ORIGINAL ARTICLE

Immunotherapy with a Ragweed–Toll-Like Receptor 9 Agonist Vaccine for Allergic Rhinitis

Peter S. Creticos, M.D., John T. Schroeder, Ph.D., Robert G. Hamilton, Ph.D., Susan L. Balcer-Whaley, M.P.H., Arouna P. Khattignavong, M.D., Robert Lindblad, M.D., Henry Li, M.D., Ph.D., Robert Coffman, Ph.D., Vicki Seyfert, Ph.D., Joseph J. Eiden, M.D., Ph.D., David Broide, M.B., Ch.B., and the Immune Tolerance Network Group

ABSTRACT

BACKGROUND

Conjugating immunostimulatory sequences of DNA to specific allergens offers a new approach to allergen immunotherapy that reduces acute allergic responses.

METHODS

We conducted a randomized, double-blind, placebo-controlled phase 2 trial of a vaccine consisting of Amb a 1, a ragweed-pollen antigen, conjugated to a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence of DNA (AIC) in 25 adults who were allergic to ragweed. Patients received six weekly injections of the AIC or placebo vaccine before the first ragweed season and were monitored during the next two ragweed seasons.

RESULTS

There was no pattern of vaccine-associated systemic reactions or clinically significant laboratory abnormalities. AIC did not alter the primary end point, the vascular permeability response (measured by the albumin level in nasal-lavage fluid) to nasal provocation. During the first ragweed season, the AIC group had better peak-season rhinitis scores on the visual-analogue scale (P=0.006), peak-season daily nasal symptom diary scores (P=0.02), and midseason overall quality-of-life scores (P=0.05) than the placebo group. AIC induced a transient increase in Amb a 1–specific IgG antibody but suppressed the seasonal increase in Amb a 1–specific IgE antibody. A reduction in the number of interleukin-4–positive basophils in AIC-treated patients correlated with lower rhinitis visual-analogue scores (r=0.49, P=0.03). Clinical benefits of AIC were again observed in the subsequent ragweed season, with improvements over placebo in peak-season rhinitis visual-analogue scores (P=0.02) and peak-season daily nasal symptom diary scores (P=0.02). The seasonal specific IgE antibody response was again suppressed, with no significant change in IgE antibody titer during the ragweed season (P=0.19).

CONCLUSIONS

In this pilot study, a 6-week regimen of the AIC vaccine appeared to offer long-term clinical efficacy in the treatment of ragweed allergic rhinitis. (ClinicalTrials.gov number, NCT00346086.)

From the Department of Medicine, Division of Allergy and Clinical Immunology, Johns Hopkins University School of Medicine, Baltimore (P.S.C., J.T.S., R.G.H., S.L.B.-W., A.P.K.); the Emmes Corporation, Rockville, MD (R.L.); the Institute for Asthma and Allergy, Wheaton, VA (H.L.); Dynavax Technologies, Berkeley, CA (R.C., J.J.E.); the Immune Tolerance Network, San Francisco (V.S.); and the Department of Medicine, Section of Allergy and Immunology, University of California, San Diego, San Diego (D.B.). Address reprint requests to Dr. Creticos at the Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Cir., Baltimore, MD 21224, or at pcretic@jhmi.edu.

N Engl J Med 2006;355:1445-55.

Standard allergen immunotherapy has been a cornerstone of the allergist's therapeutic options since its introduction in 1911.[1] However, this approach is limited by the potential for systemic allergic reactions, including anaphylaxis, caused by the relatively large doses of allergen required for efficacy.[2-4] Furthermore, standard allergen immunotherapy is logistically difficult to administer because it requires regular, frequent dosing, typically over a period of 3 to 5 years,[5,6] and thus frequently results in noncompliance.[7,8] Therefore, there is a need for new immunotherapeutic agents that have decreased risks of serious adverse events and involve shorter regimens that are more easily followed.

One possibility is an immunotherapeutic compound in which the ragweed-pollen antigen Amb a 1 is conjugated to a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence of DNA containing a CpG motif.[9] The immunostimulatory sequence binds to toll-like receptor 9 (TLR9), which is predominantly expressed in plasmacytoid dendritic cells, and this interaction is associated with the inhibition of immune responses mediated by type 2 helper T (Th2) cells.[9,10] In addition, when peripheral-blood mononuclear cells from patients who are allergic to ragweed are exposed to the Amb a 1–immunostimulatory oligodeoxyribonucleotide conjugate (AIC) in vitro, production of the Th2 cytokines interleukin-4 and interleukin-5 is decreased,[11] suggesting a potential therapeutic role for AIC in these patients. We conducted a small, placebo-controlled clinical trial in which patients who were allergic to ragweed were immunized with a six-injection regimen of the AIC vaccine before the first ragweed season and evaluated during the next two ragweed seasons.

METHODS

PATIENTS

Twenty-five patients who were 23 to 60 years of age and had a history of seasonal (fall) allergic rhinitis, a positive puncture skin test (as reflected by erythema [sum of the longest diameter and the orthogonal diameter intersecting the midpoint of the longest diameter] ≥30 mm) in response to a licensed, standardized ragweed extract (Greer Laboratories), and an immediate positive response to ragweed nasal challenge (three sneezes more than were elicited in response to the diluent nasal challenge and a doubling of the albumin level) were enrolled at the Johns Hopkins Asthma and Allergy Center. Further inclusion and exclusion criteria are given in the Supplementary Appendix, available with the full text of this article at www.nejm.org. The protocol was approved by the institutional review board of Johns Hopkins Bayview Campus, and all patients provided written informed consent.

STUDY DESIGN

The study was a randomized, double-blind, placebo-controlled, phase 2 clinical trial of AIC in patients with ragweed-induced seasonal allergic rhinitis. At enrollment, an attempt was made to balance the numbers of patients in the treatment groups according to their response to the ragweed puncture skin test, with eligible patients assigned to receive either AIC or placebo according to a random block design. Study personnel who were unaware of patients' treatment assignments administered a total of six injections at weekly intervals before the onset of the first ragweed season (2001). The planned sizes of the six doses were 0.06, 0.3, 1.2, 3.0, 6.0, and 12.0 μg of AIC; these doses were adjusted in the event of a local or systemic reaction to an injection or a missed injection. After undergoing a clinical and immunologic evaluation during the first ragweed season, the patients were asked to give written informed consent to continue in the study, and 17 of the 25 did so. No additional study injections were administered, and the patients were followed through the subsequent ragweed season (2002) and evaluated for serious adverse events, symptom scores, medication use, and immune responses.

STUDY MATERIALS

AIC was prepared by Primedica for Dynavax Technologies. The placebo was phosphate-buffered saline. The same lot of standardized extract of ragweed (*Ambrosia artemisiifolia*), containing approximately 300 μg of Amb a 1 per milliliter, was used throughout the study. Ragweed-pollen counts were measured in Baltimore with the use of a standard rotorod sampling device. The patients were given medication to be used for relief of moderate-to-severe nasal or eye symptoms that they were unwilling to tolerate (see the Supplementary Appendix). Dynavax Technologies sup-

plied the study medication and helped design the trial and reviewed the manuscript but did not collect or analyze the data or fund the trial.

CLINICAL AND MECHANISTIC ASSESSMENTS

The primary end point of the study was the effect of treatment on change from baseline in the albumin level in nasal-lavage fluid after nasal provocation and was based on previous observations that the level of albumin, a marker of vascular leakage and inflammation, is lowered by standard allergen immunotherapy.[12] Ragweed provocation procedures were conducted before the injections were begun and 2 weeks and 2 months after the injections were finished. After the challenge, the number of sneezes was counted and the degree of nasal congestion, rhinorrhea, postnasal drip and itchiness of the ears, nose, and throat were also scored on a scale of 0 (no symptoms) to 100 (the worst possible symptoms).

Other secondary clinical end points recorded during the ragweed season included the rhinitis visual-analogue score, which was self-reported and ranged from 0 mm (no symptoms) to 100 mm (the worst possible symptoms)[13]; the daily nasal symptom diary score, ranging from 0 (no symptoms) to 5 (very severe symptoms)[14]; the use of relief medication[14,15] (see the Supplementary Appendix for details on scoring); the score on the standardized Juniper rhinoconjunctivitis quality-of-life questionnaire, with scores ranging from 0 (best quality) to 6 (worst)[16]; adverse events; and skin-test sensitivity (see the Supplementary Appendix).

Serum Amb a 1–specific and ragweed-specific IgG and IgE levels were measured by enzyme immunoassays[17] at baseline, after treatment, before the first ragweed season (in 2001), 2 weeks and 2 months after the end of the first ragweed season, before the second ragweed season (in 2002), and at the end of the second ragweed season. To follow the antibody response to treatment, we used a functional antigen presentation (FAP) assay that measures the ability of postimmunotherapy serum to inhibit the binding of allergen–IgE complexes to B cells at the same time points.[18] At various times, the levels of interleukin-4 in T cells and basophils and the intracellular levels of interferon-γ and interleukin-10 as well as the secreted levels were measured to assess immune responses.[19,20] Similarly, peripheral-blood mononuclear cells were analyzed by TaqMan (Applied Biosystems) reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay for immune response genes.[21] Standard safety blood assays were performed at screening (or baseline) and during treatment (see the Supplementary Appendix).

STATISTICAL ANALYSIS

Peak ragweed season was defined as the period starting when ragweed pollen counts were at least 11 grains per cubic meter on 2 consecutive days and ending on the last day before ragweed pollen counts were less than 11 grains per cubic meter for 2 consecutive days (that were not followed by 2 days of higher pollen counts). Differences between the groups in post-treatment albumin levels in nasal-lavage fluid, the primary end point, were assessed on an intention-to-treat basis (see the Supplementary Appendix) by regression modeling adjusted for baseline values, with a P value of less than 0.05 for the primary outcome and less than 0.01 for all secondary outcomes used to indicate statistical significance; the P values were not adjusted for multiple comparisons. Descriptive statistics for daily nasal symptom diary scores and seasonal rhinitis visual-analogue scores are based on the patients' median scores during the specified seasonal periods (preseason and peak season).

Repeated-measures analysis was used to examine differences between the AIC and the placebo groups in symptoms, medication use, and scores on the rhinoconjunctivitis quality-of-life questionnaire (2002 results) and was used to test for main effects of treatment group, time, and interactions between treatment and time. An analysis-of-covariance model that included the preseason score as a covariate was also used to analyze midseason scores on the rhinoconjunctivitis quality-of-life questionnaire (2001 results). The Wilcoxon rank-sum test was used to compare median scores at given times for serum immune responses, some cellular immune responses, and nasal challenge outcomes, including both the raw scores and the difference from baseline. The Wilcoxon signed-rank test was used for within-group comparisons of serum immune responses. The chi-square test was used to analyze basophil results. Analyses were performed by the Emmes Corporation, Johns Hopkins University, and the Immune Tolerance Network. Additional information is provided in the Supplementary Appendix.

RESULTS

PATIENTS

Twenty-five patients who were allergic to ragweed were enrolled in the trial in May and June of 2001 (Fig. 1). At the time of randomization, there were no significant differences between the study groups in age or in sensitivity to ragweed, as determined by the puncture skin test (Table 1). Of the 14 patients assigned to AIC treatment, 1 voluntarily withdrew after a serious adverse event related to previous surgery and unrelated to treatment, 1 relocated, 1 was lost to follow-up, and 1 withdrew because of a work commitment before beginning immunotherapy. Of the 11 patients assigned to placebo, 2 dropped out before the first ragweed season: 1 because of noncompliance with the study protocol and 1 because of scheduling conflicts. Of the 19 patients in the study at the end of the first ragweed season, 17 consented to continue in the study, and 15 of these patients (9 assigned to placebo and 6 to AIC) remained in the trial through the end of the second ragweed season. Despite dropouts, there continued to be no significant differences in demographic characteristics between the AIC and placebo groups (see Table 2 in the Supplementary Appendix).

NASAL ALLERGEN CHALLENGE

AIC failed to affect the primary end point of the study, the increase in albumin levels in nasal secretions after post-treatment ragweed challenge ($P=0.47$). Furthermore, such increases were not diminished by AIC in the two postseason challenges, nor were there post-treatment differences in histamine levels in nasal-lavage fluids.

Several clinical end points of nasal allergen challenge were improved at the post-therapy challenge. The median number of sneezes increased from baseline by 3 in the placebo group and decreased from baseline by 1.5 in the AIC group ($P=0.03$ for the difference between the groups

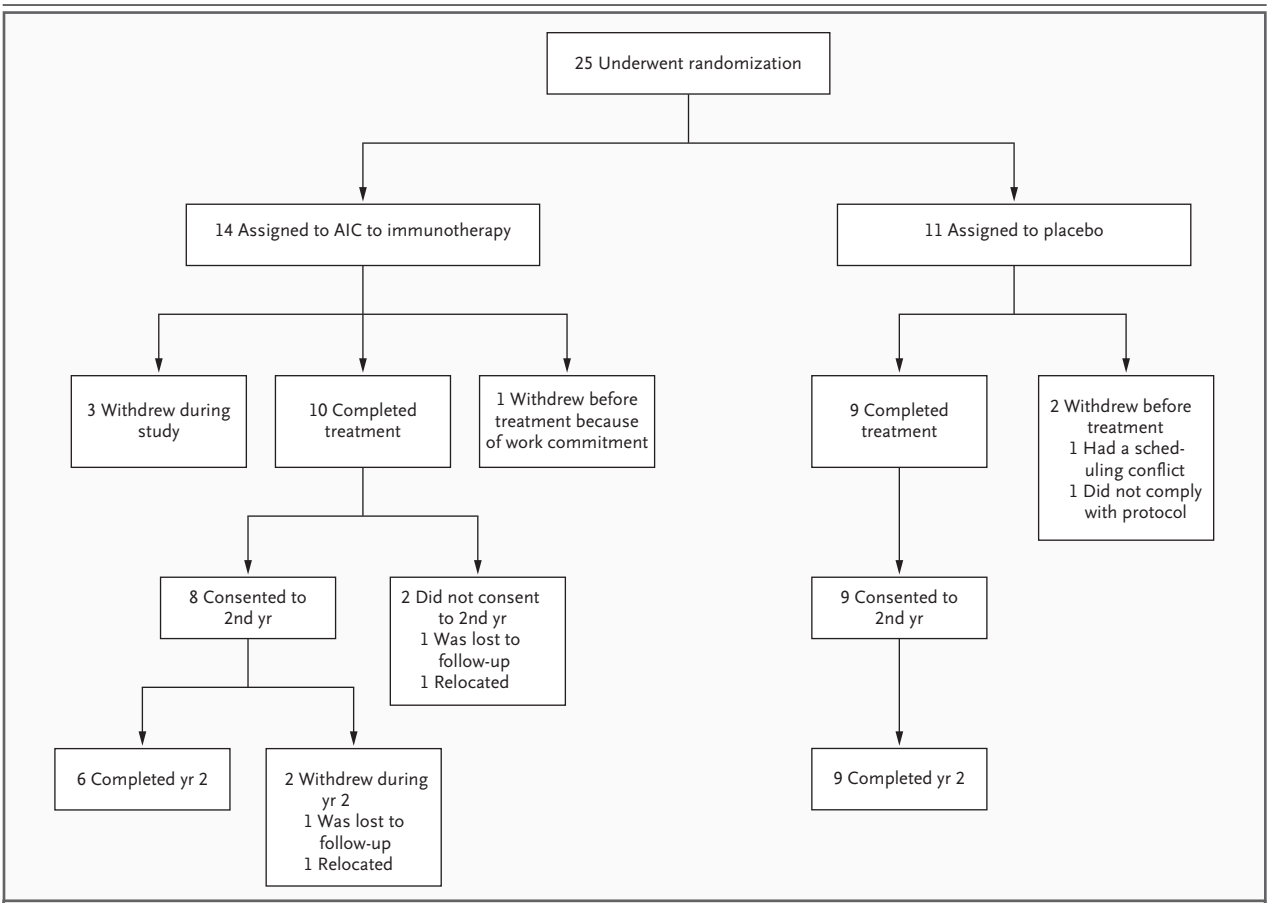

Figure 1. Disposition of Study Patients in Years 1 and 2.

in the change from baseline). As compared with the placebo group, the AIC group also had improvements in the total post-challenge symptom score, including the sneeze count (median, 37 vs. 70; P=0.03); the total post-challenge nasal symptom score, excluding the sneeze count (median, 33 vs. 60; P=0.02); and the postnasal drip score (median, 24 vs. 57; P=0.007).

CLINICAL OUTCOMES DURING THE FIRST RAGWEED SEASON (2001)

Rhinitis symptoms, as measured by the visual-analogue scale, were significantly improved in the AIC group as compared with the placebo group (Fig. 2A). The mean peak-season rhinitis visual-analogue score in the AIC group was one third of that in the placebo group (13.2 vs. 40.8, P=0.006 by repeated-measures analysis of the interaction between treatment and time). The difference in least-squares means on the visual-analogue scale was significant for the third week of the peak season (P=0.007), with treatment differences for the second and fourth weeks of the peak season (P=0.02 and P=0.04, respectively). When the data for the full ragweed season were analyzed, a similar pattern was observed: the mean score was 13.8 in the AIC group as compared with 35.1 in the placebo group (P=0.01 for the interaction between treatment and time).

The peak-season daily nasal symptom diary scores were also lower in the AIC group than in the placebo group (mean, 1.8 vs. 4.0; P=0.02 by repeated-measures analysis), with a similar treatment effect observed for the full season (P=0.03 by repeated-measures analysis) (Fig. 2B). In addition, the mean (weighted) use during the peak season was lower in the AIC group (median, 0.1 vs. 0.6) (see the Supplementary Appendix). At midseason, the total score on the rhinoconjunctivitis quality-of-life questionnaire (adjusted for baseline) was lower in the AIC group than in the placebo group (raw mean, 0.5 vs. 1.6; P=0.05 by analysis of covariance), as were the subscores for eye symptoms (0.5 vs. 1.8, P=0.03) and sleep (0.3 vs. 2.1, P=0.04).

Table 1. Demographic Characteristics of the Patients.*

Characteristic	AIC (N=14)	Placebo (N=11)
Male sex — no. (%)	6 (43)	4 (36)
Race or ethnic group — no. (%)†		
White	9 (64)	7 (64)
Black	4 (29)	4 (36)
Mixed	1 (7)	0
Age — yr		
Mean	37.6±2.7	41.7±2.6
Median	38	39
Range	23–58	33–60
No. of years with seasonal rhinitis	26±4	33±3
Ragweed IgE — U/ml		
Mean	12.0±2.8	9.8±3.5
Median	9.0	7.0
Range	0.2–30.1	0.4–33.9
Puncture skin test for ragweed sensitivity — mm of erythema‡	72	72
Sensitivity to other allergens — no. (%)		
Grass pollen	11 (79)	11 (100)
Tree pollen	11 (79)	11 (100)
Dust mites	10 (71)	6 (55)
Mold	10 (71)	6 (55)
Cat dander	9 (64)	7 (64)
Dog dander	6 (43)	4 (36)
Cockroaches	1 (7)	0
Coexisting conditions — no. (%)		
Asthma	4 (29)	2 (18)
Conjunctivitis	13 (93)	11 (100)
Sinusitis	2 (14)	4 (36)
Eczema	3 (21)	1 (9)

* Plus–minus values are means ±SE.
† Race or ethnic group was self-reported.
‡ Millimeters of erythema was defined as the sum of the longest diameter and the orthogonal diameter intersecting the midpoint of the longest diameter.

CLINICAL OUTCOMES DURING THE SECOND RAGWEED SEASON (2002)

Although the patients in the AIC group received no further treatment after 2001, the improvements in clinical outcome measures in this group as compared with the placebo group were maintained through the subsequent 2002 ragweed season. During the peak ragweed season of 2002, the mean rhinitis visual-analogue score in the AIC group was approximately one third of that in the placebo group (13.9 vs. 49.4, P=0.02 by repeated-measures analysis) (Fig. 3A). During 6 of the 8 weeks of the full ragweed season in 2002, the weekly mean rhinitis visual-analogue scores reported by AIC-treated patients were lower than the mean score for the baseline week.

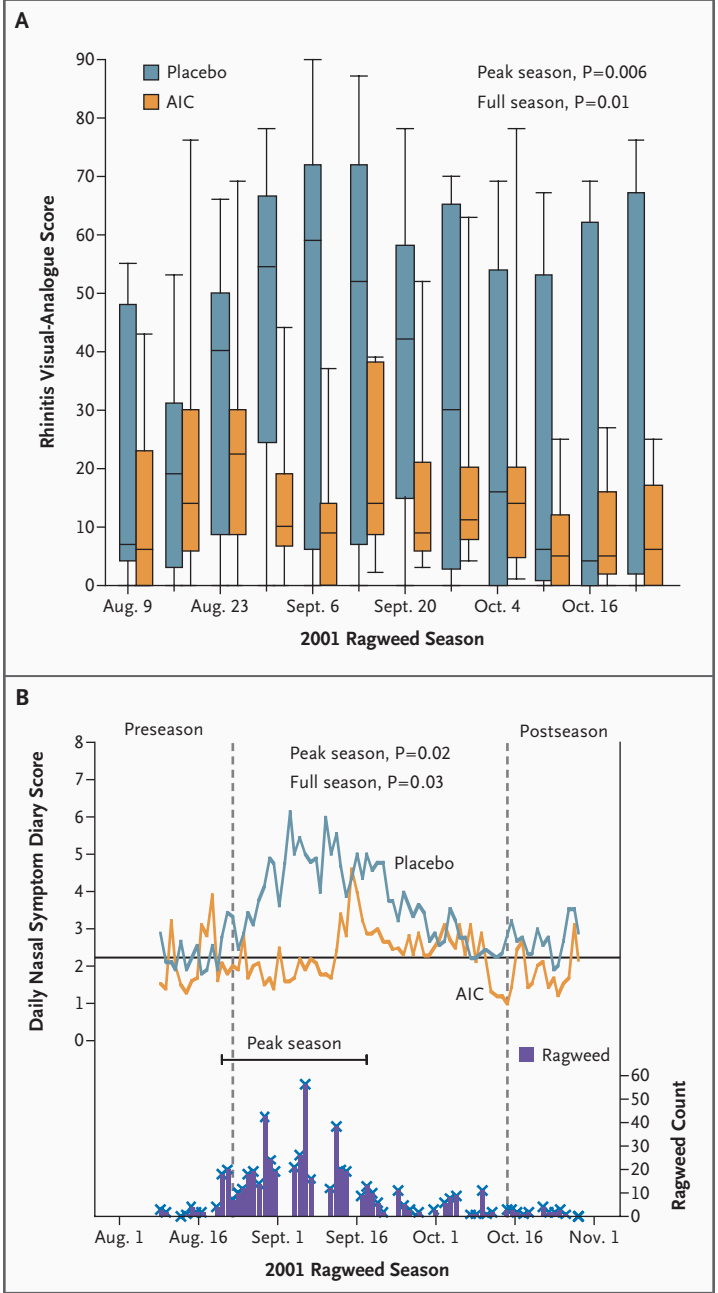

Figure 2. Results for the First Ragweed Season (2001).

Panel A shows box plots of self-reported overall rhinitis visual-analogue scores according to treatment group and week. Scores can range from 0 to 100, with higher scores indicating more severe symptoms. Box plot "whiskers" show the minimum and maximum values; the horizontal line in each box plot shows the median, and the colored segment shows the interquartile range. Dates refer to the actual start of each week. P values are for the interaction between treatment and time. Panel B shows the mean daily nasal symptom diary score according to treatment group and day. Scores can range from 0 to 20, with higher scores indicating more symptoms. The broken vertical lines indicate the beginning and end of the full season. The long horizontal line indicates the mean preseason nasal symptom diary score. P values are for the treatment effect.

In 2002, the mean nasal symptom diary score during the peak season was reduced by 53% in the AIC group, as compared with the placebo group (2.8 vs. 5.9, P=0.02 by repeated-measures analysis), a result similar to that observed in 2001. The nasal symptom diary scores for the full season showed a similar pattern, but the difference was not significant (P=0.06 by repeated-measures analysis) (Fig. 3B). As compared with placebo, AIC treatment also lowered 2002 peak-season scores for the use of relief medication (median, 0 vs. 0.6; P=0.08 by repeated-measures analysis), and resulted in fewer days of antihistamine use (median, 0 vs. 8 days) and decongestant use (median, 0 vs. 4 days). The rhinoconjunctivitis quality-of-life questionnaire scores were improved in the AIC group in 2002, with a treatment effect in the activity subscore (P=0.04 by repeated-measures analysis), the nasal subscore (P=0.03 by repeated-measures analysis), the trends-in-practical-problems subscore (P=0.06 by repeated-measures analysis), and the total score (P=0.06 by repeated-measures analysis).

SAFETY

There was no pattern of vaccine-associated systemic adverse reactions or clinically significant laboratory abnormalities. No new formation or increase in the titers of antinuclear, anti–double-stranded DNA or anti–single-stranded DNA was observed. Patients in both groups reported local reactions to injections: erythema ranging from 0 to 75 mm in diameter and wheals from 0 to 44 mm in diameter in the AIC group, and erythema from 0 to 27 mm in diameter and wheals from 0 to 6 mm in diameter in the placebo group. Erythema or wheals occurred in response to AIC in 45 of 71 injections (63%). All local reactions were self-limited, and none required medication or a change in treatment dose. One AIC-treated patient had severe itching at the injection site. One patient in the placebo group had systemic symptoms (rhinitis) after an injection.

Overall, 135 adverse events were observed in 23 patients during the first year: 73 (54%) in the AIC group and 62 (46%) in the placebo group. Of these, 112 (83%) were mild or moderate and 23 (17%) were severe; 12 of the severe adverse events occurred in the AIC group, and 11 in the placebo group. Ten of the 135 adverse events (5 in each group) were classified as possibly related to study medication. Two serious adverse events were reported in the AIC group during the first year:

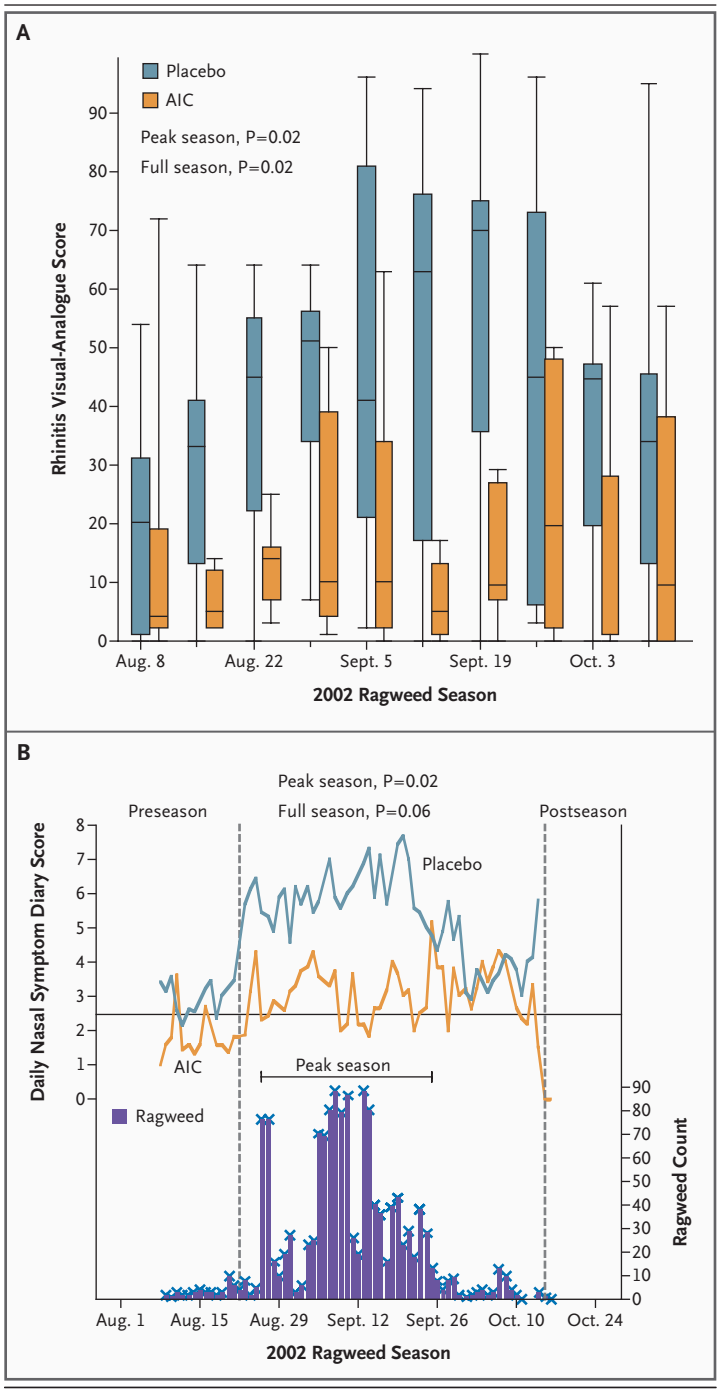

Figure 3. Results for the Second Ragweed Season (2002).

Panel A shows box plots of self-reported overall rhinitis visual-analogue scores according to treatment group and week. Scores can range from 0 to 100, with higher scores indicating more severe symptoms. Box plot "whiskers" show the minimum and maximum values; the horizontal line in each box plot shows the median, and the colored segment shows the interquartile range. Dates refer to the actual start of each week. P values are for the interaction between treatment and time. Panel B shows the mean daily nasal symptom diary score according to treatment group and day. Scores can range from 0 to 20, with higher scores indicating more severe symptoms. The broken vertical lines indicate the beginning and end of the full season. The long horizontal line indicates the mean preseason nasal symptom diary score. P values are for the treatment effect.

a postsurgical abdominal skin infection occurred in one patient, who was withdrawn from the study, and another patient had a right ovarian cyst removed. Both of these events were considered to be unrelated to treatment. One serious adverse event was reported in the placebo group during the second year. The dose was modified or temporarily stopped in two placebo recipients because of severe allergic rhinitis and fatigue in one patient and in the other, two episodes of mild upper respiratory tract infection: one with asthmatic bronchitis and one with postnasal drip and cough. The dose was modified in two AIC recipients because of a mild, influenza-like syndrome in one and a moderate upper respiratory tract infection or allergic rhinitis with asthma flare in the other.

ANTIBODY TITERS

Standard allergen immunotherapy results in a significant increase in IgG antibody against an immunized allergen.[22,23] The patients were immunized against the major protein moiety of ragweed (Amb a 1). AIC induced a transient increase in Amb a 1–specific and ragweed-specific IgG. No post-treatment increases in Amb a 1–specific or ragweed-specific IgG titers were observed in the placebo group. Furthermore, the FAP assay showed no evidence that IgG in serum from AIC-treated patients inhibited the binding of the ragweed allergen–IgE complex to B cells (see the Supplementary Appendix).

AIC induced a rise in the mean (±SE) level of Amb a 1–specific IgE antibody from a baseline value of 94.3±34.3 U per milliliter to 162.8±70.1 U per milliliter after treatment; the median difference was 10.3 U per milliliter (P=0.008). The post-treatment titers in the placebo group were unchanged. AIC treatment suppressed the increase in Amb a 1–specific IgE that usually is observed during the ragweed allergy season: the mean IgE levels were 180.0±90.2 U per milliliter before the 2001 ragweed season and 97.0±37.0 U per milliliter 2 months after the season.[24,25] Patients in the placebo group had a typical seasonal rise in Amb a 1–specific IgE, from 60.4±22.0 U per mil-

liliter before the 2001 ragweed season to 105.7±38.5 U per milliliter 2 weeks after the season, with a median difference of 18.0 U per milliliter (P=0.008) (Fig. 4). Similar results were seen in 2002 during the second season, with a rise in the placebo group from 66.8±25.8 U per milliliter before the season to 87.3±28.0 U per milliliter after the season (P=0.02), but no such rise in the AIC group (82.1±35.2 and 52.4±26.9 U per milliliter before and after the season, respectively; P=0.19) (Fig. 4). Measurements of whole ragweed-specific IgE antibodies in the placebo group produced similar results (data not shown).

SKIN-TEST SENSITIVITY

Suppression of the immediate skin-test responses (at 15 minutes) and the late-phase responses (at 24 hours), measured as a decrease in either puncture or intradermal skin-test reactivity, also serves as a marker of successful standard allergen immunotherapy.[15,26-29] AIC decreased immediate intradermal skin-test reactivity: the mean decrease in wheal size after treatment with AIC was 8 mm, as compared with 3 mm with placebo (P=0.04 for the difference from baseline). The AIC group also had a larger reduction in immediate (puncture) and late-phase (intradermal) skin-test erythema (see the Supplementary Appendix).

CELLULAR IMMUNE RESPONSES

No clear correlations were detected between AIC treatment and intracellular levels of interleukin-4 or interferon-γ in activated CD4+CD69+ T cells, as measured by flow cytometry or an enzyme-linked immunosorbent assay. Interferon-γ was detected in the positive control samples (peripheral-blood mononuclear cells stimulated with tetanus toxoid) by both methods. Increased levels of interleukin-10 were spontaneously secreted by peripheral-blood mononuclear cells cultured in medium alone for 16 hours, but the levels did not differ significantly between cells from patients in the AIC group and cells from patients in the placebo group. This effect appeared to be driven by seasonal allergen exposure, since interleukin-10 secretion was most evident in peripheral-blood mononuclear cells

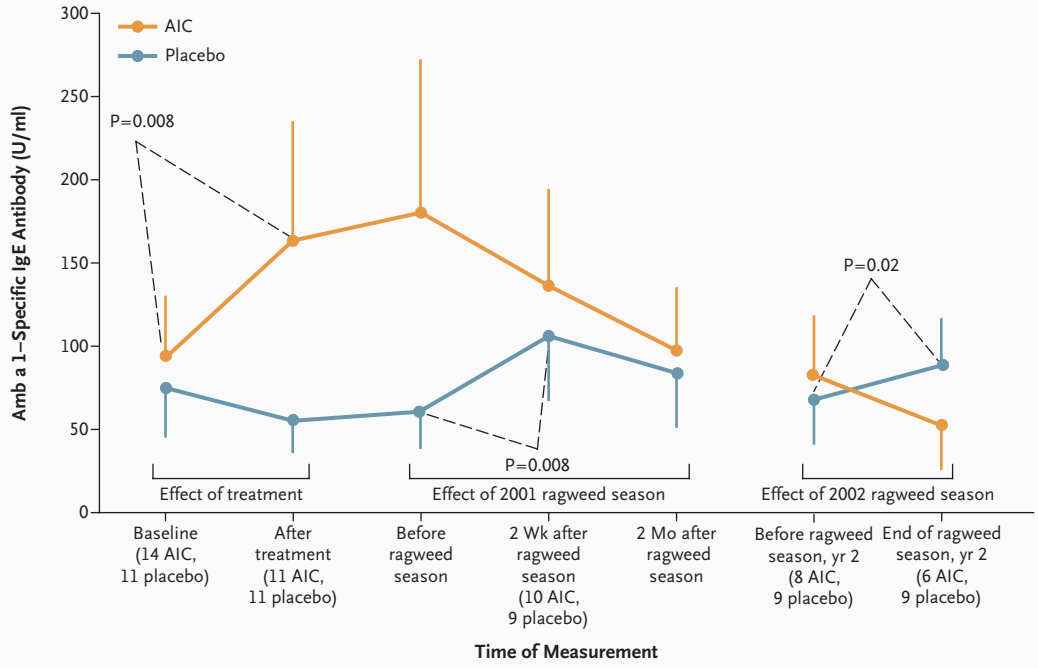

Figure 4. Effect of Treatment on Amb a 1–Specific IgE Antibody.

Antibody measurements were performed at defined times over the 2-year study period: 2 weeks (near November 1) and 2 months (near December 15) after the end of the 2001 ragweed season, and at the end of the 2002 ragweed season (near October 14). There was a significant rise in Amb a 1–specific IgE in the placebo group in each ragweed season. Although AIC induced a rise in the titer of Amb a 1–specific IgE, the increase was transient. AIC treatment blunted the usual seasonal rise in IgE during each ragweed season.

taken from patients in either group 2 weeks after the end of the ragweed season (see the Supplementary Appendix). Similarly, the levels of expression of 184 cytokine, chemokine, and immune-response genes analyzed by a TaqMan RT-PCR assay showed no consistent differences before and after treatment (see the Supplementary Appendix).

Two weeks after the end of the 2001 ragweed season, the placebo group had a mean increase from baseline in the frequency of interleukin-4–positive basophils by a factor of 5.0±2.7 (P=0.02) (see the Supplementary Appendix). In contrast, this effect was not observed in half the AIC-treated patients. This difference between the two groups was not significant (P=0.06). However, there was a correlation between diminished frequencies of interleukin-4–positive basophils and lower rhinitis visual-analogue scores for the two groups (r=0.49, P=0.03).

DISCUSSION

Although we did not demonstrate that AIC affected the primary end point (nasal vascular permeability as assessed by nasal-lavage albumin), we did demonstrate that a once-weekly, six-injection regimen of AIC substantially reduced allergic rhinitis symptoms during the ragweed season. Furthermore, the protection afforded by the administration of AIC before the first ragweed season was sustained during the second ragweed season. In contrast, several studies of standard allergen immunotherapy (consisting of 14 to 27 injections administered before the beginning of the season) only showed an improvement of 35 to 40% in ragweed seasonal symptom scores,[30,31] with no lasting benefit,[5,32,33] and a long-lasting benefit persisting after the discontinuation of standard allergen immunotherapy has been shown only after 3 or 4 years of treatment.[34]

In our study, no serious adverse events attributable to AIC occurred, no injections were discontinued because of local reactions, and dose adjustments were similar in frequency and type in the AIC and placebo groups. In contrast, systemic reactions occur in 1 to 20% of patients receiving standard allergen immunotherapy[2-4] and in up to 73% of patients receiving "cluster" or "rush" immunotherapy.[35,36] In standard allergen immunotherapy, a slow buildup (two injections per week for 10 to 12 weeks) is generally used to minimize the risk of allergic reactions to injections.[5,37] AIC may therefore also offer a safer route of allergen administration that does not sacrifice efficacy.

The limitations of our study include the small number of patients enrolled, the lack of effect of AIC on the primary end point, and the unknown long-term safety of the treatment. Hence, additional clinical trials with longer-term follow-up are needed to assess the safety and clinical effectiveness of AIC.

The mechanism by which AIC induces clinical tolerance of an inhaled allergen is incompletely understood. Like standard allergen immunotherapy, AIC blunts the seasonal rise in ragweed-specific and Amb a 1–specific IgE that is generally observed in persons with atopy.[23,25,38] Our finding that the six-injection regimen suppressed the rise in ragweed-specific IgE during the subsequent allergy season provides evidence that this regimen had immunomodulatory effects associated with a clinical benefit lasting through two ragweed seasons. The data are consistent with our observation that AIC inhibits the cutaneous response to challenge with ragweed, a defined hallmark of successful immunotherapy.[15,26,29] In contrast to standard allergen immunotherapy, successful vaccination with AIC appears to be independent of the formation of allergen-specific IgG, since AIC induced only a moderate and transient rise in Amb a 1–specific and ragweed-specific IgG antibody and did not inhibit allergen–IgE complexes from binding to B cells.[5,13,18,39]

In conclusion, this study provides preliminary evidence that a six-injection regimen of AIC reduces allergic rhinitis symptoms during the ragweed season. Furthermore, the clinical effects of AIC appear to be associated with the induction of long-lasting immune modulation. Although the mechanisms underlying the clinical benefit require further investigation, AIC vaccine has properties that make it qualitatively superior to standard allergen immunotherapy. Large-scale phase 3 studies will be needed to determine the role of AIC as a therapeutic option in ragweed-induced allergic disease.

Supported by a subcontract with the Immune Tolerance Network, a project of the National Institute of Allergy and Infectious Diseases, the National Institute of Diabetes and Digestive and Kidney Diseases, and the Juvenile Diabetes Research Foundation.

Dr. Creticos reports having received grant support from the National Institutes of Health Immune Tolerance Network, Dynavax Technologies, Curalogic, and Genentech–Novartis and lecture fees from Genentech; having served on ad hoc scientific advisory boards for Dynavax Technologies, Genentech, and

Greer Laboratories; and having served as a consultant to Curalogic. Dr. Coffman reports being an employee of Dynavax Technologies, owning equity and stock options in the company, and receiving consulting fees from the National Institute of Allergy and Infectious Diseases Board of Scientific Counselors. Dr. Eiden, a former employee of Dynavax Technologies, reports owning equity and stock options in the company. Dr. Broide reports having received grant support from the National Institutes of Health Immune Tolerance Network and royalties from the University of California Office of Technology Transfer for being a coinventor of methods and devices for immunizing a host. No other potential conflict of interest relevant to this article was reported.

We are indebted to Jeffrey Matthews, Ph.D., Larry M. Lichtenstein, M.D., Ph.D., Eyal Raz, M.D., and Philip S. Norman, M.D., for their critical review of the manuscript; to Anja Bieneman, B.S., and Kristin Chichester, M.S., for technical assay support; to James Francis, M.D., and Stephen Durham, M.D., for FAP assay measurements; to Beth Blackwell, Sc.D., for statistical guidance; and to Sharon Ritter for assistance in manuscript preparation.

REFERENCES

1. Noon L. Prophylactic inoculation against hay fever. Lancet 1911;1:1572-3.
2. Lockey RF, Benedict LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy (IT) and skin testing (ST). J Allergy Clin Immunol 1987;79:660-77.
3. Du Buske LM, Ling CJ, Sheffer AL. Special problems regarding allergen immunotherapy. Immunol Allergy Clin North Am 1992;12:145-75.
4. Bukantz SC, Lockey RF. Adverse effects and fatalities associated with subcutaneous allergen immunotherapy. Clin Allergy Immunol 2004;18:711-27.
5. Creticos PS. Immunotherapy with allergens. JAMA 1992;268:2834-9.
6. Walker SM, Varney VA, Gaga M, Jacobson MR, Durham SR. Grass pollen immunotherapy: efficacy and safety during a 4-year follow-up study. Allergy 1995;50:405-13.
7. Lower T, Henry J, Mandik L, Janosky J, Friday GA Jr. Compliance with allergen immunotherapy. Ann Allergy 1993;70:480-2.
8. Tinkelman D, Smith F, Cole WQ III, Silk HJ. Compliance with an allergen immunotherapy regime. Ann Allergy Asthma Immunol 1995;74:241-6.
9. Tighe H, Takabayashi K, Schwartz D, et al. Conjugation of immunostimulatory DNA to the short ragweed allergen Amb a 1 enhances its immunogenicity and reduces its allergenicity. J Allergy Clin Immunol 2000;106:124-34.
10. Tighe H, Takabayashi K, Schwartz D, et al. Conjugation of protein to immunostimulatory DNA results in a rapid, long-lasting and potent induction of cell-mediated and humoral immunity. Eur J Immunol 2000;30:1939-47.
11. Marshall JD, Abtahi S, Eiden JJ, et al. Immunostimulatory sequence DNA linked to the Amb a 1 allergen promotes T(H)1 cytokine expression while downregulating T(H)2 cytokine expression in PBMCs from human patients with ragweed allergy. J Allergy Clin Immunol 2001;108:191-7.
12. Baumgarten CR, Togias AG, Naclerio RM, Lichtenstein LM, Norman PS, Proud D. Influx of kininogens into nasal secretions after antigen challenge of allergic individuals. J Clin Invest 1985;76:191-7.
13. Eng PA, Reinhold M, Gnehm HP. Long-term efficacy of preseasonal grass pollen immunotherapy in children. Allergy 2002;57:306-12.
14. Norman PS, Ohman JL Jr, Long AA, et al. Treatment of cat allergy with T-cell reactive peptides. Am J Respir Crit Care Med 1996;154:1623-8.
15. Creticos PS, Reed CE, Norman PS, et al. Ragweed immunotherapy in adult asthma. N Engl J Med 1996;334:501-6.
16. Juniper EF, Thompson AK, Ferrie PJ, Roberts JN. Validation of the standardized version of the Rhinoconjunctivitis Quality of Life Questionnaire. J Allergy Clin Immunol 1999;104:364-9.
17. Hamilton RG, Adkinson NF Jr. Immunological tests for diagnosis and management of human allergic disease: total and allergen-specific IgE and allergen-specific IgG. In: Rose NR, de Macario EC, Folds JD, Lane HC, Nakamura RM, eds. Manual of clinical laboratory immunology. 5th ed. Washington, DC: ASM Press, 1997:881-92.
18. Wachholz PA, Soni NK, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. J Allergy Clin Immunol 2003;112:915-22.
19. Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. J Immunol Methods 1995;188:117-28.
20. Schroeder JT, Lichtenstein LM, Roche EM, Xiao H, Liu MC. IL-4 production by human basophils found in the lung following segmental allergen challenge. J Allergy Clin Immunol 2001;107:265-71.
21. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402-8.
22. Lichtenstein LM, Norman PS, Winkenwerder WL, Osler AG. In vitro studies of human ragweed allergy: changes in cellular and humoral activity associated with specific desensitization. J Clin Invest 1966;45:1126-36.
23. Creticos PS, Van Metre TE, Mardiney MR, Rosenberg GL, Norman PS, Adkinson NF Jr. Dose response of IgE and IgG antibodies during ragweed immunotherapy. J Allergy Clin Immunol 1984;73:94-104.
24. Creticos PS. Immunologic changes associated with immunotherapy. Immunol Allergy Clin North Am 1992;12:13-37.
25. Lichtenstein LM, Ishizaka K, Norman PS, Sobotka AK, Hill BM. IgE antibody measurements in ragweed hay fever: relationship to clinical severity and the results of immunotherapy. J Clin Invest 1973;52:472-82.
26. Walker SM, Pajno GB, Lima MT, Wilson DR, Durham SR. Grass pollen immunotherapy for seasonal rhinitis and asthma: a randomized, controlled trial. J Allergy Clin Immunol 2001;107:87-93.
27. Pienkowski MM, Norman PS, Lichtenstein LM. Suppression of late-phase skin reactions by immunotherapy with ragweed extract. J Allergy Clin Immunol 1985;76:729-34.
28. Parker WA Jr, Whisman BA, Apaliski SJ, Reid MJ. The relationships between late cutaneous responses and specific antibody responses with outcome of immunotherapy for seasonal allergic rhinitis. J Allergy Clin Immunmol 1989;84:667-77.
29. Nish WA, Charlesworth EN, Davis TL, et al. The effect of immunotherapy on the cutaneous late phase response to antigen. J Allergy Clin Immunol 1994;93:484-93.
30. Lichtenstein LM, Norman PS, Winkenwerder WL. A single year immunotherapy for ragweed hay fever: immunologic and clinical studies. Ann Intern Med 1971;75:663-71.
31. Norman PS, Winkenwerder WL, Lichtenstein LM. Immunotherapy of hay fever with ragweed antigen E: comparisons with whole pollen extract and placebos. J Allergy 1968;42:93-108.
32. Lowell FC, Franklin W. "Double-blind" study of treatment with aqueous allergenic extracts in cases of allergic rhinitis. J Allergy 1963;34:165-82.
33. Lowell FC, Franklin W. A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. N Engl J Med 1965;273:675-9.
34. Durham SR, Walker SM, Varga E-M, et al. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999;341:468-75.
35. Van Metre TE Jr, Adkinson NF Jr, Amodio FJ, et al. A comparison of immunotherapy schedules for injection treat-

ment of ragweed pollen hay fever. J Allergy Clin Immunol 1982;69:181-93.
36. Hejjaoui A, Dhivert H, Michel FB, Bousquet J. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. IV. Systemic reactions according to the immunotherapy schedule. J Allergy Clin Immunol 1990;85:473-9.
37. Frew AJ. Immunotherapy of allergic disease. J Allergy Clin Immunol 2003;111: Suppl:S712-S719.
38. Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. J Allergy Clin Immunol 1982;70:261-71.
39. Nouri-Aria KT, Wachholz PA, Francis NJ, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol 2004;172:3252-9.