
Nasal immunotherapy in weed-induced allergic rhinitis

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Background: Nasal immunotherapy with single allergen extracts, following premedication with cromolyn, has been reported to be effective in treating seasonal and perennial allergic rhinitis.

Methods: We conducted a double-blind, placebo-controlled study to assess the efficacy, tolerability, and mechanism of action of nasal immunotherapy for allergic rhinitis caused by weed pollens from three unrelated families. Twenty-seven weed-allergic patients underwent baseline nasal provocation and titrated skin test with a mixed weed extract containing ragweed, sage, and Chenopod extracts. Patients were randomized to receive either mixed weed extract or placebo. Nasal immunotherapy was self-administered daily to alternate nostrils preceded by 5.2 mg intranasal cromolyn. Beginning with 1:2500 wt/vol the concentration was increased to 1:10 wt/vol over an average period of 36 days. The maintenance dose (1:10 wt/vol) was administered daily for 12 to 16 weeks through the weed pollen season. Patients recorded nasal and eye symptoms and the use of rescue medications throughout the study. A nasal lavage for cytokine levels and nasal scraping with Rhinoprobe for nasal cytology were performed at the peak of the weed season. Nasal provocation and titrated skin tests with mixed weed extract were repeated after the weed season. Nasal lavage and scraping were also performed before and 24 hours after the final nasal provocation.

Results: During the peak weeks of the weed season the group receiving mixed weed extract by nasal instillation, compared with those treated with placebo, had significantly lower total nasal symptom scores, total eye symptom scores, and symptom medication scores. There were no significant differences in the nasal cytology or cytokines levels between the two groups, except for elevated IL-10 in the nasal lavage in the treated group at the peak of the season. Nasal symptoms and medication use were higher preseasonally in the active treatment group.

Conclusion: Nasal immunotherapy with aqueous mixed weed extract administered with cromolyn sodium pretreatment for 17 to 21 weeks was effective in reducing both nasal and ocular symptoms of weed pollen-induced allergic rhinitis. There were increased nasal symptoms in the treated group preseasonally.

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INTRODUCTION

Local nasal immunotherapy with an aqueous extract of ragweed was shown to be clinically effective for treating ragweed-induced seasonal allergic rhinitis but was associated with signifi-

cant adverse local reactions.^{1,2} Glutaraldehyde polymerized ragweed extract administered intranasally caused fewer local adverse reactions but is not commercially available.^{1,3} More recently, clinically effective nasal immunotherapy with grass,⁴ Parietaria,⁵ and house dust mite⁶ extract in powder form was found to be well tolerated through the use of intranasal cromolyn as pre-medication. This pretreatment reduced local immediate reactions and possibly reduced the priming of the nasal mucosa.

Nasal immunotherapy with cromolyn pretreatment has the advantages over the conventional immunotherapy of ease of administration, paucity of systemic side effects, and reduced cost of administration making it an attractive alternative. It also differs from conventional immunotherapy in that the immunologic response appears to be local rather than systemic.^{4–6} The aim of our study was to evaluate the efficacy and tolerability of nasal immunotherapy employing an extract containing multiple, unrelated weed allergens that are clinically important in our region in the late summer. Efficacy was assessed by reduction in clinical symptom scores and use of rescue medications during the weed pollen season. Tolerability was assessed by the report of symptoms and medication requirements prior to the specific pollen season. We evaluated mechanisms of action by analysis of cytokines in nasal washes and cells in nasal scrapings following challenges and during the height of the specific pollen season.

METHODS

Subjects

Subjects who responded to advertisements for volunteers were required to have (1) a history of allergic rhinitis symptoms during the weed pollen season for at least 2 years; (2) at least one positive prick skin test, with a wheal diameter 5 mm greater than control, to concentrated extracts of one of the dominant weeds of the region (short ragweed 1/10 wt/vol, western ragweed 1:10 wt/vol, common sagebrush 1/10 wt/vol, Kochia 1:10 wt/vol, or Russian thistle 1/20 wt/vol (Greer Laboratories, LeNoir, NC); and (3) a positive nasal provocation with a mixture of these

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weed extracts. None of the patients was a smoker or had sufficiently severe asthma to require daily therapy. Patients with histories of allergy to animals who were regularly exposed to the animal were excluded. Patients who had received immunotherapy with any weed pollen extract within the last 2 years or who were receiving corticosteroids, cromolyn, or nedocromil intranasally or by inhalation were also excluded. The study was approved by the IRB of the National Jewish Medical and Research Center and all the patients gave informed consent.

Study Design

The study was a randomized, double-blind, placebo-controlled parallel trial of nasal immunotherapy with a mixed weed extract. At the initial visit, a complete medical history and physical examination, baseline spirometry, titrated prick skin tests, and nasal provocation test were performed and blood was drawn to determine the humoral response to representative weeds (IgE for western ragweed and Russian thistle and IgG for western ragweed). Patients were then randomly assigned by the Pharmacy Service to receive aqueous mixed weed extract (Table 1) or placebo to be self-administered intranasally. Both investigators and patients were blinded to the content of their extract. The treatment phase began between March and May, 1995, was continued through the season terminating at the end of August after the peak of the weed season. At the peak of the weed pollen season, a nasal lavage and

nasal scraping were performed. In late September, 1995 all the patients had blood drawn for the weed-specific IgG and IgE, and underwent repeat titrated prick skin tests and nasal provocation with mixed weed extract. Nasal lavage and nasal scraping were done prior to and 24 hours after the nasal provocation.

Pollen Counts

Pollen counts were performed five days per week throughout the study employing a Burkard sampler (Burkard Manufacturing Company, Rickmansworth, Hertfordshire, England). Weed pollen was identified by botanical family.

Prick Skin Testing

Skin testing was performed by pricking through the drop of extract at a 45° angle using a solid-bore needle. Titrated skin tests were done in duplicate with negative (glycerin-saline) and positive (histamine phosphate, 1.0 mg/mL histamine base) controls and several 3-fold dilutions of the concentrated mixed weed extract (Table 1). The results were recorded by tracing with ink and transferring the outlines with transparent tape to a permanent record. A mean diameter was calculated from the largest and the midpoint orthogonal diameters. For analysis the reactions to 1:300 wt/vol were compared since all subjects were tested with that concentration.

Nasal Provocation Tests

Nasal provocation tests were performed between 8:00 AM and 12:00 noon to limit the effect of circadian variation. As a safety measure, oral peak expiratory flow rates were measured before and after the testing. Nasal provocation was performed by insufflation of mixed weed extract into both nostrils. Dose increments were made as follows: normal saline, then 1:1000 wt/vol, 1:300 wt/vol, 1:100 wt/vol, 1:30 wt/vol, and 1:10 wt/vol at 10-minute intervals. Mixed weed extract was diluted with 0.9% saline. Patients recorded symptom scores before nasal challenge and prior to each subsequent dose using a previously vali-

dated scoring system.⁷ The endpoint was the amount of antigen required to produce a total symptom score of 5 out of a possible 12 based on the occurrence of sneezing (3–4 1 point, 5 or more 3 points), pruritus (nose, palate or ear 1 point each), rhinorrhea (1–3 points), blockage (1 to 3 points), and ocular symptoms (1 point). For 24 hours after nasal challenge the only medication permitted was intranasal oxymetazoline. The postseason nasal challenge differed from the pre-season in that all subjects received all doses of mixed weed extract in order to ensure equal allergen stimulation. Once the endpoint was reached, oxymetazoline administration was permitted to facilitate completion of the nasal challenge.

Treatment

Mixed weed extract or placebo (buffered saline colored with caramelized sugar) was administered every morning to alternating nostrils after premedication with 5.2 mg intranasal cromolyn sodium to prevent side effects. Treatment solution was dispensed in opaque plastic nasal spray bottles with each actuation delivering 0.1 mL of a droplet mist. For the active patients, the immunotherapy dose was increased progressively as 1, 2, and 4 sprays from 1:2500 wt/vol, 1:250 wt/vol, and 1:25 wt/vol, and 1 and 2 sprays from the 1:10 wt/vol concentration were administered. Each dose was repeated four days before progressing to the next, thus 40 days were required to reach the maintenance dose. The highest concentration (1:10 wt/vol) was prepared as in Table 1. The maintenance dose of 0.2 mL of 1:10 wt/vol was administered daily to alternating nostrils for 12 to 16 weeks through the pollen season. In some patients, slight dose modifications were necessary because of local side effects. Loratadine (10-mg tablet) and an albuterol metered dose inhaler were provided to be used as rescue medications. Their use was recorded in their diary cards. All patients were provided with a Medihaler-Epi to be used in the event of local throat or generalized allergic symptoms.

Table 1. Composition of 1:10 wt/vol of Mixed Weed Extract*

Allergen	mL	wt/vol
Short ragweed	1.5	1:10
Western ragweed	1.5	1:10
Common sagebrush	3.0	1:10
Russian thistle	2.0	1:20
Kochia	2.0	1:10

* The highest concentration of mixed weed extract used for skin testing, nasal immunotherapy, and nasal provocation testing was prepared by mixing the aqueous extracts of these weeds in the specified quantities. This mixture was designated 1:10 wt/vol.

Histamine was not added to the placebo since preliminary studies suggested most patients had no immediate symptoms when allergen extract was preceded by intranasal instillation of cromolyn spray.

Assessment of Symptoms

Patients completed symptom diary cards twice daily. The morning report reflected the symptoms during the previous night, and the bedtime report reflected the daytime symptoms. Specific symptoms recorded were nasal congestion, sneezing, runny nose, itchy nose, eye watering or irritation and an asthma symptom score. Patients graded the severity of each of the six symptoms twice daily from 0 to 3 (0 = none, 1 = mild, 2 = moderate, and 3 = severe). The maximum possible 24-hour symptom score was 36. The amount of rescue medication, ie, loratadine (or occasionally other antihistamines that patients used out of protocol) was recorded daily. Each loratadine tablet used was scored as 4 as this was the approximate reduction in symptoms in patients treated with loratadine when compared with placebo in previous seasonal allergic rhinitis studies employing similar symptom grading systems. On completion of the study, while still blinded, patients provided a global rating of the effectiveness of treatment on a scale of (–) 5 for very ineffective to (+) 5 for highly effective.

Peak expiratory flow rates were determined as a safety measurement. They were done every morning three times and the best of three efforts was recorded.

Sampling Methods

Sampling for cells and cytokines was done at the peak of the season and before and 24 hours after the postseason nasal challenge. Nasal lavage was performed to recover cytokines and nasal scraping were used for cell recovery. Nasal lavage was performed by gradual infusion of 10 mL of 0.9% saline into each nostril, with the subjects head reclined at a ~45° angle. Subjects allowed the saline to remain

for 10 seconds prior to expelling it into a collection vessel. Rhinoprobe (nasal mucosal curette, Arlington Scientific, Texas) was used for nasal scrapings. The Rhinoprobe was gently shaken in a tube containing 1 mL of filtered phosphate buffered saline.

Morphologic Techniques

One milliliter of the cell suspension was centrifuged at 1500 rpm for one minute. The supernatant was decanted and the cells were resuspended in 500 μ L of filtered PBS. Fifty microliters was mixed with an equal volume of Trypan Blue, placed in a hemocytometer, and the total number of cells were counted. The remaining 450 μ L were divided equally and processed in a cytospin yielding two slides, one each for differential and mast cell stain. Diff Quick (Baxter Healthcare Corporation, McGraw Park, IL) stain was used for staining neutrophils, eosinophils, lymphocytes, monocytes, and epithelial cells. Astra Blue was used to stain mast cells as described.⁸ Differential counts were performed by counting a total of 100 consecutive cells including epithelial cells under oil immersion and cell numbers were expressed as percentages. For metachromatic cells, ten high power fields were reviewed under oil immersion and total metachromatic cells were counted under each field. The number of mast cells in each field was then added and reported as total metachromatic cells.

Cytokine Assays

Nasal lavage specimens were concentrated approximately 5-fold by using a Centriprep concentrator (Amicon Div, W R Grace & Co, Beverly, MA). The protein content in the specimens was measured by using a modified Lowry assay (BioRad, Richmond, CA). Cytokine assays were performed for tumor necrosis factor- α , interleukin (IL)-4, IL-10, interferon- γ , and RANTES. Commercially available ELISA kits (BioSource International, Camarillo, CA) were used according to the manufacturer's instructions. These are solid phase sandwich ELISA assays with two monoclonal antibodies spe-

cific for different epitopes of the cytokine being assayed.

Weed-Specific IgG and IgE

Blood samples were obtained on entry into the study and at study termination. IgE specific to western ragweed and Russian thistle were measured using the Pharmacia CAP System RAST FEIA Test Procedure (Pharmacia AB, Uppsala, Sweden). The allergen of interest, covalently coupled to ImmunoCap, reacts with specific IgE in the patient serum specimen. After washing away nonspecific IgE, enzyme-labeled antibodies against IgE are added to form a complex. After incubation, unbound enzyme anti-IgE is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured in fluoroCount 96. Fluorescence units (FU) in the samples are compared directly with FU for standards run in parallel. Western ragweed-specific IgG was also measured using the same procedure except that the enzyme-labeled antibodies were directed against IgG.

STATISTICAL ANALYSIS

Mixed models were used to determine whether the pattern of mean symptom scores across time differed for the active and placebo-treated groups. The mixed model used adjusted for both higher correlation of observations on a subject than between subjects and for observations close together in time being more highly correlated than observations far apart in time.⁹ This was done using the SAS Proc Mixed software with a between subjects compound symmetric error structure and a within subject autoregressive error structure of order 1.¹⁰ The mixed model accommodates missing data on a subject, provided it is missing at random. Tests for the interaction of weed and treatment group were performed. This test was followed by pairwise contrasts of the active and placebo group means at each week. Bonferroni's multiple comparison procedure

was applied to the pairwise comparisons at the .05 significance level.

Wilcoxon's rank sum test was used to compare distributions of placebo and active treatment response variables at the peak of the season, as well as comparing distributions of preseason to postseason changes in outcome variables, and prechallenge to postchallenge measures.⁹ Wilcoxon's rank sum test was used rather than the Student's *t* test since the data were not normally distributed.

For normally distributed data, means and standard errors are reported as summary statistics. For non-normally distributed data, either the data were log transformed or medians and interquartile ranges are reported. For log transformed data, geometric means (the anti-log of the mean on the log scale) and the standard error on the log scale are reported. All tests were 2-sided and performed at the .05 significance level unless otherwise noted.

RESULTS

Of the 29 patients who were enrolled, 27 completed the study (see Table 2). One patient withdrew due to personal reasons while the second patient dropped due to adverse events. The latter developed hives on her neck approximately 28 hours after the initial nasal challenge and four hours after the first dose of intranasal study drug. There were no respiratory symptoms. Later that day the patient also developed a "sore throat." She was to restart immunotherapy at one tenth the previ-

ous dose but decided to withdraw from the study. In addition, two patients experienced significant local side effects during the step from 1:250 wt/vol to 1:25 wt/vol and were given 1:100 wt/vol mixed weed extract as an intermediate dose. All 27 who completed the study received the projected maintenance dose.

Pollen counts were performed throughout the study and are shown as total weed counts in Figure 1. The Chenopod-Amaranth (*Kochia*, Russian thistle) counts rose steeply after mid-July, while all three weed groups rose throughout the month of August, peaking towards the end of that month. Peak counts for sage were approximately twice as high as for ragweed and the Chenopod-Amaranth. Counts fell precipitously after their peak, reaching low levels by mid-September.

The patients who received active treatment had significantly lower nasal symptom scores than those receiving placebo during 3 weeks of high pollen counts. The symptom scores in the active treatment group for the weeks of 20 and 27 August and 3 September were (geometric mean and SEM) 3.15 ± 0.18 , 2.89 ± 0.18 , and 2.70 ± 0.20 , compared with the scores in placebo treated subjects of 7.13 ± 0.15 , 8.07 ± 0.16 , and 6.45 ± 0.22 ($P = .0001$ to $.0013$). Subjects in the active treatment cohort had higher nasal symptom scores in the early course of nasal immunotherapy, however, with correction for multiple comparisons, the differences did not attain statistical

significance. This excess of symptoms was accounted for primarily by increased AM symptoms (data not shown). Analysis of the first 5 weeks of treatment for each subject revealed that the increased symptoms in the treated group began the third week. Beginning shortly after reaching maintenance, the symptom scores for the active treatment cohort began to decline. This decline continued throughout the period of preseasonal treatment and the nasal symptoms of the two treatment groups were not different immediately prior to the onset of weed season.

Patients in the active treatment group experienced significantly fewer eye symptoms during 3 weeks of high weed pollen counts compared with placebo. The geometric mean (SEM) for these weeks in the active treatment groups were 1.31 ± 0.12 , 1.24 ± 0.11 , and 1.43 ± 0.12 while in the placebo group the values were 2.91 ± 0.15 , 2.85 ± 0.16 , and 2.35 ± 0.15 ($P = .0001$ to $.0081$) (Fig 2). There was no difference in symptoms between the two groups during the period of pre-seasonal treatment.

Medication scores tended to be higher in the placebo-treated patients during the peak of weed pollen season and higher in the actively treated patients early in the course of therapy. Neither difference was statistically significant.

The combined total symptom-medication scores revealed that placebo-treated patients had approximately four times higher total scores of the active therapy group during the three highest pollen count weeks than the active therapy group during the 3 weeks with highest pollen counts. Geometric means (SEM) for the active treatment group were 4.29 ± 0.26 , 3.74 ± 0.26 , and 3.62 ± 0.25 while in the placebo group they were 14.21 ± 0.20 , 14.47 ± 0.22 , and 12.42 ± 0.11 ($P = .0001$ to $.0005$). Figure 1 shows these differences in mean natural log values, hence the apparent only 2-fold difference between groups in the figure.

At the conclusion of the study, while still blinded, subjects gave a global

Table 2. Patient Demographics*

	Active Treatment	Placebo Treatment
Age, mean (range)	44.8 (29–59) years	41.1 (22–63) years
Gender, M/F	6/6	9/6
Asthma	6	5
Use of albuterol	3	3
Skin tests		
1 positive	1	2
2 positive	1	2
3 positive	10	11

* Baseline and study characteristics of the active and placebo treatment groups are presented. Use of albuterol is any use during the course of the study. Skin tests are the number of botanical families to which the patients had positive prick skin tests.

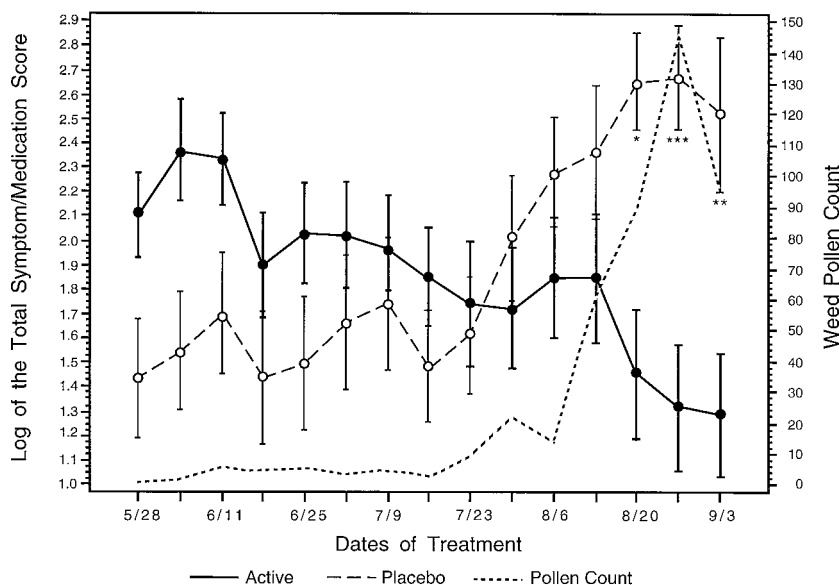


Figure 1. Combined total nasal and ocular symptom medication scores. Presented on the background of the combined ragweed, sage, and Chenopod-Amaranth pollen counts (grains/M³) are the mean combined nasal and ocular symptom scores corrected for medication use for the two study groups. Note the scale is the natural log of the total symptom/medication score. With Bonferoni's correction, the only weeks showing significant differences were 8/20, 8/27 and 9/3. The level of significance is: *.0005, **.0003, and ***.0001. The number of subjects were active, 13 and placebo, 14.

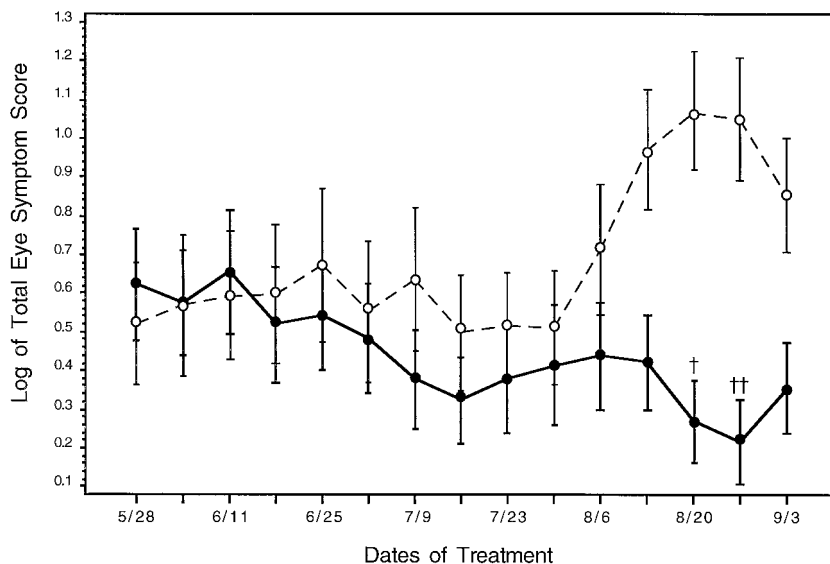


Figure 2. Total ocular symptom score. Presented are the mean natural logs of the total eye symptom scores for the two treatment groups. With Bonferoni's correction, only 2 weeks were significantly different, 8/20 and 8/27. The levels of significance were *.0002 and **.0001. The number of subjects were active 13 and placebo 14.

evaluation of the effectiveness of treatment on a scale of plus 5 (highly effective) to minus 5 (very ineffective). The global rating indicated a signifi-

cantly more favorable rating for active treatment compared with placebo ($P = .04$). Median rating (quartiles) for the active group was 3.0 (1.0, 4.0) com-

pared with the placebo groups rating of 0.0 (−3.0, 2.0).

Compared with baseline, titrated prick skin tests performed after the weed pollen season revealed a significant decrease in reactivity to the 1:300 wt/vol concentration of the mixed weed extract in the patients who received active treatment (median (quartiles) change in wheal size of treated group = −2.00 (−3.25, −2.75) mm but not in the placebo group (+0.25 (−1.50, 1.25) mm). The difference between groups was statistically significant ($P = .04$).

The threshold in the nasal provocation test at baseline was 3.0 (the third dilution) in both groups. When repeated following the pollen season, the threshold was significantly increased in the actively treated subjects (change in dilution in the treated group = +1.00 (25th, 75th quartile −1, +2) compared with the placebo-treated subjects who had slightly increased sensitivity to the mixed weed extract (change in dilution −1.00 (25th, 75th quartiles −1, 0); $P = .04$).

Western ragweed-specific IgG increased significantly in the treated patients at the end of nasal immunotherapy compared with the placebo-treated patients (change in IgG in treated group = 2.99 (quartiles 2.18, 4.14) mg/L versus placebo = 0.15 (quartiles −0.50, 0.47) mg/L; $P = .012$). There was no difference in the change in the western ragweed-specific IgE or Russian thistle-specific IgE for the two treatment groups.

The number of metachromatic cells, eosinophils, and neutrophils from nasal scrapings obtained at the peak of the season or 24 hours following post-seasonal nasal challenge showed no difference between treatment groups.

Tumor necrosis factor- α , interleukin-4, interferon- γ , and RANTES in the fluid from nasal lavage performed at the peak of the season and 24 hours after postseasonal nasal challenge also showed no difference between treatment groups. Interleukin-10 (standardized for protein content) in nasal lavage fluid was significantly elevated in the actively treated patients at the peak

of the season (median of the active group = 0.04 pg/mL, versus placebo = 0.01 pg/mL; $P = .03$). There was no difference in IL-10 in lavage fluid in the two groups following the postseason challenge.

No oropharyngeal or systemic adverse effects or asthma exacerbations were observed. All adverse effects were local and included sneezing, nasal congestion, and rhinorrhea. The results of this study confirm the previously reported safety of nasal immunotherapy.

DISCUSSION

Local application of allergen extract to treat allergic rhinitis has been studied extensively.¹⁻⁶ Using cromolyn sodium to reduce the development of nasal symptoms, nasal immunotherapy has proven to be an effective and well-tolerated form of immunotherapy.^{4-6,11}

Our study has demonstrated that nasal immunotherapy resulted in blocking the seasonal rise in nasal and ocular symptoms, with a trend for decreased use of rescue medication. We also documented an increase in the nasal threshold to weed pollen extract challenge, which is consistent with the reduced symptoms during seasonal exposure to weed pollen. Although not statistically significant, patients randomized to active treatment experienced more symptoms during the build-up and initial maintenance phase compared with the placebo-treated subjects. This increase in symptoms in the actively treated group during the preseasonal period was not observed in some previous studies.^{4,6,11} Our dose escalation was considerably more rapid than that employed in the studies that did not observe this phenomenon.

We observed a rise in ragweed-specific IgG in our study which has not been reported in several recent studies of nasal immunotherapy.⁵ This evidence of systemic effect may be related to gastrointestinal absorption of the extract as has been seen with oral immunotherapy.^{12,13} This may be a result of the high total dose of allergen extract that we administered (0.2 mL of 1:10 wt/vol extract daily at mainte-

nance). We also observed a decrease in the prick skin test reactivity following immunotherapy, probably also reflecting systemic absorption of weed extract. Evidence of a systemic immune response raises the issue of whether the reduction in clinical symptoms during the weed season resulted from the systemic or the topical response to the allergen extract. We favor the latter since the reduction in symptoms was considerably greater than has been reported with oral therapy.^{12,14}

We did not identify differences in cytokine production by T_{helper} lymphocytes or in cellular infiltrate secondary to the immunotherapy. We hoped to demonstrate with nasal immunotherapy decreased production of IL-4 indicative of induced non-responsiveness (tolerance) of allergen-specific T_{helper} lymphocytes. It is possible that immunotherapy mediates different influences on immune responses at different times. Studies that have demonstrated decreased production of IL-4 and IL-5 by T_{H2} -like cells involved long-term administration of immunotherapy.^{15,16} Early in the course of immunotherapy, at a time when clinical benefit is clearly demonstrable, there is an increase in allergen-specific IgE concentrations which may reflect increased allergen-specific IL-4 (and possibly IL-13) production. Repeating these studies after a more prolonged course of nasal immunotherapy and at a time when IgE concentrations were beginning to decline may have demonstrated decreased IL-4. A longer course of immunotherapy may also have been required for significant decreases in mast cell or eosinophil numbers to have been demonstrated. The mitigation of allergy symptoms in the presence of unchanged inflammatory cell numbers, however, suggests that immunotherapy may have acted to inhibit cellular release of some inflammatory mediators.

In contrast to the lack of influence on T_{helper} cell cytokine production, we did demonstrate an influence of nasal immunotherapy on production of the anti-inflammatory cytokine IL-10 in the actively treated group at the peak

of the season. Interleukin-10 is primarily produced by mononuclear phagocytes and has a broad spectrum of anti-inflammatory activities including inhibition of IFN- γ secretion by T_{H1} lymphocytes,^{17,18} IL-4 and IL-5 secretion by T_{H2} lymphocytes,¹⁹ and in an autocrine fashion inhibition of IL-1, IL-6, TNF, and IL-12 production by mononuclear phagocytes.²⁰⁻²³ The most important anti-inflammatory function of IL-10 may be its ability to inhibit antigen-processing by mononuclear phagocytic cells by decreasing expression of cell surface proteins such as MHC class II, CD23, and B7.1/B7.2.^{24,25} Inhibition of B7 expression by IL-10 is responsible for its ability to induce tolerance in responding T_{helper} lymphocytes. The prevention and mitigation of allergic inflammation by IL-10 is supported by its ability to inhibit IgE production,²⁶ shorten eosinophil survival,²⁷ and induce allergen-specific tolerance.^{25,28,29} Previous studies have demonstrated decreased IL-10 production in allergic inflammation secondary to decreased transcription.³⁰ The induction of IL-10 by nasal immunotherapy may therefore have a dampening effect on allergic inflammation and supports our hypothesis that nasal immunotherapy may lead to the development of immune nonresponsiveness towards allergens. This increase in IL-10 correlated with induction of allergen-specific IgG, consistent with the role of this cytokine as the IgG isotype switch factor.³¹

In conclusion, this study demonstrates that local nasal immunotherapy is safe and effective in reducing the nasal and ocular symptoms of seasonal allergic rhinitis. Actively treated patients did experience increased symptoms and requirement for medication during the preseasonal buildup, however other studies suggest that these preseasonal symptoms may be avoided by a slower dose escalation.^{4,6}

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