



Quantitation of canine regulatory T cell populations, serum interleukin-10 and allergen-specific IgE concentrations in healthy control dogs and canine atopic dermatitis patients receiving allergen-specific immunotherapy

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Abstract

Canine atopic dermatitis (AD) shares many clinical and immunological similarities with human AD. Regulatory T cells (Treg) are a distinct lineage of T lymphocytes with various immunosuppressive properties including the down-regulation of allergic inflammation associated with IgE production. Antigen-induced Treg typically regulate immune homeostasis via productions of cytokines such as interleukin-10. Given the immunological similarities with human AD, it is likely that Tregs and the cytokines they produce play an important role in diseases of dogs as well. A cross-reactive FoxP3 antibody was used to identify a subset of CD4⁺ T cells in the blood of both healthy dogs and dogs with atopic dermatitis undergoing immunotherapy over a year period. There was no significant difference in the Treg percentage over time in the healthy dogs. The immunotherapy group showed a significant increase in Treg percentage at 6, 9, and 12 months when compared to the healthy dogs. For the immunotherapy group, the mean Treg percentage at the beginning of the study was 4.94 ± 0.71 and 10.86 ± 2.73 at the completion.

A commercially available ELISA kit was also used to quantitate the concentration of IL-10 in the serum of the same subsets of dogs. There was no significant difference in the IL-10 concentrations over time in the healthy dogs. The immunotherapy group showed a significant increase in serum IL-10 concentrations at 6, 9, and 12 months when compared to the control group. The mean serum IL-10 concentration at the initiation of immunotherapy was 20.40 ± 3.52 ng L⁻¹ and 37.26 ± 15.26 ng L⁻¹ at the completion of the study. The immunotherapy group also showed a significant decrease in serum IgE levels over the 1-year treatment period for specific allergens identified during ASIT. We conclude from these studies that similar to humans undergoing immunotherapy, increasing Treg populations likely play a significant role in the success of this particular type of therapy for atopic dermatitis and other allergic conditions.

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1. Introduction

Atopic dermatitis (AD) is one of the most common allergic skin diseases of dogs. This entity has recently been defined as a genetically predisposed, inflammatory

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and pruritic allergic skin disease with characteristic clinical features (Olivry et al., 2001). It is most commonly associated with immunoglobulins of the E class (IgE) to environmental allergens (Hillier and Griffin, 2001). Numerous other factors that are known to play a role include defective epidermal barrier function, processing of allergens by epidermal Langerhans' cells, polarization of T-lymphocyte cytokine responses, increased releasability of cutaneous mast cells, and susceptibility to secondary bacterial and yeast infections (Tarpataki, 2006). The exact prevalence of AD is not known, but in recent literature it is suggested that approximately 10% of the canine population is affected by this pruritic skin disease (Griffin, 1993; Scott, 2001). The diagnosis of canine AD is based upon clinical history, physical examination, and the exclusion of other diseases including flea allergic dermatitis, adverse food reactions, dermatophytosis, and other ectoparasites (Prélaud et al., 1998; Willemse, 1986). Diagnostic tests including intradermal testing (IDT) or allergen-specific IgE serology (ASIGES) are commonly used to select the relevant allergens for allergen-specific immunotherapy (ASIT).

Allergen-specific immunotherapy (ASIT) is defined as the practice of administering gradually increasing quantities of an allergen extract to an allergic subject to ameliorate the symptoms associated with subsequent exposure to the causative allergen (Olivry et al., 2001). This represents one of the main treatments for canine AD and appears to be between 50 and 100% effective according to numerous published reports (Mueller and Bettenay, 1996; Rosser, 1997). The mechanism of action of ASIT in canine AD is currently unknown, however several theories related to changes in the immune reaction to allergens in humans have been hypothesized. The various theories that have been proposed include (1) humoral desensitization (reduced levels of IgE), (2) cellular desensitization (reduced reactivity of mast cells and basophils), (3) immunization (induction of blocking antibody), (4) tolerization (generation of allergen-specific suppressor cells), and (5) some combination thereof (Middleton, 1993; Reedy and Miller, 1989).

An optimal balance between the Th1 and the Th2 responses is essential for the maintenance of healthy skin on exposure to potential allergens. A further subset of T cells termed T regulatory (Treg) cells, which have an immunosuppressive function and cytokine profile distinct from that of either Th1 or Th2 cells, has been described (Chen et al., 2003). Intracellular detection of the transcription factor FoxP3 has been shown to uniquely identify a highly

enriched Treg population in rodents (Fontenot et al., 2003). A cross-reactive murine FoxP3 antibody was recently validated to also detect dog FoxP3 and to identify canine Treg *in vivo* (Biller et al., 2007). It was also demonstrated that FoxP3 expressing CD4⁺ T cells in dogs behaved functionally as Treg (Biller et al., 2007). Treg cells are able to inhibit the development of allergic Th2 cell responses, as well as down-regulate allergic inflammation associated with IgE production and therefore play an important role in ASIT. ASIT has also been shown to induce the antigen-specific suppressive activity of Treg cells in allergic individuals (Taylor et al., 2006).

The present study was designed to investigate the generation of suppressor cells (Treg) and cytokines, specifically IL-10, in canines treated with ASIT compared to healthy control dogs. Recent human studies revealed that the generation of allergen-specific Treg cells and increased production of IL-10 have been found to be essential events in allergen-specific immunotherapy (Jutel et al., 2003). Our results from these studies suggest that similar to humans undergoing immunotherapy, increasing Treg populations likely play a significant role in the success of this particular type of therapy in dogs with atopic dermatitis.

2. Materials and methods

The study was approved by the Institutional Animal Care and Use Committee of the School of Veterinary Medicine's Animal Care Committee. All dogs entered the study with the owners' informed consent.

2.1. Inclusion criteria

Dogs with clinical signs and laboratory and other diagnostic tests consistent with previously reported diagnostic criteria for AD were included. Ectoparasitic diseases were ruled out based on negative results of multiple skin scrapings and all dogs underwent a 6-week trial of selamectin (Revolution, Pfizer Animal Health, Exton, PA) applied every 2 weeks. All dogs underwent a food trial of 8 weeks' duration using an appropriate novel protein home-cooked or commercial diet to rule-out food allergic dermatitis with subsequent provocative testing before starting ASIT. All dogs had positive results on IDT performed according to a published protocol (Greer Veterinary Laboratories, Lenoir, NC). In the atopic dermatitis group, 27 female spayed (median age 3.1 years) and 26 male castrated (median age 3.4 years) dogs were included.

Healthy control dogs were included in the study if they had no previous history of skin disease, no history or evidence of systemic illness, and had not received any form of glucocorticoid or other immunosuppressive agent at least 6 weeks prior to participation. In the control group, 14 female spayed (median age 3.5 years) and 11 male castrated (median age 3.6 years) dogs were included in the study.

2.2. ASIT treatment group

An sterile aqueous (0.9% sodium chloride, 0.4% phenol) vaccine (Greer Veterinary, Lenoir, NC) containing a mixture of allergens specific for individual dogs and formulated based on the results of an IDT was used in all cases. The number of allergens in each vial varied and contained one to twelve allergens with a final concentration of 10,000 pnu (protein nitrogen units) per mL once on maintenance dosing. All animals went through an initial induction phase in which the allergen concentration progressively increased from 100 pnu mL⁻¹ to 10,000 pnu mL⁻¹ over the first ten weeks of immunotherapy until reaching maintenance dosing (Table 1). After the initial induction phase, all animals received 1.0 mL weekly at 10,000 pnu mL⁻¹ for the remainder of the treatment protocol.

2.3. Concurrent treatments

Ectoparasite control with fipronyl (Frontline PlusTM, Merial Limited, Duluth, GA) was instituted in all dogs at 1-month intervals before and subsequently during the study to prevent flea or other infestations during the study. Secondary infections (superficial pyoderma, *Malassezia* dermatitis, and *Malassezia*/bacterial otitis externa) were treated, if necessary, before and throughout the study. Oral antibiotic/antifungal therapy and topical antibacterial/antifungal shampoos and conditioners were allowed during the study. Any form of glucocorticoid or immunosuppressive therapy was not allowed during the initial work-up period (selamectin mite trial, 8-week food trial) or throughout the immunotherapy treatment trial.

2.4. Exclusion criteria

Dogs were withdrawn from the study if there was unacceptable discomfort in the opinion of the owner or veterinarian, unacceptable adverse effects of immunotherapy, or poor owner compliance. Both immunotherapy and healthy control dogs were withdrawn if they required any form of glucocorticoid or other immunosuppressive agent for specific symptoms at any point during the treatment trial.

2.5. Analysis of Treg and flow cytometric analysis

For analysis of Treg in normal ($n = 25$) and dogs being treated with immunotherapy ($n = 62$), approximately 5 mL of anticoagulated whole blood was obtained via jugular venipuncture and PBMC were separated using Ficoll density centrifugation. PBMC were added at 1×10^6 to micro-centrifuge tubes and then immunostained for surface expression of CD4, using appropriate concentrations of FITC-conjugated anti-dog CD4 (clone YKIX302.9; Serotec, Raleigh, NC) as described previously (Walter et al., 2006). The antibodies were suspended in FACS buffer (PBS with 2% fetal bovine serum and 0.1% sodium azide) and the staining was incubated at 4 °C for 30 min. Washing of the cells was then performed to remove unbound antibodies.

Intracellular detection of FoxP3 was then carried out using a cross-reactive, directly conjugated murine FoxP3 antibody (PE conjugated, anti-mouse/rat FoxP3, clone FJK-16s, eBioscience, San Diego, CA) and the permeabilization and fix/perm buffers that accompany the antibody (FoxP3 Staining Set, eBioscience). An isotype control consisting of a directly conjugated rat IgG2a antibody (PE) was used in addition for each blood sample tested (eBioscience). Optimal intracellular staining was achieved when the cells were resuspended in fix/perm buffer overnight at 4 °C following surface staining. The following day, cells were washed consecutively with permeabilization buffer and incubated with anti-mouse/rat FoxP3 antibody (1 µg per 1×10^6 cells) diluted in FACS buffer for 30 min at 4 °C.

Table 1

Induction Protocol (initiation of immunotherapy starting with vial I and progressively increasing in concentration through vial III until maintenance therapy)

	Vial I (100 pnu/mL)	Vial II (1000 pnu/mL)	Vial III (10,000 pnu/mL)
Interval between injections	Every other day	Every other day	Every 3rd day
Dose increments (mLs)	0.2, 0.4, 0.6, 0.8	0.1, 0.2, 0.3, 0.5, 0.7, 0.8	0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 0.9, 1.0

Flow cytometric analysis was performed using an Epics XL flow cytometer (Beckman Coulter, Miami, FL) and analysis was done using Summit software (Dako-Cytomation). Typical forward and side scatter characteristics set on the live lymphocyte population was used for the analysis gates (Faldyna et al., 2001). For lymphocytes obtained from PBMC, the percentage of FoxP3⁺CD4⁺ cells within the overall gated T cell population was calculated.

2.6. Quantitation of IL-10 using ELISA

For quantitation of canine IL-10, 3 mL of whole blood was obtained via jugular venipuncture and serum was collected after centrifugation using serum separator collector tubes. For the quantitative determination of canine IL-10 concentrations in serum samples, a commercially available canine-specific IL-10 ELISA was used (QuantikineTM canine IL-10 immunoassay, R&D Systems, Minneapolis, MN). All serum samples were analyzed in duplicate according to the manufacturer's instructions. The assay function is a controlled direct sandwich ELISA with calibration material and control consisting of recombinant canine IL-10.

2.7. Quantitation of allergen-specific IgE

Serum samples were also submitted for allergen-specific IgE ELISA measurements (Greer Veterinary Laboratories, Lenoir, NC) in the immunotherapy group prior to initiation of allergen-specific immunotherapy and at the conclusion of the study after 1 year of treatment.

2.8. Recheck examination

Dogs were evaluated initially and at 3, 6, 9, and 12 months after initiating immunotherapy. At each recheck examination blood was obtained via jugular venipuncture for both Treg analysis and quantitation of IL-10. At the initiation of immunotherapy, owners were questioned as to the severity of pruritus exhibited by their dog. To help owners assess the severity of pruritic manifestations, a visual analog scale was used. This

pruritus visual numerical scoring system was graded from (0) to (10), with the left most corner underlined by the statement 'not itchy, no scratching, chewing, rubbing, or licking observed' and the rightmost end with the statement 'extremely itchy, scratching, chewing, rubbing or licking constantly'. This survey was completed by the owners at each consecutive recheck examination through the final visit at 1 year after initiation of immunotherapy.

2.9. Statistical analyses

Differences between the control and immunotherapy groups over time were compared using a mixed regression ANOVA. These groups were compared over time for both Treg percentage and IL-10 quantitation. The immunotherapy group was also compared for allergen-specific IgE concentrations. Comparison of the pruritus scores between groups and among the immunotherapy group were done using one-way analysis of variance for non-parametric data (Kruskal–Wallis test). Statistical analyses were done using Systat 12.0 software (Systat Software Inc., San Jose, CA). For all comparisons, a p -value ≤ 0.05 was considered significant.

3. Results

3.1. Dogs

Sixty-two dogs were initially enrolled in the study. Nine dogs (14.3%) did not complete the study. Five dogs were lost to follow-up during the study, including three dogs that were lost due to client relocation to a different area of the country. Four dogs were withdrawn due to lack of owner compliance with the treatment protocol.

3.2. Treg populations in the control and immunotherapy groups over time

Changes in Treg percentage throughout the study in both the control (healthy dogs) and the immunotherapy groups are summarized in Table 2. There was no

Table 2

Treg populations (% Treg in peripheral blood) in the control and immunotherapy groups over time

Patient population	Number of dogs	0 months ^a	3 months ^a	6 months ^a	9 months ^a	12 months ^a
Healthy Dogs	25	4.84 $\pm$ 0.64	4.65 $\pm$ 0.67	4.76 $\pm$ 0.52	4.90 $\pm$ 0.52	4.98 $\pm$ 0.57
Immunotherapy	53	4.93 $\pm$ 0.71	5.19 $\pm$ 0.74	8.11 $\pm$ 1.43	10.07 $\pm$ 2.25	10.86 $\pm$ 2.73

^a Mean ($\pm$ S.D.) percentage of FoxP3⁺CD4⁺ T cells (Tregs).

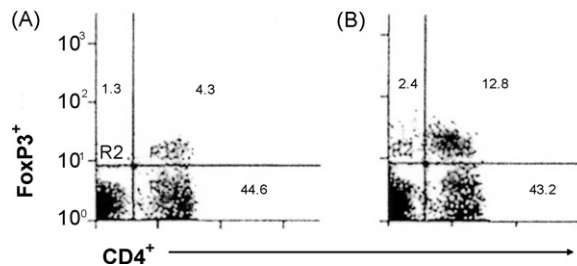


Fig. 1. Identification of canine Tregs using FoxP3 antibody. Lymphocytes were obtained from blood and immunostained for expression of FoxP3 and CD4 as described in Section 2. Representative dot plots of FoxP3 expression by CD4⁺ T cells from (A) blood from atopic dog before initiation of ASIT; (B) blood from same atopic dog after 1 year of ASIT. The percentages of lymphocytes within each quadrant are given.

significant difference in the Treg percentage over time in the control group. The mean Treg percentage at the beginning of the study was 4.84 ± 0.64 in the control group and 4.98 ± 0.57 at the completion. The immunotherapy group showed a significant increase in Treg percentage at 6, 9, and 12 months when compared to the control group ($p < 0.001$). The mean Treg percentage was not significantly different from the control group at the initiation of therapy. For the immunotherapy group, the mean Treg percentage at the beginning of the study was 4.94 ± 0.71 and 10.86 ± 2.73 at the completion (Fig. 1).

3.3. IL-10 quantitation in the control and immunotherapy groups over time

Changes in IL-10 serum concentrations throughout the study in both the control and the immunotherapy groups are summarized in Table 3. There was no significant difference in the IL-10 concentrations over time in the control group. The mean serum IL-10

concentration at the beginning of the study was $19.06 \pm 3.44 \text{ ng L}^{-1}$ in the control group and $19.03 \pm 3.27 \text{ ng L}^{-1}$ at the completion. The immunotherapy group showed a significant increase in serum IL-10 concentrations at 6, 9, and 12 months when compared to the control group ($p < 0.001$). The mean serum IL-10 concentration at the initiation of immunotherapy was $20.40 \pm 3.52 \text{ ng L}^{-1}$ and $37.26 \pm 15.26 \text{ ng L}^{-1}$ at the completion of the study.

3.4. Pruritus visual analog scores in the immunotherapy group over time

Changes in the pruritus scores throughout the study in the immunotherapy group are summarized in Table 4. There was a significant difference in the pruritus scores at 6, 9, and 12 months when compared to the pruritus scores at the initiation of the study. T regulatory populations and IL-10 concentrations were also compared between subsets of immunotherapy patients with differing reductions in their pruritus scores over time. Immunotherapy dogs with a reduction of 5 or greater in their pruritus score (responders) were compared with dogs that had a 4 or less reduction in their pruritus score (non-responders) over the study period. There was no significant difference between the two groups when comparing Treg percentages at the initiation of therapy, but a significant difference when comparing the two groups at the end of the study ($p < 0.001$). The mean Treg percentage at the initiation of therapy was 4.85 ± 0.75 for the responder group and 5.06 ± 0.66 for the non-responders. After 1 year of treatment, the responder group had an increased percentage of 13.26 ± 0.30 and the non-responder group had increased to 8.17 ± 1.51 . There was also no significant difference between the two groups when comparing IL-10 concentrations at the initiation of

Table 3
Serum interleukin-10 (IL-10) concentrations in the control and immunotherapy groups over time

Patient population	Number of dogs	0 months ^a	3 months ^a	6 months ^a	9 months ^a	12 months ^a
Healthy dogs	25	19.06 ± 3.44	18.07 ± 3.25	17.90 ± 3.25	18.94 ± 2.85	19.03 ± 3.27
Immunotherapy	53	20.40 ± 3.52	20.85 ± 3.80	24.82 ± 5.97	29.39 ± 9.13	37.26 ± 15.26

^a Mean ($\pm$ S.D.) serum interleukin-10 concentrations (ng/L).

Table 4
Pruritus visual analog scores for immunotherapy group over time

Patient population	Number of dogs	0 months ^a	3 months ^a	6 months ^a	9 months ^a	12 months ^a
Immunotherapy	53	8.77 ± 0.93	8.06 ± 1.00	6.35 ± 1.27	5.35 ± 1.37	4.60 ± 1.91

^a Mean ($\pm$ S.D.) pruritus visual analog scores (0)—least severe to (10)—most severe.

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Table 5

Comparing the most commonly identified allergens using IDT and the respective changes in the serum allergen-specific IgE concentrations in the immunotherapy group over time

Immunotherapy Patient Population and % identified on IDT	Most common allergens identified on IDT	Allergen-specific IgE levels prior to ASIT ^a	Allergen-specific IgE levels 1-year after ASIT ^a
Responders (<i>n</i> = 27)			
85%	House dust mite	156.33 ± 23.40	56.15 ± 21.45
74%	Penicillium	135.62 ± 25.89	55.78 ± 30.90
70%	Alternaria	121.95 ± 20.23	63.55 ± 24.38
63%	Rhizopus	119.67 ± 21.45	62.95 ± 25.12
Non-responders (<i>n</i> = 26)			
73%	Ragweed	142.85 ± 25.30	115.95 ± 28.56
69%	Alfalfa grass	129.34 ± 20.50	100.45 ± 30.37
65%	Rye grass	115.32 ± 26.21	105.41 ± 21.40
62%	Oak tree	91.56 ± 28.76	93.25 ± 19.88

M.A.U. = Modified Absorbance Units.

^a Mean (±S.D.) serum allergen-specific IgE concentrations (M.A.U.).

therapy, but a significant difference when comparing the two groups at the completion of the study ($p < 0.001$). The mean serum IL-10 concentration at the initiation of therapy was $20.49 \pm 1.26 \text{ ng L}^{-1}$ for the responder group and $19.91 \pm 1.66 \text{ ng L}^{-1}$ for the non-responders. After 1 year of treatment, the responder group had an increased mean serum concentration of $50.54 \pm 2.40 \text{ ng L}^{-1}$ and the non-responder group had increased to $23.47 \pm 1.81 \text{ ng L}^{-1}$.

3.5. Intradermal skin test results

The allergens identified by intradermal skin testing before starting treatment in the immunotherapy group were also compared. The most commonly identified allergens were found to be significantly different when comparing the responder group to the non-responders (Table 5). The most common allergens identified in the responder group through intradermal testing in order of frequency were: house dust mites (85%), penicillium (74%), alternaria (70%), and rhizopus (63%). The most common allergens identified in the non-responder group before starting treatment included: ragweed (73%), alfalfa grass (69%), rye grass (65%), and oak tree (62%).

3.6. Allergen-specific IgE results

When comparing the allergen-specific IgE results, the responder group showed a significant decrease in their serum IgE levels after 1 year of therapy; while no significant difference was found in these levels for the non-responders ($p < 0.001$, Table 5). The most significant reduction in allergen-specific IgE was found for house dust mite allergen.

4. Discussion

In this study, we found that Treg numbers were significantly higher in the peripheral blood of dogs undergoing allergen-specific immunotherapy (ASIT) and that these populations increased over time from the initiation of therapy. In addition, a significant increase in serum IL-10 concentrations was also evident over time in this subset of patients. Dogs that had the most significant reductions in pruritus during immunotherapy treatment, also had the most significant increases in both peripheral Treg numbers, as well as serum IL-10 concentrations. Recent human studies have shown that peripheral T cell tolerance induced by Treg cells is of major importance for the immune systems' responses to allergens and the success of ASIT (Thunberg et al., 2007). ASIT has recently been shown to generate allergen-specific regulatory T cells and increase production of IL-10 protein in both the blood and the tissues following ASIT (Akdis et al., 1998; Jutel et al., 2003). Due to the significant increases in both peripheral Treg numbers and serum IL-10 concentrations in canine ASIT patients; it is possible that these same regulatory mechanisms play a significant role in the success of canine immunotherapy as well.

At the present time, assessment of FoxP3 expression is the best available method of enumerating Treg populations in dogs (Biller et al., 2007). Treg analysis using multiple surface makers is precluded at this time in dogs, due to lack of antibody reagents for assessing expression of these molecules. In mice, FoxP3 is expressed by most Treg and can be used to distinguish CD4⁺CD25⁺ Treg cells from CD4⁺CD25⁺ non-regulatory T cells (Fontenot et al., 2003). The specificity of human FoxP3 expression for identification of Tregs is

still debated. In one study, TCR stimulation of naïve T human CD4+CD25– T cells failed to elicit FoxP3 expression and found this expression to be limited solely to regulatory T cells (Yagi et al., 2004). Other investigators, however have shown increased FoxP3 expression in non-regulatory T cells as well (Morgan et al., 2005; Walker et al., 2003). It is possible that in dogs there is FoxP3 expression in both subsets, however it is believed that its expression occurs predominantly within the canine Treg population. This assertion is further strengthened by the recent finding of concurrent significant increases in IL-10 and TGF- β mRNA expression in appropriately activated cells, a result not expected if activation were to lead to the development of cells without a Treg phenotype (Biller et al., 2007). The availability of a diagnostic reagent to reliably identify Treg in dogs should greatly facilitate future investigations into the role of Treg in important diseases of dogs (Biller et al., 2007).

During this study, significant increases in serum interleukin-10 concentrations occurred concurrently with increases in peripheral blood Treg concentrations. In clinical trials, ASIT has been shown to increase the production of IL-10 by APCs, including B cells, monocytes, and macrophages, a phenomenon that leads to increased generation of IL-10 secreting Treg cells (Larché et al., 2006). It is likely that the significant increases in serum IL-10 concentrations in this study, can also be attributed to the same immunological regulatory phenomenon. Moreover, IL-10 that is produced and progressively secreted during ASIT appears to function in counter-regulating synthesis of antigen-specific IgE and IgG4 antibodies (Akdis et al., 1998). IL-10 potently suppresses both total and allergen-specific IgE, and simultaneously, increases IgG4 production in cultures (Akdis et al., 1998; Punnonen et al., 1993). Therefore, IL-10 not only generates T cell tolerance but also regulates specific antibody isotype formation (Akdis, 2006).

Chronic environmental allergens, such as house dust mites and molds, were the most prominent allergens identified in the responder group which had the most significant reduction in pruritus during treatment with immunotherapy. These patients also had the most significant reduction in allergen-specific IgE for the respective allergens for which they were receiving ASIT. The magnitude of the reduction in allergen-specific IgE does not always correlate with the severity of clinical signs, but warrants further study in light of the group's increasing Treg production and mean serum IL-10 concentrations. Seasonal allergens such as pollens, grasses, and trees were the most common

allergens in the non-responder group which had a less significant reduction in pruritus when comparing the two groups receiving ASIT. It is possible that chronic non-seasonal allergy patients which elicit a more cell-mediated immune response, could respond more favorably to ASIT, given the probability that immunotherapy increases both Treg production and their immunosuppressive cytokine products such as IL-10. Further research and investigation of the success for specific allergens in long-term ASIT patients is warranted.

The immunological mechanisms of ASIT are becoming increasingly well defined and elucidated through recent research. Various studies have described the modulation of APC, T-cell and B-cell responses to allergen exposure. Successful ASIT has been shown to be associated with several features, including increases in allergen-specific serum antibodies (particularly IgG1 and IgG4), increased ratios of Th1 cell responses to Th2 cell responses, and the induction of functional regulatory T cells (Larché et al., 2006). Increased expression of IL-10, seems to be associated with augmentations in regulatory T-cell populations and changes in serum-antibody profiles. It is possible that adjuvants favoring the production of regulatory cytokines and Treg populations may be identified in the near future. In addition, specific types of pharmacotherapy may favor induction of optimal clonal expansion of regulatory T cells when used in combination with ASIT. To optimize clinical response, future therapeutic strategies, including ASIT, will hope to employ such immunological mechanisms. Thus, the application of current knowledge regarding Treg cells and related mechanisms of peripheral tolerance may lead to more appropriate and successful treatment strategies for the prevention and cure of allergic disease.

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