LONG-TERM CLINICAL EFFICACY OF GRASS-POLLEN IMMUNOTHERAPY

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ABSTRACT

Background Pollen immunotherapy is effective in selected patients with IgE-mediated seasonal allergic rhinitis, although it is questionable whether there is long-term benefit after the discontinuation of treatment.

Methods We conducted a randomized, double-blind, placebo-controlled trial of the discontinuation of immunotherapy for grass-pollen allergy in patients in whom three to four years of this treatment had previously been shown to be effective. During the three years of this trial, primary outcome measures were scores for seasonal symptoms and the use of rescue medication. Objective measures included the immediate conjunctival response and the immediate and late skin responses to allergen challenge. Cutaneous-biopsy specimens obtained 24 hours after intradermal allergen challenge were examined for T-cell infiltration and the presence of cytokine-producing T helper cells (T_H2 cells) (as evidenced by the presence of interleukin-4 messenger RNA). A matched group of patients with hay fever who had not received immunotherapy was followed as a control for the natural course of the disease.

Results Scores for seasonal symptoms and the use of rescue antiallergic medication, which included short courses of prednisolone, remained low after the discontinuation of immunotherapy, and there was no significant difference between patients who continued immunotherapy and those who discontinued it. Symptom scores in both treatment groups (median areas under the curve in 1995, 921 for continuation of immunotherapy and 504 for discontinuation of immunotherapy; P=0.60) were markedly lower than those in the group that had not received immunotherapy (median value in 1995, 2863). Although there was a tendency for immediate sensitivity to allergen to return late after discontinuation, there was a sustained reduction in the late skin response and associated CD3+ T-cell infiltration and interleukin-4 messenger RNA expression.

Conclusions Immunotherapy for grass-pollen allergy for three to four years induces prolonged clinical remission accompanied by a persistent alteration in immunologic reactivity. (N Engl J Med 1999; 341:468-75.)

DESPITE advances in pharmacotherapy for grass-pollen allergy, there has been a marked increase in the prevalence of summer hay fever in countries with a Western lifestyle.[1] Although topical nasal corticosteroids and the new nonsedating antihistamines are highly effective in treating hay fever,[2] there remains a group of patients who have a poor response to these treatments and for whom immunotherapy is currently recommended.[3] An important question is whether allergen immunotherapy exerts a prolonged effect after it is discontinued. Such an effect would make this form of therapy attractive for prophylaxis and for early intervention.

We previously demonstrated the usefulness of immunotherapy in a cohort of patients with severe summer hay fever that could not be controlled by antiallergic drugs.[4] We initially followed these patients for a four-year period. During the first year (1989), patients were randomly assigned to receive injections of either grass-pollen vaccine or placebo. The vaccine was highly effective in reducing symptoms and the need for rescue drugs. Efficacy was accompanied by decreased sensitivity of the conjunctiva and skin to allergen and by inhibition of the late skin response.[4] Clinical improvement was maintained with continued immunotherapy during the ensuing three years.[5]

In the current, placebo-controlled study, we examined the effects of the discontinuation of immunotherapy for three years in the same group of patients. We also followed a matched group of patients who never received immunotherapy as a control for the natural history of the disease during this phase. Objective measures of outcome included immediate sensitivity of the conjunctiva and early and late skin responses to grass-pollen extract. Immunologic responsiveness was determined by assessing the late infiltration of CD3+ T lymphocytes and production of interleukin-4 in skin specimens 24 hours after intradermal grass-pollen challenge. We tested the hy-

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pothesis that in selected patients with IgE-mediated grass-pollen allergy, immunotherapy has a long-term effect and can modify the course of the disease.

METHODS

Patients

In 1988, 40 patients were recruited from the Royal Brompton Hospital Allergy Clinic or through an advertisement in a local newspaper; these patients had a history of severe seasonal allergic rhinitis, poor control of symptoms in previous years despite regular use of antiallergic drugs, and a positive skin-prick test (wheal, >5 mm) to timothy grass-pollen extract. Patients were excluded if they had a clinical history of other allergies or important medical illnesses or if they had chronic asthma. Patients with mild seasonal asthma were included, provided their symptoms were controlled by inhaled sympathomimetic β_2-adrenergic–agonist bronchodilators. Thirty-seven patients completed the initial one-year, double-blind, placebo-controlled study (1989).[4] For patients who received placebo injections during that year, immunotherapy with grass-pollen extract was then initiated over a six-to-eight-week period, and subsequently 32 of the 37 patients completed maintenance therapy with grass-pollen injections for the following three years (1990 through 1992).[5] In 1992, 15 matched patients with hay fever who had never received immunotherapy were recruited as a control group; the inclusion and exclusion criteria were identical to those used for the patients who received immunotherapy.

Study Design

The study was performed with the approval of the Royal Brompton Hospital ethics committee, and all the patients gave written informed consent. In 1992, 32 patients remained in the group receiving immunotherapy; analyses of data on these patients were stratified according to whether they had received three or four years of active immunotherapy before the current, double-blind randomization to either continued maintenance immunotherapy with depot grass-pollen vaccine (the maintenance group) or matched placebo injections (the discontinuation group). Injections of vaccine or placebo were given monthly for three years. The 15 matched patients who had never received immunotherapy (the control group) received no injections and were monitored in parallel. Patients in all three groups had equal access to the same rescue medication and underwent the same follow-up assessments.

Immunotherapy

A standardized, aluminum hydroxide–adsorbed, depot grass-pollen vaccine (Alutard SQ, ALK Abelló, Horsholm, Denmark) was used for subcutaneous-injection immunotherapy. Each monthly 1-ml maintenance injection contained 100,000 SQ units (equivalent to 10,000 biologic units[6] and containing 20 μg of the phleum [timothy] allergen P5).[6] Placebo injections consisted of identical vials of diluent, including aluminum hydroxide and 0.01 mg of histamine per milliliter. For three years, 1-ml injections were given monthly in the upper arm, except during the pollen seasons, when the maintenance dose was reduced by 40 percent. Patients were observed for one hour after each injection.

Assessments

Primary outcome measures were the presence of symptoms and the need for rescue medication. Patients recorded symptom scores and drug requirements every day from May through September of each year. Individual symptoms in the nose (sneezing, blockage, and running), eyes (itching, redness, tears, and swelling), mouth and throat (itching and dryness), and chest (breathlessness, cough, wheezing, and tightness) were recorded on a scale of 0 to 3 (with a score of 0 indicating no symptoms and 1, 2, and 3 indicating mild, moderate, and severe symptoms, respectively). Patients were given cromolyn sodium eye drops (Opticrom, Rhone-Poulenc, West Malling, United Kingdom), aqueous nasal spray (cromolyn sodium, Rynacrom, Laboratoires Fisons, Le Trait, France), a short-acting, nonsedating antihistamine, acrivastine (8-mg capsules, Semprex, Glaxo Wellcome, Greenford, United Kingdom), and an albuterol inhaler (Ventolin, Allen and Hanbury's, Stockley Park, United Kingdom) as rescue medications. If symptoms were not controlled, patients were advised to take, in addition, a seven-day course of prednisolone tablets (5-mg tablets; dosage, 30 mg per day for two days, with the dose successively reduced by 5 mg on each of the following five days).

Patients' diaries were scored by totaling individual symptom scores for each week, with a maximal possible score of 21 for each symptom. Drugs were scored as follows: each eye drop, dose of nasal spray, or inhalation of albuterol was given a score of 1, and each acrivastine capsule or prednisolone tablet was given a score of 2. Patients were asked every two weeks to record a visual-analogue score (on a scale of 0 to 10, where 0 indicated minimal symptoms and 10 indicated maximal symptoms) in response to the question, "How has your hay fever been during the past week?"

Objective measures of outcome were the immediate and late skin responses and the immediate conjunctival response to allergen challenge. They were assessed at the end of the study in November 1995. Skin-prick tests were performed in duplicate, with allergen concentrations of 100 to 100,000 SQ units per milliliter applied to the flexor aspect of the forearm. Immediate responses were recorded after 15 minutes and were expressed as the allergen concentration that caused a 6-mm wheal. Intradermal testing was performed on the extensor surface of the forearm with an injection of 10 SQ units of allergen in 0.02 ml of diluent and a control injection of diluent alone. Late responses were recorded as the mean diameter of the swelling at 24 hours.

Tests of the conjunctival response were performed by instilling half-log (approximately threefold) incremental concentrations of grass-pollen extract (100 to 100,000 SQ units per milliliter) into alternate eyes at 10-minute intervals. Immediate conjunctival sensitivity was recorded as the dose that induced a minimum of two of four symptoms (itching, redness, tears, or swelling). In both the skin and conjunctival tests, if there was no response at the highest concentration of allergen tested (100,000 SQ units per milliliter), the outcome was arbitrarily assigned a value of 300,000 SQ units per milliliter.

Skin Biopsies

Punch-biopsy specimens 3 mm in diameter were taken 24 hours after intradermal injection from both the site of allergen injection and the site of diluent (control) injection. CD3+ T cells and cells containing interleukin-4 messenger RNA (mRNA) were identified by immunohistochemical analysis and in situ hybridization of 6-μm cryostat sections of the biopsy specimens with appropriate negative controls, as previously described.[7] All analyses were performed in a blinded manner.

Statistical Analysis

Symptom and medication scores were expressed as the area under the curve for the 11-week period that corresponded to the peak pollen season. The primary outcome was analyzed by comparing symptom and rescue-medication scores over the three-year period of the study for the maintenance group with scores for the discontinuation group. Visual-analogue scores during the pollen season, the results of skin and conjunctival tests, and cell counts in skin-biopsy sections were analyzed in the same way with use of the two-tailed Mann–Whitney U test[8,9] (Minitab statistical software, State College, Pa.). P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

The three groups of patients were matched for sex and age. They were also matched for wheal size in

TABLE 1. CLINICAL CHARACTERISTICS OF THE 47 PATIENTS STUDIED FROM 1992 THROUGH 1995.

CHARACTERISTIC	IMMUNOTHERAPY 1989–1992		NO IMMUNOTHERAPY (N=15)
	MAINTENANCE 1992–1995 (N=16)	DISCONTINUATION 1992–1995 (N=16)	
Sex (M/F)	11/5	8/8	10/5
Age in 1992 (yr)			
Median	38	42	33
Interquartile range	32–48	33–50	26–47
Diameter of wheal on skin-prick testing (mm)*			
Median	10	11	10
Interquartile range	8–12	9–14	9–12

*Values represent the size of the wheal in response to skin-prick testing with timothy allergen at the time of enrollment.

response to skin-prick testing at enrollment (1988 for both immunotherapy groups and 1992 for the control group) (Table 1). Throughout the current trial, weekly pollen counts in London peaked consistently in the average or above-average range (60 [±24] SE grains per cubic meter for the years 1989 to 1998) in June and July (Fig. 1). Scores for total hay fever symptoms, rescue medication, and the visual-analogue scale for both the maintenance group and the discontinuation group were temporally related to pollen counts, remained low, and were similar to those recorded during the preceding three years, when all the patients in these two groups had received active immunotherapy.[5] There were no significant differences in any of these scores between these two groups throughout the three-year period. Individual nose, eye, chest, mouth, and throat symptoms also remained similar between these two groups (Table 2). In contrast, symptom and rescue-medication scores in both groups were markedly lower than those in patients in the control group (Fig. 1 and Table 2). The need for one or more courses of prednisolone tablets during the three-year period was also markedly lower in the maintenance and discontinuation groups (3 of 16 patients in each group) than in the control group (9 of 15 patients).

There was a tendency for immediate sensitivity to grass pollen to return three years after the discontinuation of immunotherapy. The concentration of grass-pollen extract required to cause a 6-mm wheal on skin-prick testing was significantly lower in the group that discontinued immunotherapy (median, 40,000 SQ units per milliliter; range, 3000 to 300,000) than in the group that received maintenance immunotherapy (median, 300,000 SQ units per milliliter; range, 60,000 to 300,000; $P=0.005$). There was also a trend in the discontinuation group toward a decrease in the grass-pollen concentration that elicited an immediate conjunctival response (median, 30,000 SQ units per milliliter; range, 300 to 300,000, as compared with 100,000 SQ units per milliliter; range, 3000 to 300,000, in the maintenance group; $P=0.06$). In the control group, however, the concentrations of allergen that caused an immediate wheal (median, 3000 SQ units per milliliter; range, 1000 to 10,000) and immediate conjunctival symptoms (median, 3000 SQ units per milliliter; range, 300 to 30,000) remained markedly lower than those in both immunotherapy groups (maintenance and withdrawal).

The control group had large (>3 cm) late skin responses 6 to 48 hours after the intradermal injection of grass pollen (Fig. 2). Immunohistochemical analyses of biopsy specimens from the site of allergen injection, as compared with control sites, revealed marked infiltration at 24 hours by CD3+ T lymphocytes and an increase in cells containing interleukin-4 mRNA. In contrast, in both the maintenance group and the discontinuation group, the late skin response was virtually absent and there were fewer infiltrating CD3+ T cells and markedly fewer infiltrating cells containing interleukin-4 mRNA than in the control group (Fig. 2). No differences were observed between these two groups in the late skin response as assessed on the basis of the size of the swelling ($P=0.16$) or in the numbers of CD3+ T cells ($P=0.57$) or cells containing interleukin-4 mRNA ($P=0.87$). In both immunotherapy groups, there was no correlation between the late skin responses and the corresponding immediate skin responses after intradermal injection of grass pollen (data not shown).

Immunotherapy was well tolerated by the patients who received it throughout the three-year period. Less than 2 percent of injections resulted in early or delayed local reactions larger than 3 cm in diameter. No substantial immediate or late systemic reactions were observed after allergen injections. Thirty-nine of the 47 patients completed the study. During the second and third years of the study, 2 of the 16 patients in the maintenance group, 3 of the 16 in the discontinuation group, and 3 of the 15 in the control group dropped out. These patients withdrew for reasons unrelated to the study protocol. Blinding of the trial was checked at the end of the study by asking the patients and the investigator to guess which treatments (active [maintenance] or placebo [discontinuation]) the patients in the two immunotherapy groups had received. Sixteen of the 27 patients remaining in these two groups guessed correctly, whereas the investigator was correct in 15 of the 27 cases. These results were not significantly different from those that would have occurred by chance ($P=0.5$ for the patients' guesses, and $P=0.8$ for the investigator's guesses, by the chi-square test).

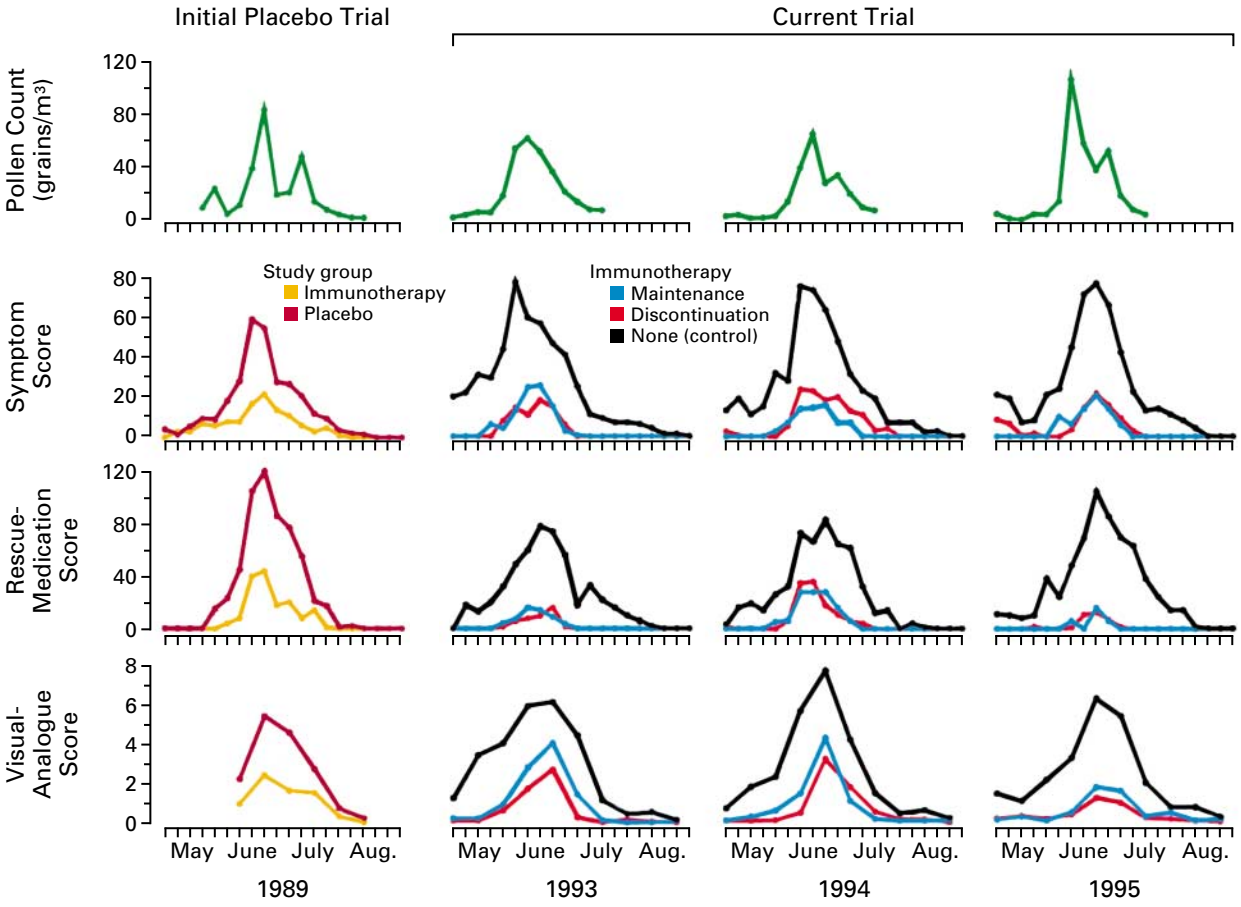

Figure 1. Median Weekly Pollen Counts and Symptom, Rescue-Medication, and Visual-Analogue Scores for the Initial Placebo Trial (1989) and for the Current Trial (1993 through 1995).

Analysis of the area under the curve with the Mann–Whitney U test revealed no significant differences between the maintenance group and the discontinuation group (see Table 2); the median scores in both of these groups were markedly lower than those in the control group. For comparison, results are also shown for the initial placebo-controlled trial of immunotherapy (1989). Tick marks on the x axes indicate one-week intervals. Data for 1989 are from Varney et al.[4]

DISCUSSION

We have shown, under double-blind conditions, that three to four years of grass-pollen immunotherapy remains effective for at least three years after the discontinuation of the injections. In both the group that received maintenance immunotherapy and the group that discontinued immunotherapy, clinical improvement was accompanied by a marked decrease in the late skin response to allergen challenge, blunting of the accompanying CD3+ T-cell infiltrate, and fewer interleukin-4 mRNA–expressing cells than in the control group. The results demonstrate prolonged clinical benefit and provide evidence of decreased immunologic reactivity for at least three years after the discontinuation of immunotherapy for the treatment of hay fever.

In contrast to the late skin response, there was a tendency for immediate sensitivity to grass pollen to return three years after discontinuation of immunotherapy, as reflected by the appearance of a small but significant difference in the immediate response to skin-prick testing and a trend toward an increase in conjunctival sensitivity (measured 15 minutes after allergen challenge) in the discontinuation group as compared with the maintenance group. However, this tendency was not accompanied by a return of symptoms, perhaps indicating that late responses have greater relevance than early responses to the clinical expression of hay fever.

The efficacy of grass-pollen immunotherapy in patients with seasonal hay fever has been confirmed in many controlled trials.[3-5,7,10-16] Immunotherapy is also effective, although less so, in patients with seasonal asthma.[14,17,18] In contrast, patients with perennial disease associated with sensitivity to multiple allergens are less responsive to this form of treatment.[19]

TABLE 2. SCORES FOR SYMPTOMS, RESCUE-MEDICATION USE, AND THE VISUAL-ANALOGUE SCALE DURING THE 1993, 1994, AND 1995 POLLEN SEASONS.

OUTCOME MEASURE	IMMUNOTHERAPY		POINT ESTIMATE*	P VALUE†	NO IMMUNOTHERAPY (N=15)
	MAINTENANCE GROUP (N=16)	DISCONTINUATION GROUP (N=16)			
	median (range)		value (95% CI)		median (range)
Symptoms‡					
Nose					
1993	392 (0–1974)	367 (0–1750)	−74 (−325 to 266)	0.65	1389 (469–3346)
1994	453 (0–2107)	490 (28–2205)	67 (−287 to 490)	0.68	1358 (165–3360)
1995	679 (0–1274)	422 (28–2208)	−5 (−462 to 462)	0.98	1225 (241–3066)
Eyes					
1993	140 (0–1554)	56 (0–665)	−42 (−301 to 98)	0.52	959 (42–3024)
1994	119 (0–1526)	142 (0–1729)	7 (−266 to 290)	0.81	1054 (0–4984)
1995	82 (0–1015)	98 (0–1407)	21 (−164 to 308)	0.55	1099 (77–4077)
Chest					
1993	0 (0–861)	0 (0–315)	0 (0 to 14)	0.60	290 (0–3517)
1994	0 (0–1365)	7 (0–774)	0 (0 to 49)	0.42	262 (0–3766)
1995	0 (0–749)	12 (0–1316)	0 (0 to 28)	0.30	84 (0–3353)
Mouth and throat					
1993	35 (0–357)	42 (0–546)	0 (−56 to 133)	0.58	301 (0–1491)
1994	35 (0–564)	63 (0–1183)	7 (−70 to 199)	0.47	416 (0–1841)
1995	116 (0–865)	39 (0–966)	0 (−217 to 112)	0.94	452 (0–1690)
Total					
1993	626 (70–3528)	798 (0–2289)	−70 (−613 to 634)	0.85	2615 (609–10,416)
1994	779 (0–5030)	745 (28–4277)	229 (−487 to 1141)	0.53	3220 (1106–13,951)
1995	921 (0–2299)	504 (45–4567)	133 (−690 to 1656)	0.60	2863 (774–12,033)
Rescue-medication use‡					
1993	756 (0–4553)	633 (0–7903)	54 (−724 to 2009)	0.85	3672 (1029–10,832)
1994	1134 (0–5820)	742 (0–11,130)	4 (−1064 to 2121)	0.96	4088 (1141–10,808)
1995	672 (0–1827)	357 (0–7637)	11 (−689 to 1488)	0.88	4729 (1197–8505)
Visual-analogue scale§					
1993	2.9 (0.2–8.2)	1.8 (0–5.0)	−1 (−2.6 to 0.3)	0.13	6.0 (0.7–8.1)
1994	4.4 (0–8.0)	3.4 (0–8.8)	0 (−3 to 3.1)	0.92	7.8 (1.1–9.0)
1995	1.9 (0–8.5)	1.4 (0.4–8.4)	0.2 (−1.9 to 1.6)	0.87	6.4 (0–8.6)

*The point estimates and 95 percent confidence intervals (CIs) were calculated with Minitab software.[9]

†P values are for the comparison between the maintenance group and the discontinuation group.

‡Data shown are the medians and ranges for the area under the curve (the scores) during the 11-week pollen season.

§Data shown are median scores for a one-week period during the peak of the pollen season.

Previous studies have suggested that immunotherapy has a long-term effect.[20-25] In a retrospective study of children who were sensitive to house-dust mites, short-term (12-month) immunotherapy was associated with a greater rate of relapse than was treatment for more than 3 years. In the only blinded study of the discontinuation of pollen immunotherapy, patients with sensitivity to ragweed were followed for one year; in a finding consistent with our own, a recurrence of immediate sensitivity to allergen was observed.[26]

T-cell–derived cytokines play a key part in allergic inflammation. Grass-pollen–specific T cells from patients with atopy produce greater quantities of cytokines such as interleukin-4, interleukin-13, and interleukin-5 (and thus can be identified as type 2 T helper cells [T_H2])[27,28] than do cells from control subjects without atopy, which favor the production of interferon-γ (T_H1 cells).[27,29] Interleukin-4[30] and interleukin-13[31] stimulate IgE production by B cells and therefore promote the sensitization of high-affinity IgE receptors on the surface of mast cells and basophils, whereas interleukin-5 has specific pro-eosinophilic properties.[32] IgE-dependent activation of mast cells results in an immediate response to allergen and may contribute to the development of the late response.[33]

Previous studies found decreases in serum IgE concentrations,[34] increases in IgG,[35] and inhibition of recruitment or activation of effector cells such as mast cells[36,37] and eosinophils[38,39] in the target organ in response to immunotherapy. Since each of these processes is thought to be largely T-cell–dependent, one possibility is that immunotherapy exerts a prolonged effect by altering the T-cell response to subsequent allergen exposure. Our earlier studies, performed in the same group of patients after they had received one year of immunotherapy, demonstrated inhibition of the late response in both nose and skin, accompanied by an increase in T_H1 responses

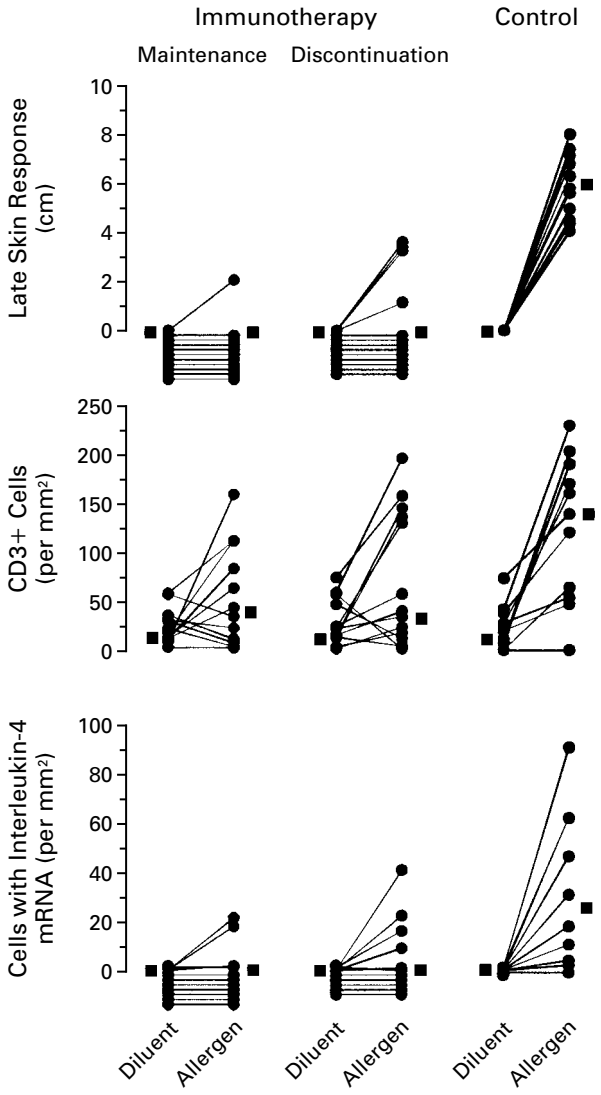

Figure 2. Late Skin Responses and Number of CD3+ T Cells and Cells Containing Interleukin-4 mRNA in Skin-Biopsy Specimens Obtained 24 Hours after Intradermal Injection of Allergen or Diluent.

Late skin responses were assessed by intradermal injection of either grass-pollen extract or the diluent alone as a negative control at the end of the study. Late skin responses are expressed as the size of the swelling as measured 24 hours after intradermal allergen challenge. Twelve patients in the maintenance-therapy group, 13 in the group that discontinued therapy, and 11 in the control group underwent skin biopsy. Cell counts are expressed per square millimeter of skin. Values for individual patients (circles) and median values (squares) are shown. Data on interleukin-4 mRNA were not obtained for one patient in the maintenance-therapy group, two in the group that discontinued therapy, and two in the control group because the tissue architecture of their biopsy specimens was distorted during processing for in situ hybridization.

as detected by an increase in interferon-γ mRNA expression.[7,40]

Further studies of cutaneous-biopsy specimens obtained at 24 hours suggested that this T_H1 response may have been driven by interleukin-12, since inhibition of the late skin response was accompanied by a marked increase in cells expressing interleukin-12 mRNA, predominantly tissue macrophages.[41] Studies of T-cell responses in the peripheral blood of patients undergoing immunotherapy with pollen extract have revealed a corresponding reduction in T_H2 responses, as shown by a decrease in interleukin-4.[42,43] Taken together, these studies suggest that pollen immunotherapy may act either by inducing immune deviation of T_H2 and T_H0 T-cell responses in favor of T_H1 responses or by diminishing T_H2 and T_H0 T-cell responses.[44]

In contrast to our earlier findings, the current finding of a decrease in the number of cells expressing interleukin-4 mRNA suggests that persistent suppression of T_H2 responses may be responsible for sustained clinical improvement, as reflected by an inhibition of the late response, whereas immediate mast-cell–dependent responses to allergen may return several years after discontinuation of treatment. Since we did not identify the cellular source of interleukin-4 mRNA, it is possible that cells other than T cells, including basophils,[45] mast cells,[46] or eosinophils,[47] contribute to the expression of this cytokine. Irrespective of the precise mechanism, these data provide objective evidence of a long-term immunologic effect after the discontinuation of immunotherapy.

The usefulness of allergen immunotherapy is highlighted in a recent World Health Organization report,[3] which advocates its use in selected patients with specific IgE antibodies to clinically relevant allergens. Selection of patients is extremely important; the risk–benefit ratio is less favorable for patients with asthma than for those with allergic rhinitis. The rationale for prescribing allergen immunotherapy depends on the degree to which symptoms can be alleviated by medication and whether effective avoidance of allergen is possible. The quality of allergen vaccines is also critical, and an optimal maintenance dose of 5 to 20 μg of major allergen per injection (as in the current study) correlates with clinical efficacy.[3]

An important question has been whether immunotherapy has the potential to modify the long-term course of allergic disease after discontinuation.[19-25] The current findings suggest that it does and raise the question of whether allergen-injection immunotherapy should be considered earlier in the course of allergic disease to prevent progression or, as suggested by another study,[48] the development of multiple allergies. Further studies with long-term follow-up, particularly in children with limited allergic sensitivities, could address this possibility.

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