

Clinical aspects of allergic disease

A double-blind study of the discontinuation of ragweed immunotherapy

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Background: Immunotherapy effectively treats the symptoms of allergic rhinitis and improves its pathophysiology. We studied whether the effects of immunotherapy on the early response to nasal challenge with antigen and seasonal symptoms persist after discontinuation.

Methods: Twenty subjects with ragweed allergy who were receiving immunotherapy and who had nasal challenges performed before initiation of treatment were selected. The patients had been receiving maintenance therapy with aqueous ragweed extract at a dose of 12 µg of Amb a 1 equivalent for a minimum of 3 years, at which point they were randomized to receive either placebo injections or to continue with the maintenance dose. Nasal challenges were performed before and 1 year after randomization. Nasal challenges were monitored by counting the number of sneezes and measuring histamine, N-α-tosyl-L-arginine methyl ester-esterase activity, and kinins in recovered nasal lavages. In the same year symptom diaries were collected during the ragweed season. **Results:** The initial immunotherapy significantly reduced responses to nasal challenge in both groups. The group continuing to receive active treatment showed no significant changes from the response before randomization. In contrast, the group randomized to placebo treatment showed a partial return of histamine, kinins, and N-α-tosyl-L-arginine methyl ester-esterase in nasal secretions and the numbers of sneezes. IgG antibodies to ragweed declined only in the group switched to placebo treatment. Seasonal rises of IgE antibodies to ragweed did not return during the first season after treatment was stopped. Symptoms reported during the ragweed season were not different between the groups.

Conclusions: One year after discontinuation of ragweed immunotherapy, nasal challenges showed partial recrudescence of mediator responses even though reports during the season appeared to indicate continued suppression of symptoms. (J Allergy Clin Immunol 1997;100:293-300.)

Key words: Ragweed immunotherapy, discontinuation of immunotherapy, IgE antibodies, IgG antibodies, allergic rhinitis

Abbreviation used

TAME: N-α-tosyl-L-arginine methyl ester

Immunotherapy, like steroids, has effects on both early mediator release and late inflammatory responses to nasal provocation. Clinically, both treatments effectively control symptoms. The morbidity rate and rare deaths associated with immunotherapy, however, have raised questions about its role in the treatment of allergic rhinitis.¹ A major theoretic advantage of immunotherapy is its potential to alleviate more or less permanently the abnormal immunologic responses that mediate the disease.¹

Several immunologic changes appear in the peripheral blood and the nasal mucosa. In the serum, specific IgG antibody levels rise, but the degree of change shows only a weak association with clinical improvement.^{2,3} IgG₁ antibodies dominate in the early response to immunotherapy and disappear relatively slowly when immunotherapy is stopped.⁴ IgG₄, in contrast, appears relatively late in the course of immunotherapy and disappears relatively quickly when immunotherapy is discontinued.⁴ Specific IgE antibody levels rise initially and then fall with continued treatment.² Immunotherapy also blocks the seasonal increase in specific IgE antibodies after 1 year, but the seasonal increase returns at least partially 1 year after treatment is stopped.^{2,4} In nasal secretions, specific IgA and IgG antibodies increase.⁵ Changes in lymphocyte proliferative responses and in elaboration of cytokines have also been associated with successful therapy.^{6,7} The development of antigen-specific mononuclear cells and an inhibition of the production of a mononuclear cell-derived histamine-releasing factor have also been reported.⁸ In the skin 1 year of immunotherapy decreases the cellular influx of CD4⁺ cells and switches the cytokine profile to increased production of γ-interferon and IL-2.⁹ The initial response and the subsequent inflammatory events after nasal challenge with antigen are reduced.⁹⁻¹¹ The reduced early response to challenge correlates with the reduction of symptom medication scores during the pollen season.¹² Many findings after treatment with topical steroids¹³⁻¹⁵ parallel those observed after successful immunotherapy. We

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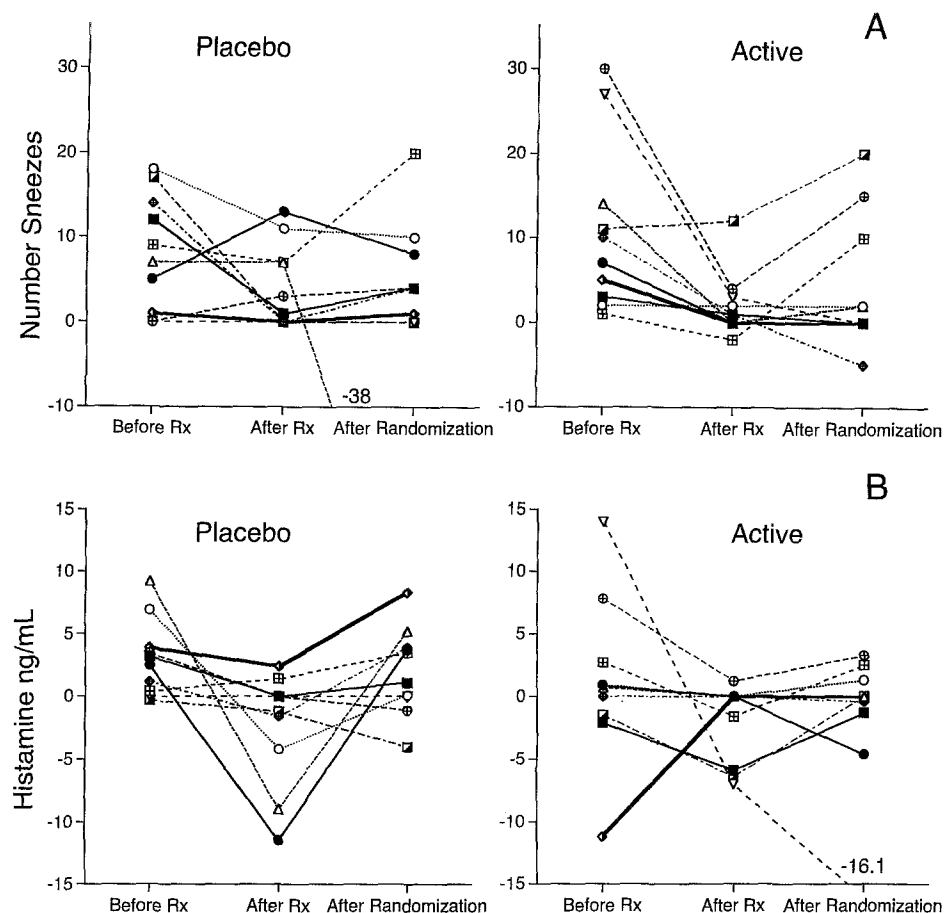


FIG. 1. Mediators of sneezes during nasal challenge with mediators or number of sneezes during preliminary saline lavages subtracted. **A**, Sneezes. **B**, Histamine. **C**, TAME-esterase. **D**, Kinin. Active refers to group that continued to receive immunotherapy, whereas placebo refers to group that was switched to placebo injections after 3 years of active therapy. Rx, Therapy.

believe that immunotherapy and topical steroids are similar in their efficacy for relieving nasal symptoms.¹⁶

If immunotherapy permanently alters the natural course of the disease, then no recrudescence of symptoms would be observed when therapy is discontinued after successful treatment. Such an alteration would have a profound effect on the long-term decision about how to manage allergic rhinitis. Clinical impressions suggest that successful treatment can be stopped after 3 to 5 years, but clinical studies addressing this question are few and the results are conflicting. Patterson et al.¹⁷ noted that when injections are stopped, patients may either have long-standing clinical improvement and experience minimal, pharmacologically manageable symptoms or reexperience severe symptoms, equivalent to those that prompted the initiation of therapy. Norman and Lichtenstein,¹⁸ in an uncontrolled study with symptom diaries as the outcome variable, found that the discontinuation of therapy in patients treated for 5 years caused a partial relapse in 1 year and, by 2 years, a return to previous symptoms. In an uncontrolled study by Mosbech and Østerballe,¹⁹ 2½ years of grass immunotherapy was still effective in controlling symptoms 6

years after treatment. A more recent controlled study by Norman et al.²⁰ showed the maintenance of clinical improvement after discontinuation of allergoid immunotherapy. These studies all involved pollen allergens. The results regarding the discontinuation of venom immunotherapy are more consistent. Golden et al.²¹ showed that 2 to 5 years after discontinuing venom immunotherapy, subjects had minimal, if any, reactions to induced stings. Other studies suggest that venom immunotherapy can be discontinued safely after 3 years.²²⁻²⁴ Another study convincingly suggests that venom immunotherapy altered the host response to insect stings and provided long-term clinical benefit.²⁵

Over the last decade, we have investigated the response to nasal challenge with ragweed antigen and the effects of immunotherapy on that response. Although immunotherapy had profound effects on many aspects of the nasal response, it consistently reduced the early reaction. Thus the early response to nasal challenge with ragweed provides one way to assess the effect of immunotherapy objectively.

We hypothesized that the inhibitory effects of ragweed immunotherapy on the response to nasal provocation

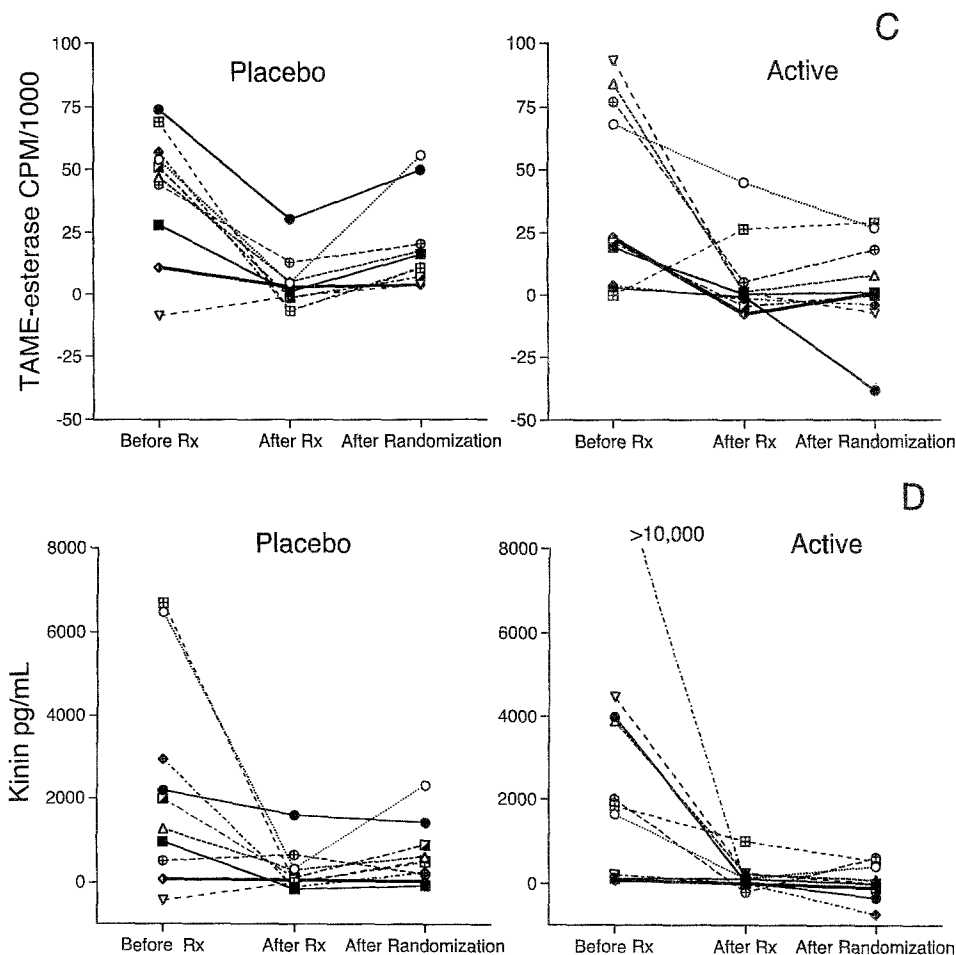


FIG. 1. Cont'd.

would be maintained when immunotherapy was discontinued after a minimum of 3 years at a high maintenance dose. We performed a double-blind, placebo-controlled, randomized study of ragweed immunotherapy to determine whether the inhibitory effects on the immediate response to antigen provocation persist after 1 year. Specific serum IgE and IgG antibodies to ragweed were also monitored. We simultaneously evaluated any change in symptoms reported during the ragweed season.

METHODS

Subjects

Volunteers were recruited through advertisements and from the allergy clinic. The subjects completed a questionnaire on nasal disease and atopic status. Nasal rhinoscopy was performed on each volunteer to rule out the presence of significant nasal septal deviation, other anatomic abnormalities, or pathologic conditions. We selected volunteers whose ages ranged between 18 and 55 years and who were in good health, other than having a history of allergic rhinitis or mild asthma. Seasonal ragweed allergic rhinitis was defined by a history of symptoms of rhinorrhea, nasal congestion, or sneezing during seasonal exposure. We confirmed the diagnosis by demonstrating a wheal and erythema response to an intradermal skin test

with ragweed extract. The study protocol was approved by the institutional review board, and participants signed approved consent forms.

Study plan

The patients had been receiving immunotherapy for ragweed-induced hay fever for 3 or more years in a study of the changes that occur in nasal challenge responses to ragweed extract provocation. They were asked to participate in a further study in which some would be switched to placebo treatment and others would continue to receive maintenance injections. After a fall ragweed season monitored by symptom diaries, the subjects had a control "after treatment" nasal challenge in December or early January. They were then randomized either to continue active treatment or to switch to placebo injections. These injections were continued through the following fall ragweed season during which symptom diaries were again used. Another "post randomization" nasal challenge was carried out in December. Blood samples for IgG and IgE antibodies to ragweed were taken before each season began in August and again after the season ended in October.

Nasal challenge

Standardized extracts and the diluent for the extract, phenol buffered saline, were purchased from Greer Laboratories (Lenoir, N.C.). The same extracts were used for immunotherapy

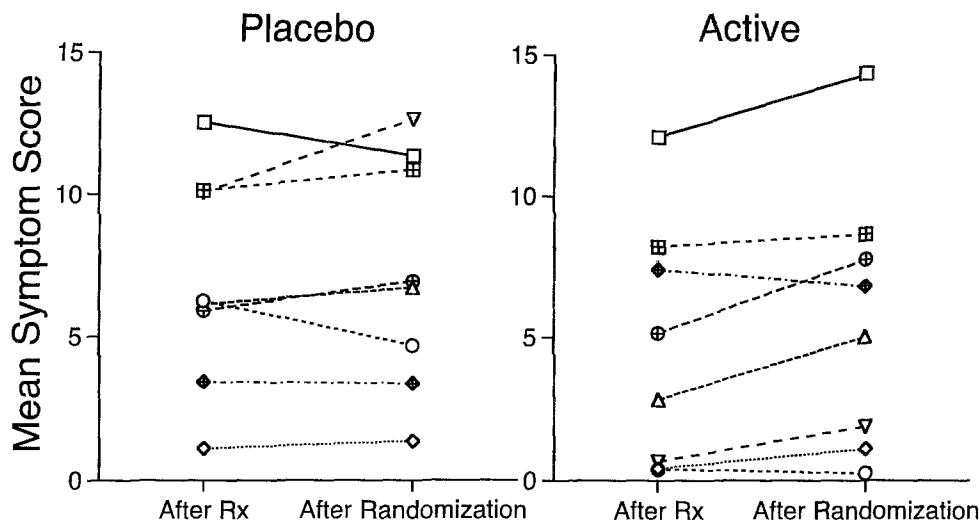


FIG. 2. Mean daily symptom score during ragweed season after 3 or more years of ragweed immunotherapy and 1 year after randomization to continue treatment or change to placebo (8 patients in each group). Rx, Therapy.

and for skin and nasal testing. The extracts were delivered by spraying 0.1 ml from a metered-dose bottle. Challenges were carried out according to a standard lavage protocol.²⁶ After a series of lavages to bring the concentration of nasal mediators to baseline, oxymetazoline hydrochloride was applied topically to the nasal mucosa to prevent mucosal congestion, which interferes with lavage. Prior experiments have shown that oxymetazoline does not interfere with sneezing or histamine release. A report by Watase et al.²⁷ also showed that α -adrenergic agents affect nasal congestion but do not affect sneezing or rhinorrhea after nasal provocation. Others have shown that oxymetazoline does not affect the changes in vascular permeability induced by histamine provocation.²⁸ Our data, obtained by using localized disk challenge, further support the absence of effect of oxymetazoline on mast cell activation.²⁹

After two control challenges with the diluent used for the allergen extract, increasing doses of antigen (1, 10, 100, and 1000 allergen units) were sprayed into the nose. Ten minutes after each administration of diluent or antigen, nasal lavage was performed.

Five milliliters of warmed (37° C) lactated Ringer's solution was instilled into each nostril, and after 10 seconds, expelled into a plastic collection vessel. The returned volume ranged between 75% and 85% of that instilled.²⁶ All samples were first vigorously shaken to homogenize the mixture of sol and gel phases and were stored on ice in plastic tubes until the conclusion of the experiment, when they were centrifuged at 3000 *g* for 15 minutes at 4° C. Aliquots (0.4 ml) for histamine analysis by the spectrofluorometric assay were mixed with 8% HClO₄ in a ratio of 4:1 and centrifuged at 1000 *g* for 10 minutes. Supernatants were stored at 4° C until assayed. Aliquots for N- α -tosyl-L-arginine methyl ester (TAME) were immediately stored at -20° C. Aliquots obtained for kinin determination were made 40 mmol/L in ethylenediaminetetraacetic acid and stored at -80° C until they were assayed. Assays for each mediator were performed within months of completing the challenge.

In lavages, because sensitivity is not the major problem, histamine was assayed by an automated fluorometric method³⁰ capable of detecting as little as 1 ng/ml. The assay has an

accuracy of $\pm 5\%$. The identity of histamine in nasal lavages was confirmed by showing linear addition and recovery of standard histamine and by verifying destruction by diamine oxidase. We have shown an excellent correlation between the histamine content of nasal lavages measured by the fluorometric and radioenzymatic assays ($r = 0.96$, $p < 0.001$).

The assay for measuring proteases with arginine esterase activity is essentially that of Imanari et al.,³¹ with tritiated TAME (Amersham). Proteases possessing arginine esterolytic activity hydrolyze the TAME, liberating tritiated methanol. In the immediate response to antigen, approximately 75% of the activity represents plasma kallikrein complexed to α_2 -macroglobulin, 20% mast cell tryptase, and less than 5% glandular kallikrein.^{32, 33}

The levels of kinins present in lavages were measured by using a radioimmunoassay sensitive to 20 pg of bradykinin per milliliter.³⁴ Interassay and intraassay coefficients of variation are 2% and 5%, respectively. The assay does not distinguish, on a molar basis, between bradykinin and lysyl bradykinin.

During the experiment, subjects recorded the number of sneezes. The prevalence of a positive response, with this challenge protocol, for subjects with a history of ragweed-induced hay fever and a positive intradermal skin test result is over 90%.³⁵

Other measurements

Serum ragweed-specific IgE and IgG antibodies were measured by the methods of Hamilton and Adkinson.^{36, 37}

Symptom-medication diaries were kept by subjects during the seasons preceding the nasal challenges according to the technique of Lichtenstein et al.³⁸

Immunotherapy injections

Twenty subjects who had received maintenance ragweed immunotherapy with approximately 12 μ g of Amb a 1 (5000 allergy units) for a minimum of 3 years were entered into the study. Maintenance injections were continued every 2 weeks at this dose. If a subject missed 6 weeks of therapy, he or she was dropped back one dilution. Placebo therapy consisted of injection of saline solution mixed with histamine and colored with

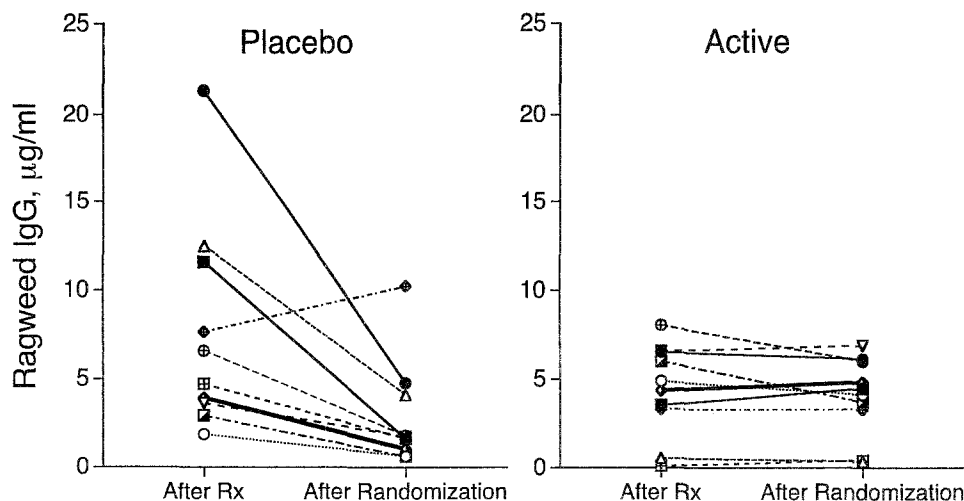


FIG. 3. Serum IgG antibodies to ragweed after immunotherapy and 1 year after randomization to continue treatment or change to placebo. Rx, Therapy.

caramel concentrate. The highest concentration of histamine was 100 µg/ml, and this solution was diluted in the same manner as the active solutions. Injections and modifications of immunotherapy and treatment of immunotherapy reactions were carried out in a double-blind fashion. Subjects stayed for 30 minutes after injection to be observed for systemic reactions. Physicians who assumed responsibility for immunotherapy were separate from those assuming responsibility for the nasal challenges and all other aspects of subject care.

Statistical methods

Nonparametric statistical methods were used to analyze results. For evaluation of the changes in both mediator levels and sneezes, differences from baseline were analyzed. The Wilcoxon matched-pairs signed-rank test was applied for paired data analysis, such as comparison of the mediators and symptom scores after antigen challenges in different years. Unpaired observations were made by using the Mann-Whitney U test. Statistical tests were performed with software packages for the Macintosh computer.

RESULTS

Twenty subjects (10 in each group) completed nasal challenges 1 year after being randomized. Nasal challenge responses when subjects were initially entered into the study (i.e., before ever receiving immunotherapy) showed no significant differences between the groups for any of the parameters evaluated. Similarly, posttreatment responses to nasal challenge with antigen were not different. The posttreatment response, however, for the 20 patients was significantly reduced compared with the initial response (Fig. 1). The median number of sneezes was reduced from 7 to 1 ($p = 0.005$), the median TAME value (counts per minute $\times 10^{-3}$) was reduced from 44 to 0.9 ($p = 0.0004$), the median histamine value (in nanograms per milliliter) was reduced from 0.9 to 0.0 ($p = 0.008$), and the median kinin value (in picograms per milliliter) was reduced from 1839 to 39 ($p = 0.0004$).

The group continuing to receive active treatment with ragweed showed no significant changes from the re-

sponses before randomization (Fig. 1). In contrast, the group changed to placebo treatment began to show partial return of responsiveness to nasal challenge. The net change for the response after antigen challenge between before and 1 year after placebo treatment was (median) for sneezes (2 vs 4, $p = 0.57$), TAME esterase activity (2.1 vs 13.5 cpm $\times 10^{-3}$, $p = 0.005$), histamine (-0.6 vs 2.3 ng/ml, $p = 0.04$), and kinins (82 vs 500 pg/ml; $p = 0.07$). The responses were, however, considerably less than those that occurred before initiating immunotherapy. The negative numbers in Fig. 1 result from either a high response to diluent (i.e., sneezes after diluent) or from postchallenge lavages continuing to wash out a mediator, which occurs frequently with histamine.

Symptom diaries the season before and the season after randomization were available from 16 of the patients (8 in each group). Comparison of the two groups showed no significant change after randomization in either group (Fig. 2) in contrast to the changes in response to nasal challenge. However, a power calculation for this observation indicated that for a 5% level of significance there was a greater than 90% chance that a significant difference could have been missed (a type II error).

IgG antibodies to ragweed had declined in all but one patient in the placebo group by the end of the first season after randomization (Fig. 3). On the other hand, there was no evidence of greater seasonal increases in IgE antibodies in the placebo group than in the active group (Fig. 4).

DISCUSSION

Our study confirms the considerable effect of ragweed immunotherapy on the immediate mediator response to nasal challenge, showing almost complete suppression of the response after 3 years at a maintenance dose. This effect was greater than the effect of only 1 year of

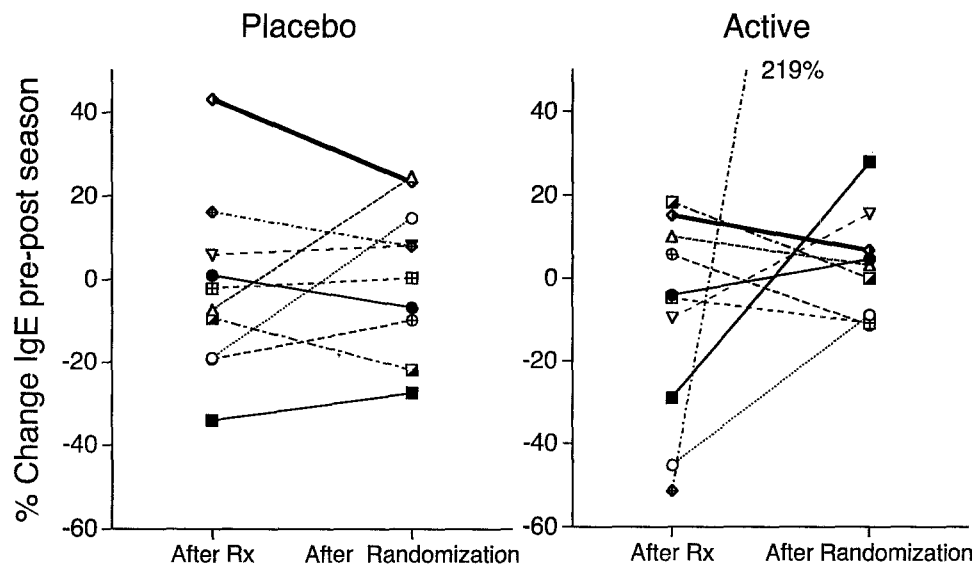


FIG. 4. Percent change in serum IgE antibodies to ragweed from before ragweed season (August) to after ragweed season (October). Rx, Therapy.

therapy at a lower treatment dose.¹⁰ (Many of the subjects in this study had participated in the earlier trial.) However, 1 year after discontinuing successful aqueous ragweed immunotherapy, the patients began to regain their responsiveness to nasal challenge.

We chose to monitor changes in mediator levels after challenge as a quantifiable index of the reaction. The nasal lavage model emphasizes such changes in levels of mediators rather than symptoms. Increases in histamine levels during the immediate response to antigen probably represent mast cell activation. TAME-esterase activity probably reflects several aspects of the allergic response (mast cell activation, plasma leakage, and glandular activation). Kinins are proinflammatory mediators with effects on blood vessels and nerves. An increase in kinins most likely reflects a decrease in vascular permeability caused by histamine or vasoactive mediators. The combined results with the three mediators provide strong evidence for some return of reactivity, although the kinin data alone do not reach statistical significance.

Symptoms reported during the season, on the other hand, were not apparently increased in the patients switched to placebo. The wide individual variation in the recording of symptoms, along with the small number of subjects, reduces the power to detect a difference. Hedlin et al.³⁹ have recently reported similar results in patients with dog and cat allergy 5 years after a 3-year course of immunotherapy had been discontinued. Bronchial reactivity to challenge with hair and dander extract indicated a return of PC₂₀ toward pretreatment levels, although asthmatic symptoms remained decreased.

Eventually, one might anticipate that symptoms would regress under such circumstances, although the data from venom immunotherapy suggest the duration of discontinuation does not affect the response to a

sting.^{21,22} If symptoms were not different and the early response to nasal challenge had continued to worsen, then the importance of the early reaction to the overall symptoms of allergic rhinitis would become downgraded.

Whether immunotherapy would continue to be effective on another aspect of the response to nasal challenge with antigen, such as the late reaction or the pattern of cellular infiltration, was not evaluated. Whether the response to nasal challenge with antigen would plateau at the current level, despite the further discontinuation of immunotherapy, could only be shown in subsequent follow-up. The skin testing data reported by Mosbech and Østerballe¹⁹ might suggest that a plateau is reached and that additional years without treatment do not lead to a return of reactivity comparable to that before the initiation of immunotherapy. Unfortunately, lack of funding precluded further study of our group of patients.

Our observations were made with ragweed immunotherapy. We suspect that our results would apply to other seasonal and perennial allergens. Why our results differ from those obtained with venom immunotherapy is not apparent. The route of antigen exposure in hay fever and venom anaphylaxis is, of course, different.

Our results can be compared with those previously reported. The statement by Patterson et al.¹⁶ that approximately one third of patients experience worsening of symptoms after discontinuing immunotherapy would not be consistent with the results of our objective assessment because a change in only one third of patients would not be detectable in our model with the size of the groups reported. The observation by Norman and Lichtenstein¹⁸ that diaries indicated some worsening of symptoms after 1 year and even more 2 years after therapy would be consistent with our nasal challenge data but not with our symptom data. In another study in

which allergoid was used, these investigators found that the effects on symptoms appeared to persist for 2 years after therapy and were comparable to those in subjects continuing with treatment.²⁰ Whether the different preparations of antigen have a profound effect on the immunologic response is an important issue. We studied treatment with aqueous extracts (most commonly used in the United States), whereas allergoid treatment is not available to physicians in the United States.

Our results appear to contrast most with those of Mosbech and Østerballe.¹⁹ They found that the effect persisted 6 years after 2½ years of therapy with either of two grass extracts. The two extracts differed in their initial efficacy, but both had persistent effects on symptoms and medication as recorded in diaries. The symptom-medication scores were corrected for pollen counts by a technique that assumes a linear relationship between pollen exposure and symptoms. Although the scores did not statistically worsen, there was a trend in that direction. The fact that both groups subjectively felt that their symptoms were declining raises the question of whether the natural history of grass-induced allergic rhinitis is for symptoms to regress with time. Levels of specific IgE antibodies, however, showed no significant decline. Immediate responses to skin testing became greater after discontinuation, even though they did not return to pretreatment values, a finding that is entirely consistent with our observations with nasal challenge.

The mechanism for the effectiveness of immunotherapy remains obscure, although many potential benefits have been described. Because we cannot explain why immunotherapy is effective, we likewise cannot determine which mechanisms are responsible for any reduction in efficacy after discontinuation. Studies of the discontinuation of venom immunotherapy show no relationship to specific IgG and IgE levels or the time from discontinuation of therapy. The disappearance of specific IgE antibodies and complete nonresponsiveness to skin testing have been considered good prognostic factors, although these events occur rarely. Also, some studies show a relationship between the severity of the initial reaction and the ability to discontinue therapy.^{21, 22}

Whether increasing the duration of active treatment from 3 to 5 or more years would have altered the results is unknown. Studies with venom indicate that 5 years of immunotherapy is better than 3, whereas the study of grass pollen by Mosbech and Østerballe¹⁹ suggests that either duration leads to persistent effects.

Our data make one hesitant to discontinue successful immunotherapy, a decision that would have a significant impact on the cost of health care. The impact would be greatest on the treatment of children and young adults, who could conceivably be committed to immunotherapy for decades. On the other hand, if immunotherapy cannot cure allergic rhinitis, then the decision to initiate immunotherapy may be weighed differently. Innovative study designs will be required to satisfactorily address the question of when to discontinue successful inhalant

immunotherapy because our approach would require years to duplicate.

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