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Evaluation of subcutaneous versus mucosal (intranasal) allergen-specific rush immunotherapy in experimental feline asthma

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ABSTRACT

Rush immunotherapy (RIT) is effective for the treatment of experimental feline allergic asthma. In humans, the safety profile of immunotherapy is improved by delivering allergen by a mucosal route. We hypothesized that mucosal (intranasal) RIT would have similar efficacy to subcutaneous RIT with improved safety.

Twelve cats sensitized and challenged with Bermuda grass allergen (BGA) were randomized to receive subcutaneous (SC) or intranasal (IN) RIT. Increasing doses of BGA (20–200 µg) were administered over 24 h followed by 200 µg BGA weekly as maintenance. Adverse reactions were recorded. Clinical respiratory scores after BGA aerosol challenge, bronchoalveolar lavage fluid (BALF) % eosinophils, and cytokine concentrations were measured before RIT (day 1) and at months 1, 3 and 6 (M1, M3, M6).

More adverse events were recorded with SC RIT ($n = 12$) compared with IN RIT ($n = 6$). Respiratory scores were lower by M6 compared with D1 in both the groups. The % BALF eosinophils declined significantly after RIT for both groups (mean $\pm$ SEM, SC RIT D1 62 ± 12 , M6 9 ± 4 ; IN RIT D1 54 ± 9 , M6 14 ± 6). The BALF IL-4:IFN- γ ratio significantly decreased over time in the IN RIT group (mean $\pm$ SEM, D1 2.4 ± 0.2 , M6 1.0 ± 0.2).

While both protocols decreased eosinophilic airway inflammation, the SC RIT protocol did not cause life-threatening adverse events and demonstrated more consistent resolution of clinical signs after allergen challenge. Either protocol could be considered for the treatment of feline allergic asthma.

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1. Introduction

Feline allergic asthma results from an aberrant immune response to inhaled allergens. Allergen-specific activation of CD4+T helper 2 (Th2) cells leads to production of pro-inflammatory cytokines such IL-4, IL-5, and IL-13 causing eosinophilic airway inflammation, airway hyperresponsiveness, and airway remodeling (Bousquet et al., 2000; Fixman et al., 2007). Pathologic changes manifest with a wide range of clinical signs from a mild, intermittent cough to an acute, life-threatening onset of respiratory distress.

Glucocorticoids and bronchodilators have been the cornerstone of asthma therapy. However, these drugs do not address the underlying pathophysiology of the disease and only suppress events late in the inflammatory cascade. An ideal treatment should be directed at shutting off the abnormal immune response at the early stages; in other words, inducing tolerance to the allergen.

Currently, the only curative treatment for allergic disease is allergen-specific immunotherapy (ASIT). This involves the administration of gradually increasing doses of allergen over time (weeks to months with conventional immunotherapy versus days with abbreviated or “rush” immunotherapy (RIT)). Allergen-specific immunotherapy is most commonly used in the veterinary field for the treatment of atopic dermatitis (Griffin and Hillier, 2001). In

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human medicine, it has been effective as a treatment for allergic rhinitis and, less commonly, asthma (Kohno et al., 1998; Bousquet et al., 2001; Khinchi et al., 2004). Because of its potential to achieve a cure, protocols for ASIT were developed and evaluated in cats using an experimental model of allergic asthma (Reinero et al., 2006). These studies investigated the use of ASIT using an abbreviated subcutaneous/intranodal RIT protocol or using an adjuvanted (CpG motifs) RIT protocol (Reinero et al., 2006, 2008). In this animal model, allergen-specific RIT significantly decreased eosinophilic airway inflammation with or without an adjuvant, respectively. Although efficacy was demonstrated, at least some adverse reactions were noted (especially in the non-adjuvanted protocol) making improvement of the safety profile important prior to routine clinical use in asthmatic pet cats. In humans, an alternative route of ASIT administration, mucosal immunotherapy, has shown equal or superior efficacy with a reduction in adverse events (Rossi and Monasterolo, 2002; Khinchi et al., 2004; Wilson et al., 2005).

The purpose of the current study was to compare safety and efficacy of the established subcutaneous RIT protocol directly with a novel mucosal (intranasal) RIT protocol using Bermuda grass allergen (BGA) in an experimental model of feline asthma. We hypothesized that mucosal RIT would have similar efficacy, but have fewer adverse side effects than subcutaneous RIT.

2. Materials and methods

2.1. Animals

Twelve specific pathogen free, mixed breed cats (4 intact females and 8 intact males) were bred from a high-responder asthmatic cat research colony (University of Missouri, Columbia, MO) and were cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The cats ranged in age from 3 to 9 months and weighed from 2.1 to 4.6 kg at the time of enrollment in the study. This study was approved by the University of Missouri Animal Care and Use Committee.

2.2. Allergen sensitization and aerosol challenge

Prior to induction of an asthmatic phenotype, all cats were tested to confirm they had not previously been sensitized to BGA by performance of intradermal skin testing (IDST); additionally, they were screened to ensure they did not have preexisting eosinophilic airway inflammation by analysis of bronchoalveolar lavage fluid (BALF). Cats were sensitized and challenged as previously described (Reinero et al., 2008). Briefly, on day 0, cats received a subcutaneous (SC) injection of 12 µg of BGA in 10 mg of alum and an intramuscular injection of 10⁷ *Bordetella pertussis* organisms; on day 14, 75 µg BGA in 0.2 ml of PBS intranasally; and on day 21, another SC injection of 12 µg of BGA in 10 mg of alum. On day 28, IDST was repeated to confirm sensitization to BGA. Although wheals to BGA were seen in all cats, three cats were boosted with a third SC injection of 12 µg of BGA in 10 mg of alum due to marginally positive reactions.

After parenteral sensitization, aerosol challenges of BGA were conducted on awake, spontaneously breathing cats in a sealed chamber. Allergen solution (100 µg of BGA dissolved in PBS) was aerosolized using an air compressor attached to a nebulizer. A total of seven challenges were administered within 2 weeks following parenteral sensitization (at which time eosinophilic airway inflammation was confirmed by BALF collection), and were given once weekly thereafter. The first aerosol challenge was administered for 15 min, and remaining aerosol challenges were administered for a maximum duration of 10 min. However, the duration of challenge varied between cats dependent upon the demonstration of clinical signs of asthma and respiratory distress. The allergen challenge was immediately discontinued and albuterol was administered when coughing, increased expiratory effort, and/or respiratory distress was noted. A respiratory scoring system was employed by the same investigator (TML-F) to evaluate clinical signs of airway hyperreactivity during the aerosol challenges (Table 1). Each cat was assigned a score based on these parameters, and scores were recorded for day 1 (time period after induction of the asthmatic phenotype but prior to rush immunotherapy), and months 1, 3, and 6 after rush immunotherapy.

2.3. Rush immunotherapy (RIT) protocol

Cats were randomized into two groups of 6 cats each based on a table of random numbers. Group 1 received SC injections (SC RIT), and group 2 received IN drops (IN RIT) of BGA in a dose escalating fashion (Table 2). Intravenous cephalic catheters were placed prior to RIT for vascular access if needed. All cats were continuously monitored for vomiting, diarrhea, agitation, labored breathing, and pruritus. On an hourly basis, the heart rate (HR), respiratory rate (RR) and character, temperature (T), capillary refill time (CRT), mucus membrane color, and

Table 1

Respiratory scoring system developed to evaluate clinical response to allergen challenges by aerosol.

Respiratory scoring system (0–5 points)	
Respiratory rate changes	
0 points	<50% increase in baseline RR
0.5 points	>50% increase in baseline RR
1.0 point	>100% increase in baseline RR
Clinical signs (post-challenge)	
0 points	No signs
0.5 points	Mild increase in respiratory effort but not enough to affect activity or mentation; spontaneous cough
1.0 point	Moderate signs of respiratory difficulty (expiratory push or rapid and shallow breathing)
1.5 points	Moderate to severe signs of respiratory difficulty (nostrils flaring, impaired activity, obvious distress)
2.0 points	Very severe signs (open mouth breathing, lateral collapse, obtunded)
Time in chamber	
0 points	10 min
0.5 points	5 to <10 min
1 point	2 to <5 min
1.5 points	1 to <2 min
2.0 points	<1 min

Table 2

Schedule of the rush immunotherapy (RIT) protocol used in an experimental model of feline asthma.

RIT protocol		
Day of treatment	Time	Dose
Day 1	8 am	20 µg
	10 am	40 µg
	12 pm	80 µg
	2 pm	100 µg
	4 pm	200 µg
Day 2	8 am	200 µg

Both the subcutaneous (SC) or intranasal (IN) groups received the same doses (µg) of allergen; allergen was administered in a volume of 1 ml for the SC group and 200 µl for the IN group. The maximum dose (200 µg) was continued weekly in the maintenance phase of allergen-specific immunotherapy for the full 6 months of study.

pulse quality were recorded. If cats showed any of the following abnormalities, the next injection of allergen was delayed until the parameters normalized: HR or RR increase by >30% over baseline, $T > 103.5^\circ\text{F}$, $\text{CRT} > 2\text{ s}$, pale mucus membranes and poor or bounding pulse quality. Cats were monitored until 1 h after their last injection of BGA. After the RIT was completed, a weekly maintenance treatment was administered at the dose of 200 µg BGA in PBS. Group 1 received this as a SC injection with a total final volume of 1 ml and group 2 received an IN treatment with a total final volume of 0.2 ml.

2.4. Bronchoalveolar lavage fluid collection and cell counts

Bronchoalveolar lavage fluid (BALF) was collected in a blind fashion (i.e., without bronchoscopic guidance) on day 1 (immediately prior to RIT) and months 1, 3, and 6 following RIT. Cats were anesthetized with ketamine HCl (5–10 mg/kg IV; Fort Dodge Animal Health, Fort Dodge, IA) and intubated. A 7-french polypropylene catheter was introduced through an endotracheal tube and advanced gently until the catheter became wedged and could no longer be advanced. A 15 ml aliquot of PBS was instilled through the catheter and immediately aspirated. All samples were placed on ice during the time between collection and analysis (within 2 h). A total nucleated cell count was performed on each BALF sample using a coulter counter (Z1 particle counter, Beckman Coulter, Fullerton, CA). Cytological evaluation and differential cell counts were performed on samples prepared by cytocentrifugation (Shandon Cytospin 4, ThermoElectron Corporation, Waltham, MA). Differential cell counts were performed by evaluating 200 nucleated cells per slide and reporting cell percentages. The remaining BALF was centrifuged at $300 \times g$ for 10 min, and the supernatant harvested in aliquots for storage at -20°C .

2.5. Cytokine analysis on BALF supernatant

Banked aliquots of BALF supernatant from day 1 and months 1, 3, and 6 stored at -20°C were thawed and used undiluted for determination of feline-specific IL-4, IFN- γ , and IL-10 concentrations. All samples were run in duplicate using commercially available ELISA kits (R&D

systems, Minneapolis, MN) according to manufacturer's instructions. The ranges of detection of these assays are 62.5–4000, 62.5–4000, and 125–8000 pg/ml, respectively. To evaluate Th2 to Th1 cytokine balance, IL-4 to IFN- γ ratios were determined. When evaluating absolute amounts of cytokine in BALF, samples were normalized to total protein concentration to account for the effects of dilution. Samples for total protein analysis were run in duplicate using a commercially available Bradford protein assay according to manufacturer's instructions (BioRad Laboratories, Hercules, CA); the range of detection was 0.05–0.5 mg/ml.

2.6. In vitro cytokine restimulation assay

Whole blood was collected by jugular venipuncture into EDTA tubes at day 1 and at months 1, 3, and 6. Peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque density centrifugation and were resuspended in complete RPMI (RPMI 1640 with 10% FBS, 5 ml 1 M Hepes, 0.35 µl diluted beta-mercaptoethanol (7 µl of 14.3 M BME in 10 ml PBS), 5 ml penicillin–streptomycin–glutamine (Gibco, Invitrogen Corporation, Grand Island, NY)) to a concentration of 5×10^6 cells/well. Cells were then cultured at 37°C in humidified 5% $\text{CO}_2/95\%$ air (Hera Cell 150, Kendro Laboratory Products, Langenselbold, Germany) for 24 h in a 24-well flat bottomed plate (Becton Dickinson Labware, Franklin Lakes, NJ). Two wells per cat were plated in a final volume of 800 µl: one well contained cells with cRPMI only and the second well contained cells incubated with 20 µg BGA in cRPMI. Supernatant was harvested after 24 h and banked at -20°C . Cytokine analyses for IL-4, IL-10, and IFN- γ were performed on banked samples as previously described for BALF supernatant.

2.7. Statistical analysis

A commercially available software program (SAS, version 9.1, SAS Institute, Cary, NC) was used to perform the Shapiro-Wilk test for normality. Differences in percentage BALF eosinophils, clinical scores of airway hyperreactivity, cytokine (IL-4, IFN- γ) concentrations in restimulated cell culture supernatants, and BALF IL-4: IFN- γ ratios among treatments were assessed by use of a mixed-model ANOVA to adjust for the effects of individual cat variation. All variables met criteria suitable for normality assumptions. Data are presented as mean $\pm$ SEM. For all comparisons, a p value < 0.05 was considered significant.

3. Results

3.1. Adverse reactions to RIT

Documented adverse reactions in both groups are shown in Fig. 1. No cat in either group had a severe (i.e., life-threatening) reaction. At the monitoring investigator's discretion, diphenhydramine was administered to two cats after vomiting/lethargy were noted on day 1; both these cats were premedicated with diphenhydramine on day 2

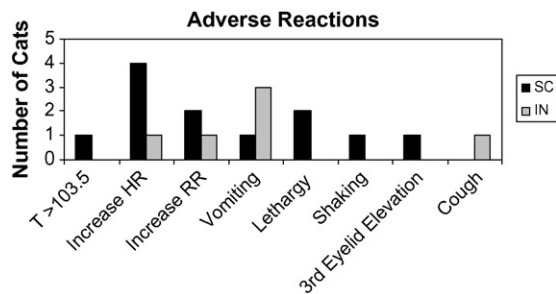


Fig. 1. Adverse reactions recorded during RIT. Fewer reactions were noted in the IN RIT group ($n = 6$) in comparison to the SC RIT group ($n = 12$). No life-threatening reactions were observed.

prior to completion of RIT. No adverse reactions requiring further therapy were noted on day 2 in either cat. During weekly maintenance treatments using SC injections or IN drops, no cat had a visually observable adverse reaction at any time over the 6 month treatment period.

3.2. Respiratory scores

Grading of clinical signs of airway hyperreactivity in response to aerosolized allergen challenge was performed at D1, M1, M3 and M6; the group mean $\pm$ SEM respiratory scores are shown in Fig. 2. Within the SC RIT group, the respiratory scores were significantly higher at D1 compared with M1 ($p = 0.009$), M3 ($p = 0.009$), and M6 ($p < 0.0001$). The respiratory scores in the IN RIT group were significantly higher at D1 compared with M6 ($p = 0.0091$). All 6 cats in the SC RIT group had complete resolution of clinical signs after BGA aerosol treatment after 6 months therapy (i.e., they had a score of 0). In comparison, 3 of 6 cats in the IN RIT group had complete resolution of clinical signs, 1 had worsening of signs, one had no change in signs, and one never displayed signs at any time point (even prior to IN RIT).

3.3. Bronchoalveolar lavage fluid (BALF) eosinophil percentages

In evaluating BALF eosinophil percentages, there was no significant treatment-time interaction ($p = 0.3499$),

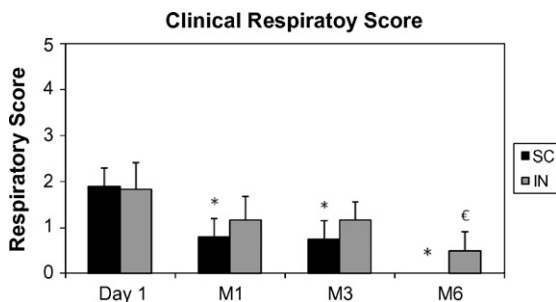


Fig. 2. Respiratory scores were assigned based on a respiratory scoring system (Table 1) during BGA aerosol challenges that took place 48 h prior to sample collection for D1 (prior to RIT), M1, M3, and M6 post-RIT. The SC RIT group had significant decreases in respiratory scores at M1, M3 and M6 compared to D1. The IN RIT group experienced a significant decrease in respiratory scores during aerosol challenges by month 6 compared with D1. * $p < 0.05$ in the SC group; [€] $p < 0.05$ in the IN group.

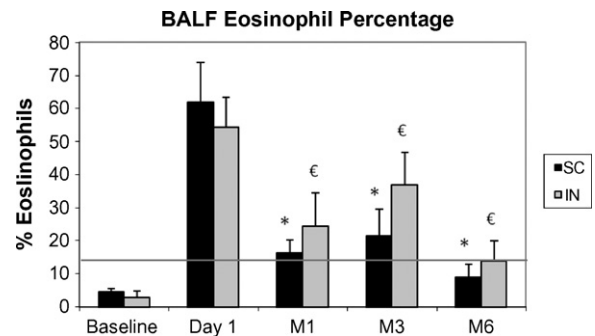


Fig. 3. Group mean $\pm$ SEM percentage of bronchoalveolar lavage fluid (BALF) eosinophils from baseline (prior to allergen sensitization), D1 (post-allergen sensitization and immediately prior to RIT), months 1, 3, and 6 post-RIT. The horizontal line across this graph represents a 16% BALF eosinophil percentage considered to be the reference range for healthy cats. Both groups had significant reduction in airway eosinophilia over the 6 month period ($p < 0.0001$). When compared to respective baseline values, there was no significant difference in mean eosinophil percentages at month 6 for either group. * $p < 0.05$ compared with D1 in the SC group; [€] $p < 0.05$ compared with D1 in the IN group.

suggesting that the two groups behaved similarly over time. Additionally, there was no effect of treatment ($p = 0.6212$) on the BALF eosinophil percentage; that is, neither SC or IN treatment had a clear advantage over one another in terms of reduction of eosinophilic airway inflammation. However, there was a significant ($***p < 0.0001$) difference in response to the treatment over time: both the SC and IN groups had a significant reduction in the group mean BALF eosinophil percentage over the 6 month period (Fig. 3). Individual group analyses showed that in the SC RIT group, the mean BALF eosinophil percentage was lower at M1, M3 and M6 compared with D1 ($p < 0.0001$ for all), and in the IN RIT group, the mean BALF eosinophil percentage was lower at M1 ($p = 0.009$), M3 ($p = 0.0420$) and M6 ($p < 0.0001$) compared to D1. Additionally, there was no significant difference between mean BALF eosinophil percentage between M6 and the eosinophil percentage in the cats prior to induction of asthma ($p = 0.6059$ SC RIT group; $p = 0.1752$ IN RIT group). After RIT, ten of twelve cats in this study had BALF eosinophil percentages that returned to the reference

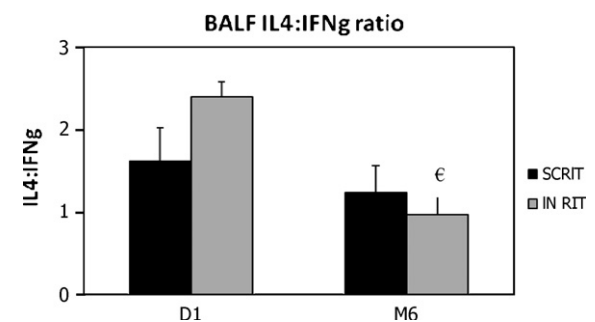


Fig. 4. Group mean $\pm$ SEM BALF IL-4:IFN- γ ratio at D1 (prior to RIT) and M6 post-RIT. There was no significant difference within the SC RIT group between the two time points. There was no significant difference noted between the SC and IN RIT groups at month 6. However, there was a significant reduction in the IL-4:IFN- γ ratio when D1 and M6 were compared. [€] $p < 0.05$ compared with day 1 in the IN group.

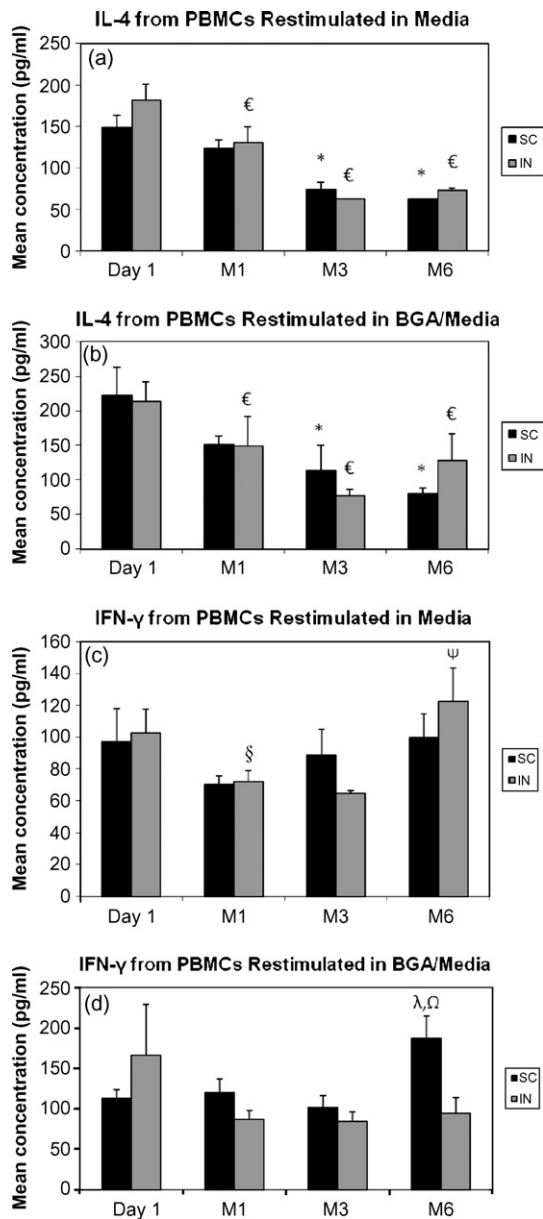


Fig. 5. Group mean $\pm$ SEM IL-4 and IFN- γ concentrations from restimulated peripheral blood mononuclear cells (PBMCs) at D1 (prior to RIT), and months 1, 3, and 6 post-RIT. (a) IL-4 concentrations from PBMCs incubated in media alone. A significant difference ($p < 0.0001$) in response to treatment over time was seen in both groups with a significant reduction in the group mean concentration of IL-4 over the 6 month study period. There were no significant differences between treatment groups. * $p < 0.05$ compared with D1 in the SC group; € $p < 0.05$ compared with D1 in the IN group. (b) IL-4 concentrations from PBMCs incubated in BGA. A significant difference ($p < 0.0001$) was demonstrated in response to both treatments over time. A significant reduction in mean IL-4 concentration was seen over the course of the 6 month study. No statistically significant differences were found between treatment groups * $p < 0.05$ compared with D1 in the SC group; € $p < 0.05$ compared with D1 in the IN group. (c) IFN- γ concentrations from PBMCs incubated in media alone. There was a difference in response to the treatment groups over time with only the IN RIT group mean IFN- γ concentration significantly increasing over the 6 month study. In the IN RIT group, there was a significantly higher mean IFN- γ concentration at M6 compared with M1 and M3, and at M1 compared with M3. However, there were no significant differences in mean IFN- γ concentrations between the

range for healthy cats ($<16\%$) by month 6 (Hawkins et al., 1990). Increased BALF eosinophil percentages persisted in one cat from each group.

3.4. Cytokine analysis (BALF supernatant and in vitro restimulated PBMCs)

There was a significant decrease in the BALF IL-4:IFN- γ ratio at month 6 compared with baseline only in the IN RIT group (Fig. 4; $p = 0.03$; mean $\pm$ SEM, D1 2.4 ± 0.2 , M6 1.0 ± 0.2). There was no significant difference ($p > 0.05$) in the BALF IL-4:IFN- γ ratio between groups at baseline or at month 6, and there was no significant decrease in the BALF IL-4:IFN- γ ratio within the SC RIT group at month 6 compared with baseline (D1 1.6 ± 0.4 , M6 1.2 ± 0.2).

Interleukin-10 was not detected in the majority of BALF and PBMC supernatant samples, precluding meaningful statistical analysis.

The IL-4 concentration in supernatant harvested from PBMCs incubated in media alone showed no significant treatment-time ($p = 0.2893$) or treatment ($p = 0.3748$) effects. There was a significant ($p < 0.0001$) difference in response to the treatment over time; both the SC and IN groups had a significant reduction in the group mean IL-4 concentration over the 6 month study (Fig. 5a). In the SC RIT group, the mean IL-4 concentration was significantly lower at M3 and M6 compared with D1 ($p < 0.0001$). Comparatively, in the IN RIT group, the mean IL-4 concentration was lower at M1 ($p = 0.0019$), M3 ($p < 0.0001$) and M6 ($p < 0.0001$) compared to D1. There was no significant difference in the group mean IL-4 concentration between the SC RIT and IN RIT groups at the start (D1, $p = 0.0643$) or end ($p = 0.5581$) of immunotherapy.

The IL-4 concentration in supernatant harvested from cells incubated in BGA showed no significant treatment-time ($p = 0.2798$) or treatment ($p = 0.8735$) effects. There was a significant ($p < 0.0001$) difference in response to the treatment over time; both the SC and IN groups had a significant reduction in the group mean IL-4 concentration over the 6 month study (Fig. 5b). In the SC RIT group, the mean IL-4 concentration was lower at M3 ($p < 0.0009$) and M6 ($p < 0.0001$) compared to D1. In the IN RIT group, the mean IL-4 concentration was lower at M1 ($p = 0.0276$), M3 ($p < 0.0001$) and M6 ($p = 0.0021$) compared to D1. There was no significant difference in the group mean IL-4 concentration between the SC RIT and IN RIT groups at the start (D1, $p = 0.9875$) or end ($p = 0.2991$) of immunotherapy.

The IFN- γ concentration in supernatant harvested from cells incubated in media alone showed no significant treatment-time ($p = 0.3897$) or treatment ($p = 0.9277$)

treatment groups at the start or end of RIT (day 1 or month 6). $\psi p < 0.05$ compared with M1 and M3 in the IN RIT group; $\S p < 0.05$ compared with M3 in the IN RIT group (d) IFN- γ concentrations from PBMCs incubated in BGA. Individual group analysis revealed a significantly higher group mean IFN- γ concentration in the SC group at M6 when compared to the IN group. $\lambda p < 0.05$ compared with M6 in the IN RIT group. The mean IFN- γ concentration was significantly higher at M6 than at all other time points in the SC RIT group. $\Omega p < 0.05$ compared with D1, M1 and M3 in the SC group.

effects. There was a significant ($p = 0.0168$) difference in response to the treatment over time. This difference was due to an increase in the group mean IFN- γ concentration in the IN RIT group over the 6 month study ($p = 0.0157$, Fig. 5c). In the IN RIT group, the mean IFN- γ concentration was higher at M6 compared with M1 ($p = 0.0148$) and M3 ($p = 0.0049$), and M1 compared with M3 ($p = 0.0416$). There was no significant difference in the group mean IFN- γ concentration between the SC RIT and IN RIT groups at the start (D1, $p = 0.7079$) or end ($p = 0.3140$) of immunotherapy.

Overall, there were significant treatment-time ($p = 0.0210$), treatment ($p = 0.0258$) and time ($p = 0.0125$) effects in the mean IFN- γ concentration in supernatant harvested from cells incubated in BGA. Individual group analysis showed that the mean IFN- γ concentration was significantly higher at M6 in the SC RIT group compared with the IN RIT group ($p = 0.0004$). Additionally, in only the SC RIT group was there a significant ($p = 0.0006$) effect of time on the IFN- γ concentration. Specifically, the mean IFN- γ concentration at M6 was significantly higher than at D1 ($p = 0.0007$), M1 ($p = 0.0019$) and M3 ($p = 0.0002$) (Fig. 5d). No significant differences across time were appreciated in the IN RIT group ($p = 0.7159$; Fig. 5d). There was no significant ($p = 0.8052$) difference in the group mean IFN- γ concentration at D1 in the SC RIT versus the IN RIT groups.

4. Discussion

The goal of allergen-specific immunotherapy in allergic asthma is to reverse the underlying aberrant immune response driving pathologic changes of eosinophilic airway inflammation, airway hyperreactivity, and airway remodeling. In the current study, we demonstrated that protocols for both SC and IN RIT with weekly maintenance injections significantly dampened eosinophilic airway inflammation over a 6 month period in an experimental model of feline asthma. Additionally, clinical signs of airway hyperreactivity in response to inhaled allergen challenge (Fig. 4) were completely abolished in all 6 cats receiving SC RIT over a 6 month period; comparatively, 3 of 5 cats demonstrating clinical signs in the IN RIT had complete resolution of clinical signs during BGA aerosol challenge. Evaluation of permanent architectural changes in the lung was not assessed in this study, but since airway inflammation leads to airway remodeling (Bousquet et al., 2000; Fixman et al., 2007), further studies could be performed to determine if the dampening of eosinophilic inflammation subsequently minimized remodeling in this model.

By definition, allergens are antigens which selectively activate Th2 cells to drive an IgE-mediated immune response (Janeway et al., 2001). Effective allergens are generally delivered transmucosally at very low doses. The rationale behind using mucosal immunotherapy is to deliver high concentrations of allergen directly to the mucosal surfaces that initially encounter, process and present allergen to Th2 cells with the intent of inducing tolerance. Immunotherapy by injection has been effective for humans with hypersensitivities to stinging insects (e.g.,

wasps) as it likely alters hypersensitivity reactions that are primarily maintained by systemic lymphocytes. However, injectable immunotherapy classically produces weak mucosal immune responses (Horner et al., 1998) which perhaps explains in part why this treatment has not been routinely advocated in human asthmatics. Non-injectable immunotherapy may be administered via oral, sublingual and intranasal routes. Local lymphocytes in the respiratory mucosa are postulated to be of more importance in maintaining airway hypersensitivities, and theoretically, mucosal immunotherapy should more effectively target this immune compartment (Boyaka et al., 2003). Additionally, mucosal immunotherapy has been shown to elicit both mucosal and systemic immune responses (Horner et al., 1998; Czerkinsky et al., 1999). Aside from murine models of asthma which document mucosal tolerance to allergen with IN ASIT (Wiedermann et al., 2001; Takabayashi et al., 2003), the vast majority of the scientific literature focuses on IN ASIT for rhinitis/rhinoconjunctivitis and sublingual immunotherapy (SLIT) for asthma in humans. Administration of sublingual immunotherapy in cats would be technically challenging; therefore, an intranasal route of administration was chosen for our study.

In this study, there was no clear advantage between SC RIT or IN RIT in terms of effectively decreasing the percentage of eosinophils in the BALF. Both protocols had a dramatic impact on reducing eosinophilic airway inflammation with the group mean % eosinophils in BALF not significantly different from cats prior to induction of experimental asthma and falling within an established reference range for healthy cats (Hawkins et al., 1990) by 6 months. Airway inflammation contributes both to airway hyperreactivity and remodeling in asthma (Cohn et al., 2004). Therefore, decreasing local eosinophilic inflammation is an essential end-point for successful treatment of allergic asthma.

Direct or indirect (e.g., barometric whole body plethysmography) measures of airway hyperreactivity in response to a bronchoprovocant were not measured in this study. Instead, we developed a respiratory scoring scheme that takes into account clinical signs of airway hyperreactivity based on duration of allergen challenge and induction of respiratory signs of bronchoconstriction (Table 1). All 6 cats receiving SC RIT had scores of 0 by month 6, suggesting resolution of clinical evidence of airway hyperreactivity in response to aerosol challenges of BGA. The response to clinical signs of airway hyperreactivity in cats administered IN RIT was more variable, with only 3 of 5 cats displaying clinical signs showing complete resolution by the end of the study. In cats with naturally developing asthma, clinical signs relating to bronchoconstriction (including status asthmaticus, a severe, life-threatening acute manifestation of bronchoconstriction) can be the main reason why veterinary attention is sought. Therefore one of the main goals of therapy in asthmatic cats is to diminish clinical signs driven by airway hyperreactivity. Based on this study, SC RIT has a more consistent beneficial effect on clinical signs of airway hyperreactivity.

In selecting a protocol for ASIT, the safety profile must be given serious consideration as treatment for allergy

should not induce complications worse than the disease itself. Although infrequent, serious systemic adverse reactions to SC ASIT have been reported in humans and veterinary patients (Lockey et al., 2001; Scott et al., 2001). In humans, an increased risk of serious adverse reactions has been reported with ASIT in asthmatic patients and with accelerated protocols (Lockey et al., 2001; Cox, 2006). Mucosal immunotherapy has been investigated in people as a less invasive and safer means of immune modification (Passalacqua and Canonica, 2001; Wilson et al., 2005). Few studies directly comparing mucosal immunotherapy with subcutaneous immunotherapy have been performed; however, one study focusing on humans with allergic rhinoconjunctivitis demonstrated comparable efficacy and improved safety of sublingual immunotherapy compared with subcutaneous immunotherapy (Khinchi et al., 2004). Accelerated protocols for mucosal immunotherapy have also been associated with a favorable safety profile. For example, ultra-rush sublingual-swallow immunotherapy in 679 humans with allergic rhinitis and/or asthma showed that aside from transient pruritus of the buccal cavity, the most severe reaction was urticaria which was reported in 3 people (Rossi and Monasterolo, 2002, 2005). In our study, no cat had a life-threatening reaction to RIT; however, marked lethargy ($n = 2$ SC RIT group), vomiting ($n = 1$ SC RIT group, $n = 3$ IN RIT group) and fever ($n = 1$ SC RIT group) were notable adverse events. Cats intentionally were not prophylactically treated on day 1 of RIT with antihistamines (H1 blockers) so that the number of adverse effects could be recorded without being masked. However, cats that experienced reactions during RIT received treatment with diphenhydramine. This could have masked further signs of reactions in these cats. Since it would be ideal to prevent adverse reactions in patients and histamine released from mast cells is responsible for many acute reactions, premedicating cats with antihistamines could reduce the number or severity of side effects to RIT.

The mechanisms by which ASIT attenuates the harmful immune response to allergen are not completely known, and may differ somewhat between injectable and mucosal immunotherapy. For injectable immunotherapy, proposed mechanisms include modulation of antigen presenting cell function and T cell responses (i.e., increasing the ratio of Th1 to Th2 cytokines, induction of T cell anergy, induction of T regulatory cells, and increasing the production of regulatory cytokines like IL-10 and TGF- β), and production of “blocking antibodies” such as IgG1, IgG4, and IgA (Till et al., 2004; Moingeon et al., 2006). The differences between how the immune system at systemic versus mucosal sites responds to ASIT are not resolved. There is speculation that local antigen presenting cells (dendritic cells) and the cytokines in the mucosal microenvironment are better suited to tolerizing against allergen since these surfaces normally provide a tolerogenic function especially in handling bacteria (Allam et al., 2003, 2006, 2008).

Since our group previously documented that SC RIT significantly attenuates eosinophilic airway inflammation (Reinero et al., 2006), the goal of the current study was to do a side by side comparison of efficacy and safety for SC and IN protocols. We therefore did not have an untreated (i.e., no RIT) asthmatic group as a control. However, in our

prior studies investigating SC RIT (Reinero et al., 2006) or adjuvanted SC RIT (Reinero et al., 2008) in this same model, we showed that a control group of cats not receiving ASIT failed to have significant decreases in the % BALF eosinophils over time.

In this study, the cats subjectively appeared to tolerate the subcutaneous injections better than the intranasal drops. Occasionally, cats would sneeze after administration of the intranasal drops making it difficult to administer exact doses via the intranasal route. It is possible that this could have altered the efficacy of the IN RIT. There is also the possibility that the dose of allergen required may be different for SC compared to IN RIT. The literature documents that anywhere between 3 and 10 times the dose of allergen is used in mucosal immunotherapy when compared to subcutaneous immunotherapy in humans. However, the highest allergen concentration (i.e., the maintenance concentration of 200 μ g) that was used in our study for both groups is roughly equivalent to the highest maintenance dose used in human mucosal immunotherapy studies (Lima et al., 2002; Pajno et al., 2003; Marogna et al., 2004; Moingeon et al., 2006).

5. Conclusion

While both the SC and IN RIT protocols were safe and were associated with decreased eosinophilic airway inflammation in this experimental asthma model, the SC RIT protocol was associated with a more consistent reduction in clinical signs of feline asthma after allergen challenge. Therefore, it appears that the SC RIT protocol may have a slight advantage over the IN protocol. It is likely that the experimentally asthmatic cats responded optimally to RIT because the allergen to which they were sensitized was used for treatment. Before using RIT routinely in pet cats with asthma, future studies should be performed to evaluate the best diagnostic test for identifying clinically relevant allergens in cats with airway allergies.

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