

Original article

A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections

Background: We present data showing that a Th1-inducing adjuvant can reduce the number of injections required for allergy vaccination. Allergy vaccination is the only treatment for type 1 hypersensitivity that can alter the underlying disease process. A switch of specific T-cell activity from Th2 > Th1 to Th1 > Th2 is believed to be an important change seen after long-term vaccination therapy. An immunologic adjuvant that enhances such a switch could be used to reduce the number of injections required. This would improve compliance with the treatment and provide pharmacoeconomic advantages. Such an adjuvant is 3-deacylated monophosphoryl lipid A (MPL[®] adjuvant, Corixa).

Methods: A multicentre, placebo-controlled, randomized, double-blind clinical study was performed with a new standardized allergy vaccine comprising a tyrosine-adsorbed glutaraldehyde-modified grass pollen extract containing MPL[®] adjuvant. Four subcutaneous injections of the active product were given preseasonally to 81 grass pollen-sensitive subjects, and 60 received placebo injections (tyrosine alone). Diary cards were used to record symptoms and medication taken during approximately 30 days of the grass pollen season.

Results: There was a statistical advantage in favour of the active treatment for nasal ($P=0.016$) and ocular ($P=0.003$) symptoms and combined symptom and medication scores ($P=0.013$). Titrated skin prick testing revealed a significant reduction of skin sensitivity in the active group compared to placebo ($P=0.04$). Grass-pollen-specific IgG antibody was raised by active treatment ($P<0.01$). A rise in IgE antibody was seen in the placebo group during the season ($P<0.01$). The first year's treatment rise of IgE was not seen in the active group, and no rise occurred during the pollen season. More local adverse events were seen in the active group. There was no difference in generalized adverse events.

Conclusions: A new, well-tolerated allergy vaccine, incorporating a Th1-inducing adjuvant, MPL[®], was efficacious and after only four preseasonal injections produced antibody changes normally associated with long injection schedules. This may encourage wider application of allergy vaccination. The vaccine is now available in a number of countries as Pollinex Quattro[®].

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Preseasonal (1) and coseasonal (2) specific allergy vaccinations (AV) have been proven to ameliorate the symptoms of respiratory allergy in a number of well-controlled clinical studies, and both are accepted as valid means of treatment in the WHO position paper on allergy vaccination (3).

It is recognized that AV is able to modify the underlying disease process in allergy rather than just treating the symptoms, as is currently true of most antiallergy drugs and possibly also of certain other new forms of treatment currently being developed (4). It has also been shown that AV can bring about long-term

benefits after several years of treatment without the need for further allergy vaccination (5) and can prevent the progression from a relatively mild form of the disease, such as rhinoconjunctivitis, to asthma if used early in the onset of disease (6). There is a pharmacoeconomic advantage of AV, even with long courses of treatment (7). Thus, it is likely that a short course of AV such as we describe would be particularly advantageous.

Considering all these benefits, it is surprising that the treatment of respiratory allergy by AV is not employed to a greater extent. Perceptions of the potential side-

effects and lack of efficacy of older vaccines may persist. These doubts may be countered by the availability of modern, standardized vaccines, particularly when administered by suitably trained clinicians. Regarding safety, the risk of a fatal systemic reaction from immunotherapy is now regarded as extremely small (3). The length of injection regimens may inhibit more frequent application; therefore, inducements to extend the use of AV would be to incorporate shorter injection regimens and also to enhance safety and efficacy profiles.

There is no total consensus regarding the mechanism of successful AV apart from the view that there is a modulation of the activity of T helper cells. One hypothesis of the essential events is that there is an induction of a tolerizing effect or anergy of allergen-specific T cells (8) possibly via the induction of IL-10, which may also downregulate mast-cell activity. Another possibility is that there is a switch, changing cytokine profiles of allergen-specific T cells from a more Th2-like to a more Th1-like profile, and leading to downregulation of the late-phase reaction, associated inflammation, and an eventual reduction in IgE antibody (9). We speculated that if a means were developed of enhancing this switch, for example by the addition of an adjuvant known to direct a Th1 response, this might potentially increase the efficacy of AV.

A glycolipid adjuvant derived from the cell walls of *Salmonella minnesota* has been purified and detoxified (10). This new adjuvant, 3-deacylated monophosphoryl lipid A (MPL[®] adjuvant), has recently been shown in both animal (11, 12) and human (13, 14) studies to induce Th1-like cytokine profiles. Many studies involving MPL[®] have demonstrated a lack of toxic effects, and this adjuvant was found to be well tolerated in different populations for the treatment of infectious diseases (15–17) and cancer (18). It is a component of a new therapeutic vaccine for the treatment of melanoma (Melacine[®] vaccine, Corixa, Inc./Schering Plough) (18), which recently received marketing authorization in Canada.

It follows that addition of MPL[®] adjuvant might enhance the effectiveness of AV, and this improvement could be exploited to provide shorter courses than those currently used. It was thus necessary to use a formulation in which the top dose of injected allergen extract was achieved very quickly in a few doses; accordingly, a modified allergen extract was used.

The allergy vaccine studied in this communication incorporated a partially purified and standardized grass pollen extract that had been treated with glutaraldehyde, thus rendering it safer to use because of a reduced reactivity with IgE antibody (19). Other properties, such as induction of specific IgG (20, 21) and T-cell reactivity (22), are not similarly reduced by modification. The natural amino acid L-tyrosine, which is very sparing in solubility (23), was employed as a depot base

to adsorb the allergoid and otherwise soluble MPL[®] adjuvant. The top dose of major group 1 allergens to be administered in the third and fourth injections was the equivalent of 24 µg. The product was manufactured in an audited Good Manufacturing Practice (GMP) facility and controlled and standardized as required by the regulatory authorities (24–26).

Extensive toxicology studies were performed as agreed with a regulatory agency, the Paul Ehrlich Institute; these studies showed no abnormalities preventing use of the new product in clinical trials. Phase 1 and 2 clinical studies were completed successfully, and the ethics committees agreed that there were no adverse events that would prevent further evaluation in phase 3, and hence gave approval to carry out the study. This was a multi-centre, randomized, double-blind, placebo-controlled, phase 3 study carried out in Germany and Austria in 1999. The study was performed according to GCP principles and the Declaration of Helsinki.

Material and methods

Subjects

Patients were selected from 13 study centres in Germany and one in Austria. Suitable subjects were randomized in the proportions of active:placebo 2:1 in Germany and 1:1 in Austria, according to the requirements of local ethical review committees. The selection criteria were as follows: men or women aged 18–60 years; patients with relevant symptoms of seasonal allergic rhinitis who had not received previous AV therapy; a positive skin prick test wheal of >3 mm in diameter (when challenged using a mixture of pollens from 12 temperate-zone grasses, with Allergy Therapeutics Limited [ATL] B2 grass mixture and *Secale cereale* prick test solutions), and a positive RAST or equivalent test (≥ class 2) to grasses and *S. cereale*. Subjects sensitized to perennial allergens were excluded if they had perennial symptoms or showed symptoms during exposure. In most cases, the connection between exposure and symptoms was evident from their clinical history; however, if this was not clear, a provocation test was performed. Also excluded were those with contraindications for AV, disturbances of tyrosine metabolism, and atopic dermatitis and other skin diseases which could influence the skin test results.

In total, 141 patients were treated and evaluated for safety. A computer-generated randomization code was used to allocate the active treatment to 81 subjects and the placebo to 60 subjects. Of these 141 patients, the number that could be appropriately analysed for efficacy was 124. Of the 17 subjects not evaluated for efficacy, there was one dropout due to treatment. After the first injection (active), a female patient experienced hypotension, which was treated with ephedrine, and a full recovery followed. Without knowing which group she was in, the investigator decided to withdraw her for psychological reasons. The other subjects were not evaluated owing to nondelivery of diary cards, moving to another geographic area, or spending summer holidays in a noncomparable area. Thus, the efficacy analysis was based on the 124 patients, comprising 74 receiving active treatment and 50 on placebo.

Products

The allergy vaccine contained semipurified glutaraldehyde-modified extracts from a mixture of pollens from 12 temperate zone grasses (B2) and *S. cereale* (cultivated rye). These were adsorbed to L-tyrosine (2%) essentially as described previously (23). The MPL

adjuvant (10) was also adsorbed to the L-tyrosine at 50 µg/ml in all vaccine dose levels. The product was standardized according to European requirements (25, 26). The active product was presented as three strengths of modified allergen extract: 300, 800, and 2000 standardized units (SU)/ml. An amount of 2000 SU is approximately equivalent to 24 µg of group 1 grass pollen allergen (19, 24). Thus, the cumulative dose from the course was approximately 60 µg group 1 grass pollen allergen.

The placebo injections consisted of L-tyrosine (2% w/v) in excipient solution.

Study design and dosing

- The trial was carried out as a randomized, double-blind, placebo-controlled multicentre study.
- The first three 1.0-ml increasing strength injections were given subcutaneously in the middle third of the posterior aspect of the upper arm at 1-week intervals. An interval of 1–4 weeks was permitted before the last top-strength injection.
- The last injection was given approximately 2–4 weeks before the start of the main grass pollen season.
- Subjects were studied during 1 month of the main grass pollen season.
- Subjects filled in diary cards daily according to a standard system. This consisted of recording whether the symptoms from the eyes, nose, and lungs that day scored none (0), mild (1), moderate (2), or severe (3).
- The rescue medications used were provided and were those used typically to control seasonal allergic rhinoconjunctivitis. These included local and systemic antihistamines, bronchodilators, topical steroids, and cromoglycates; some medications were in combination. Systemic corticosteroids and long-acting antihistamines were excluded. A medication scoring system allocated one point per organ treated; for example, for an oral antihistamine, both nose and eyes would be treated, giving a score of 2 points. For the combined symptom/medication score, the medication was equated to a further symptom. Since randomization was successful, inequalities would balance out.
- Blood samples were taken before treatment, after treatment, in the middle of the assessment period, and after the pollen season. Serum samples were prepared and assayed at a central laboratory.

Skin prick titration

A titrated skin prick test was performed before treatment and 2 weeks after the last injection with ten 1:2 serial dilutions of a standardized grass-pollen extract (B2 grasses, ATL). Tests were performed in duplicate on the volar aspect of both forearms. Histamine 0.1% and dilution medium served as positive control and negative control, respectively. Both threshold concentrations (visible wheal elicited, larger than negative control) and total wheal areas were recorded by applying an adhesive tape over the test area and outlining the wheal with a fine-tip pen. Data were evaluated by computerized planimetry.

Antibody assays

Serum samples were analysed for allergen-specific antibodies at a central laboratory by a liquid-phase immunoassay system with biotin/avidin quantification (Diagnostic Products Corporation AlaSTAT) (27). Specific IgE and specific IgG antibodies against a grass pollen mixture and *S. cereale* were determined.

Pollen counts

Atmospheric grass pollen counts for each day were provided by Deutscher Pollenflug Informationsdienst and AKH Vienna. The pollen traps were located near the study centres.

Data analysis and statistics

All data were analysed by an independent statistician. An analysis plan was agreed before unblinding, and the combined symptom/medication score from the patients' diary cards was considered the main target variable for efficacy. As the graphical data inspection showed asymmetric distributions and therefore median values which remarkably differed from related mean values, a nonparametric approach was chosen, with medians as primary descriptive statistics together with suitable significance tests (Wilcoxon signed rank test, Wilcoxon two-sample test). Group comparisons were performed with stratified test procedures (Wilcoxon two-sample test with stratification over study centres = Cochran-Mantel-Haenszel test).

All significance tests were performed as two-sided tests on an explorative basis with a type I error alpha of 0.05.

Results

Proof of randomization

It was shown statistically that there were no pretreatment differences between groups in demographic variables, age at symptom onset, duration of disease, symptom type and severity, other sensitivities, RAST class, skin prick test sensitivity to grass pollen, medications taken during the previous year, and time off work due to allergy. Table 1 shows the demographic variables, and Table 2 the distribution of organ-related symptoms recorded from the previous pollen season.

Skin sensitivity

There were changes in skin test activity recorded before and after therapy. Activity was significantly reduced in the active compared with placebo group for both the threshold values ($P=0.03$, stratified Wilcoxon two-sample test) and the total wheal areas ($P=0.04$,

Table 1. Patient demography: sex, age, height, and weight

		Placebo group	Active group	Significance value
Sex	F	23	34	$P=0.99^a$
	M	27	40	$P=0.99^b$
Age (years)	Mean $\pm$ SD	27.4 ± 7.8	29.6 ± 8.4	$P=0.13^c$
	Median	25.5	27	$P=0.24^d$
	(Minimum, maximum)	[18, 56]	[19, 54]	
Height (cm)	Mean $\pm$ SD	174.8 ± 9.0	175.7 ± 9.5	$P=0.75^c$
	Median	176	175.5	$P=0.67^d$
	(Minimum, maximum)	[143, 193]	[154, 202]	
Weight (kg)	Mean $\pm$ SD	70.8 ± 13.1	69.5 ± 13.9	$P=0.52^c$
	Median	72	68.5	$P=0.30^d$
	(Minimum, maximum)	[48, 100]	[45, 110]	

a: Fisher's exact test, b: Mantel-Haenszel test, c: Wilcoxon two-sample test, d: stratified Wilcoxon two-sample test. Numbers: active $n=74$, placebo $n=50$.

Table 2. Distribution of organ-related symptoms (average of previous pollen season)

Intensity of symptoms	Eyes		Nose		Lungs	
	Placebo no. of patients	Active no. of patients	Placebo no. of patients	Active no. of patients	Placebo no. of patients	Active no. of patients
No	1	—	—	—	25	42
Mild	8	17	2	4	16	21
Moderate	22	37	23	36	7	6
Severe	19	20	25	34	2	5
Total	50	74	50	74	50	74
Wilcoxon two-sample test	$P=0.24$		$P=0.63$		$P=0.50$	
Stratified Wilcoxon two-sample test	$P=0.50$		$P=0.91$		$P=0.97$	

stratified Wilcoxon two-sample test). The mean wheal areas of both groups before and after therapy are shown as a bar chart in Fig. 1.

Symptoms and medications

Table 3 shows the significant differences apparent between the two subject groups. The symptoms recorded for both eyes and nose were significantly fewer in the active group. This was not the case for symptoms in the lungs, probably because very few subjects recorded such symptoms. The lower medication scores in the active group did not reach significance but are shown in Fig. 2. All combinations of symptom scores and medication scores were significantly improved in the active over the placebo groups, as illustrated graphically in Fig. 3. The pollen counts are represented on all figures.

The effect size (Table 3) was found to be moderate to large for the symptom score and the combined score.

Antibody assays

As might be expected, the blood levels of pollen-specific IgG antibody were raised significantly ($P \leq 0.01$ for all data points except baseline) by active treatment, and another such rise was seen at the middle of the

assessment period (Fig. 4). However, the active treatment did not increase levels of grass-pollen-specific IgE antibody (Fig. 5). In the placebo group, at the middle of the assessment period, a rise was found which was not seen in the active group ($P=0.002$). Slight falls were observed in both groups after the end of the pollen season.

Taking the median, specific IgG was over 300% in the active group compared to baseline. At all time points after treatment, the IgG/IgE ratio was in favour of the active group, and the same was apparent for differences from the baseline ($P < 0.01$, stratified Wilcoxon two-sample test).

Tolerance and safety

All adverse events (AE) reported by the patients or observed by the investigators were recorded.

Local reactions. Local reactions (LR) were defined as redness/swelling (> 5 cm in diameter) and/or pain and/or itching at the injection site. As expected, LR were more frequent in the active group (Table 4). None of these reactions needed treatment beyond physical measures such as cooling. All reactions

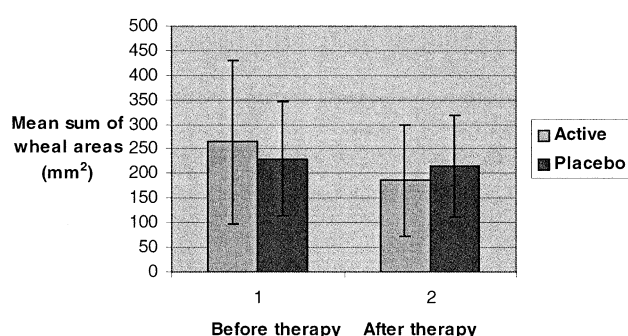


Figure 1. Titrated skin prick test of active and placebo patients. Bars indicate mean sum of wheal areas (mm^2) of subjects before and after treatment with immunotherapy or placebo. Numbers: active $n=74$, placebo $n=50$. Significance (stratified Wilcoxon two-sample test): Baseline (before therapy) $P=0.41$. After therapy, comparing to baseline $P=0.04$. Dispersion bars indicate standard deviation.

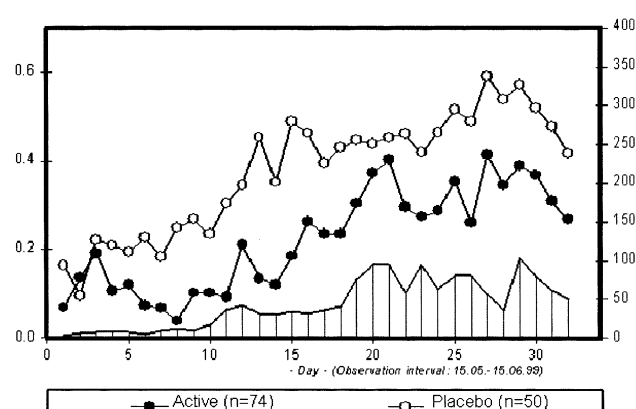


Figure 2. Median medication scores of actively treated and placebo patients. Observation days are shown on x-axis. Medication scores (left y-axis) are indicated by black spots for actively treated patients and white spots for those on placebo. Pollen count (right y-axis) is given as bars corresponding to daily mean (count/m^3) of all pollen traps.

Table 3. Comparison of patients treated with active agent and those receiving placebo. Statistical results for organ-related symptoms, use of medication, and combined symptom/medication scoring. Patient numbers: $n=74$ (active treatment); $n=50$ (placebo)

Significance level	Eyes $P=0.003$		Nose $P=0.016$		Medication $P=0.28$		Eyes, nose, and lungs $P=0.003$		Eyes, nose, lungs, and medication $P=0.013$	
	Placebo	Active	Placebo	Active	Placebo	Active	Placebo	Active	Placebo	Active
Mean	1.12	0.82	1.46	1.21	0.71	0.54	0.95	0.75	0.83	0.65
$\pm$ SD	0.52	0.58	0.51	0.65	0.77	0.71	0.41	0.44	0.47	0.48
Median	1.13	0.71	1.43	1.09	0.34	0.23	0.9	0.65	0.71	0.54
Difference of medians							-28%		-24%	
Effect size							-0.46		-0.38	
95% Confidence limits of effect size							-0.10	-0.83	-0.01	-0.74

disappeared within a few days. The intensity and frequency of local reactions were found by the investigators to be comparable to those resulting from SIT with other products.

Systemic reactions. Systemic reactions (SR) consisted mainly of mild symptoms of rhinoconjunctivitis (Table 5) and were equally distributed between the groups. No serious or severe SR occurred.

In four patients from the active group, the third dose was reduced to 0.4 ml due to a local reaction after the second dose. The subsequent dose increase was without problems. One patient from the placebo group reported tinnitus 3 days after the second dose. The third dose was reduced to 0.15 ml and further treatment proceeded without adverse events.

Other adverse events assessed as unlikely to be related or unrelated to treatment were also evenly distributed between the groups.

Discussion

Although allergy vaccination is considered by many allergists to be the only means of treating allergic

disease that can modify the underlying pathologic mechanisms, this form of treatment is used relatively infrequently, as described in the introduction. There is a need for a vaccine formulation which provides improved efficacy with a short injection schedule; therefore, the choice of an effective adjuvant is of paramount importance in the pursuit of this goal.

A change in the allergen-specific T-cell cytokine profile from a Th2 to a Th1 type may be one of the important results of the therapy (28). Thus, an adjuvant was sought that could induce preferentially a Th1 response, and this was found in MPL[®]. The immunologic properties of MPL[®] adjuvant have been studied in depth, and it has been particularly shown to activate antigen-presenting cells (APC); it upregulates MHC expression and encourages phagocytosis, causing release of IL-12, IL-1, TNF- α , and GM-CSF. IL-2 and IFN- γ are released from Th1 cells, probably indirectly via the effects of IL-12 (11). Studies in mice have confirmed that MPL[®] adjuvant has a propensity to induce a Th1 response, as indicated by the profile of antibody isotypes produced; in rats, it also prevented a boost of IgE antibody (29). It was of particular significance that tyrosine, acting as a depot for an immunogen, and monophosphoryl lipid A were syner-

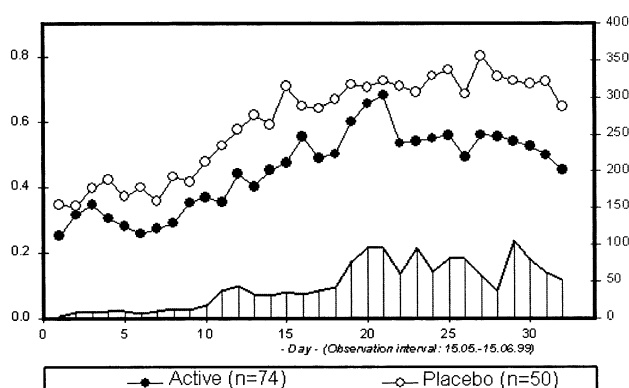


Figure 3. Combined median symptom and medication scores of actively treated and placebo patients. Observation days are shown on the x-axis. Combined symptom and medication scores (left y-axis) are indicated by black spots for actively treated patients and white spots for those on placebo. Pollen count (right y-axis) is given as bars corresponding to daily mean (count/m³) of all pollen traps.

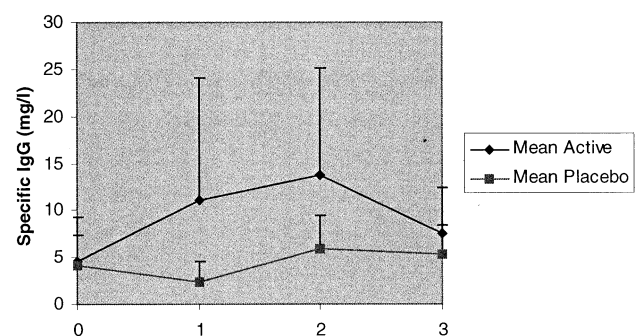


Figure 4. Specific IgG profiles comparing patients on active immunotherapy with those on placebo. Mean active levels are shown as diamond points and mean placebo levels as square points. Specific antibody measured as mg/l on y-axis. X-axis: 0=baseline, 1=after therapy, 2=middle of assessment period, 3=after pollen season. Significance values (stratified Wilcoxon two-sample test): 0, $P=0.55$. 1, 2, 3, $P<0.01$. Dispersion bars are + standard deviation.

Table 4. Comparison of patients treated with active agent and those receiving placebo. Local adverse events categorized with statistical results

Local reaction		Placebo	Active	Significance
Redness and swelling	Patients with event	15	61	$P < 0.01$
	Patients without event	45	20	
Pain	Patients with event	8	19	$P = 0.19$
	Patients without event	52	62	
Itching	Patients with event	2	23	$P < 0.01$
	Patients without event	58	58	
Total number of patients with local reactions		20	65	$P < 0.01$
Total number of patients treated		60	81	

gistic in their Th1-inducing properties (30). Other investigators have reported that MPL[®] adjuvant causes activation of APC and stimulation of IL-12 (14, 31), leading to the preferential induction of a Th1 cell response to an accompanying antigen.

In order to achieve safely the top dose of allergen extract in only four injections, it was necessary to use a modified allergen extract. Therefore, grass pollen extract was modified with glutaraldehyde for use in the product. This treatment provides an added safety bonus (1, 32). The modified grass pollen extract was adsorbed to L-tyrosine as a depot because it has been observed that alum salts promote Th2 cell induction (33). MPL[®] adjuvant was incorporated into each dose of the product. This vaccine was standardized, as reported previously (24), according to European requirements and incorporating a new HPLC method to assay the MPL[®] adjuvant (34).

The clinical data reported from this study show that only four injections are required to achieve a satisfactory clinical response in terms of reduction of symptom/medication scores. This was particularly due to the statistically significantly lower nasal and ocular symptom scores in the active group. An interesting finding was that the difference in diary card scores between the groups appeared generally to become greater as the

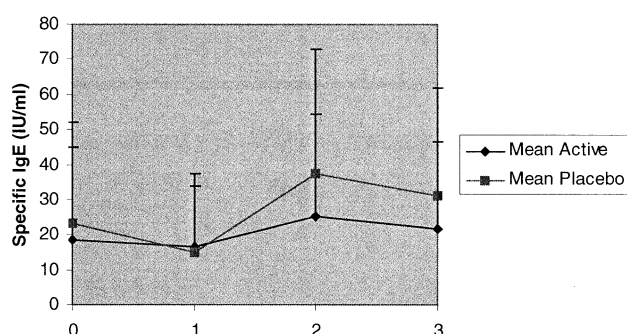


Figure 5. Specific IgE profiles comparing patients on active immunotherapy with those on placebo. Mean active levels are shown as diamond points and mean placebo levels as square points. Specific antibody is measured as IU/ml on y-axis. X-axis: 0=baseline, 1=after therapy, 2=middle of assessment period, 3=after pollen season. Significance value (stratified Wilcoxon two-sample test) comparing point 2 to 1, $P = 0.002$.

Table 5. Total numbers of generalized adverse events, comparing patients treated with active ($n = 14$) agent and those receiving placebo ($n = 7$). Symptoms in combination were all counted separately

Symptoms	Placebo no. of events	Active no. of events
Conjunctivitis	6	9
Rhinitis	8	9
Itching	1	4
Nausea	—	4
Sneezing	3	—
Headache	—	2
Pain in joints (arm, hands)	—	2
Migraine	1	1
Hot flushes	—	1
Cough	1	—
Tickling (of lower lip)	—	1
Hypotension	—	1
Chills	—	1
Tachycardia	—	1
Total number of events	20	36
Number of patients with events	7	14
Comparison of groups (Fisher's exact test)	$P = 0.47$	

pollen season progressed. Moreover, the baseline symptom scores indicated that subjects were recording symptoms before grass pollen was measured in the pollen traps. Therefore, it is unlikely that specific treatment could reduce scores to zero. Although no differences were apparent between the groups as regards symptoms in the lungs, very few subjects reported any such symptoms; therefore, there was no possibility of achieving significant results. There was a large difference in the amount of medication taken by subjects to control any symptoms between the actively treated and placebo groups, but this did not reach significance due to the large variability within the placebo group and the unequal number of patients. However, when the symptom and medication scores were combined, a significant difference was apparent.

The rise in specific IgG antibody was very rapid compared with other studies of tyrosine allergoids (35). Specific IgE antibody changes were of particular interest, because it is often seen that a specific IgE rise is induced by the first few years of AV treatment even though the patient may receive some relief (36). However, after several years, the specific IgE level can sometimes fall. In the study reported here, not only was there no rise in specific IgE resulting from the therapy but also the rise due to the seasonal exposure to pollen seen in the placebo group was prevented by treatment. This finding is consistent with the view of a change in the ratio of Th1:Th2 activity. This clearly bodes well for the patient and the use of the new treatment in later years.

The safety profile of the new product was good, as might be expected with an allergoid. Local swelling and some pain, which was greater in the actively treated subjects, were seen but considered by the trialists to be

no worse than normally seen with many allergy vaccine regimens. Some generalized, possibly treatment-related adverse events were reported but were not of a serious nature and were equally divided between the two groups.

The data we have reported here and elsewhere show that the new product is effective in the first year of therapy and tend to support the hypothesis that a T-cell switch is occurring. It is also possible that T-cell anergy via IL-10 induction was an eventual outcome of treatment. Of course, the beneficial changes we have reported have been reported with other allergy vaccines, but longer courses of therapy, sometimes several years, are required. We believe that our preseasonal four-injection regimen, which may be required to be repeated in a further year, has the potential to transform allergy vaccination into a first- rather than a last-choice therapy for the well-diagnosed grass pollen-allergic patient.

Work is continuing to assess the effect of a second year's treatment with the new product and to extend the studies to the treatment of allergy to other pollens and to house-dust mites.

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