

Grass pollen immunotherapy: efficacy and safety during a 4-year follow-up study

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S. M. Walker, V. A. Varney, M. Gaga,
M. R. Jacobson, S. R. Durham

Grass pollen immunotherapy is effective, although efficacy must be balanced against side-effects. In a double-blind, placebo-controlled trial of 40 adult patients with summer hay fever, immunotherapy with a depot grass pollen extract (*Phleum pratense*, Alutard SQ) reduced symptoms and medication requirements with an acceptable minimal level of side-effects (31). The original placebo group, as well as the actively treated group, have now received active immunotherapy in an open fashion for a further 3 years. An important question was whether continued injection treatment was accompanied by maintained clinical improvement. By analysis of diary symptoms, rescue medication, and visual analogue scores during the pollen season, we show that efficacy was maintained throughout the 3–4-year study period. Clinical improvement was accompanied by a sustained and marked decrease in immediate conjunctival allergen sensitivity and a further significant decrease in the size of the allergen-induced late cutaneous response. In contrast, an initial decrease in the allergen-induced immediate cutaneous response was not maintained at 3–4 years. Of the patients, 37/40 completed the first year, 33/40 the second year, and 32/40 the third year of treatment. Patients dropped out for reasons other than the outcome of immunotherapy. During a total of 2598 injections, five immediate systemic reactions were observed, all during the induction (not maintenance) phase, and all occurred within 10 min of injection and responded promptly to adrenaline. Grass pollen immunotherapy is effective and safe, provided it is performed on carefully selected patients by trained physicians with immediate access to resuscitative measures.

Key words: grass pollen; immunotherapy.

Dr Stephen R. Durham
Allergy Clinic
Royal Brompton Hospital
Sydney Street
London SW3 6NP
UK

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Several studies using double-blind, placebo-controlled protocols have confirmed the efficacy of grass pollen immunotherapy in patients with pollen allergy (4, 5, 14, 21, 29, 31). We previously reported that with a biologically standardized, partially purified grass pollen extract (*Phleum pratense*, ALK Denmark) grass pollen immunotherapy was effective in patients with severe summer hay fever previously uncontrolled by antiallergic drugs (31). Use of immunotherapy within the UK has declined following a report from the Committee on Safety of Medicines in 1986 which expressed doubt about its efficacy and concern about a number of deaths from severe bronchospasm and anaphylaxis over the preceding 30 years (8). Following a recent review of data from the UK since 1986, current recommendations are that immunotherapy is indicated in patients who develop systemic reactions following bee or wasp stings and in patients with grass pollen hay fever unresponsive

to antiallergic drugs (6, 15). Grass pollen immunotherapy is effective, although the potential for immediate systemic reactions limits its use to specialist centres. Patient selection is extremely important, and patients with chronic perennial asthma should be specifically excluded. An important question is whether continued treatment with immunotherapy is associated with maintained improvement and reduction in allergen sensitivity. In the present study, we report results of efficacy and data relating to adverse events in patients receiving continued monthly maintenance injections of grass pollen extract over a 4-year period.

Material and methods

Patients

Forty patients were selected according to 1) a history of severe summer hay fever; 2) poor symptom

control in previous seasons despite regular anti-allergic treatment which included antihistamines, intranasal corticosteroids, and sodium cromoglycates; and 3) a positive skin prick test (wheal diameter greater than 5 mm) to timothy grass pollen extract (*Phleum pratense*, Soluprick ALK, Denmark) (31). Patients were excluded if they gave an appreciable clinical history of other allergies, had received immunotherapy in the preceding 5 years, or gave a history of cardiovascular or other medical or immunologic diseases. Patients with chronic asthma and all patients with methacholine PC₂₀ (provocation concentration of methacholine that caused a 20% decrease in FEV₁) less than 2 mg/ml were specifically excluded. Patients with mild seasonal asthma were included, provided that their symptoms were controlled by an inhaled beta-sympathomimetic drug. All patients were stratified prior to randomization, as previously reported. Clinical data on the patients are presented in Table 1.

Study design

The study was performed with the approval of the Royal Brompton and National Heart Hospital ethics committee and the patients' written informed consent. The first year of treatment was performed double-blind. During years 2–4, the group of patients who initially received immunotherapy was continued on monthly maintenance injections for 3 years. After the double-blind, placebo-controlled phase of the trial, the original placebo-treated patients also received active treatment with grass pollen immunotherapy during years 2–4. The induction phase was performed with twice weekly injections of Alutard SQ *P. pratense* over 6–8 weeks, increasing to maintenance treatment with 1 ml of a depot

preparation of *P. pratense* (100 000 SQ units) containing 4% aluminium hydroxide. Adjustments in the schedule were made on an individual basis. If patients developed a delayed local reaction greater than 8 × 8 cm, the same dose was repeated at the next attendance. Injections were postponed if the patients were unwell. Maintenance injections were reduced by 40% during the pollen season. The maintenance dose was given once per month.

Local reactions were recorded 30 min after injections, and the patient was asked to record any swelling over the injection site during the following 24 h. All patients were observed for 2 h after injections and asked to record any general symptoms in the following 24 h.

Assessments

Assessments were performed as previously described. All patients recorded daily symptom scores and medication during May through August. The severity of symptoms was assessed on a scale of 0–3 for chest (breathlessness, cough, wheeze, chest tightness), nose (sneeze, blocking, and running), eyes (itching, redness, streaming, and swelling), and mouth and throat symptoms (itch and dryness). Antiallergic drugs in the form of sodium cromoglycate eye-drops and nasal spray were allowed freely along with the short-acting antihistamine acrivastine (8 mg every 6–8 h as required) and the inhaled beta-agonist salbutamol. Topical corticosteroids were not allowed. If symptoms developed that were uncontrolled by the above drugs taken regularly, patients received a 7-day course of oral prednisolone (30 mg for 2 days reducing by 5 mg daily to 0 over 1 week). Diary cards were scored by totalling the symptoms individually for each week, giving a maximum possible score of 21 for each symptom. Drugs were scored as follows: each eye-drop, nasal spray, or inhalation of salbutamol was scored 1, and each acrivastine (8-mg) or prednisolone (5-mg) tablet was scored 2. Results were expressed as an area under curve analysis (for both seasonal symptoms and medication use) during 11 weeks, corresponding to increases in the pollen count (Fig. 1).

During the season, patients were asked at 2-week intervals to indicate their overall hay fever symptoms as a visual analogue score (scale 0–10) in response to the question, "How has your hay fever been this week?"

At the end of the season, patients were asked to make an overall assessment of their hay fever severity compared with previous years (prior to immunotherapy treatment) on a scale from –3 "a lot worse" to +3 "a lot better".

Skin prick tests were performed on the extensor aspect of the forearm with an aqueous extract of

Table 1. Clinical data of patients

	Alutard SQ	Placebo
Number	21	19
Sex		
Female	7	11
Male	14	8
Age (years)	34	37
(range)	(19–51)	(23–52)
Duration of allergy (years)	15.0	13.5
(range)	(2–30)	(4–30)
* Severity score A ("extremely hypersensitive")	9	9
* Severity score B ("very hypersensitive")	12	10

* Severity score A = skin prick test > 16 mm; positive conjunctival provocation test at > 300 BU/ml; history of symptoms for > 10 weeks associated with asthma and previous oral and/or injected corticosteroids.

* Severity score B = patients with moderate to severe hay fever who did not fulfil all of the above criteria.

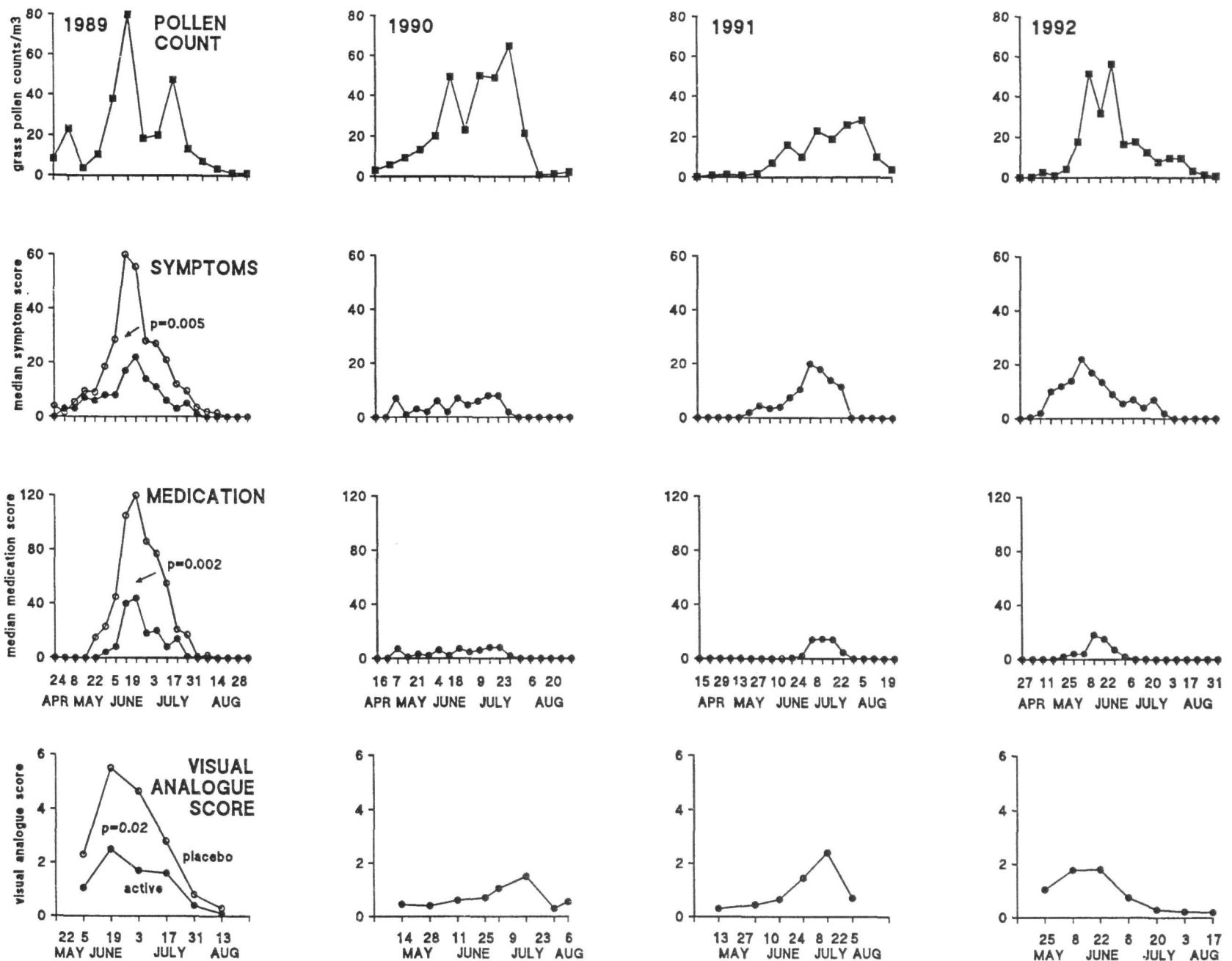


Fig. 1. Average weekly grass pollen count (1989–92), symptom and medication scores, and visual analogue scores (scale 0–10). Median values for immunotherapy-treated patients ($n = 21$) and placebo-treated patients ($n = 19$) during 1989 are shown. In subsequent years, values represent data for combined groups of patients who received active immunotherapy (closed circles).

P. pratense (Aquagen SQ; ALK, Denmark). Tests were performed during the winter months each year. The size of the immediate (15-min) and late (24-h) skin responses to 30 biological units (BU) (in 0.02 ml) and diluent (0.02 ml) was assessed. Reaction sizes were recorded in two dimensions, from which the mean diameter was calculated.

Conjunctival tests were performed according to the method of Moller et al. (23). Half-log incremental concentrations from 10 to 100 000 BU/ml *P. pratense* were applied (one drop) to alternate eyes at 10-min intervals. The control test was performed with Aquagen diluent (0.03% human serum albumin, 0.5% phenol in 0.9% sodium chloride).

Pollen counts were provided courtesy of Dr Jean Emberlin (Polytechnic of North London, London, UK) by use of a Burkard spore trap.

Analysis of results

Results were analysed using a statistical package (Minitab). Results for the whole group (original placebo and actively treated groups combined) during the years 1990, 1991, and 1992 were compared to data from the original placebo-treated group during 1989 (year 1) by use of unpaired analysis with the Mann-Whitney U test.

Results

Clinical efficacy

Results for the double-blind, placebo-controlled phase of the study (1989) have been reported previously (31). These data together with the combined data for symptoms, medication use, and visual analogue scores for both groups of patients treated with

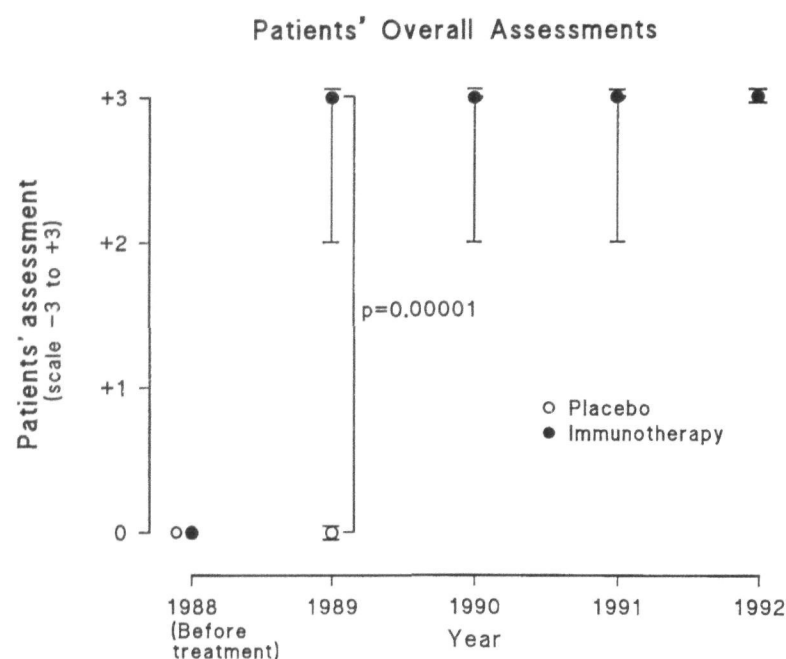


Fig. 2. Postseasonal assessments (median values $\pm$ interquartile ranges) by patients on scale from -3 ("a lot worse") to $+3$ ("a lot better").

active immunotherapy for the years 1990 through 1992 were compared and related to seasonal pollen counts (Fig. 1). During year 1, there was a highly significant reduction in symptoms ($P = 0.001$), medication ($P = 0.002$), and visual analogue scores ($P = 0.02$) for immunotherapy-treated compared with placebo-treated patients. This clinical improvement was maintained throughout the 4-year study period. Median symptom and medication scores and visual analogue scores corresponding to 11 weeks during the peak season during the 4 years 1989–1992 were compared using an area under curve analysis (Fig. 1; Table 2). When data for all patients (original placebo- and immunotherapy-treated patients

combined) during years 2, 3, and 4 were compared with data from placebo-treated patients during year 1, symptoms and medication scores and visual analogue scores remained significantly low (all $P < 0.001$, Table 2). There was a consistent reduction in visual analogue scores and a striking reduction in medication requirements. There was some variability in median symptom scores, although these also remained low compared with the original placebo-treated group in 1989.

When patients were asked, "How has your hay fever been this year compared with previous years before treatment?" in 1989, there was a highly significant difference, over 50% of patients being very much better ($+3$) ($P = 0.00001$). This result was maintained for both groups of patients during years 2, 3, and 4.

Provocation tests

Immediate and late-phase skin responses were assessed before treatment and during the winter months 1988–92 (Figs. 3 and 4; Table 2). As previously reported, when Alutard SQ- and placebo-treated patients were compared after 9 months' treatment, there was a significant reduction in the size of the early response, and in particular the late-phase response, after intradermal injection of 30 BU grass pollen extract. For the early response, these differences were not maintained during years 3 and 4 when values were similar to those observed before and after treatment in the placebo-treated group during 1989. In contrast, the highly significant reduction in the size of the late-phase response was maintained with a further reduction in the size of the late skin

Table 2. Summary of clinical data and provocation tests during 4-year study. Results represent median values (area under curve analysis for 11 weeks during grass pollen season) and 95% confidence intervals

Outcome measure		1989			1990	1991	1992
Total symptoms	Placebo	291.5	(227.1, 386.3)	All patients	82.0 (28.0, 131.5)	89.0 (42.2, 174.1)	126.8 (50.1, 87.1)
	Active	125.5	(59.2, 184.4)				
Total medication	Placebo	607.0	(362.1, 832.1)	All patients	48.8 (14, 87)	45.0 (21.0, 142.8)	53.5 (20.3, 212)
	Active	128.0	(100.1, 313.5)				
Visual analogue scores (scale 1–10)	Placebo	5.5	(3.6, 6.0)	All patients	1.1 (0.4, 1.7)	0.7 (0.3, 1.1)	1.8 (0.6, 2.3)
	Active	2.5	(1.0, 4.5)				
Patients' overall assessments (−3 to +3)	Placebo	0.0	(0.0, 0.0)	All patients	+3.0 (2.0, 3.0)	+3.0 (3.0, 3.0)	+3.0 (3.0, 3.0)
	Active	+3.0	(2.0, 3.0)				
Skin tests responses (mm)							
Early (15 min)	Placebo	17.0	(15.1, 20.5)	All patients	15.0 (13.5, 16.6)	19.0 (18, 20.5)	18.5 (16.6, 20.0)
	Active	11.25	(10.5, 15.1)				
Late (24 h)	Placebo	66.3	(56.7, 74.4)	All patients	22.0 (16.8, 23.8)	Not done	7.0 (0.0, 11.4)
	Active	25.2	(18.4, 31.03)				
Conjunctival Immediate allergen sensitivity BU/(ml)	Placebo	1000	(561, 1000)	All patients	30 000 (10 000, 100 000)	10 000 (10 000, 100 000)	30 000 (26 685, 100 000)
	Active	30 000	(10 000, 30 000)				

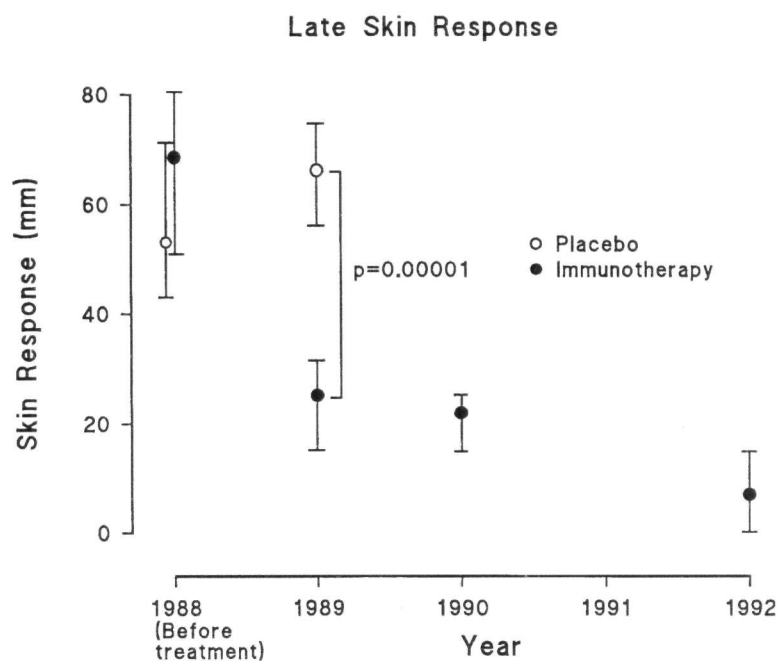


Fig. 3. Size of late skin response (median values $\pm$ interquartile ranges) 24 h after intradermal injection of grass pollen extract in placebo (open circles)- and immunotherapy (closed circles)-treated patients. Measurement of late skin response was omitted in 1991.

response when results for 1992 were compared with those for 1990 ($P = 0.00001$). Although the decrease in the late response paralleled clinical improvement, there was no significant correlation between the size of the late response (or the magnitude of change in the late response) and seasonal symptoms or medication requirements during the study period.

Immediate conjunctival allergen sensitivity was assessed after local application of increasing concentrations of grass pollen extract. The highly

significant 1–1.5 log increment in grass pollen concentration required to provoke an immediate conjunctival response after the first year was maintained throughout the study period ($P = 0.00001$ during years 2, 3, and 4 when immunotherapy-treated patients were compared with the results for the original placebo group).

Drop-outs

In general, patients dropped out for reasons unrelated to the clinical protocol. During the first year, three patients (all placebo-treated) dropped out before the pollen season. One developed a systemic reaction to a placebo injection, and one moved away from London. The third patient's work commitments changed (to a night shift), preventing attendance for injections. During year 2, there were four drop-outs. Two patients emigrated, and one declined further attendance owing to the distances involved and commitment to a young family. The fourth patient developed inflammatory bowel disease which was considered to be unrelated to immunotherapy. During year 3, one patient withdrew for social reasons.

Safety

Adverse events which occurred during induction and maintenance therapy during the 4-year study are presented in Table 3. A total of 2598 injections were given. Five immediate systemic reactions were confined to the induction phase of treatment. No systemic reactions were observed during maintenance

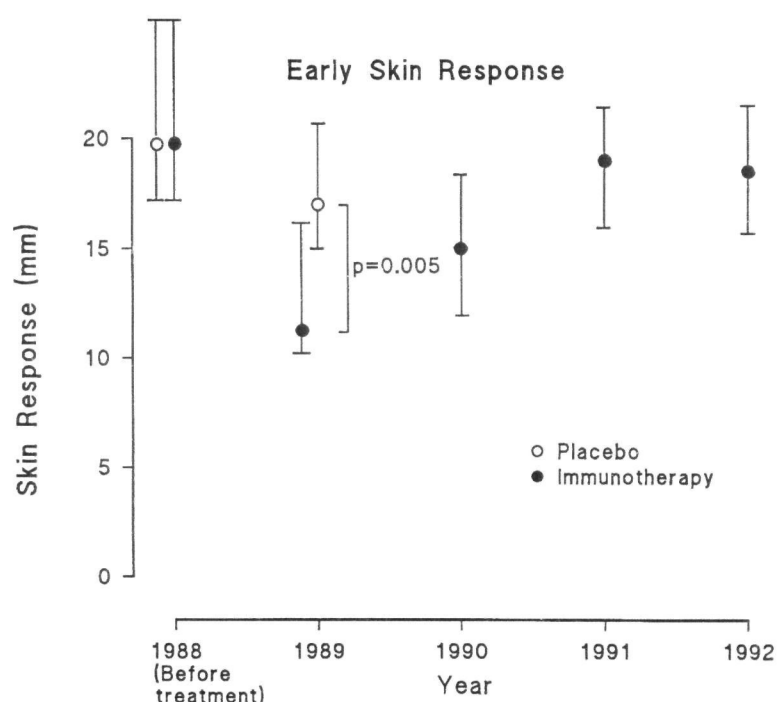


Fig. 4. Size of early skin response (median values $\pm$ interquartile ranges) 15 min after intradermal grass pollen extract in placebo (open circles)- and immunotherapy (closed circles)-treated patients.

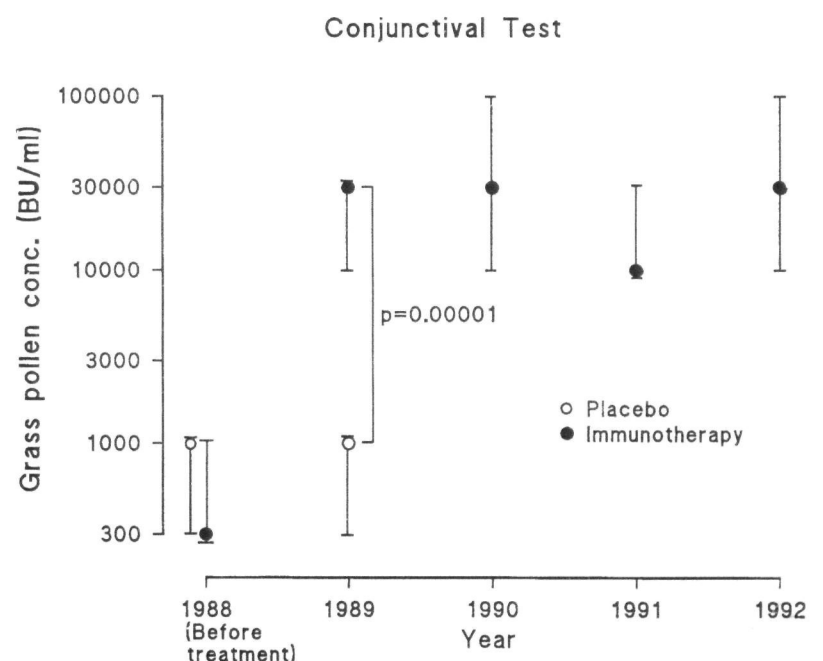


Fig. 5. Immediate conjunctival allergen sensitivity (15 min after topical grass pollen extract) in placebo (open circles)- and immunotherapy (closed circles)-treated patients. Results refer to lowest concentration of grass pollen that provoked redness and conjunctival swelling at 15 min.

Table 3. Adverse events (April 1989–February 1993). Results for “induction” (i.e., up dosing phase of study) are presented separate from these during monthly maintenance injection treatment

	Induction	Maintenance	Total
No. of injections	1121	1477	2598
Immediate systemic	5	0	5
Delayed mild systemic (> 2.5 h)	6	0	6
Large local reactions (> 8 × 8 cm)	28	6	34

Table 4. Details of immediate systemic reactions and treatment received. Immediate systemic reactions were confined to induction phase and occurred within 20 min of injection

Patient	Time after immunotherapy (min)	Symptoms	Treatment
AM	10	Flushing, chest tightness, PEFR 550	Adrenaline 1:1000 Oxygen
HD	5	Itching, pallor, nausea, bradycardia, hypotension	Adrenaline 1:1000 Oxygen, IV fluids
MB	20	Flushing, dry throat, agitation	No treatment
SK	20	Itching, flushing	Adrenaline 1:1000
MB	2	Itching, flushing	Adrenaline 1:1000

treatment. Large local reactions were also more frequent during induction treatment. They were not troublesome, and no patients required specific treatment for local reactions. Details of symptoms and treatment given following the five immediate systemic reactions are summarized in Table 4. All occurred within 20 min of injection and responded promptly to treatment. None of the immediate reactions were due to dosage errors. In retrospect, there were no obvious precipitating triggers except that the reactions occurred during the usual up dosing regimen of immunotherapy. Delayed systemic reactions (occurring 1 h after injection) were minor and took the form of itching, rhinitis, sneezing, or eye symptoms. Reactions occurred within 2.5–24 h of injection. These reactions did not require treatment other than antihistamines, although one patient who developed urticaria at 2.5 h was given adrenaline as a precaution.

Discussion

In adult patients with severe summer hay fever, we have shown that clinical improvement with grass pollen immunotherapy was maintained during 3–4 years of continued monthly injections. We observed marked reductions in symptoms, medication require-

ments, and visual analogue scores, which were accompanied by a very marked decrease in immediate (15-min) conjunctival allergen sensitivity and in the size of the late (24-h) skin response. The decrease in conjunctival sensitivity was maintained, whereas a further highly significant decrease in the size of the late skin response was observed between years 2, 3, and 4. Immediate systemic reactions occurred in only five of 2598 allergen injections, were confined to the induction-phase of immunotherapy, occurred within 20 min, and responded promptly to adrenaline. Despite the patients' previous failure to respond to antiallergic drugs, they considered immunotherapy both highly effective and acceptable.

In view of the clinical response after the first year, we considered that it was unethical to continue placebo injections for years 2–4, during which all patients received active treatment. None the less, comparisons were possible for clinical and provocation test results for the whole group compared with the placebo group during the first year. Furthermore, the time course of symptoms, medication, and visual analogue scores paralleled the pollen counts (i.e., exhibited dose-response characteristics) and were accompanied by similar very marked and sustained changes in objective tests, including skin and conjunctival provocation tests. In view of the absence of continued placebo measurements, statistical analysis was kept to a minimum, and descriptive data were presented as a time course (Fig. 1) and as median and 95% confidence intervals (Table 2) in order to convey a measure of the scatter of the data.

A striking finding in this study was the decrease in the size of the late skin response at 24 h after intradermal grass pollen. Inhibition of the late response after immunotherapy has previously been shown (13, 30), although this is the first report of a prolonged and continued reduction over a time course of years which paralleled clinical improvement. This raises the possibility that inhibition of the late skin response may represent an early clinical marker of response to immunotherapy. In this regard, we did not find a significant relationship between the size of the late response (or the magnitude of decrease after immunotherapy) and seasonal symptoms or medication requirements. However, in this selected group of patients with severe disease, the large majority experienced substantial improvement in symptoms and a marked reduction in the late response such that there may not have been adequate “scatter” of data to detect a significant correlation. Further studies, possibly in patients representing a wider spectrum of hay fever severity, are required to examine the relation between the late skin response and the clinical efficacy of immunotherapy.

The reason for the return of immediate skin test reactivity after the first year with continued treatment during years 2–4 is unclear. The observation is consistent with clinical experience which indicates a variable relationship between the size of the immediate skin wheal and clinical symptoms. In a previous report (33), by use of immunohistology and *in situ* hybridization studies of cutaneous biopsies in the same group of patients, we showed that there was a significant decrease in mast-cell numbers after 9 months' treatment. After allergen provocation, we identified a marked reduction in CD4+ T lymphocytes and a trend for reduction in eosinophils. *In situ* hybridization demonstrated no change in the so-called TH2-type cytokines, interleukin-4 and interleukin-5, which are characteristically associated with late cutaneous responses, whereas an increase in the so-called TH1-type cytokines (interleukin-2 and interferon-gamma) was observed at 24 h after challenge following successful immunotherapy. We postulated that suppression of the immediate skin response may occur as a consequence of a reduction in mast-cell numbers, whereas suppression of the late response results from modification of the T-lymphocyte response to allergen after successful immunotherapy. Late-phase skin responses are more relevant to clinical disease. For example, subjects who develop late-phase skin responses to small doses of allergen are those who develop late-phase responses to bronchial provocation challenge (2). Corticosteroids also inhibit late nasal responses with less effect on the immediate response to allergen challenge (1, 2, 9, 11, 22, 26, 28). For these reasons, alterations in the late skin response (and associated modification of T lymphocytes) seem likely to underlie the mechanism of successful immunotherapy, in contrast to early changes in mast-cell numbers, which are not sustained during prolonged treatment and which, in contrast to the late response, do not parallel clinical improvement.

A major objective of this follow-up study in patients with rhinitis was to document the risk or benefit and side-effects of grass pollen immunotherapy. Local side-effects were minimal and required no specific treatment. There was an acceptably low prevalence of systemic reactions, which were confined to the induction phase. Maintenance monthly injections over a 3–4-year period proved highly acceptable, and no further systemic reactions were observed. It is important that immunotherapy be performed by trained persons with immediate access to resuscitative equipment. Patients should be observed after injections. The current recommended minimum period in the UK is 60 min (6, 9), whereas European guidelines state that 30-min observation is adequate, provided the patient remains asymptomatic (21).

Several previous controlled trials have confirmed the efficacy and tolerance of pollen immunotherapy for grasses (4, 5, 14, 29, 31), ragweed (34), tree pollens (35), and moulds (20). Biologically standardized extracts for mould immunotherapy are currently not available in the UK. In contrast, few studies of the long-term effects of pollen immunotherapy have been performed. In a 14-year follow-up study which involved unstandardized extracts in children, Johnstone et al. demonstrated a prolonged effect, lower numbers of children developing asthma compared to an untreated control group (17). Although of interest, a whole range of different allergen extracts, including bacterial enzymes and unstandardized preparations, was employed. In a further study, clinical improvement was observed for up to 6 years in patients treated with grass pollen extract (24). However, the efficacy of the extract was not verified under placebo-controlled conditions before initiating prolonged treatment. In mite-sensitive asthmatic children, immunotherapy was effective in improving symptoms and inhibiting the late asthmatic response (34). However, withdrawal of treatment was accompanied by a recurrence of symptoms within 1 year (27). This is in contrast to the present study, which employed high-dose prolonged therapy with a potent, biologically standardized depot preparation in grass pollen-sensitive patients.

Our results suggest that grass pollen immunotherapy is a viable alternative in patients with severe summer hay fever unresponsive to antiallergic drugs (16). Further studies are required in order to assess the role of immunotherapy in mild to moderate disease. Double-blind comparator studies involving topical corticosteroids and immunotherapy would also be of interest. A single previous report (18) which favoured topical corticosteroids employed a low potency extract, and few patients achieved effective maintenance treatment. Of more interest, "add on" studies in which either immunotherapy is added to topical corticosteroids or, conversely, topical corticosteroids are added to a pre-existing immunotherapy regimen would yield valuable information. It is also important to establish whether withdrawal of immunotherapy is associated with prolonged clinical improvement. Three recent reports of immunotherapy for patients with perennial rhinitis and house-dust mite sensitivity demonstrated clinical improvement, although further studies are required (3, 10, 12). It is hoped that in future studies the use of nonstimulatory peptides (peptide fragments of allergens representing T-lymphocyte epitopes) may induce prolonged altered T-lymphocyte reactivity and clinical improvement without side-effects of IgE-associated, mast-cell-mediated untoward reactions (7). Finally, it is essential that immunotherapy is performed by trained

doctors with ready access to resuscitative equipment. Continuous safety monitoring and reporting of untoward effects are essential in the development of novel forms of immunotherapy (25).

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