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Oral glucocorticoids diminish the efficacy of allergen-specific immunotherapy in experimental feline asthma

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ABSTRACT

Allergen-specific rush immunotherapy (RIT) shows promise in treating asthma; however, pet cats will likely require at least initial concurrent glucocorticoids (GCs) to control serious clinical signs. How the immunosuppressive effects of GCs would impact RIT in cats is unknown. The hypothesis of this study was that oral, but not inhaled GCs will diminish the efficacy of RIT in experimental feline asthma. Cats ($n = 6/\text{group}$) were sensitized using Bermuda grass allergen (BGA) and randomized to receive BGA-specific RIT for 9 months with an oral GC (prednisolone 10 mg daily), inhaled GC (fluticasone 220 μg twice daily), or placebo administered for the first 6 months. Bronchoalveolar lavage fluid (BALF) percent eosinophils and other immunological assays were performed.

Eosinophilic airway inflammation was suppressed in all groups at month 6 of RIT (group mean $\pm$ SD, $5 \pm 2\%$, $13 \pm 4\%$, and $7 \pm 2\%$ for oral GC, inhaled GC, and placebo, respectively; $P = 0.291$). BALF percent eosinophils significantly increased over time only in oral GC/RIT cats between months 6 and 9 ($P = 0.031$). Placebo/RIT cats had significant decreases over time in BGA-specific serum IgE ($P = 0.031$). Concentration of interleukin (IL)-5 in BALF significantly increased over time in inhaled GC/RIT cats ($P = 0.031$). No significant differences were found between groups at month 6 or over time in each group for BGA-specific lymphocyte blastogenesis, percent blood T regulatory cells, or number of IL-10-producing cells. Given the significant increase of airway eosinophilia over time in RIT cats initially treated with an oral GC, inhaled GCs might be better for dampening eosinophilic inflammation until RIT normalizes the dysregulated immune system.

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Introduction

Feline allergic asthma is most commonly treated with glucocorticoids (GCs) and bronchodilators, although neither addresses the underlying aberrant immune response. Furthermore, GCs are contraindicated with concurrent diseases such as diabetes mellitus. To minimize these systemic effects, inhaled GCs have been applied in asthmatic cats (Cohn et al., 2010; Leemans et al., 2012; Reinero et al., 2005). In place of palliative treatment, allergen-specific immunotherapy (ASIT) has been advocated with the potential to cure allergic asthma.

Using an experimental feline asthma model, we developed a protocol for an injectable abbreviated form of ASIT called rush immunotherapy (RIT) (Reinero et al., 2006b) and compared its safety and efficacy with an adjuvant (Reinero et al., 2008) and with mucosal RIT (Lee-Fowler et al., 2009b). These studies demonstrated promising results in dampening eosinophilic airway inflammation via alterations in allergen-specific immunoglobulins, induction of

hyporesponsive lymphocytes, and modulation of cytokine profiles. Recently we documented that RIT could blunt eosinophilic inflammation even when the allergen(s) used for RIT were unrelated to or were just a partial repertoire of allergen(s) implicated in sensitization (Reinero et al., 2011). These data are especially important in application of RIT in pet cats, because accurate allergen identification can be challenging due to limitations of allergen-specific IgE assays (Lee-Fowler et al., 2009a), concurrent drug therapy (Chang et al., 2011) or intermittent allergenic exposure.

A primary goal of asthma treatment is reduction of airway inflammation, since inflammation contributes to airway hyperresponsiveness and remodeling. In experimental feline asthma, RIT reduces inflammation over several months. Asthmatic pet cats will require concurrent GC therapy until RIT becomes effective. Consequently, determining the impact of GCs on RIT efficacy is prerequisite before RIT can be translated to asthmatic pet cats. The current investigation was designed to determine how the immunosuppressive effects of GCs will impact RIT by simulating the clinical situation where RIT is used with concurrent oral or inhaled GC treatment before weekly RIT becomes a sole treatment. Because inhaled GC has shown less systemic immunosuppressive effects than

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oral GC (Allen et al., 2003; Reinero et al., 2006a), we hypothesized that oral but not inhaled GC would diminish the efficacy of RIT in experimentally induced asthmatic cats.

Materials and methods

Animals

Eighteen purposely bred domestic cats (11 males, 7 females; 6 months to 1.5 years old) were used and cared for in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and with approval by the University of Missouri Animal Care and Use Committee (ACUC protocol number: 6912).

Induction of asthma

Naïve cats were sensitized with Bermuda grass allergen (BGA), as previously described (Reinero et al., 2011), receiving weekly BGA aerosol challenges until study completion. Briefly, on day 0, cats received a subcutaneous (SC) injection of 12 µg of BGA/10 mg of alum and 100 ng *Bordetella pertussis* toxin SC; on day 14, intranasal BGA (75 µg of BGA in 200 µL of PBS); and on day 21, 12 µg of BGA/10 mg alum SC. On day 28, sensitization was confirmed with a positive intradermal skin test (IDST). During the next 2 weeks, cats were challenged seven times with nebulized BGA (up to 500 µg, titrated to effect). Following the intensive aerosol challenges, the asthmatic phenotype was confirmed (defined as bronchoalveolar lavage fluid [BALF] % eosinophils >17%).

Treatments

As previously described (Lee-Fowler et al., 2009b), all cats received SC RIT for 9 months. For acute escalation of the allergen dose for RIT, cephalic intravenous (IV) catheters were placed and cats were monitored closely for allergic complications. Subsequently, weekly maintenance SC RIT injections (200 µg of BGA in 1 mL of PBS) were given to all cats. Cats were randomly divided into three groups of six to receive simultaneous treatment with oral GC (10 mg prednisolone daily), inhaled GC (220 µg fluticasone twice daily), or gelatin capsules as placebo for the first 6 months of the study.

Airway sampling

BAL samples were collected using a blind technique at month 6 (M6) and month 9 (M9) as previously described (Reinero et al., 2011). Cyto centrifugation was used to prepare a slide for staining with a modified Wright's stain. A 200 cell differential count was performed. Remaining BALF was centrifuged at 300 g for 10 min and the supernatant harvested and stored at –20 °C until analysis.

Serum BGA-specific IgE

Serum was harvested at M6 and M9 and banked at –20 °C until analysis. An enzyme-linked immunosorbent assay (ELISA) using polyclonal chicken anti-feline IgE antisera (developed and validated in a similar fashion to the previously published protocol using polyclonal rabbit antisera) was conducted to measure serum BGA-specific IgE. Pooled sera from experimentally induced asthmatic cats used in a different study with strong IDST reaction to BGA was used as a positive control; PBS and pooled sera from non-sensitized cats were used as negative controls. The following modifications to the original protocol were made, however, the times of incubation remained the same and all volumes were 100 µL unless noted. Washes were performed between each step. The 96-well plate after initial coating with 1 µg of BGA was blocked with 1% bovine serum albumin (BSA) in PBS–Tween 0.5%, and incubated with each of the following in a step-wise fashion: 1:5 diluted serum samples in duplicate (in PBS–Tween 0.5%/0.5% BSA), 1:15,000 diluted polyclonal chicken anti-feline IgE, 1:20,000 diluted biotinylated donkey anti-chicken antibody (Jackson ImmunoResearch Laboratories), 1:1000 diluted peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories), and 200 µL of substrate (O-phenylenediamine dihydrochloride, Sigma) for 45 min. A spectrophotometer (SpectraMax Plus 384, Molecular Devices) was used to read the plate at a dual wavelength of 450–650 nm.

The optical density (OD) of each well was measured and the OD of background wells (PBS–Tween 0.5%) subtracted. To normalize the OD value between different plates and days, the mean OD of positive control from all plates run was divided by the OD of positive control of the plate and the number was multiplied by the OD value of each sample on that plate. Consequently, the results of serum BGA-specific IgE were recorded as the percentage of a normalized positive control.

BALF interleukin-5

Banked BALF supernatants from M6 and M9 were used to measure the concentration of interleukin (IL)-5 using a commercially available ELISA kit (R&D systems) following the manufacturer's instructions. Samples were run neat and in duplicate. The range of detection was 0.0156–1 ng/mL.

Lymphocyte blastogenesis

Using density gradient centrifugation, peripheral blood mononuclear cells (PBMC) were isolated from whole blood in EDTA and resuspended in complete RPMI (cRPMI; 1640 with 10% FBS, 5 mL 1 M Hepes, 5 mL penicillin–streptomycin–glutamine, and 0.35 µL diluted beta-mercaptoethanol). To a 96-well plate (3595 Costar, Corning), 1×10^5 PBMC were added in triplicate with media alone or BGA (50 µg/mL). The plate was incubated at 37 °C with 5% CO₂ for 6 days (media replaced at 3 days). A commercially available kit for lymphocyte proliferation was used according to instruction (Cell Proliferation ELISA, Roche Diagnostic). The results are represented as a stimulation index (SI) for each cat: $SI_{BGA} = OD_{BGA}/OD_{media\ alone}$.

T regulatory cells

Whole blood in EDTA (100 µL) was added to 12 × 75 polystyrene tubes and red blood cells were lysed using ACK lysis buffer (8.26 g NH₄Cl, 1 g KHCO₃, 0.037 g Na₂EDTA, in 1 L deionized distilled water, pH 7.2). Cell pellets were added to a 96-well plate (3799 Costar, Corning) and stained for surface markers (CD4 and CD25) and an intracellular marker (FoxP3) with appropriate isotype controls. In brief, anti-feline CD4 (clone 3-4F4, Southern Biotech) and anti-feline CD25 (clone 9F23, provided by Dr. W.A. Tompkins, North Carolina State University) were incubated with cells and FACS buffer (PBS with 3% FBS and 0.09% sodium azide) on ice for 30 min. Using the FoxP3/Transcription Factor Staining Buffer Set (eBioscience), cells were fixed and permeabilized on ice for 30 min and stained with FoxP3 (clone FJK-16, eBioscience) for 60 min.

After washing and resuspension in FACS buffer, the cells were analyzed with a Cyan ADP Flow Cytometer (Becton Dickinson). Lymphocytes were gated on a forward vs. side scatter plot and then on a CD4 vs. CD25 plot. The CD4+CD25+ T cells were then applied to a histogram of FoxP3+, so the CD4+CD25+FoxP3+ lymphocytes could be quantified. To determine % CD4+CD25+FoxP3+ T regulatory cells (Tregs), the quantified numbers were divided by the number of peripheral blood lymphocytes in the forward vs. side scatter gate.

IL-10 ELISPOT

An ELISPOT kit (Feline IL-10 Development Module, R&D Systems) was used according to the manufacturer's instructions with minor modifications. Diluted (1:120) feline IL-10 capture antibody was added at 100 µL to a 96-well filtration plate and incubated overnight at 4 °C. Blocking buffer (200 µL) was added after washing and the plate was incubated at room temperature for 2 h. Next, 5×10^4 PBMC in cRPMI were added in triplicate. Recombinant feline IL-10 and sterile media were used as positive and negative controls, respectively. The plate was incubated at 37 °C with 5% CO₂ for 2 days and 100 µL of diluted (1:120) detection antibody were added after washing with subsequent overnight incubation at 4 °C. After washing, ELISPOT Blue Color Module (R&D System) was added and the plate was rinsed with deionized water and dried. Spots in each well were quantitatively measured using a CTL Immunospot Analyzer (Cellular Technology).

Statistical analysis

A commercially available software program (SigmaStat, Systat Software) was used. Given the small sample size, non-parametric statistical tests were used. For each parameter (% BALF eosinophils, BGA-specific serum IgE, BALF IL-5, proliferating lymphocytes, % Tregs, IL-10 producing cells), Kruskal–Wallis one-way analysis of variance on Ranks was used to compare the data among groups at M6. Additionally, to compare the data over time between M6 and M9 in each group, a Wilcoxon signed rank test was performed. $P < 0.05$ was considered statistically significant.

Results

Percent BALF eosinophils

At M6 of RIT while still receiving concurrent oral or inhaled GC or placebo, similar suppression of eosinophilic airway inflammation was shown in all groups (oral GC 0–14%; inhaled GC 3–29%; placebo 1–12%; Table 1) with no significant difference between groups ($P = 0.291$; Fig. 1). However, after discontinuing oral or inhaled medications, a significant increase in % BALF eosinophils over

Table 1

Percentage of eosinophils in bronchoalveolar lavage fluid from four different time points (pre-sensitization with BGA, post-sensitization with BGA, month 6 [M6] of rush immunotherapy [RIT], and month 9 [M9] of RIT) are shown for each cat in each treatment group (oral glucocorticoid [GC]/RIT, inhaled GC/RIT, placebo/RIT).

	Pre-sensitization	Post-sensitization	M6 of RIT	M9 of RIT
<i>Oral GC/RIT</i>				
Cat 1	1	88	5	53
Cat 2	0	42	3	8
Cat 3	5	49	7	16
Cat 4	9	83	14	46
Cat 5	8	33	1	5
Cat 6	0	61	0	19
<i>Inhaled GC/RIT</i>				
Cat 7	1	85	29	19
Cat 8	0	89	6	23
Cat 9	4	54	3	11
Cat 10	1	77	6	1
Cat 11	1	77	20	20
Cat 12	0	84	11	3
<i>Placebo/RIT</i>				
Cat 13	1	40	12	18
Cat 14	0	70	7	29
Cat 15	4	27	9	2
Cat 16	0	43	2	0
Cat 17	0	32	1	2
Cat 18	2	96	8	16

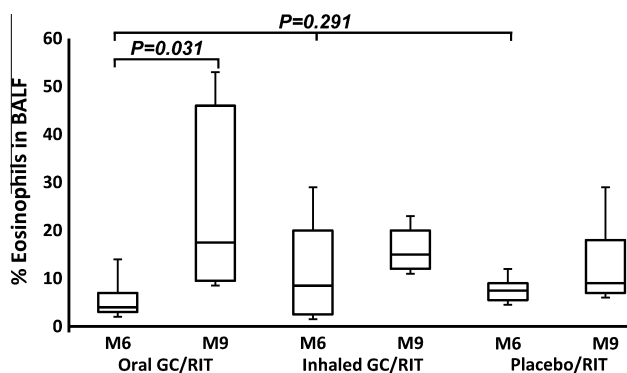


Fig. 1. Box and whisker plots of the percentage of bronchoalveolar fluid (BALF) eosinophils at month 6 (M6) and month 9 (M9) in each treatment group (oral glucocorticoid [GC]/rush immunotherapy [RIT], inhaled GC/RIT, or placebo/RIT). The upper and lower edges of the box represent the 75th and 25th percentiles respectively. The line inside the box shows the median value and the whiskers represent the range. There was no significant difference in the % BALF eosinophils at M6 between treatments ($P = 0.291$). After withdrawal of oral or inhaled GC or oral placebo at month 6 and with continued weekly maintenance RIT for the full 9 months, the impact of GC therapy on the efficacy of RIT could be assessed. Only cats receiving oral GC/RIT had significant increases in % BALF eosinophils over time between M6 and M9 ($P = 0.031$), suggesting that oral GC negatively impacted the efficacy of RIT.

time between M6 and M9 was found in oral GC/RIT treated cats ($P = 0.031$), but not in cats treated with inhaled GC/RIT ($P = 1.000$) or placebo/RIT ($P = 0.438$).

Serum BGA-specific IgE

Although serum BGA-specific IgE was not significantly different between groups at M6 ($P = 0.830$), serum BGA-specific IgE decreased significantly over time between M6 and M9 in the placebo/RIT group ($P = 0.031$; Table 2). Neither inhaled GC/RIT, nor oral GC/RIT showed a significant change between M6 and M9 ($P = 0.156$ and $P = 0.438$, respectively).

BALF IL-5

The BALF IL-5 concentration was not significantly different between groups at M6 ($P = 0.437$; Table 2). However, a significant increase ($P = 0.031$) in IL-5 over time between M6 and M9 was noted in inhaled GC/RIT treated cats. Though not statistically significant, increased IL-5 over time was also found in cats receiving oral GC/RIT ($P = 0.063$), contrasting to decreased IL-5 over time ($P = 1.000$) in placebo/RIT cats.

Lymphocyte blastogenesis

Lymphocyte proliferative response to BGA in vitro was not significantly different between groups at M6 ($P = 0.849$; Table 2). No significant difference between M6 and M9 was detected for any treatment group.

Percent T regulatory cells

The % CD4+CD25+FoxP3+ Tregs did not show a significant difference between groups at M6 ($P = 0.949$; Table 2) and a significant change between M6 and M9 was not found in any group.

IL-10 ELISPOT

The number of IL-10 producing cells determined by ELISPOT was not significantly different between groups at M6 ($P = 0.83$; Table 2). While all three groups showed an increase in the number of IL-10-producing cells over time after RIT, the difference was not statistically significant.

Adverse effects

Two cats developed vomiting and watery diarrhea during acute administration of RIT. As part of the RIT protocol, both cats were administered diphenhydramine (2 mg/kg, IM), which alleviated the clinical signs and allowed for the continuation of the dose escalation. No adverse signs were noted after any of the maintenance injections in any cat.

Discussion

The results of this study demonstrated that oral GC (administered at clinically relevant doses for cats with spontaneously developing asthma) negatively impacted the efficacy of RIT in experimental feline asthma. After 6 months of RIT with or without GCs, all cats in each group demonstrated decreased % eosinophils in their airways representing the anti-inflammatory effect by GC, RIT, or the combined treatments. After 9 months of RIT, the last three without either GC or placebo administration, only the oral GC/RIT group demonstrated a significant increase of % BALF eosinophils between M6 and M9. Considering RIT has shown impressive efficacy in dampening eosinophilic inflammation in several prior studies, increased % BALF eosinophils in cats treated with concurrent oral GC and RIT treatment suggests a negative impact of oral GCs on the efficacy of RIT in dampening eosinophilic airway inflammation in asthmatic cats.

A recent study (Majak et al., 2009) evaluating asthmatic children demonstrated similar suppressive effects of GCs on early clinical and immunological effects of ASIT. In that study, asthmatic children who received placebo with ASIT used a reduced dose of inhaled GC to control their clinical signs compared with asthmatic children who had oral GC treatment concurrent with ASIT, suggesting suppressive effects of oral GC on the efficacy of ASIT. Monitoring the need to use inhaled GC to control clinical signs has been

Table 2

Results (median with range, *P* values over time between month 6 [M6] and month 9 [M9]) for several immunoassays (BGA-specific serum IgE by ELISA, BALF IL-5 concentration by ELISA, lymphocyte blastogenesis by ELISA, % Tregs by flow cytometry, and IL-10 producing cells by ELISPOT) redundant in each treatment group (oral glucocorticoid [GC]/rush immunotherapy [RIT], inhaled GC/RIT, or placebo/RIT).

	BGA-specific serum IgE (%)	BALF IL-5 (ng/mL)	Lymphocyte blastogenesis (SI)	Tregs (%)	IL-10 producing cells
<i>Oral GC/RIT</i>					
M6	20 (11–355)	0.016 (0.016–0.174)	1.5 (0.9–2.6)	23.7 (13.6–34.8)	188 (115–242)
M9	7 (4–69)	0.063 (0.016–0.398)	1.5 (1.0–3.3)	22.6 (18.2–32.7)	224 (133–350)
<i>P</i>	0.438	0.063	0.563	1.000	0.313
<i>Inhaled GC/RIT</i>					
M6	19 (11–70)	0.018 (0.016–0.090)	1.8 (1.0–3.1)	21.5 (14.2–34.0)	212 (115–261)
M9	7 (3–25)	0.121 (0.020–0.235)	2.6 (0.6–3.4)	29.4 (19.8–40.2)	277 (175–319)
<i>P</i>	0.156	0.031	0.219	0.156	0.063
<i>Placebo/RIT</i>					
M6	27 (13–73)	0.047 (0.016–0.186)	1.6 (1.1–3.0)	30.6 (21.1–39.8)	211 (83–279)
M9	3 (2–7)	0.019 (0.016–1.155)	2.0 (0.8–3.4)	29.8 (19.7–39.4)	273 (28–372)
<i>P</i>	0.031	1.000	0.844	0.688	0.219

mentioned as the best approach to evaluate the clinical efficacy of ASIT in asthmatic children (Pham-Thi et al., 2007). Additionally, pulmonary function testing has been used in this regard in human asthma (Stelmach et al., 2008). However, evaluating improvement or exacerbation of clinical signs with GC treatment and RIT in our study was not feasible because experimentally asthmatic cats are symptomatic only when directly challenged with allergen. To determine the effect of GCs on clinical efficacy of RIT, future studies in pet cats with naturally developing asthma, where clinical signs can be monitored on a daily basis, would be warranted. As a surrogate for clinical signs, pulmonary function testing with broncho-provocants to assess airway hyperresponsiveness in experimentally asthmatic cats treated with GC and RIT could also be performed.

Serum BGA-specific IgE was not significantly different between groups after 6 months of oral or inhaled GC, or placebo treatment with concurrent RIT. However, serum BGA-specific IgE in placebo/RIT significantly decreased at M9 compared to M6, suggesting that RIT is effective in decreasing allergen-specific IgE in serum, consistent with several studies of humans with asthma (Bahceciler et al., 2005; Cosmi et al., 2006; Eifan et al., 2010). Although a prior study in experimentally asthmatic cats treated with RIT for 6 months did not demonstrate a decrease in serum BGA-specific IgE (Reinero et al., 2006b), this could be due to the fact that this change takes longer to manifest. Interestingly, serum BGA-specific IgE was not significantly different after 9 months in either oral or inhaled GC/RIT group, suggesting that GC can interfere with this type of immunomodulation.

Local IL-5, a representative Th2 cell cytokine, did not differ between groups after 6 months of treatment. Between M6 and M9, IL-5 in BALF increased significantly only in cats treated with inhaled GC/RIT, although IL-5 concentration also increased over time in cats treated with oral GC/RIT. This was in contrast to the placebo/RIT groups (decreased, but not statistically significant). A study has shown significantly decreased IL-5 after 3 months of oral GC and ASIT in asthmatic children (Majak et al., 2009) and these contrasting results might be due to the different kinds of samples assayed (BALF vs. peripheral mononuclear blood cells).

There was no positive relationship between % eosinophils and IL-5 in the BALF of the inhaled GC/RIT group, as only the IL-5 concentrations were significantly higher over time. Although mRNA for feline IL-5 in experimental asthma has been previously shown to be increased in untreated asthmatic cats compared to cats after 6 months of RIT (Reinero et al., 2006b), this was not statistically significant, most likely because the study was underpowered for this outcome parameter. In the current study, we were also limited by a small sample size. Additionally, assaying cytokines in BALF is subject to dilution and there has not been a universally accepted

analyte in the cat that can be used to normalize for this dilution. Future studies should focus on alternative methods of IL-5 protein analysis, such as by intracellular flow cytometry or culture of BALF lymphocytes to be used in an ELISPOT assay to determine if this is a repeatable finding.

Treatment with GC concurrent with RIT did not significantly impact allergen-specific lymphocyte blastogenesis. While there was no statistically significant difference in the SI between groups, it is noteworthy that the median SI at M9 both in inhaled GC/RIT and placebo/RIT groups was ≥ 2 , whereas the median SI was < 2 in all groups at M6 and in the oral GC/RIT group at M9. A SI < 2 is reflective of an insignificant lymphocytic proliferative response (i.e. a 'negative' result) (Fujiwara et al., 2003). The in vitro lymphocyte proliferative response to BGA was measured by an ELISA that is relatively insensitive to quantify changes in the small numbers of cells that are allergen-specific (likely 1/100–1/1000 CD4+ T cells) (Abbas et al., 2007). A recent flow cytometric assay tailored to measure lymphocyte proliferation to BGA has been shown to be highly sensitive and flexible (Reinero et al., 2012). Future studies with this newly developed flow cytometric assay might be more effective in evaluating the proliferative response of lymphocytes to specific allergens like BGA.

Many studies in humans and animals (Gross et al., 2011; Larche et al., 2006; Radulovic et al., 2008; Reinero et al., 2011) have shown that ASIT upregulates Tregs, which might dampen Th2-driven immune responses in allergic asthma. In asthmatic children, one 0.5–1 mg/kg dose of prednisone during of the first treatment of ASIT decreased FoxP3 expression and % Tregs induced by ASIT over 9 months (Majak et al., 2009). Reports on the effects of GCs on Tregs are conflicting with the evidence that they can limit (Stock et al., 2005) or stimulate (Kang et al., 2008; Peek et al., 2005) Treg development. In our study, no significant difference in the number of Tregs was found between groups at M6 or between M6 and M9 in any group. As the number and function of Tregs are not always correlated (Lee et al., 2007; Smyth et al., 2010), future studies could investigate the function of Tregs.

IL-10 is secreted by Tregs and might be a beneficial mediator in ASIT by down-regulating airway inflammation (Hawrylowicz and O'Garra, 2005; Larche et al., 2006; Reinero et al., 2011). In human asthma, GCs decreased the production of IL-10 in the supernatant of cultured PBMC with ASIT (Majak et al., 2009). In the current study, the number of IL-10-secreting cells was measured by ELISPOT and no significant difference was found between groups at M6 or between M6 and M9 in each group. Additional studies on local IL-10 production in the airways, perhaps using ELISA or flow cytometry, are warranted.

Several limitations should be noted for this study. Firstly, a small number of cats were used in each group. Secondly, the cur-

rent study did not evaluate functional (airway hyperresponsiveness) or anatomical (remodeling) changes with each GC treatment or RIT. Thirdly, our study used a model of asthma and future studies will need to be performed in pet cats with spontaneously developing asthma to confirm these findings.

Conclusions

Initial treatment with oral GC, but not with inhaled GC or placebo with concurrent RIT led to increases in airway eosinophilia over time after withdrawal of GC or placebo. However, a significant increase in BALF IL-5 concentration over time without a concurrent significant increase of % eosinophils was found in inhaled GC/RIT treated cats, a finding which further needs to be evaluated in future studies. Inhaled GC appeared to be a better option for control of clinical signs in experimentally induced asthmatic cats during the time it takes RIT to induce immunological tolerance.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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