum dosage, 64 mg/mL), which was followed by 4 minutes of data collection. The dose-response study was terminated when there was **an increase in airway resistance to 200% of the value obtained after aerosol challenge with the nebulized saline solution ($EC200R_L$)** or oxygen saturation of arterial blood decreased to ≤ 75%. The $EC200R_L$ was determined by linear interpolation on the log-log plot of the dose-response curve.

Collection of blood samples—Blood samples were collected by jugular venipuncture; a minimum of 1.5 mL was placed in a tube that contained EDTA for lymphocyte phenotype analysis, and 2 mL was placed in a tube without additives and allowed to clot at 24°C. The EDTA-blood samples were placed directly on ice until analysis; all samples were analyzed within 1 hour after collection. Clotted blood samples were centrifuged at 1,730 X *g* for 30 minutes. Serum was harvested and stored at –20°C until the time of assay; all serum samples were assayed with 6 months after harvest.

Collection of BALF—Collection of BALF was performed after airway hyperresponsiveness testing by gently inserting a 7-F polypropylene catheter through the endotracheal tube and advancing it until the catheter was wedged in a small airway. Three 10-mL aliquots of warm 0.9% PBS solution were sequentially lavaged through the catheter and retrieved by use of gentle suction. Samples of BALF were placed on ice until processed; all BALF samples were processed within 2 hours after collection.

A cytocentrifuge was used to prepare slides of BALF for cytologic examination; slides were stained with a modified Wright stain. Differential cells counts were determined by evaluating 200 nucleated cells/slide, and the number of eosinophils was expressed as a percentage. The remaining BALF was centrifuged at 300 X *g* for 20 minutes, and the supernatant was harvested and stored at –20°C until the time of assay.

Flow cytometric analysis of lymphocytes—Phenotypic analysis of peripheral lymphocytes was performed by use of flow cytometry on EDTA-blood samples.[1] Briefly, 100 µL of blood was placed in 5-mL polystyrene round-bottom tubes.[n] Several murine monoclonal antibodies (ie, anti-feline CD4, anti-feline CD5 [pan-T cell marker], anti-feline CD8, and anti-canine CD21 [pan-B cell marker]) were obtained[o] for use in identifying subpopulations of lymphocytes, and 25 µL of each antibody was added to the tubes. Tubes were allowed to incubate at 24°C for 15 minutes. Lysis buffer was added to the cells, and tubes were allowed to incubate for an additional 5 minutes. Cells were washed in **fluorescent-activated cell sorting (FACS)** buffer (PBS solution with 1% fetal bovine serum and 0.09% sodium azide) and centrifuged at 300 X *g* for 5 minutes. Fifty microliters of 1:100 R-phycoerythrin conjugated $F(ab')_2$ fragment donkey anti-mouse IgG (H + L)[p] was added to the cell pellet, and the tubes were incubated for another 15 minutes at 24°C. Cells were then washed and centrifuged to form a pellet, as described previously. The pellet was resuspended in 200 µL of FACS buffer and 200 µL of 1% formalin. Samples were stored at 4°C for up to 24 hours. Samples were then analyzed by use of a flow cytometer[q] and commercially available software.[r] The lymphocyte population recognized on the forward- and side-scatter plots was gated, and this gate was used for identification of the fluorescein-conjugated secondary antibody attached to the aforementioned cell surface markers. Results were reported as a percentage of lymphocytes for each marker.

Serum content of BGA-specific IgE—Serum content of BGA-specific IgE was measured by use of ELISAs that involved use of a polyclonal rabbit anti-feline IgE antibody developed by our laboratory group.[16] Briefly, gel filtration and affinity chromatography were used to purify feline IgE in pooled sera of experimentally induced asthmatic cats. Purified IgE was used to immunize rabbits, and the polyclonal rabbit anti-feline IgE was optimized in an ELISA. Negative and positive control samples consisted of pooled samples from research cats before or after allergen sensitization, respectively, and were frozen in aliquots that were thawed prior to each assay. Results were reported as the **optical density (OD)**. The OD of the background well (ie, PBS solution and Tween 0.2%) was subtracted from the OD for each sample, and results of samples were then expressed as a percentage of the positive control sample.

Serum and BALF content of BGA-specific IgG and IgA—Measurement of BGA-specific IgG and IgA in serum and BALF was performed by use of ELISAs optimized by our laboratory group.[17] Positive control samples were obtained from pooled serum and pooled BALF obtained from cats with experimentally induced asthma in an unrelated study after allergen sensitization to BGA. Results of BALF were referenced to **total protein (TP)** concentrations obtained by use of a protein assay.[s] Values were calculated as follows:

$$(OD_{\text{unknown sample}}/TP_{\text{unknown sample}})/(OD_{\text{positive control sample}}/TP_{\text{positive control sample}}).$$

Statistical analysis—Univariate repeated-measures ANOVAs were performed by use of commercially available software[t] to determine whether measured variables differed significantly among the treatments. When appropriate, the critical value of *F* was adjusted for a lack of sphericity by use of the Huynh-Feldt estimate of epsilon.[18] Post hoc pairwise comparisons were performed by use of the Tukey **honestly significantly different (HSD)** procedure to limit the overall error rate to 5%. Significance was defined as values of $P < 0.05$.

Results

Cellular composition of BALF—Percentage of eosinophils in BALF differed significantly (repeated-measures ANOVA; $P_{\text{unadjusted}} = 0.007$ and $P_{\text{Huynh-Feldt}} = 0.013$) among treatments for the 6 cats. Mean ± SE percentages of eosinophils for the treatments were 5.0 ± 2.3% for prednisone, 2.5 ± 1.7% for flunisolide, 19.0 ± 8.0% for zafirlukast, 20.3 ± 7.3% for cyproheptadine, and 33.7 ± 11.1% for the control substance. Mean eosinophil percentages observed during the prednisone and inhalant flunisolide treatments were significantly (Tukey HSD) lower than the percentage during treatment with the control substance. Values observed for zafirlukast and cyproheptadine were intermediate and did not differ significantly from values for any of the other treatments (**Figure 1**). We did not detect significant differences among treatment groups for the percentages of macrophages, neutrophils, or lymphocytes.

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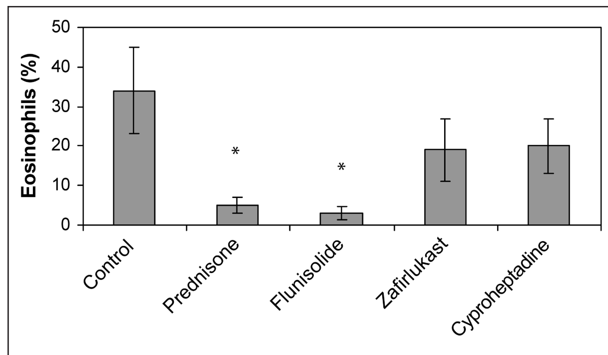

Figure 1—Mean ± SE percentage of eosinophils detected in bronchoalveolar lavage fluid (BALF) obtained from 6 asthmatic cats following treatment for 2 weeks with a control substance, orally administered prednisone, inhaled flunisolide, orally administered zafirlukast, and orally administered cyproheptadine. *Value differs significantly ($P < 0.05$) from the value for the control treatment.

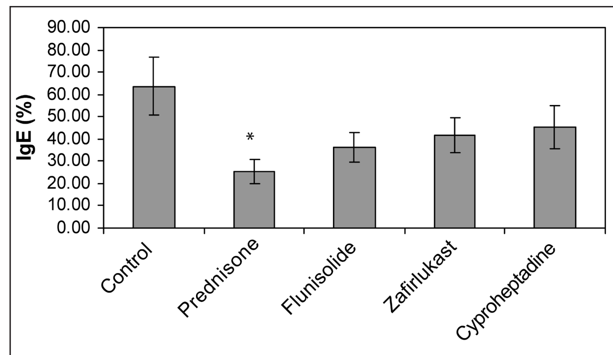

Figure 3—Mean ± SE amount of Bermuda grass allergen–specific IgE in serum obtained from 6 asthmatic cats after 2 weeks of treatment with a control substance, orally administered prednisone, inhaled flunisolide, orally administered zafirlukast, and orally administered cyproheptadine. Values are expressed as a percentage of the values for a positive control sample. *See* Figure 1 for remainder of key.

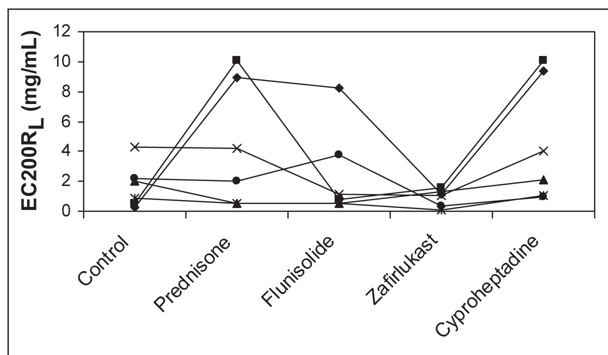

Figure 2—Values for an increase in airway resistance to 200% of the value obtained after aerosol challenge with the nebulized saline (0.9% NaCl) solution ($EC200R_L$) for each of 6 asthmatic cats after 2 weeks of treatment with a control substance, orally administered prednisone, inhaled flunisolide, orally administered zafirlukast, and orally administered cyproheptadine. Each symbol represents results for 1 cat. No significant differences in $EC200R_L$ were detected among treatments.

Airway hyperresponsiveness—The $EC200R_L$ did not differ significantly among treatments for the 6 cats. Mean ± SE $EC200R_L$ for the treatments was 4.4 ± 4.2 mg/mL for prednisone, 2.5 ± 3.0 mg/mL for flunisolide, 0.9 ± 0.6 mg/mL for zafirlukast, 4.6 ± 4.1 mg/mL for cyproheptadine, and 1.7 ± 1.5 mg/mL for the control treatment. Despite the lack of significant differences among treatment groups, 2 of 6 cats receiving prednisone, 2 of 6 cats receiving cyproheptadine, and 1 of 6 cats receiving inhaled flunisolide had dramatic increases in the $EC200R_L$ (ie, a decrease in airway hyperreactivity), compared with values for the control treatment **(Figure 2)**.

Lymphocyte phenotype—Flow cytometric analysis of lymphocyte markers did not reveal significant differences among treatment groups for CD4+, CD5+, CD8+, and CD21+ markers. Mean ± SE percentages of surface markers were calculated **(Table 1)**.

Serum content of allergen-specific IgE—Serum content of IgE differed significantly (repeated-measures ANOVA; $P_{unadjusted}$ = 0.014 and $P_{Huynh\text{-}Feldt}$ = 0.033) among treatments for the 6 cats. Mean ± SE values of positive results for IgE were 25.5 ± 5.4% for prednisone, 36.2 ± 6.5% for flunisolide, 41.8 ± 7.9% for zafirlukast, 45.3 ± 9.6% for cyproheptadine, and 63.6 ± 12.9% for the control treatment. Group mean values of IgE measured after treatment with prednisone were significantly (Tukey HSD) lower than those measured after the control treatment. Values measured after treatment with flunisolide, zafirlukast, and cyproheptadine were intermediate and were not significantly different from values for any other treatments **(Figure 3)**.

Serum and BALF content of allergen-specific IgG and IgA—We did not detect significant differences in serum content of BGA-specific IgG or IgA or BALF content of BGA-specific IgG or IgA among the treatments. Mean ± SE OD values for immunoglobulin content in serum and immunoglobulin-to-TP ratios for BALF were calculated **(Table 2)**.

Table 1—Mean ± SE percentages of lymphocytes that had positive results for various T- and B-cell surface markers during flow cytometric analysis of peripheral blood lymphocytes obtained from 6 asthmatic cats after 2 weeks of treatment with a control substance, orally administered prednisone, inhaled flunisolide, orally administered zafirlukast, and orally administered cyproheptadine.

Treatment	CD4+	CD5+	CD8+	CD21+
Control	28.5 ± 3.1	51.5 ± 3.2	22.1 ± 2.1	17.0 ± 4.2
Prednisone	24.8 ± 2.7	42.8 ± 8.6	18.7 ± 3.3	14.1 ± 1.8
Flunisolide	29.1 ± 3.3	57.3 ± 3.8	19.6 ± 4.0	15.5 ± 1.7
Zafirlukast	29.1 ± 2.8	60.0 ± 7.1	28.6 ± 5.8	19.9 ± 3.7
Cyproheptadine	25.6 ± 2.9	48.1 ± 10.0	20.0 ± 3.6	23.7 ± 3.9

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Table 2—Mean ± SE values for serum and bronchoalveolar lavage fluid (BALF) content of Bermuda grass allergen–specific IgG and IgA in 6 asthmatic cats after 2 weeks of treatment with a control substance, orally administered prednisone, inhaled flunisolide, orally administered zafirlukast, and orally administered cyproheptadine.

Treatment	Serum IgG*	Serum IgA*	BALF IgG†	BALF IgA†
Control	0.322 ± 0.034	0.169 ± 0.055	0.379 ± 0.144	0.311 ± 0.104
Prednisone	0.271 ± 0.041	0.095 ± 0.019	0.566 ± 0.320	0.469 ± 0.191
Flunisolide	0.251 ± 0.042	0.317 ± 0.148	0.417 ± 0.104	0.756 ± 0.330
Zafirlukast	0.264 ± 0.042	0.221 ± 0.098	0.399 ± 0.086	0.348 ± 0.113
Cyproheptadine	0.349 ± 0.050	0.272 ± 0.158	0.567 ± 0.121	0.699 ± 0.185

*Results are reported as adjusted optical density. †Results are reported as adjusted optical density divided by total protein concentration.

Discussion

In the veterinary scientific literature, remarkably little attention has been paid to novel therapeutic options for cats with naturally developing asthma. Orally administered or injectable corticosteroids and bronchodilators have been the mainstay of treatment for decades.[5,6] Response to treatment has traditionally been measured by resolution of clinical signs and radiographic abnormalities and, for 1 study,[5,6] improvement in pulmonary function measurements. To our knowledge, the study reported here is the first to compare immunologic and physiologic variables in response to orally administered and inhaled corticosteroids, an LT-receptor antagonist, and an antiseroitonergic drug in cats with asthma.

We chose to use cats with experimentally induced asthma to compare immunologic and physiologic variables in response to various treatments. On exposure to allergen, cats with experimentally induced asthma develop coughing, wheezing, tachypnea, increased expiratory effort, or a combination of these clinical signs, which are clinically reversible with inhaled albuterol. Cats with naturally developing asthma would be expected to have waxing and waning clinicopathologic changes, depending on time and duration of exposure and the number and types of allergens to which they have been sensitized. The cats in this study were sensitized to a single allergen (BGA), housed in the same environment, and administered a standard dose of BGA on a weekly basis. The experimental setting also eliminated owner compliance with treatment administration as a confounding factor.

The percentage of eosinophils in BALF, which is a marker for the amount of airway inflammation in patients with asthma, was significantly lower for cats when treated with orally administered and inhaled corticosteroids. It has been hypothesized that the beneficial effects of corticosteroids for the treatment of asthmatic patients are attributable, at least in part, to the effects of these drugs on eosinophils. Only orally administered prednisone caused significant decreases in serum content of BGA-specific IgE; all other evaluated immunologic and physiologic variables were not significantly different among treatments. One possible reason for the lack of significant differences in other measured variables (eg, airway hyperreactivity) could have been the relatively low power to detect changes in subgroups of animals associated with the small number of cats in this study. It is expected that outbred animals (such as the cats of our study) have more variation in responses than genetically identical animals such as mice, which are commonly used for studies of experimentally induced asthma. Despite this, the study of experimentally induced asthma in cats reported here is useful because the experimental technique caused results similar to those for cats with naturally developing disease (ie, it used a relevant allergen [BGA] and created clinicopathologic changes similar to those of cats with naturally developing disease). In addition, environmental influences were carefully controlled, and repeated invasive procedures (eg, anesthetic episodes with BALF collection) were more feasible than would be possible with client-owned cats.

Corticosteroids are considered the most effective treatment for patients with asthma.[19] In humans, inhaled corticosteroids have replaced orally administered corticosteroids for treatment of persistent asthma because the inhaled corticosteroids result in fewer systemic adverse effects and have considerable efficacy.[20] However, adverse effects can be seen, especially with high-dose, long-term treatment. In addition, corticosteroids would potentially be contraindicated in cats with concurrent infections, predisposing factors for infection (eg, urinary calculi), certain types of heart disease, or diabetes mellitus. The goal of inhalant treatment is to maximize local efficacy and minimize systemic bioavailability. Local delivery to the bronchi of 20 cats was documented[21] by use of an inhalant radiopharmaceutical. Administration of this radiopharmaceutical was performed in awake, untrained cats and was tolerated well in all cases. Although to our knowledge similar studies have not been performed with inhalant flunisolide, the blunting of eosinophilic inflammation with this treatment would provide support that it does reach the bronchi and exert anti-inflammatory effects.

In the study reported here, we determined that delivery of inhalant flunisolide by use of a pediatric holding chamber (spacer) significantly reduced the percentage of eosinophils in BALF. Eosinophils play a critical role in the pathogenesis of asthma through release of preformed mediators stored within secretory granules and synthesis of lipid mediators and reactive oxygen species.[22] Although a reduction in the percentage of eosinophils in BALF was detected, the inhaled corticosteroid did not significantly decrease airway hyperresponsiveness among all cats.

The importance of LTs in the pathogenesis of asthma in cats has been questioned, with studies[1,13] docu-

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menting a lack of increased excretion of cysteinyl LTs in urine (considered a marker of total body LT excretion) from cats with naturally developing bronchial disease or experimentally induced asthma and no substantial detection of cysteinyl LTs in BALF from the cats with experimentally induced asthma. An in vitro study[9] that used a selective 5-lipoxygenase inhibitor to block LT synthesis from tracheal and bronchial (third generation) smooth muscle strips from antigen-sensitized cats did not reveal effects on the type I immediate hypersensitivity reaction. Not all humans receiving antileukotriene medications respond, perhaps because LTs do not play a critical role in the inflammatory cascade in these individuals.[12] In the study reported here, there was no significant beneficial effect of zafirlukast on any of the evaluated variables, and considered together with data from other studies, these results would suggest that we cannot recommend use of this drug for treatment.

Evidence for the role of serotonin in the airways of cats was reported in an aforementioned study[9] in which investigators evaluated tracheal and bronchial smooth muscle from cats with experimentally induced asthma attributable to sensitization with *Ascaris suum*. Serotonin was detected in the perfusate of tissues after (but not before) addition of *A suum* antigen. Additionally, blockade of serotonin receptors by use of cyproheptadine was documented by a decrease in the maximal contraction of smooth muscle in response to antigen. Although we did not detect a significant decrease in airway hyperreactivity after cyproheptadine treatment in the study reported here, a subgroup (2 of 6) of the asthmatic cats had noticeable increases in their $EC200R_L$. Although we administered a commonly used dose of cyproheptadine, it is possible that the lack of a significant difference could have been attributable to the fact that the dose was too low to achieve a blunting of airway hyperreactivity in all cats. Aside from a requirement for an increased dose of cyproheptadine, another reason for the lack of consistent decrease in airway hyperreactivity could have been the mechanism of testing this variable (ie, bronchoprovocation by use of the nonspecific bronchoprovocant methacholine). In humans, specific bronchial challenge testing with the allergen used to induce hyperresponsiveness is considered the criterion-referenced standard for diagnosis.[23] In another study[1] conducted by our laboratory group, we determined that challenge with BGA is a better method of documenting airway hyperreactivity than is challenge with methacholine (eg, instead of the use of methacholine as the bronchoprovocant, challenge exposure with aerosols of BGA could be used to induce an increase in airway resistance in a specific manner). Unfortunately, at the time the current study was performed, only methacholine was used. Finally, the small number of cats in the study reported here may have been responsible for the lack of significant differences.

Contrary to our hypothesis, orally administered corticosteroids did not have a significant effect on measured variables of systemic immunity, aside from a reduction in content of BGA-specific IgE. However, although the percentage of lymphocytes stained with B- and T-cell markers did not change among treatments, the absolute numbers of each of these cell populations were not evaluated and could have been decreased. In humans, administration of corticosteroids has been associated with peripheral lymphopenia and a preferential decrease of CD4+ cells, compared with that for CD8+ cells.[24] Additionally, because the washout period between drugs was only 4 weeks and the half-life of IgG is approximately 28 days, decreased production of allergen-specific IgG may not have been detected. The reason for a significant decrease in content of BGA-specific IgE but a lack of a significant change in IgA content, both of which have a half-life (typically a few days) that is substantially shorter than the half-life of IgG, is unknown.

Orally administered and inhalant corticosteroids appeared to suppress eosinophilic inflammation of the airways in cats with experimentally induced asthma. Inhaled corticosteroids can be considered as an alternate treatment for decreasing inflammation of airways in cats with asthma. Oral administration of prednisone decreased serum content of BGA-specific IgE, but other classes of antibodies were not significantly affected. Airway hyperreactivity was not consistently diminished by any of the drugs evaluated in this study; however, a subpopulation of cats had noticeable decreases in airway hyperresponsiveness when treated by oral administration of prednisone or cyproheptadine. Additional studies are warranted to evaluate inhalant steroids and cyproheptadine in cats with naturally developing asthma.

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j. Series 8300, Hans Rudolph Inc, Kansas City, Mo.
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l. Model DP15-24, Validyne Engineering, Northridge, Calif.
m. Po-Ne-Mah, Gould Instrument Systems, Valley View, Ohio.
n. Falcon 352054, Becton, Dickinson & Co, Franklin Lakes, NJ.
o. Leukocyte Antigen Biology Lab, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, Calif.
p. Donkey anti-mouse IgG (H + L), Jackson Immunoresearch Laboratories, West Grove, Pa.
q. FACScan, Becton Dickinson Biosciences, San Jose, Calif.
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