

Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response

Meri K. Tulic, PhD,^a Pierre-Olivier Fiset, BSc,^a Pota Christodouloupolos, PhD,^a
Patrice Vaillancourt, MSc,^a Martin Desrosiers, MD,^b François Lavigne, MD,^b
Joseph Eiden, MD,^c and Qutayba Hamid, MD, PhD^a Montreal, Quebec, Canada, and
Berkeley, Calif

Background: Amb a 1-immunostimulatory phosphorothioate oligonucleotide conjugate (AIC) is a novel immunotherapeutic compound consisting of purified Amb a 1 from short ragweed proteins covalently linked to an immunostimulatory phosphorothioate oligodeoxyribonucleotide. In sensitized animals AIC can stimulate an Amb a 1-specific T_H1 response and decrease pulmonary reactivity to ragweed challenge. Clinical trials have documented reduced allergic response to AIC in comparison with licensed ragweed extract.

Objectives: We sought to determine the *in vivo* effect of short-course immunotherapy with AIC on eosinophilia and cytokine mRNA expression in the nasal mucosa of ragweed-sensitive patients.

Methods: Ragweed-sensitive patients with allergic rhinitis were treated with 6 escalating doses of AIC (0.06–12 µg, n = 28) or placebo (n = 29) at weekly intervals immediately before the 2001 ragweed season. Symptom scores and medication use were recorded for the 2001 and 2002 ragweed seasons for all patients. A subset of patients (12 receiving AIC and 7 receiving placebo) consented to have nasal biopsy specimens taken before immunization and before and after the first ragweed season. The preseason and postseason biopsy specimens were taken 24 hours after ragweed allergen challenge and compared with the initial unchallenged biopsy specimen to assess cytokine and inflammatory cell responses by using immunocytochemistry and *in situ* hybridization.

Results: AIC was safe and well tolerated by all patients. There was no difference between the AIC and placebo groups in the number of allergen-induced major basic protein-, IL-4-, IL-5-, or IFN-γ-positive cells in the mucosa in the first weeks

after AIC immunization. On rechallenge and rebiopsy after the end of the 2001 ragweed season, however, AIC-treated patients had a significantly reduced increase in eosinophils and IL-4 mRNA-positive cells and an increased number of IFN-γ mRNA-positive cells compared with placebo-treated patients. No difference between treatment groups was observed in symptom scores or medication use during the first ragweed season. During the second ragweed season, however, there was a significant decrease in chest symptoms and a trend toward reduced nasal symptoms in the AIC-treated group.

Conclusion: Short-course immunotherapy with AIC can modify the response of nasal mucosa to allergen challenge by increasing T_H1 cytokine production and decreasing T_H2 cytokine production and eosinophilia. This modification was not immediate but was observed 4 to 5 months after completion of immunotherapy and seasonal ragweed-pollen exposure. The T-cell subset shift after immunization and seasonal exposure was followed by evidence of clinical efficacy in the second ragweed season without additional AIC immunizations. (*J Allergy Clin Immunol* 2004;113:235–41.)

Key words: Amb a 1-immunostimulatory phosphorothioate oligonucleotide conjugate, immunotherapy, rhinitis, ragweed

Seasonal allergic rhinitis is a common clinical problem affecting between 20 and 40 million individuals in North America. Allergic rhinitis is a chronic inflammatory condition of the nasal mucosa and is characterized by infiltration of inflammatory cells, particularly CD4⁺ T lymphocytes and eosinophils.¹ Currently, treatment of these patients largely involves allergen avoidance and pharmacotherapy. Although pharmacotherapy, such as antihistamines, corticosteroid nasal sprays, and nasal sprays with a cromolyn derivative, can provide relief, it must be used on a daily basis for control of the disease and does not provide sustained relief of symptoms after completion of the course of medical treatment.

Allergen immunotherapy is a third option for a selected group of patients with IgE-mediated allergic rhinitis in whom the seasonal symptoms cannot be controlled by optimal use of pharmacotherapeutic agents. Immunotherapy often involves weekly or biweekly administration of increasing doses of the allergen extract, which offers a specific antidote for allergen-specific immune responses. Previous studies have shown grass-pollen immunotherapy

From ^aMeakins-Christie Laboratories, McGill University, and ^bCentre Hospitalier de l'Université de Montréal, Montreal, and ^cDynavax Technologies Corp, Berkeley.

Supported by a grant from Dynavax Technologies Corporation, Berkeley, Calif. M. K. Tulic is a GlaxoSmithKline/CIHR/Canadian Lung Association Fellow, P. O. Fiset is a recipient of an FRSQ-FCAR Santé Master's Studentship, and Q. Hamid is a recipient of the Senior FRSQ Chercheur-Boursier Award.

Received for publication June 30, 2003; revised October 16, 2003; accepted for publication November 3, 2003.

Reprint requests: Qutayba Hamid, MD, PhD, Meakins-Christie Laboratories, McGill University, 3626 St Urbain St, Montreal, Quebec Canada H2X 2P2.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2003.11.001

Abbreviations used

AIC: Amb a 1-immunostimulatory phosphorothioate
oligonucleotide conjugate
ICC: Immunocytochemistry
ISH: In situ hybridization
ISS: Immunostimulatory sequence

to be effective in reducing both the early- and late-phase allergic responses to grass, as well as associated inflammation.²⁻⁴ The therapeutic basis for immunotherapy might result through upregulation of T_H1 cytokines, such as IL-12 and IFN- γ .^{5,6} More recently, Durham et al⁵ have demonstrated that immunotherapy for 3 to 4 years with a grass-pollen extract induces a clinical remission that persists for at least 3 years after treatment is discontinued. This clinical remission is accompanied by persistent immunologic changes, including cellular desensitization and attenuated late-phase inflammatory responses on cutaneous challenge.⁵ Although effective, frequent injection over several years associated with this therapy is inconvenient for the patient, and the current methods of immunotherapy do not provide a complete cure for most patients with rhinitis. Immunotherapy would be an acceptable alternative for an increased proportion of allergic patients if the effectiveness were increased or if the regimen of administration was more convenient, associated with fewer adverse reactions, or both.

Amb a 1-immunostimulatory oligodeoxyribonucleotide conjugate (AIC) is a novel immunotherapeutic compound prepared by covalently linking the purified short ragweed-pollen antigen Amb a 1 conjugated to a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence (ISS).⁷ In vitro studies have demonstrated that AIC can promote a switch from T_H2 to T_H1 cytokine production in PBMCs from patients with ragweed allergy.⁸ In addition, preclinical animal experiments have shown AIC to preferentially induce a T_H1 response and downregulate the T_H2 response to Amb a 1 antigen.⁷ The amount of AIC required to produce this effect was 50- to 100-fold lower relative to the amount of free ISS mixed with Amb a 1. AIC induced a T_H1 response and suppressed Amb a 1-induced IgE production in mice primed for a T_H2 response and induced high-titer anti-Amb a 1 IgG antibodies in rabbits and cynomolgus monkeys when compared with Amb a 1 alone.⁷ In a mouse model of ragweed-induced asthma, AIC treatment was effective in completely reversing the established airway hyperresponsiveness.⁹

AIC is less allergenic than Amb a 1 alone. Tighe et al⁷ have shown that with AIC there is a 30-fold lower in vitro histamine release from human basophils of patients with ragweed allergy when compared with Amb a 1 alone. The covalent presentation of ISS and Amb a 1 was more effective in inducing IgG2a antibodies (surrogate for T_H1 response) in mice when compared with the coinjection of mice with both components at the same doses.⁷ In addition, skin test reactivity results in ragweed-sensitive patients suggest that approximately 184-fold more AIC

was needed to induce a skin test response in those patients compared with response induced by licensed ragweed extract.¹⁰ These data and the in vitro histamine release data indicate that AIC not only has potential for enhanced modification of allergic response compared with licensed ragweed extract, but this novel form of immunotherapy might be considered for administration with a shorter regimen of administration, higher doses than used with licensed ragweed extract, or both.

The aim of the current study was to examine the in vivo effect or effects of short-course immunotherapy with AIC in the nasal mucosa of ragweed-sensitive subjects on (1) eosinophil counts and changes in cytokine profiles (IL-4, IL-5, and IFN- γ), (2) chest and nasal symptoms after ragweed nasal challenge, and (3) safety and tolerability of AIC injections before and after allergen challenge. Our results suggest that a short-course immunotherapy with AIC has potential in inducing long-term beneficial effects, even after the ragweed season. AIC might offer a more effective and safer approach for allergen immunotherapy than currently available methods.

METHODS

Subjects and study design

Fifty-seven patients between 27 and 55 years of age (39.9 ± 2.4 [SEM]) with ragweed allergy and a history of seasonal symptoms of rhinoconjunctivitis were recruited into this study. The inclusion criterion was a positive skin prick test response to standardized ragweed extract with at least Σ_{30} erythema response to ragweed (Σ_{30} is the sum of erythema that is defined as the sum of the longest diameter [A] of the erythematous area and the diameter [B] orthogonal to A at the midpoint of A). Many patients also responded to grass allergen extract, suggesting they were not monosensitized to ragweed.

Subjects were randomized to receive AIC ($n = 28$) or placebo ($n = 29$, PBS). Patient demographics, baseline sensitivities to allergen, and IgG-IgE antibody responses to Amb a 1 were well matched between the 2 study groups (Table 1). The study was conducted according to an observer-blinded protocol whereby subjects and investigators were unaware of the randomization. Informed consent was given before the procedure, and the study was approved by IRB Services (Toronto) and the Comité d'évaluation Scientifique et d'éthique de la Recherche, Hôpital Saint-Luc (CHUM).

AIC was prepared by linking purified Amb a 1 with 1018 ISS (sequence: 5'-TGACTGTGAACGTTTCGAGATGA-3'). Cross-linking was done with the heterobifunctional linker sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, which creates stable amide and thioether bonds between the ISS oligonucleotide and the Amb a 1. The average molar ratio of Amb a 1 to 1018 ISS is 1:4. A more detailed description of the production and characterization has previously been published.⁷

After randomization, 6 subcutaneous injections with increasing concentrations of AIC (0.06, 0.3, 1.2, 3.0, 6.0, and 12.0 μ g) or placebo were administered at weekly intervals, ending 2 to 4 weeks before the 2001 ragweed season (Fig 1). Subjects were examined for local reactions and adverse events during vaccine administration, and increased doses were administered as long as the injections were well tolerated by the patient. Two weeks after the last injection, subjects underwent skin testing and had blood drawn for laboratory studies for safety and immune response to ragweed allergen.

Nasal biopsies were performed on a subset of study participants, excluding individuals with a previous history of ragweed

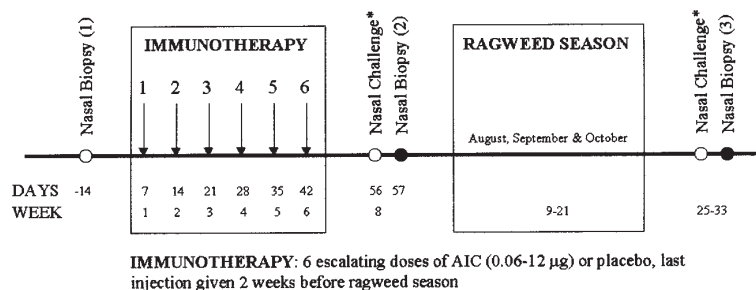


FIG 1. Dosing schedule for AIC immunotherapy in ragweed-sensitive patients. AIC (or placebo) was administered once per week for 6 weeks before ragweed season nasal biopsy specimens were taken 24 hours after allergen challenge for evaluation of the late-phase response.

immunotherapy or current immunotherapy to any allergens. A total of 19 patients (12 receiving AIC and 7 receiving placebo) consented to all 3 biopsies being performed. A baseline biopsy was performed at the initiation of the study without prior allergen challenge. Biopsy specimens were collected 24 hours after nasal allergen challenge at 2 subsequent time points (Fig 1): 2 weeks after the last injection of AIC or placebo and 1 to 2 months after the end of the first (2001) ragweed season.

Clinical efficacy of AIC: Evaluation of nasal and chest symptoms

Symptom scores and medication use were recorded in subject diaries daily at bedtime. Scores were assessed on a 5-point scale, with 0 representing no apparent symptoms and 4 representing severe symptoms (very bothersome and interfering with sleep). Nasal symptom categories were rhinorrhea, pruritis, sneezing, and nasal congestion, and the chest symptom categories were tightness, wheezing, cough, and shortness of breath. Symptoms were evaluated for all subjects undergoing AIC or placebo treatment and not only those patients who consented to nasal biopsies being performed. After the end of the 2001 ragweed season, participants were asked to remain in the study and record symptom scores and medication use during the 2002 ragweed season. No additional injections were given, and no additional nasal biopsies were performed. Twenty-three of 27 subjects in the AIC group and 21 of 28 subjects in the placebo group were evaluated during the 2002 season, and the study remained blinded to both the subjects and the investigators.

The start of the ragweed-pollen season was determined on the basis of the detection of 5 or more ragweed-pollen grains per cubic meter (for 2002, this was August 1). Two weeks after the end of the pollen season, subjects returned for safety evaluation and skin testing. The end of the ragweed-pollen season was determined on the basis of the occurrence of 3 consecutive days with a ragweed-pollen count of 0 or 1 pollen grains per cubic meter.

Nasal challenge and biopsies

Before initiation of immunotherapy, all subjects underwent a nasal challenge with ragweed antigen extract (3.59 AU; Greer Laboratories, Lenoir, NC). This was performed by applying a filter-paper disk soaked in allergen or diluent (0.4% phenol preserved saline) and applied unilaterally to the inferior turbinate for 60 seconds, as previously described.¹¹ A positive response to ragweed was defined as 3 or more sneezes than experienced with diluent challenge. This was observed in 90% of individuals with a positive skin prick test response to ragweed extract and a previous history of symptoms during ragweed season. Patients consenting to serial biopsies were challenged, as described above, 24 hours before the biopsy specimens were taken after the completion of immunization and again after the end of the 2001 ragweed season.

TABLE I. Patient demographics

	AIC	Placebo
No. of subjects	28	29
Sex		
Male	13 (46.4%)	13 (44.8%)
Female	15 (53.6%)	16 (55.2%)
Age (y)		
Median	41.2	36.7
Range	(20.5-55.1)	(24.9-54.9)
Ethnic origin		
White	26 (92.9%)	29 (100%)
Hispanic	2 (7.1%)	
Weight (kg)		
Median	72.3	71.9
Range	(48.0-105.0)	(41.7-112.0)
Baseline erythema (mm), prick-puncture		
Median	83	89
Range	(50-177)	(31-167)
Baseline IgG anti-Amb a 1 (ng/mL)		
Mean	299.9	166.6
Median	239.6	131.3
Range	(45.7-1007.5)	(58.1-447.8)
Baseline IgE anti-Amb a 1 (U/mL)		
Mean	172.9	85.4
Median	32.1	34.5
Range	(2.0-2910.6)	(2.0-664.5)

Nasal mucosa biopsy specimens of 2 mm in size were obtained from the posterior portion of the anterior third of the inferior turbinate. One set of biopsy specimens was taken for immunocytochemistry (ICC) and the other for in situ hybridization (ISH). Biopsy specimens were taken from the alternate nostril during the subsequent procedure. The number of major basic protein (MBP)-positive eosinophils was assessed by means of ICC and was used to estimate the primary efficacy end point. The secondary efficacy end points were determined by assessing expression of IL-4 mRNA, IL-5 mRNA, and IFN-γ mRNA by means of ISH.

Fixation and processing

Tissue was fixed in 4% paraformaldehyde, washed in 15% sucrose-PBS, and placed overnight at 4°C. The next day, tissue was blocked, sectioned (5 µm), and baked overnight at 37°C.

ICC

Alkaline phosphatase-antialkaline phosphatase ICC was used to identify eosinophils (anti-MBP, a gift from Dr R. Moqbel), as pre-

viously described.¹² Briefly, cryostat sections were incubated with the primary antibody (1:30). Slides were washed, incubated first with rabbit anti-mouse secondary antibody (1:60; DAKO, Carpinteria, Calif), and finally with mouse alkaline phosphatase–antialkaline phosphatase (DAKO; 1:60). Immunostaining was developed with Fast Red (Sigma Chemical Co, Toronto, Ontario, Canada) dissolved in substrate solution (0.5 mmol/L naphthol AS-MX phosphate, 2% dimethylformamide, 1 mmol/L levamisole, and 0.1 mol/L Tris base, pH 8.2). All slides were counterstained with Gill II Haematoxylin, and positive cells appeared red under bright field illumination. For negative controls, the primary antibody was replaced with an isotype-matched control antibody.

ISH

Cytokine mRNA was identified by means of ISH, as previously described.^{13,14} Briefly, tissue was prehybridized in 50% formamide (Sigma) for 15 minutes at 37°C and hybridized overnight at 42°C with sulfur 35–labeled antisense (complementary) and sense probes (identical to mRNA) recognizing RNA message encoding for human IL-4, IL-5, or IFN- γ . Nonspecific binding was removed by washing in standard saline citrate (4–0.1 $\times$) at 42°C and subsequent treatment with RNase (20 μ g/mL) to destroy excess and unbound RNA probe. Slides were dipped in emulsion (Amersham LM-2, Oakville, Canada), stored at 4°C, and developed 2 weeks later in Kodak D19 solution (Eastman Kodak, Toronto, Canada). Slides were counterstained with Gill II Haematoxylin, and cover slips were applied. Positive signal was identified as silver grains over the cells.

Quantification

Positive cells were counted blindly by 2 observers using an Olympus light microscope (Carson Group Inc, Markham, Ontario, Canada) at 200 $\times$ magnification with an eyepiece graticule of 0.202 mm². In the airway epithelium and subepithelium, the number of positive cells was averaged from 6 to 8 random, nonoverlapping grids and expressed as the mean number of positive cells per field.

Statistical analysis

Comparisons between treatment groups were made with the Fisher exact test for proportions and the Wilcoxon 2-sample test (Systat version 10; SyStat Inc, Evanston, Ill) for continuous variables. Comparisons within treatment groups were made by using the Wilcoxon 1-sample test. All statistical tests were 2-sided, with a *P* value of less than .05 regarded as statistically significant. Repeated-measures analysis was performed to examine the difference between AIC and placebo nasal and chest symptom scores (0–4). ICC and ISH results were expressed as mean values $\pm$ SEM for individuals who had all 3 biopsy specimens taken.

RESULTS

Safety of AIC and adverse events

The immunization regimen was considered safe and generally well tolerated. No serious adverse events were reported from the time of study initiation through the time at which the third nasal biopsy specimen was obtained (approximately 4–5 months after the last study injection). The majority of adverse events were mild or moderate, appeared with similar frequency in both treatment groups, and were not related to the study drug. In total, there were 24 moderate adverse events in the AIC treatment group and 22 moderate adverse events in the placebo group. These consisted primarily of headaches, rhinitis, and upper respiratory tract infections. No differ-

ence in frequency was apparent between the 2 treatment groups. Only a single serious adverse event occurred (pregnancy) in the placebo group.

Twenty-six of 28 subjects receiving AIC successfully completed the injection series, and 18 of these reached the highest dose of 12 μ g of AIC without dose adjustments. Dose adjustments were made as a result of missed study visits or injection-site reactions in accordance with protocol-specified criteria (local swelling of >20 mm at the injection site). The injection site reactions did not appear to be associated with clinically significant systemic reactions, and no dose adjustments were made because of systemic reactions. No study subject had study injections stopped as a result of either local or systemic events, and no study subject required medical treatment of local or systemic reactions within 30 minutes after study injection. Overall, local reactions were considered to be mild. Only 3 patients experienced local hives, and no local reactions required therapy. No clinically significant systemic reactions to the immunizations were observed; however, 2 patients did experience transient and mild vasovagal symptoms of dizziness and nausea. No medication was necessary to treat these symptoms. IgG directed against Amb a 1 was increased in AIC recipients but not in placebo-treated recipients during and after immunotherapy. No difference in serum IgE directed against Amb a 1 was observed between the AIC and placebo groups during the course of the study. Study injection was not associated with a pattern of complement activation or clinically significant increases in antinuclear antibodies or serum anti-DNA antibody. Clinical chemistry, hematology, and urinalysis results were all normal.

Effect of AIC on allergen-induced allergic inflammation in ragweed-sensitive patients before the ragweed season

After completion of the injection protocol and before the ragweed season had begun, patients from both the AIC- and placebo-treated groups underwent a localized nasal challenge with ragweed extract, followed 24 hours later by biopsy of the challenge site. Although the numbers of MBP-, IL-4-, and IL-5–positive cells tended to be higher in the allergen-challenged biopsy specimens compared with the baseline biopsy specimens, none of the mean values differed significantly at a *P* value of .05 or less (Fig 2). Comparison of baseline biopsy specimens between the 2 groups showed the groups to be closely matched (Fig 2). When compared with each other, no significant difference was observed between AIC- and placebo-treated subjects in the numbers of MBP-positive (Fig 2, A), IL-4–positive (Fig 2, B), IL-5–positive (Fig 2, C), or IFN- γ –positive (Fig 2, D) cells measured after allergen challenge.

Effect of AIC on allergen-induced allergic inflammation in ragweed-sensitive patients after the ragweed season

A second nasal challenge and biopsy specimen taken 2 weeks after the ragweed season and approximately 4 to 5

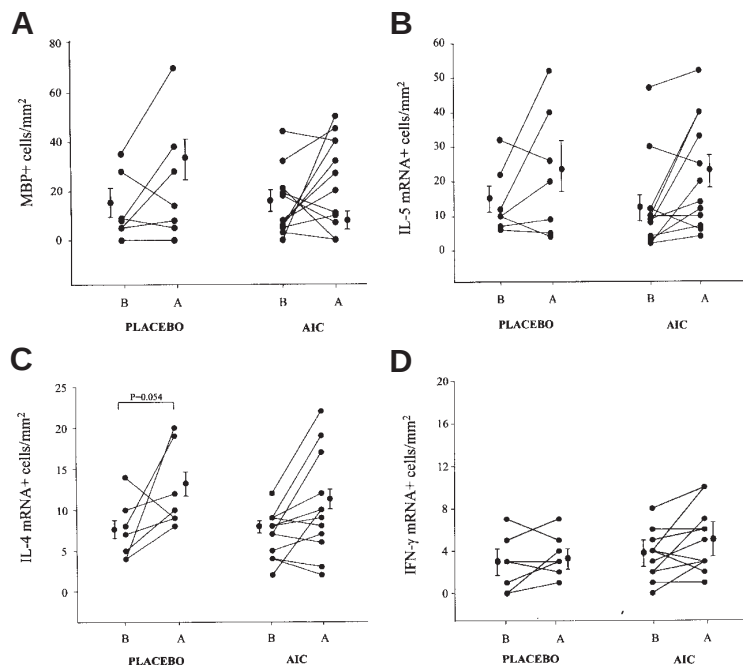


FIG 2. Effect of AIC on allergen challenge before the ragweed season. The number of MBP-positive cells (**A**), IL-5 mRNA-positive cells (**B**), IL-4 mRNA-positive cells (**C**), and IFN- γ mRNA-positive cells (**D**) at baseline (**B**) and after nasal allergen challenge with ragweed extract (**A**) in ragweed-sensitive patients 2 weeks after treatment with placebo ($n = 7$) or AIC ($n = 12$) is shown. Circles alongside the data represent mean values $\pm$ SEM.

months after the initial injections yielded results that contrasted markedly with the preseason biopsy evaluation. Nasal allergen challenge provoked significant increases in the T_H2 -associated markers, including IL-5 (Fig 3, B; $P = .03$) and IL-4 (Fig 3, C; $P = .024$) mRNA expression, as well as a strong tendency toward increased MBP immunoreactivity (Fig 3, A; $P = .067$), but no change in the T_H1 -associated IFN- γ expression (Fig 3, D). No allergen-induced increase in MBP immunoreactivity, IL-5 mRNA, and IL-4 mRNA expression was observed in the AIC-treated group; however, there was a highly significant ($P = .005$) increase in the number of IFN- γ mRNA-positive cells in the nasal mucosa after allergen challenge. Comparing the 2 treatment groups at the second nasal challenge and biopsy, the AIC-treated group had significantly lower responses of IL-4 mRNA-positive cells ($P = .008$) and MBP-immunoreactive cells ($P = .02$) and significantly higher numbers of IFN- γ mRNA-positive cells ($P = .002$) than the placebo-treated group (Fig 3). This pattern of nasal response to the postseasonal allergen challenge was consistent with the change in the balance of T_H1 and T_H2 responses to ragweed allergen that is predicted to occur in response to AIC immunization. The absence of a similar change in the preseason response to allergen challenge suggested that either additional time or additional ragweed-pollen exposure, in this case through natural seasonal exposure, was needed to achieve benefit from this mode of allergen desensitization.

Clinical efficacy of AIC

During the 2001 ragweed season, there were no significant differences between the AIC and placebo groups in

the mean symptom or medication-use scores (data not shown). The fact that a change in the balance of T_H1 to T_H2 responses in the AIC-treated group was demonstrable only after the 2001 ragweed season suggested the possibility of a greater effect on symptoms during the 2002 ragweed season. Of the patients enrolled in 2001, 44 of 53 patients were able to complete daily diary records of symptoms and medication use during the 2002 ragweed season. Indeed, chest symptom scores were significantly reduced over the 4-week period of peak pollen levels ($P = .047$; Fig 4, B) in the AIC-treated group. Nasal symptom scores tended to be reduced in the AIC group as well, especially during the period of maximum symptoms ($P = .065$; Fig 4, A); however, these values did not reach statistical significance. Total symptom scores and medication use in 2002 similarly trended lower in the AIC group compared with the placebo group, but the differences were not statistically significant (data not shown).

DISCUSSION

Although immunotherapy has previously been shown to be an effective form of treatment in allergic patients, frequent dosing and lengthy treatment courses are not only inconvenient but also have increased risk of anaphylaxis in some patients.² The current aim in immunotherapy is to improve the safety and effectiveness of agents (ie, reduce their allergenicity and increase their specific immunogenic response). Previous research with PBMCs from patients with ragweed allergy has suggested purified allergen antigen conjugated to immunostimulatory DNA sequences to be effective in vitro in

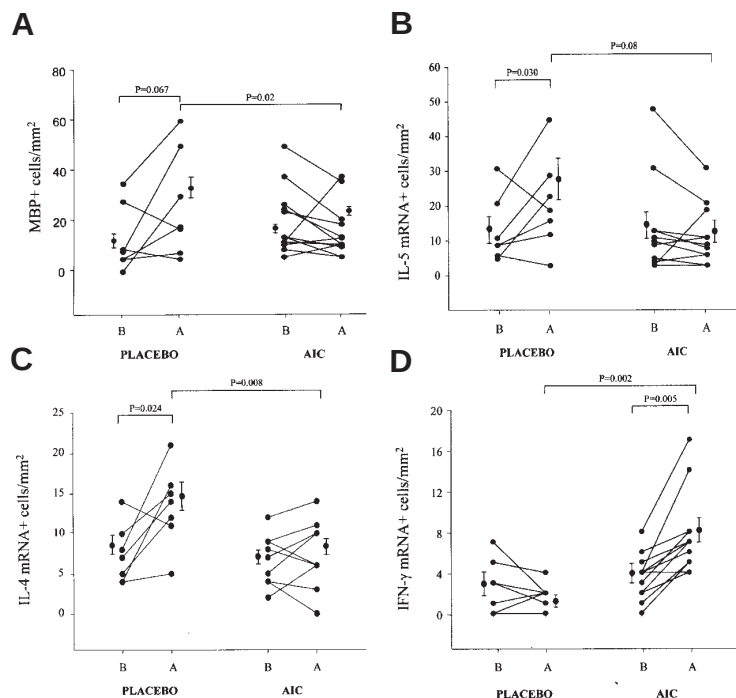


FIG 3. Effect of AIC on allergen challenge after the ragweed season. The number of MBP-positive cells (**A**), IL-5 mRNA-positive cells (**B**), IL-4 mRNA-positive cells (**C**), and IFN- γ mRNA-positive cells (**D**) at baseline (**B**) and after nasal allergen challenge with ragweed extract (**A**) in ragweed-sensitive patients 4 to 5 months after treatment with placebo ($n = 7$) or AIC ($n = 12$) is shown. Circles alongside the data represent mean values $\pm$ SEM.

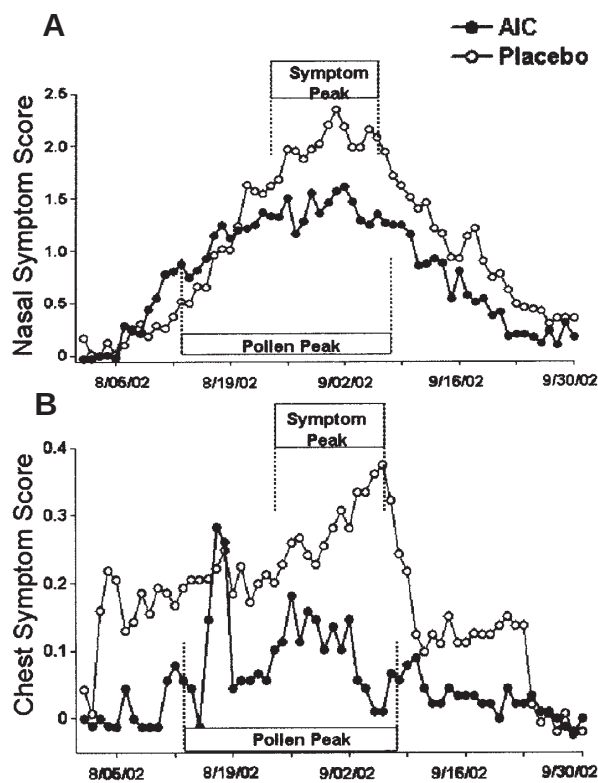


FIG 4. Clinical efficacy of AIC in ragweed-sensitive subjects. The least square means of daily nasal (**A**) and chest (**B**) symptom scores are shown for the entire ragweed-pollen season. Data are calculated as the change from preseason baseline symptom scores. Periods corresponding to the 4-week period of maximum pollen counts and the 2-week period of maximum total symptom scores are shown.

suppressing T_H2 cytokine production and stimulating T_H1 cytokine production.⁸

The present study has demonstrated for the first time that AIC treatment of individuals with ragweed allergy can result in a decrease in T_H2 and an increase in T_H1 responses to in vivo ragweed challenge. These results are consistent with our previous in vitro data demonstrating AIC to be effective in reducing T_H2 cytokine production (IL-4, IL-5, and IL-13) and increasing T_H1 cytokine production (IFN- γ) in the explanted mucosa of ragweed-sensitive patients exposed to ragweed challenge (abstract communication).¹⁵ Those experiments have also shown AIC to be much more effective in inducing a T_H1 immune response than ISS unconjugated to Amb a 1.

The change in phenotype of the ragweed-specific T-cell response was not observed after allergen challenge given a short time after the last AIC injection but was prominent 4 to 5 months later and after a season of natural ragweed-pollen exposure. It is not yet known whether the natural ragweed exposure was required to cause this shift in T-cell phenotype or whether the AIC-induced change in T-cell populations simply required longer than a few weeks to complete.

Short-course immunotherapy with AIC appeared safe and well tolerated by all the patients enrolled in our study. Twenty-six of the 28 AIC-treated study subjects enrolled successfully completed the injection series, and 18 of these reached the target dose of 12 μ g of AIC without dose adjustments and without serious systemic reactions. There was no significant difference between the AIC and placebo groups in rhinitis symptom scores or medication use during the 2001 ragweed season, which began a few weeks after the last immunizations. This is consistent with the results of nasal allergen challenges performed after the immunizations but before the beginning of the season.

In contrast, AIC treatment does lead to the predicted changes in ragweed-specific T_H1 and T_H2 responses by 4 to 5 months after the first AIC injection, suggesting a potential long-term beneficial effect of this agent in treatment of ragweed-sensitive patients. The reduction in chest symptoms and the trend toward reduced nasal symptoms in the AIC-treated group in the 2002 ragweed season, more than 1 year after the last AIC injection, is consistent with the progressive change in T_H1/T_H2 balance.

Long-term effectiveness with immunotherapy has previously been described by Durham et al⁵ using grass-pollen extract in patients with grass-pollen allergy; however, those patients were treated for 3 to 4 years. Our results with AIC have demonstrated that this agent has the potential to induce similar long-term beneficial effects in patients with seasonal allergic rhinitis after only 6 weeks of treatment. Importantly, we must emphasize that the effect of AIC was examined after antigen challenge out of ragweed season. For this reason, the effectiveness of AIC immunotherapy was based on controlled exposures to allergen and not natural exposure to ragweed allergen. Efficient rebalancing of T_H2 to T_H1 responses might require AIC immunization and seasonal

exposure. Further clinical studies are needed to delineate the influence of natural exposure and the optimal timing and regimen of administration of this promising novel form of immunotherapy.

We thank the study coordinators, Mr Elyess Khemici, Ms Dima Saba, and Mr Xavier Rodriguez, and the support staff, Pierrette Bouchard (RN) and Ms Paulette Montambault. We thank Drs Robert Coffman and Daniel Levitt, Dynavax Technologies, for help with preparation of the manuscript.

REFERENCES

1. Varney VA, Jacobson MR, Sudderick RM, Robinson DS, Irani AM, Schwartz LB, et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. *Am Rev Respir Dis* 1992;146:170-6.
2. Durham SR, Kay AB, Hamid Q. Changes in allergic inflammation associated with successful immunotherapy. *Intern Arch Allergy Immunol* 1995;107:282-4.
3. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4⁺ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J Allergy Clin Immunol* 1996;97:1356-65.
4. Durham SR, Hamid QA. The effect of immunotherapy on allergen induced late responses. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M* 1997;91:33-9.
5. Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999;341:468-75.
6. Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997;99:254-60.
7. Tighe H, Takabayashi K, Schwartz D, Van Nest G, Tuck S, Eiden JD, 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.
8. Marshall JD, Abtahi S, Eiden JJ, Tuck S, Milley R, Haycock F, et al. Immunostimulatory sequence DNA linked to the Amb a 1 allergen promotes Th1 cytokine expression while downregulating Th2 cytokine expression in PBMCs from human patients with ragweed allergy. *J Allergy Clin Immunol* 2001;108:191-7.
9. Santeliz JV, Van Nest G, Traquina P, Larsen E, Wills-Karp M. Amb a 1-linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma. *J Allergy Clin Immunol* 2002;109:455-62.
10. Creticos PS, Eiden JJ, Balcer SL, Van Nest G, Kagey-Sobotka A, Tuck S, et al. Immunostimulatory oligonucleotides conjugated to Amb a 1: safety, skin test reactivity, and basophil histamine release. *J Allergy Clin Immunol* 2000;105(suppl):S70.
11. Nakamura Y, Ghaffar O, Olivenstein R, Taha RA, Soussi-Gounni A, Zhang DH, et al. Gene expression of the GATA-3 transcription factor is increased in atopic asthma. *J Allergy Clin Immunol* 1999;103:215-22.
12. Frew AJ, Kay AB. The relationship between infiltrating CD4⁺ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous reaction in man. *J Immunol* 1988;141:4158-64.
13. Hamid Q, Wharton J, Terenghi G, Hassall CJ, Aimi J, Taylor KM, et al. Localization of atrial natriuretic peptide mRNA and immunoreactivity in the rat heart and human atrial appendage. *Proc Natl Acad Sci U S A* 1987;84:6760-4.
14. Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, et al. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991;87:1541-6.
15. Christodoulou P, Lavigne F, Marshall J, Van Nest G, Eiden J, Hamid QA. Induction of a specific Th1 response by allergen-linked immunostimulatory DNA in the nasal explant [abstract]. *J Allergy Clinical Immunol* 2002;109:A973:404-14.