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Original article

Long-term efficacy of preseasonal grass pollen immunotherapy in children

Background: In a previous controlled study we demonstrated that preseasonal grass pollen immunotherapy for three years was effective in children. In the current study we examined the same group of patients to see if there is still a benefit six years after discontinuation of treatment.
Methods: Thirteen of 14 patients with previous specific immunotherapy (SIT) and 10 out of 14 patients of the control group were prospectively followed during the grass pollen season. Outcome measures were seasonal symptom scores for eyes, nose and chest, the use of symptomatic medication and visual analog scale. Objective measures included skin prick test reactivity to seasonal and perennial allergens and conjunctival provocation testing.
Results: During the 13 week observation time scores for overall hayfever symptoms ($P<0.004$) and individual symptoms for eyes ($P<0.02$), nose ($P<0.04$) and chest ($P<0.01$) as well as combined symptom and medication scores ($P<0.002$) remained lower in the group with previous SIT. Only 23% of patients with previous pollen-asthma who had received SIT experienced pollen-associated lower respiratory tract symptoms compared to 70% in the control group ($P<0.05$). There was no significant difference in the use of pharmacological treatment during the pollen season except for asthma medication. The average visual analog scale was lower in the post-SIT group ($P<0.05$). Six years after cessation of SIT the immediate skin response to grass pollen remained decreased compared to the reaction of the controls ($P<0.01$). There was also a tendency for higher allergen concentration to provoke a conjunctival response in the post-SIT group but without reaching statistical significance. Eight years after commencement of SIT, 61% of the initially pollen-monosensitized children had developed new sensitization to perennial allergens compared to 100% in the control group ($P<0.05$).
Conclusions: There is still a significant clinical benefit six years after discontinuation of preseasonal grass pollen immunotherapy in childhood. SIT in children with pollen-allergy reduces onset of new sensitization and therefore has the potential to modify the natural course of allergic disease.

P. A. Eng, M. Reinhold, H. P. E. Gnehm
Department of Pediatrics, Kantonsspital Aarau, Switzerland

Key words: childhood; follow-up; grass pollen; hayfever; immunotherapy; natural course.

Dr P. A. Eng
Allergologie/Pneumologie
Kinderklinik
CH-5001 Aarau
Switzerland

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Many controlled studies have demonstrated that specific immunotherapy (SIT) with standardized pollen extracts is an effective treatment of seasonal allergic disease (1–8). However only limited knowledge exists about the optimal duration, age of commencement, route of administration and duration of the therapeutic response. There are only a few follow-up studies after discontinuation of pollen immunotherapy (9–14). All of them were performed in adults. Little is known also about the course of the seasonal allergic rhinoconjunctivitis and asthma after early intervention with SIT in children. Since hayfever most commonly begins in childhood and adolescence,early intervention with SIT is an important issue in this age group.

In a previous study we enrolled 28 children with IgE-mediated seasonal allergic rhinoconjunctivitis with or without seasonal asthma. All patients were sensitized to seasonal pollen-allergens only and had no history of allergic disease outside the pollen season. The children were either treated with subcutaneous grass pollen immunotherapy ($n=14$) in the years 1989, 1990 and 1991 or with standardized pharmacological treatment only ($n=14$) during the three grass pollen seasons. The study demonstrated a significant clinical benefit and decrease of skin test reactivity after discontinuation of SIT when compared with the control group (5).

In order to assess the duration of efficacy of this treatment we decided to follow-up prospectively the

two study populations six years after cessation of SIT. All patients had to record their symptoms and their need for medications during the pollen season 1997. Objective measures included immediate sensitivity of conjunctiva as well as of the skin by performing prick tests with a panel of common inhalant and food allergens. This study was designed to determine whether:

1) SIT with grass pollen allergoids in childhood is still effective six years after discontinuation

2) the natural course of the disease can be modified by early intervention with SIT, especially with regard to disease progression and onset of new sensitizations.

Material and methods

Patients

Twenty-eight children were recruited from our Allergy Clinic. They had a history of severe hayfever for at least two years and IgE-mediated sensitivity to seasonal allergens only (grass pollen with or without tree pollen) as assessed by skin prick test and radio-allergosorbent assay (RAST). Group 1 ($n=14$) was treated with immunotherapy for three consecutive years (1989–91). SIT was performed with a standardized grass pollen depot-allergoid (Allergovit®) in a preseasonal protocol according to the recommendations of the manufacturer (Allergopharma, Rheinbek, Germany). Group 2 ($n=14$) had the same clinical manifestations and fulfilled the criteria but declined having SIT. The two groups were well matched in terms of age, onset of disease and immediate skin reactivity to grass pollen (5).

All 14 patients completing the previous SIT were recruited for the follow-up study. One patient dropped out because of failure to attend clinical visits. Ten of the 14 patients in the control group entered the follow-up study. Two of the four remaining patients had moved and were impossible to trace, and two decided to have SIT in the meantime and could therefore not be included as controls.

Study design

The project was designed as a prospective controlled open follow-up study during the grass pollen season. Patients were invited for a first assessment in April 97. They were asked in a questionnaire about their hayfever symptoms since the end of the previous study in 1991. Each patient was supplied with diary cards to record symptom scores and use of medication. A 10-cm visual analog scale (VAS) was handed out. Subjects were also provided with rescue medications. During the pollen season each subject was contacted twice by telephone. In a second clinical attendance in November 1997, four months after the end of the grass pollen season, skin prick and conjunctival provocation testing (CPT) were performed. Diary cards and VAS were also evaluated.

Symptom score

Patients recorded their allergic symptoms in a standardized diary of the 'Swiss association of aerobiology' from May 1st to July 31st 1997. Individual symptoms of the eyes (itching, redness, tears and swelling), nose (sneezing, blockage, rhinorrhea) and chest (exercise intolerance, wheezing, tightness, breathlessness) were noted every day on a scale of 0–3, where 0 indicated no symptoms, and 1, 2 and 3 indicated mild, moderate and severe symptoms, respectively. For each patient, the total individual symptom score was calculated for each week (maximum weekly score = 21 for each symptom).

Medications

Patients were provided with and instructed on the use of a number of medications to control their allergic symptoms. These included antihistamine-containing eye drops and nasal spray (levocabastine), a short acting nonsedating antihistamine (cetirizine), and a β-2-agonist inhaler (terbutaline turbuhaler) as rescue medications for allergic rhinoconjunctivitis or asthma symptoms, respectively. If these were insufficient to control symptoms, patients were allowed to take nasal or bronchial steroids (budesonide). The study subjects were provided with a standardized information sheet about dosage and application of these drugs. Each dose of eye drops, nasal spray, inhalation of β-2-agonist or topical steroids was given a score of 1, each tablet of antihistamine a score of 2. A combined symptom and medication score was calculated for each patient.

Visual analog scale

During the pollen season patients were asked to record the overall severity of their hayfever symptoms during the preceding two weeks on a 10-cm VAS where 0 indicated no symptoms and 10 indicated maximal symptoms.

Skin prick test

Skin prick tests (SPT) were performed in duplicate on the volar forearms. In addition to seasonal grass and tree pollen, *Dermatophagoides pteronyssinus*, *D. farinae*, cat, dog, horse, *Alternaria*, cow's milk, and hen's egg, as well as positive (histamine) and negative (glycerol diluent) control extracts, were tested using commercial extracts (Allergopharma, Rheinbek, Germany). The immediate responses were recorded after 15 min by copying the size of wheal reaction with transparent adhesive tape onto the patient's record sheet. The area of the wheal was calculated ($A=r^2\times\pi$). SPT reactivity to each allergen was expressed as (A allergen ÷ A histamine).

Assessment of newly developed sensitization

In 1989 all patients were sensitized to seasonal pollen-allergens only as determined by SPT and RAST. Eight years later the results of the SPT were studied by using the same panel of food and aeroallergens as at the enrollment in 1989. Reactions were considered positive if the mean wheal diameter was at least 3 mm.

Conjunctival provocation test

An aqueous solution of the allergen (Allergopharma, Germany) was instilled in increasing concentrations from 0 to 5000 BU/ml (standardised biological units) into alternate eyes (lower conjunctival sac) at 10 min intervals. The diluent solution served as a negative control. Immediate conjunctival sensitivity was recorded as the dose that induced a minimum of two of the four symptoms itching, redness, tears or swelling.

Pollen count

A Burkhard spore trap was used to collect pollen grains. The trap was placed on the roof of the nearby High School and maintained by the students. The collected specimens were sent to the Swiss Institute of Meteorology for analysis and pollen count. All the study patients were living within 30 km of the spore trap.

Statistics

The two-tailed Mann–Whitney test (STATVIEW software, Cary, NC, USA) was used for comparison between groups. The assessment of the course of pollen asthma and occurrence of new sensitization were compared by means of chi-squared test. A 5% significance level was used.

Table 1. Characteristics of the 23 patients in the follow-up study

	Previous SIT 1989–91	Control group
Number of patients	13	10
Gender (M/F)	10/3	8/2
Mean age (year) at follow-up	17.9	17.1
Mean age (year) and range at beginning of SIT/observation	9.6 (5–16)	8.8 (7–13)
Mean age (year) at onset of hay fever	5.8	5.5
Patients with seasonal asthma at beginning of SIT/observation	9	8
Result of skin prick testing with grass pollen at enrolment 1989*	2.49	2.27

*Values represent the quotient of mean wheal area to grass and mean wheal area of the histamine control

Results

The demographic characteristics of the two study groups are presented in Table 1. They are matched for age, gender and age at onset of hayfever symptoms. Furthermore wheal size in response to SPT with grass pollen were similar for both group at enrolment 1989 (5).

Clinical efficacy

The weekly grass pollen count during the observation time shows a main peak at the beginning of June and a second smaller peak in mid-July (Fig. 1a). Average weekly symptom score graphs of the two groups for total hayfever and individual eye, nose and chest symptoms are shown in Fig. 1b–Fig. 1e. All symptoms were temporally related to the pollen counts throughout the season in both groups. However hayfever symptom scores were significantly lower in the group with previous SIT both for total symptom score ($P=0.0038$) and individual eye ($P=0.019$), nose ($P=0.038$) and chest symptoms ($P=0.008$). The control subjects also experienced another increase of allergic symptoms during the second pollen peak in July This was not seen in the group of patients who had had previous immunotherapy.

The average VAS during the pollen season was significantly lower in the post-SIT group ($P=0.04$). Again the sores corresponded temporally to the amount of airborne grass pollen in both group. (Fig. 1f)

There was no significant difference in use of total symptomatic medication during the pollen season. Of the patients with previous SIT 31% did not need any treatment for hayfever symptoms throughout the season compared to 20% of the control group ($P=0.08$). In patients who had previous SIT the need

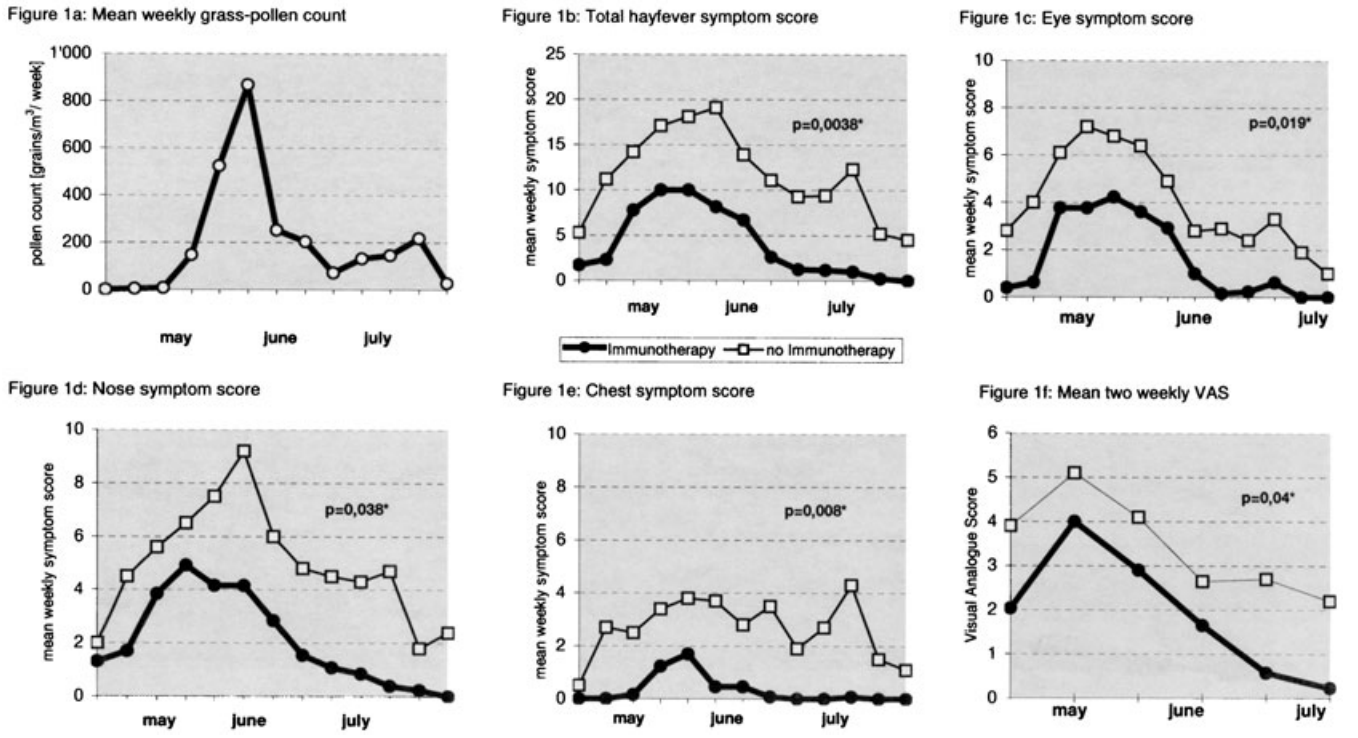

Figure 1 Mean weekly pollen count, total hayfever symptom score and individual symptom scores for eyes, nose, chest and visual analog score during the pollen season in 1997. * *P*-values are for the comparison between the two groups.

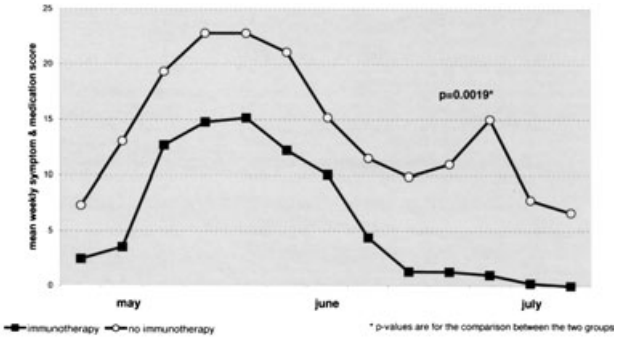

Figure 2. Total symptom and medication score.

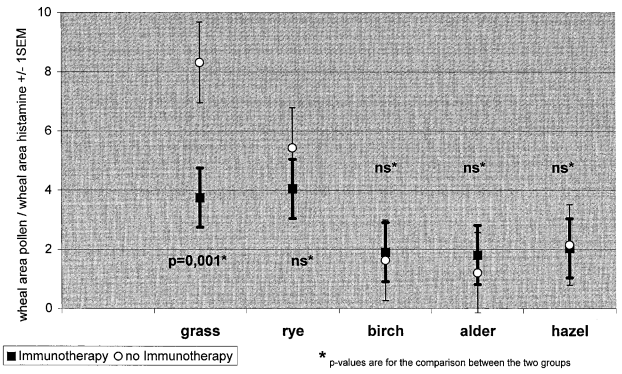

Figure 3. Skin prick testing with pollen allergens.

for asthma medication was significantly lower compared to the controls ($P=0.04$) whereas individual treatment of eye and nose symptoms was not significantly reduced compared to the control patients. However the combined symptom plus medication score remained markedly lower in patients with previous SIT ($P=0.0019$) as illustrated in Fig. 2.

Provocation tests

Wheal size in response to skin prick testing with grass pollen allergens was matched for both groups at enrolment in 1989 (Table 1). As previously reported, after 36 months of preseasonal allergen vaccination there was a significant reduction of the immediate skin test reactivity to grass pollen allergens in the immunotherapy group ($P<0.01$).

Re-evaluation of prick test sensitivity six years later revealed that the immediate skin reactivity to grass pollen remained significantly lower in the group with previous immunotherapy when compared to the control patients ($P=0.001$, Fig. 3). No difference between the two groups was seen for wheal size in response to rye, birch, alder and hazel pollens (Fig. 3).

Immediate conjunctival allergen sensitivity was assessed after the end of the pollen season in 1997 with installation of increasing grass allergen concentrations into the lower conjunctival sac. There was a tendency for higher allergen concentration to provoke a conjunctival response in the post-SIT group (Fig. 4) but without reaching statistical significance, mainly because of the low numbers of subjects. Four patients declined to undergo the conjunctival provocation test.

Assessment of the course of allergic disease

All of the patients were sensitized to seasonal inhalant allergens only at enrolment in 1989. None of the patients had a history of perennial allergic disease. At the end of this follow-up study, eight of 13 patients (61%) who previously received SIT had new sensitivities to perennial inhalants or food allergens, whereas all of the 10 children in the control group experienced new sensitivities (Tables 2, $P<0.02$ chi-squared test). The difference between the two groups was highest for onset of new sensitization to *D. pteronyssinus* and *D. farinae* ($P<0.04$).

In addition to seasonal rhinoconjunctivitis, nine (69%) children had pollen asthma before starting SIT compared to eight patients (80%) in the control group. At follow-up, eight years later, only three of the nine patients with previous pollen asthma who received SIT experienced pollen-associated lower respiratory tract symptoms compared to seven out of eight asthma patients in the control group ($P<0.05$, Table 3).

Discussion

This prospective controlled follow-up study has shown that there is still a significant clinical benefit six years after discontinuation of subcutaneous grass pollen immunotherapy in childhood. Scores for all hayfever symptoms, for symptoms plus medication, and for VAS were reduced during the pollen season compared to the control group. As an objective parameter immediate skin response to grass pollen remained decreased when compared to the reaction of the controls. Thirty percent of patients who received SIT no longer required pharmacological treatment during the pollen season. It shows that in most of the hayfever patients SIT does not cure the allergic disease, but it decreases disease

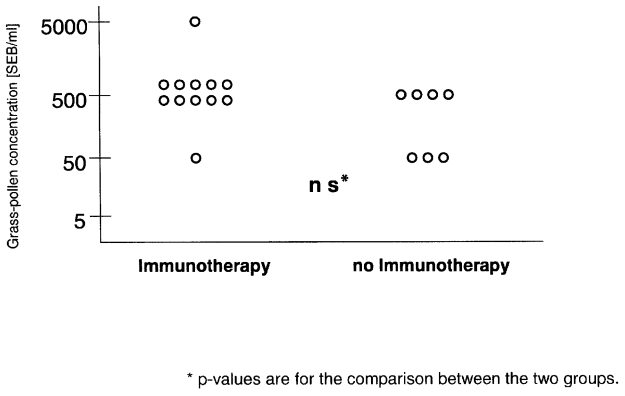

Figure 4. Conjunctival provocation test with grass pollen.

Table 2. Development of new sensitivities from 1989 to 1997

	No. of patients	Percent of patients with new sensitivities to								
		None	*D. pteronyssinus*	*D. farinae*	Cat	Dog	Horse	*Alternaria*	Cow's milk	Egg
SIT group	13	39	15	15	39	0	0	0	0	0
Control group	10	0	40	40	30	10	20	10	0	10

severity and hence has the potential to improve quality of life. This is especially important in children who participate in a lot of outdoor activities in spring and early summer.

A striking finding of this study was the reduced onset of new sensitization to perennial allergens in the SIT group and the better evolution of pollen asthma after three years of preseasonal allergen vaccination. These results suggest that SIT has also the potential to modify the long-term course of the allergic disease with regard to the development of multiple allergies and disease progression.

The selection of patients for SIT is important. We think that the good long-term results in our SIT-group are due to restrictive inclusion criteria of patients with sensitivities to seasonal allergens only, without a history of perennial allergic disease. SIT in children with multiple allergies and sensitization to perennial allergens has been shown to be less effective (15).

Though many studies have confirmed the efficacy of SIT in patients with hayfever, only a few studies exist about its long-term effect (9–14). To our knowledge this is the first controlled follow-up study of previous SIT with a standardized grass pollen extract in children. Two of the studies performed in adults also observed clinical improvement and reduced skin test reactivity six years after discontinuation of three-year SIT with grass pollen (9) and birch pollen, respectively (13). However both studies were not controlled and Mosbach and Osterballe's results may have been biased by an adjustment of the symptom and medication score for the increase in pollen exposure during the follow-up period, assuming that there is a linear relationship between pollen exposure and symptoms. Another controlled study using allergoid (ragweed) in adults showed a maintenance in clinical improvement two years after discontinuation of a five-year duration of SIT (12).

Durham et al. recruited 32 adult patients with severe hayfever symptoms unresponsive to pharmacological treatment. In a double-blind placebo-controlled trial the authors have demonstrated that three to four years of grass pollen SIT remained effective for three years after cessation (14). There was no significant difference when compared to a group of patients who continued SIT. However the population in this elegant study is different from our enrolled subjects in terms of age and disease severity. We chose SIT as an early intervention in children to prevent disease progression.

Our patients with previous SIT maintained decreased immediate skin reactivity to grass pollen compared to the control subjects. There was also a tendency for decreased reactivity to rye pollen (Fig. 3) whereas wheal sizes in response to tree pollen were the same in both groups. Since the applied allergoid extract contained 80% grass and 20% rye pollen only, we speculate that the relatively small amount of sc administered rye pollen was not sufficient to sustain decreased specific skin test reactivity after cessation of SIT.

The immunologic mechanism by which SIT exerts its effect has not been fully determined. Earlier work (16, 17) focused on changes in circulating antibodies (specific IgE and IgG) and effector cells (mast cells and eosinophils). Recent studies suggest that these changes may be secondary to the influence of SIT on allergen-specific T-cell responses. Successful SIT may be associated with a down-regulation of Th2 response (decreased IL-4 and IL-5 production) or increased Th1 response (increased IFN-γ) (19, 20). However neither changes in antibody levels, in effector cells, nor changing patterns of cytokines have been demonstrated to correlate with the clinical response to SIT.

Recent studies have discovered that T-cell immunity is directed by transcription factors (i.e. GATA-3) which have been shown to regulate the production of key cytokines (20). Further studies have to demonstrate if measurement of allergen-specific T-cell transcription factors is an effective way to monitor effectiveness of SIT.

An important finding of the present study is the result of the assessment of the natural course of the allergic disease. Thirty-nine percent of the pollen-sensitized children had not developed any new sensitization eight years after commencement of SIT compared to 0% in the control group. The most prevalent new sensitizations were to cat and house dust mites. The recent study of Des Roches et al. (21) was the first to evaluate the evolution of sensitivities

Table 3. Assessment of the course of pollen asthma from 1989 to 1997

	Percentage of patients with pollen asthma	
	1989	1997
SIT group	69	23
Control group	80	70

$P<0.05$ chi-squared test.

during SIT in children monosensitized to house dust mites. Forty-five percent of their children who received SIT with a standardized *D. pteronyssinus* extract did not develop new sensitization, compared to 0% of previously monosensitized children without SIT in a three-years follow-up survey. Our data correspond very well with their findings and demonstrate that SIT has the potential to modify the long-term course of allergy also in another group of children with early onset of pollen allergy without perennial allergic disease.

Most of our study patients in both groups presented with seasonal asthma at enrolment. The long-term outcome of asthma was significantly better in the group with SIT. This is in accordance with other studies, which showed that more children with allergic asthma have become asymptomatic after SIT compared to controls (22, 23). However it remains unanswered whether early commencement of SIT in children with hayfever suffering from allergic rhinoconjunctivitis only has the potential to decrease the incidence of asthma.

Major drawbacks of our follow-up study are the small number of subjects involved and the fact that it was not double-blind placebo-controlled. A study protocol requiring placebo injection in a randomized group of children over a prolonged time is difficult to justify to parents and ethical committees. However more studies in this age group are needed.

In conclusion this prospective follow-up study six years after discontinuation of SIT in children with seasonal allergic disease showed a prolonged clinical benefit of SIT in comparison to pharmacological treatment only. It demonstrates that SIT in children with monovalent pollen allergy prevents development of new sensitization, reduces prevalence of subsequent asthma and therefore has the potential to modify the natural course of the allergic disease.

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