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Beneficial cross-protection of allergen-specific immunotherapy on airway eosinophilia using unrelated or a partial repertoire of allergen(s) implicated in experimental feline asthma

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ABSTRACT

The study hypothesis was that in experimentally asthmatic cats rush immunotherapy (RIT) using allergens not completely matched with sensitizing allergen(s) would at least partially attenuate the asthmatic phenotype and modulate the aberrant immune response. In phase I, cats sensitized to Bermuda grass allergen (BGA), house dust mite allergen (HDMA) or placebo received BGA RIT. In phase II, cats dually sensitized to BGA and HDMA received RIT using BGA, HDMA or placebo. Efficacy of RIT was assessed using percentage bronchoalveolar lavage fluid (BALF) eosinophils. Additionally, a variety of immunologic assays were performed.

Eosinophilic airway inflammation significantly decreased over time in asthmatic cats given RIT using sensitizing allergen or unrelated allergen ($P < 0.001$). In dually sensitized cats, single allergen RIT but not placebo reduced airway eosinophilia ($P = 0.038$). Differences in allergen-specific lymphocyte proliferation, in the number of IL-10 producing cells and in the percentage T regulatory cells were detected between asthmatic cats getting RIT and controls. Cross-protection manifested by reduced airway eosinophilia was noted in cats treated with RIT allergens which did not completely match allergen used in asthma induction. However, the mechanism of immunologic tolerance may differ when improperly matched allergens to the sensitizing allergens are used in RIT.

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Introduction

Feline asthma is postulated to be an allergic response, resulting from T helper 2 (Th2) lymphocyte-polarized responses to aeroallergens (Kim et al., 2010; Norris Reinero et al., 2004; Reinero, 2010). Experimental models of feline asthma have been useful to study the pathogenesis of disease and to explore novel, promising treatments (Padrid et al., 1995; Norris Reinero et al., 2004; Kirschvink et al., 2007). In humans, allergen-specific immunotherapy (ASIT) establishes tolerance to allergen, thus having potential to cure asthma (Akdis and Akdis, 2007). In experimental feline asthma, abbreviated ASIT (rush immunotherapy or RIT) decreases airway eosinophilia when the same allergen is used for asthma induction and RIT (Reinero et al., 2006, 2008; Lee-Fowler et al., 2009).

The mechanisms of ASIT are not completely understood. Recent studies support a central role of regulatory T cells (Tregs) in ASIT (Groß et al., 2010). Allergic inflammation in healthy humans is

suppressed by Tregs; Treg defects may contribute to an allergic phenotype (Akdis et al., 2004; Ling et al., 2004; Domdey et al., 2010). Interleukin-10 (IL-10), which is secreted by a variety of cells including Tregs, dampens effector cells relevant to allergic inflammation in humans (James and Durham, 2008). Development of new feline-specific immunologic reagents now allows for mechanistic evaluation of RIT in experimentally asthmatic cats, specifically evaluation of Treg phenotype and the number of IL-10 producing cells. Induction of allergen-specific lymphocyte hyporesponsiveness, implying tolerance to allergen, has previously been shown for RIT in a feline asthma model (Reinero et al., 2006).

The hallmark features of asthma include airway inflammation, airway hyperresponsiveness and airway remodeling (Kim et al., 2010). As airway inflammation contributes to the latter two features, it is a key target of therapy and a primary indicator of efficacy. The goal of RIT is to induce immunologic tolerance, which may be in part gauged by induction of lymphocyte hypoproliferation and increases in IL-10 producing cells and in the percentage of Tregs. The effects of using allergens in RIT, that are not specifically and completely matched to sensitizing allergens, have not been studied in asthmatic cats. We hypothesized that in experimentally

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asthmatic cats Bermuda grass allergen (BGA)-specific RIT would attenuate the asthmatic phenotype by reducing eosinophilic airway inflammation and would lead to immunologic tolerance in cats that are: (1) sensitized to BGA only but not to the unrelated allergen house dust mite allergen (HDMA) and (2) dually-sensitized to BGA and HDMA.

Materials and methods

Animals

Intact mixed breed cats (phase I: 11 females, 8 males; phase II: 12 males, 5 females) bred from a high-responder asthmatic cat colony (Comparative Internal Medicine Laboratory, University of Missouri-Columbia) were used according to NIH Guide for the Care and Use of Laboratory Animals after approval by the University of Missouri Animal Care and Use Committee (protocol #4075).

In phase I, cats were assigned to be sensitized using BGA (BGA SENS) or HDMA (HDMA SENS) using a table of random numbers. Control cats received saline (PLACEBO SENS). All cats received BGA RIT for 6 months.

In phase II, cats were dually-sensitized (DS) using BGA and HDMA and were randomized to receive either placebo RIT (DS-PLACEBO RIT), BGA RIT (DS-BGA RIT) or HDMA RIT (DS-HDMA RIT) for 6 months.

Induction of asthma and RIT

The study timeline for the development of the asthma model and the treatment trial is shown in Fig. 1. Cats were sensitized and challenged with a single allergen as described below or had dual-sensitization with simultaneous administration of BGA and HDMA, each dosed as single allergen. Briefly, the single allergen protocol involved: (1) injection of 12 µg of allergen in 10 mg of alum subcutaneously (SC) and 100 ng *Bordetella pertussis* toxin SC on day 0 (Sigma Aldrich); (2) administration of 75 µg allergen in 0.2 mL of PBS intranasally on day 14; and (3) another injection of 12 µg of allergen in 10 mg of alum SC on day 21. Intradermal skin testing was performed on day 28 to confirm sensitization to specific allergen. After parenteral sensitization, aerosol challenges of 100 µg of allergen were administered to awake, spontaneously breathing cats in a sealed chamber. Seven aerosol challenges were administered over the following 2 weeks and eosinophilic airway inflammation was confirmed by BALF collection 48 h after the last allergen challenge.

Cats needed % bronchoalveolar lavage fluid (BALF) eosinophils $\geq 16\%$ for inclusion. Weekly aerosol challenge with BGA (phase I) or BGA and HDMA (phase II) were performed for 6 months. A published protocol for SC RIT administration (escalating doses from 20 to 200 µg initially followed by 200 µg/week for 6 months) was used (Lee-Fowler et al., 2009). Briefly, IV cephalic catheters were placed immediately prior to RIT for vascular access. Five doses of allergen (20 µg, 40 µg, 80 µg, 100 µg, and 200 µg) were administered every 2 h on the first day and a single dose (200 µg) was administered the following day. During RIT, cats were continuously monitored for vomiting, diarrhea, agitation, labored breathing, and pruritus, and heart rate (HR), respiratory rate (RR) and character, temperature (T), capillary refill time (CRT), mucus membrane color, and pulse quality were recorded every hour. The next

injection of allergen was delayed until the parameters normalized if cats showed any of the following abnormalities: HR or RR increase by $>30\%$ over baseline, $T > 103.5^\circ\text{F}$, CRT > 2 s, pale mucous membranes and poor or bounding pulse quality.

Sample collection and BALF analysis

Jugular venipuncture for whole blood and BALF were obtained before RIT (day 0) and 1, 3, and 6 months (M1, M3 and M6) afterwards. Collection of BALF was performed in a blind fashion using an eight FR red rubber catheter. The catheter was advanced through the endotracheal tube until gentle resistance was met as it wedged in a lower airway. Sterile saline (20 mL) was instilled from an attached syringe followed by gentle manual suction. Samples were placed on ice until analysis (within 2 h). Differential cell counts on modified Wright's stained slides prepared by cytocentrifugation of BALF were performed on 200 nucleated cells (Norris Reinero et al., 2004).

Lymphocyte proliferation

Peripheral blood mononuclear cells (PBMCs) isolated using density-gradient centrifugation were resuspended in complete RPMI (cRPMI: RPMI 1640 with 10% FBS, 5 mL 1 M Hepes, 5 mL penicillin-streptomycin-glutamine; Gibco, Invitrogen Corporation). In a 96-well plate, 1×10^5 cells were added in triplicate with each of the following: media alone, BGA (50 µg/mL) and HDMA (50 µg/mL). Cells were cultured at 37°C in humidified 5% $\text{CO}_2/95\%$ air for 5 days with media change at day 3. A kit for lymphocyte proliferation was used per instructions (Cell Proliferation ELISA, Roche Diagnostics Corporation). Results are reported as a stimulation index (SI) for each allergen: $\text{SI}_{\text{BGAorHDMA}} = \text{OD}_{\text{BGAorHDMA}} / \text{OD}_{\text{media alone}}$. An SI < 2 indicates lack of lymphocyte proliferation (i.e., negative result) (Fujiwara et al., 2003).

IL-10 ELISPOT

An ELISPOT kit (Feline IL-10 Development Module, R&D Systems) was used per manufacturer's instructions with prior optimization in our laboratory. A 96-well filtration plate was coated with capture antibody (diluted 1:120) and incubated overnight at 4°C . After washing, blocking buffer was added for 2 h at 24°C with a subsequent wash. In triplicate, 5×10^4 PBMCs in cRPMI were added. Cell numbers were optimized by comparing results from titrations of 1×10^3 – 1×10^6 cells/well (data not shown). Positive and negative controls were recombinant feline IL-10 and media, respectively. Cells were cultured at 37°C in humidified 5% $\text{CO}_2/95\%$ air for 2 days. After washing, detection antibody (diluted 1:120) was added and incubated overnight at 4°C . ELISPOT Blue Color Module (R&D Systems) was applied after wash and the plate rinsed with deionized water and dried. Spots were quantified using a CTL Immunospot Analyzer (Cellular Technology Limited).

T regulatory cell percentage

PBMCs were added (10^5 cells/well) to a 96-well plate and stained for surface expression of CD4 and CD25 and intracellular FoxP3 with appropriate isotype controls. Briefly, anti-feline CD4 (clone 3-4F4, Southern Biotech) and anti-feline CD25

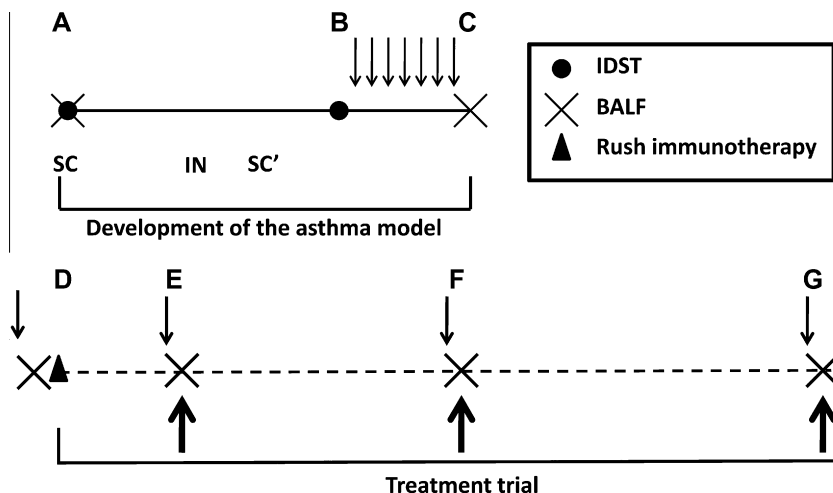


Fig. 1. Timeline of asthma induction and of rush immunotherapy (RIT) or placebo treatment. Healthy research cats were screened for pre-existing sensitization to allergen by intradermal skin testing (IDST) and for airway inflammation by collection of BALF (A). A SC injection of allergen in alum and pertussis toxin were administered (SC) at day 0, followed by intranasal allergen (IN) at day 14, and SC allergen in alum alone (SC') at day 21. Repeat IDST was performed at day 28 to confirm parenteral sensitization to allergen (B). Seven challenges of allergen by aerosol were delivered over the following 2 weeks (thin arrows), and repeat collection of BALF documented an asthmatic phenotype 24 h after the last aerosol challenge (C). Baseline assessment of blood and BALF were collected prior to the treatment trial. The treatment trial consisted of escalating doses of allergen over 2 days (D). Aerosol challenges of allergen and maintenance allergen-specific immunotherapy injections were continued weekly throughout the treatment trial (not shown). Allergen aerosols (thin arrows) were administered 24 h prior to blood and BALF collection at months 1, 3 and 6 (E–G; bold arrows).

(clone 9F23, provided by Dr. Wayne Thompkins, North Carolina State University) were suspended in FACS buffer (PBS with 3% FBS and 0.09% sodium azide) on ice for 30 min. Cells were fixed and permeabilized on ice for 30 min using a FoxP3 staining kit (eBioscience) followed by staining with FoxP3 (clone FJK-16, eBioscience) on ice for 60 min. Cells were washed and resuspended in 400 μ L FACS buffer before analysis on a Cyan ADP Flow Cytometer (Becton Dickinson). Lymphocytes were gated on a forward vs. side scatter plot and used to display CD4 and CD25 double-positive stained cells. The CD4 + CD25 + T cells were then applied to a histogram of FoxP3 + lymphocytes to quantify CD4 + CD25 + lymphocytes expressing FoxP3. This number was divided by the number of peripheral blood lymphocytes in the forward vs. side scatter gate to provide the % CD4 + CD25 + FoxP3 + Tregs.

Statistical analysis

Data are presented as mean $\pm$ SD. When necessary, natural log function was used to insure that data met criteria for normality assumptions. For most analyses, a two-way repeated measures ANOVA was used followed by a post hoc Tukey test. A post hoc Fisher LSD method was used for comparisons of IL-10 producing cell numbers. Percentage BALF eosinophils were compared between the RIT and placebo groups in phase II using a *t* test. $P < 0.05$ was considered significant.

Results

Phase I

The group mean $\pm$ SD of the % BALF eosinophils is shown in Fig. 2. Regardless of whether cats were sensitized using BGA or HDMA, there was a significant decrease in the BALF eosinophilia over time after BGA RIT ($P < 0.001$). This was not seen in the PLACEBO SENS group ($P = 0.221$).

Group mean BGA SI did not differ over time in PLACEBO SENS cats ($P = 0.932$) and the BGA SI was < 2 (biologically insignificant proliferation) at all times. There was a significant effect of group on the BGA SI ($P < 0.001$) with BGA SI decreasing over time in BGA SENS cats and increasing in HDMA SENS cats (Fig. 3a).

Group mean HDMA SI did not differ over time for PLACEBO SENS cats (< 2 at all times; $P = 0.594$). The BGA SENS cats had significantly lower ($P < 0.001$) HDMA SI than HDMA SENS cats. The group mean HDMA SI was < 2 in BGA SENS cats at all time points and the group mean HDMA SI was > 2 in HDMA SENS cats at all time points except M3 (Fig. 3b).

Numbers of IL-10 producing cells were only quantified for all cats at M6. Data were collected from nine additional experimentally asthmatic cats not receiving RIT (unrelated study) for further comparison; these untreated asthmatics had an average of 131 ± 59

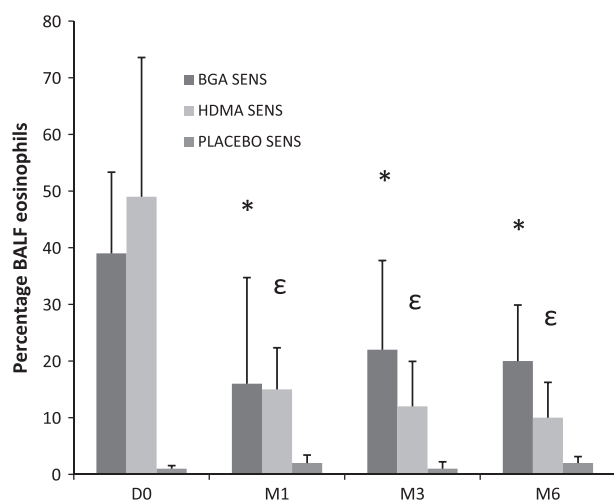


Fig. 2. Significant decreases in the group mean % BALF eosinophils in cats treated with Bermuda grass allergen (BGA) rush immunotherapy (RIT) were noted at day 0 compared with all subsequent time points in asthmatic cats. * $P < 0.001$ in the BGA SENS group; $^{\epsilon}P < 0.001$ in the house dust mite allergen (HDMA) SENS group.

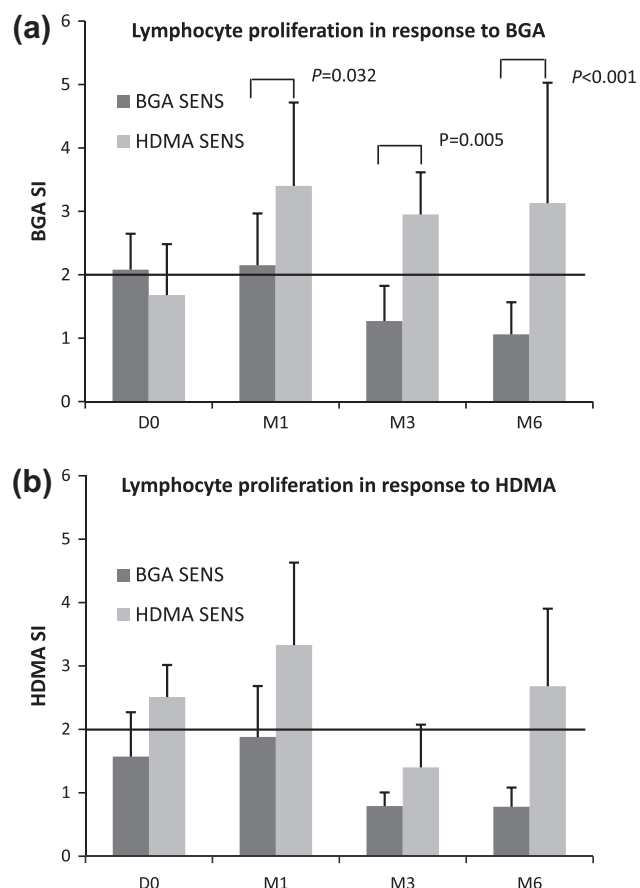


Fig. 3. Ex vivo responses of PBMCs to allergens from monosensitized cats administered Bermuda grass allergen (BGA) rush immunotherapy (RIT), represented as a group mean stimulation index (SI). The horizontal line depicts a SI = 2, below which indicates lymphocyte hypoproliferation. (a) Significant differences in the BGA SI between BGA SENS and house dust mite allergen (HDMA) SENS cats were noted at months 1, 3 and 6 of BGA RIT. (b) Significant effects of treatment ($P < 0.001$) and of time ($P = 0.004$) were observed.

IL-10 producing cells. After 6 months of BGA RIT, the number of IL-10 producing cells was significantly lower in HDMA SENS cats (115 ± 43) compared with BGA SENS cats (291 ± 155 ; $P = 0.022$) and PLACEBO SENS cats (279 ± 146 ; $P = 0.037$). The numbers of IL-10 producing cells were higher after 6 months of BGA RIT in PLACEBO SENS ($P = 0.025$) and BGA SENS ($P = 0.012$), but not in HDMA SENS ($P = 0.812$) cats when compared with untreated asthmatic cats.

Phase II

Baseline % BALF eosinophils between groups were not significantly different ($P = 0.175$). After 6 months, there was a significant ($P = 0.038$) effect of treatment (placebo RIT vs. allergen RIT; Fig. 4). More cats achieved cytologic remission of asthma (i.e., % BALF eosinophils $< 17\%$) at M6 in the allergen RIT group compared to the placebo RIT group (7/11 allergen RIT; 0/6 placebo RIT; $P = 0.035$).

There was no significant difference in the BGA SI or HDMA SI between treatments. However, the SI was reduced to < 2 over time in allergen RIT groups supporting a biological difference (Fig. 5a and b). Whereas the BGA SI was > 2 for all groups at D0, it was < 2 for the DS-BGA RIT and DS-HDMA RIT groups at M6. Ex vivo incubation with HDMA did not cause lymphocyte proliferation in PLACEBO cats at baseline making this control group poor for comparison. However, the group mean HDMA SI was > 2 in the DS-BGA RIT and DS-HDMA RIT groups at D0 and subsequently decreased < 2 at M6.

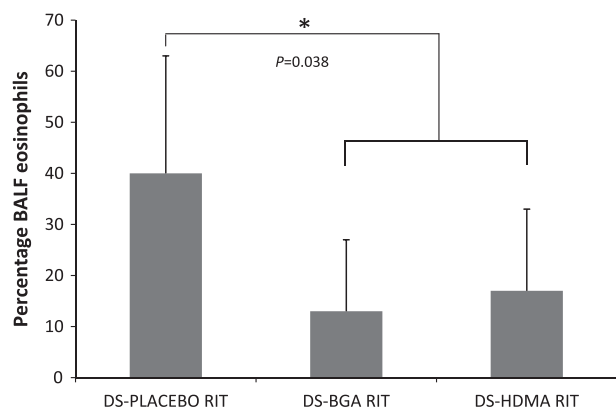


Fig. 4. Significant decreases in group mean % BALF eosinophils in cats receiving allergen rush immunotherapy (RIT) with Bermuda grass allergen (BGA) or house dust mite allergen (HDMA) (DS-BGA RIT or DS-HDMA RIT) were noted compared with cats receiving placebo RIT.

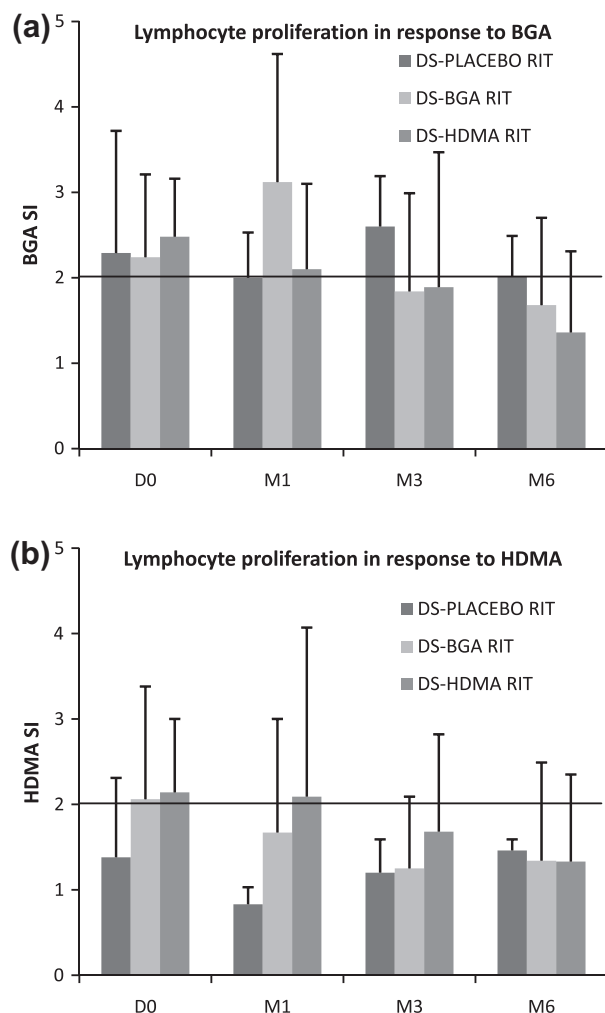


Fig. 5. Ex vivo responses of PBMCs to allergens from dual-sensitized cats, represented as a group mean stimulation index (SI). The horizontal line depicts a SI = 2, below which indicates lymphocyte hypoproliferation. (a) Group mean BGA SI > 2 at D0 in all groups but < 2 only in the allergen rush immunotherapy (RIT) groups. (b) Group mean HDMA SI > 2 at D0 and < 2 at month 6 in allergen RIT groups. DS-PLACEBO RIT cats failed to develop an ex vivo proliferative response to HDMA at baseline, making this control group poor for comparison.

The % CD4 + CD25 + FoxP3 + Tregs differed between treatments over time ($P = 0.045$), reflecting increased Tregs with allergen-RIT but not with placebo-RIT. The DS-BGA RIT group had more

CD4 + CD25 + FoxP3 + Tregs at M3 ($1.2 \pm 0.7\%$) and M6 ($1.9 \pm 1.5\%$) compared with D0 ($0.6 \pm 0.5\%$; $P = 0.020$ and 0.010 , respectively). The DS-HDMA RIT group had more CD4 + CD25 + FoxP3 + Tregs at M6 ($3.3 \pm 3.7\%$) vs. D0 ($0.9 \pm 0.3\%$; $P = 0.019$) and M1 ($0.9 \pm 0.4\%$; $P = 0.029$). In the DS-PLACEBO RIT group, there were no significant differences between CD4 + CD25 + FoxP3 + lymphocytes over time (D0, $1.0 \pm 0.3\%$; M6, $0.7 \pm 0.5\%$).

Discussion

For RIT, the use of non-sensitizing allergen or a partial repertoire of allergens used to induce experimental allergic asthma dampened eosinophilic airway inflammation and modulated the immune response to induce some degree of 'cross-protection'. However, the mechanisms behind beneficial modulation of the immune system with imprecisely matched allergens appeared to differ from those induced when sensitizing and RIT allergens are identical. Additionally, concurrent documentation of airway and peripheral blood immunologic changes supports the growing concept that although the mucosal and systemic immune tissues are anatomically and functionally independent, there is 'cross-talk' between them.

Phase I was designed to mimic a pet cat with asthma sensitized to one allergen but receiving an unrelated allergen for immunotherapy. Cats sensitized with either BGA or HDMA had a significant decrease in their BALF eosinophils when administered BGA RIT. Non-asthmatic cats administered BGA RIT did not have induction of airway eosinophilia indicative of asthma induction. This is clinically important, since it is theoretically possible that RIT might be inadvertently administered to pet cats who do not have allergic asthma (e.g., cats with chronic bronchitis). While there was not a control group of asthmatic cats receiving placebo RIT in this study, we have previously documented that placebo RIT does not diminish BALF eosinophilia (Reinero et al., 2008).

Consistent with previous results (Reinero et al., 2006), BGA-sensitized cats receiving BGA RIT had dampened lymphocyte proliferation to BGA (group mean SI < 2 at M3 and M6); this is in contrast to their baseline SI > 2, reflecting lymphocyte recognition and proliferation in response to BGA. Lymphocyte hypoproliferation may be one mechanism of allergen tolerance when sensitizing and RIT allergens are identically matched. In humans receiving ASIT, there have been similar decreases in SI (i.e., SI > 2 prior to ASIT and SI < 2 after ASIT) using relevant allergen (Akoum et al., 1996; Tarzi et al., 2006). That same phenomenon was not systematically observed when lymphocytes from HDMA sensitized cats administered BGA RIT were incubated with HDMA (HDMA SI > 2 at M1 and M6). When assessing ex vivo response to BGA, the HDMA SENS cats had hyporesponsive lymphocytes at baseline (i.e., prior to BGA RIT; group mean SI = 1.68). This was expected, given they had no prior exposure to BGA. However, intensive BGA-specific RIT led to the opposite lymphocytic proliferative response compared to what was observed in the BGA sensitized/BGA RIT group: lymphocytes subsequently recognized BGA and increased proliferation (group mean SI > 2 at M1, M3 and M6).

Humans who do not develop an allergic phenotype after allergen exposure are tolerized in part by expansion of IL-10 producing Tregs (Meiler et al., 2008). The predominant allergen-specific T cell phenotypes determined ex vivo in healthy, non-allergic individuals were IL-10 secreting T cells and in allergic individuals, IL-4 secreting T cells (Akdis et al., 2004). In humans ASIT increases IL-10 production from PBMCs (Tarzi et al., 2006). Production of IL-10 may also prevent polysensitization in monosensitized individuals (Prigione et al., 2009). In the current study, asthmatic cats had significantly fewer IL-10 producing cells than non-asthmatic cats (i.e., PLACEBO SENS cats) receiving 6 months of BGA RIT, consistent with a 'deficiency' of IL-10 in the asthmatic group. After 6 months,

BGA SENS cats had increased IL-10 producing cells which were not significantly different from non-asthmatic cats. Normalization of IL-10 production may have helped dampen eosinophilic airway inflammation in these asthmatic cats. In comparison, after 6 months of BGA RIT, HDMA SENS cats had fewer IL-10 producing cells than other asthmatic groups and were not significantly different from non-asthmatic cats. Thus, IL-10 may not play a role in 'cross-protection' induced by an unrelated allergen.

Phase II was designed to mimic a pet cat with asthma induced by a mixture of allergens (BGA and HDMA) then administered RIT using just one relevant allergen. Since cats with allergies are generally sensitized to more than one allergen, regimens that could induce cross-tolerance are desirable. Murine CD4 + CD25 + Tregs activated by their cognate antigen in vitro suppress T cells with the same or unrelated antigen specificity (Thornton and Shevach, 2000). Cross-tolerance has been demonstrated using a murine asthma model with ovalbumin (OVA) and schistosoma egg antigen sensitization (Jain et al., 2003). When given immunotherapy (OVA with adjuvant), these mice demonstrated protection against both allergens, although the protection against OVA was stronger.

There is a benefit if even one correct allergen is used for RIT. Mechanistically, lymphocyte hypoproliferation ($SI < 2$) was seen with ex vivo incubation with either BGA or HDMA at M6 in the DS-BGA RIT and DS-HDMA RIT groups, but not in the DS-PLACEBO RIT group. This demonstrates that the allergen used in RIT in a dually sensitized model was capable of inducing a tolerogenic response not only in lymphocytes specific for the RIT allergen, but also in lymphocytes recognizing allergen used for sensitization but not RIT (e.g., BGA-RIT led to both a BGA SI and HDMA SI < 2).

Increased numbers of CD4 + CD25 + FoxP3 + Tregs were documented at M6 compared with baseline values for cats treated with allergen RIT. Future studies should evaluate Treg function (in addition to number) in asthmatic cats to determine their role in restoring tolerance with RIT.

Conclusions

Allergens used in RIT do not have to be identical to sensitizing allergens to provide benefit. Alterations in the immune system induced by RIT differed between relevant and irrelevant allergens. Whereas 'allergen-matched' RIT appears to induce tolerance by promoting allergen-specific lymphocyte hypoproliferation, increasing IL-10 producing cells and increasing CD4 + CD25 + FoxP3 + lymphocytes (which may allow discontinuation of RIT), the use of an irrelevant allergen promotes lymphocyte proliferation and fails to alter numbers of IL-10 producing cells. Thus, an active immune response requiring permanent immunotherapy may be required.

Conflict of interest statement

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References

- Akdis, M., Verhagen, J., Taylor, A., Karamloo, F., Karagiannidis, C., Crameri, R., Thunberg, S., Deniz, G., Valenta, R., Fiebig, H., Kegel, C., Disch, R., Schmidt-Weber, C., Blaser, K., Akdis, C.A., 2004. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *Journal of Experimental Medicine* 199, 1567–1575.
- Akdis, M., Akdis, C.A., 2007. Mechanisms of allergen-specific immunotherapy. *Journal of Allergy and Clinical Immunology* 119, 780–791.
- Akoum, H., Tscipoulos, A., Vorng, H., Wallaert, B., Dessaint, J., Joseph, M., Hamid, Q., Tonnel, A., 1996. Venom immunotherapy modulates interleukin-4 and interferon- γ messenger RNA expression of peripheral T lymphocytes. *Immunology* 87, 593–598.
- Domdey, A., Liu, A., Millner, A., Lund, K., Jacobi, H., Malling, H., Sondergaard, I., Wurtzen, P., 2010. The T cell response to major grass allergens is regulated and includes IL-10 production in atopic but not in non-atopic subjects. *International Archives of Allergy and Immunology* 152, 243–254.
- Fujiwara, S., Yasunaga, S., Iwabuchi, S., Masuda, K., Ohno, K., Tsujimoto, H., 2003. Cytokine profiles of peripheral blood mononuclear cells from dogs experimentally sensitized to Japanese cedar pollen. *Veterinary Immunology and Immunopathology* 93, 9–20.
- Groß, R., Metzner, G., Behn, U., 2010. Mathematical modeling of allergy and specific immunotherapy: Th1–Th2–Treg interactions. *Journal of Theoretical Biology* 269, 70–78.
- Jain, V., Businga, I., Kitagaki, K., George, C., O'Shaughnessy, P., Kline, J., 2003. Mucosal immunotherapy with CpG oligodeoxynucleotides reverses a murine model of chronic asthma induced by repeated antigen exposure. *American Journal of Physiology – Lung Cellular and Molecular Physiology* 285, L1137–L1146.
- James, L., Durham, S., 2008. Update on mechanisms of allergen injection immunotherapy. *Clinical and Experimental Allergy* 38, 1074–1088.
- Kim, H., DeKruyff, R.H., Umetsu, D.T., 2010. The many paths to asthma: Phenotype shaped by innate and adaptive immunity. *Nature Immunology* 11, 577–584.
- Kirschvink, N., Leemans, J., Delvaux, F., Snaps, F., Clercx, C., Gustin, P., 2007. Functional, inflammatory and morphologic characterisation of a cat model of allergic airway inflammation. *The Veterinary Journal* 174, 541–553.
- Lee-Fowler, T., Cohn, L., DeClue, A., Reinero, C., 2009. Evaluation of subcutaneous versus mucosal (intranasal) allergen-specific rush immunotherapy in experimental feline asthma. *Veterinary Immunology and Immunopathology* 129, 49–56.
- Ling, E., Smith, T., Nguyen, X., Pridgeon, C., Dallman, M., Arbery, J., Carr, V.A., Robinson, D.S., 2004. Relation of CD4 + CD25 + regulatory T-cell suppression of allergen driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 363, 608–615.
- Meiler, F., Zumkehr, J., Klunker, S., Ruckert, B., Akdis, C.A., Akdis, M., 2008. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *Journal of Experimental Medicine* 205, 2887–2898.
- Norris Reinero, C., Decile, K., Berghaus, R., Williams, K., Leutenegger, C., Walby, W., Schelegle, E., Hyde, D., Gershwin, L., 2004. An experimental model of allergic asthma in cats sensitized to house dust mite or Bermuda grass allergen. *International Archives of Allergy and Immunology* 135, 117–131.
- Padrid, P., Snook, S., Finucane, T., Shiue, P., Cozzi, P., Solway, J., Leff, A.R., 1995. Persistent airway hyperresponsiveness and histologic alterations after chronic antigen challenge in cats. *American Journal of Respiratory and Critical Care Medicine* 151, 184–193.
- Prigione, I., Morandi, F., Tosca, M., Silvestri, M., Pistoia, V., Ciprandi, G., Rossi, G., 2009. Interferon-gamma and IL-10 may protect from allergic polysensitization in children: Preliminary evidence. *Allergy* 65, 740–742.
- Reinero, C.R., Byerly, J.R., Berghaus, R.D., Berghaus, L.J., Schelegle, E.S., Hyde, D.M., Gershwin, L.J., 2006. Rush immunotherapy in an experimental model of feline allergic asthma. *Veterinary Immunology and Immunopathology* 110, 141–153.
- Reinero, C., Cohn, L., Delgado, C., Spinka, C., Schooley, E., DeClue, A., 2008. Adjuvanted rush immunotherapy using CpG oligodeoxynucleotides in experimental feline allergic asthma. *Veterinary Immunology and Immunopathology* 121, 241–250.
- Reinero, C., 2010. Advances in the understanding of pathogenesis, and diagnostics and therapeutics for feline allergic asthma. *The Veterinary Journal*. doi:10.1016/j.tvjl.2010.09.022.
- Tarzi, M., Klunker, S., Texier, C., Verhoef, A., Stapel, S., Akdis, C., Maillere, B., Kay, A., Larche, M., 2006. Induction of interleukin-10 and suppressor of cytokine signalling-3 gene expression following peptide immunotherapy. *Clinical and Experimental Allergy* 36, 465–474.
- Thornton, A., Shevach, E., 2000. Suppressor effector function of CD4 + CD25 + immunoregulatory T cells is antigen nonspecific. *Journal of Immunology* 164, 183–190.