

Immunotherapy with an alum-adsorbed *Parietaria*-pollen allergoid: a 2-year, double-blind, placebo-controlled study

Tari MG, Mancino M, Ghezzi E, Frank E, Cromwell O. Immunotherapy with an alum-adsorbed *Parietaria*-pollen allergoid: a 2-year, double-blind, placebo-controlled study.

Allergy 1997; 52: 65–74. © Munksgaard 1997.

A double-blind, placebo-controlled study was performed in order to confirm the safety, suitability, and efficacy of an alum-adsorbed *Parietaria judaica*-pollen allergoid, Allergovit[®], for allergen-specific immunotherapy. *Parietaria* pollen is an important cause of pollinosis, particularly in the Mediterranean zone, where it may be encountered for up to 8–9 months of the year. It is an aggressive allergen, and the doses tolerated during immunotherapy are less than those achieved with grass pollen. This factor increases the desirability of using therapeutic preparations with minimal IgE-binding activity, such as allergoids, in order to reduce the risk of side-effects and enable patients to tolerate a higher dose of allergen, thereby increasing the chances of successful specific immunotherapy. Forty patients with rhinitis and/or asthma were allocated at random to active- or placebo-treatment groups at the beginning of the study. All patients received the active preparation during the second year of the study. Immunotherapy was well tolerated by all patients and the incidence of side-effects was low. Treatment resulted in significant reductions in specific cutaneous reactivity and increases in nasal tolerance. A progressive improvement in nasal inspiratory peak flow in association with the immunotherapy indicated a reduction in nasal inflammation. These objective assessments of efficacy endorsed the results from the patients' diary cards, which indicated significant improvements in symptoms and reductions in the use of medication. The immunologic activity of the therapeutic preparation was demonstrated by the induction of a significant specific-IgG antibody response, with increases in IgG4 during the second year of treatment. We conclude that the safety and efficacy of immunotherapy with the *Parietaria* allergoid make it suitable for consideration in the treatment of patients with *Parietaria*-pollen-induced rhinitis or asthma.

**M. G. Tari¹, M. Mancino¹, E. Ghezzi²,
E. Frank³, O. Cromwell³**

¹Department of Internal Medicine, Center for Study of Allergic Diseases, Arienzo, Italy;

²Bracco S.p.A., Milan, Italy; ³Allergopharma Joachim Ganzer KG, Reinbek, Germany

Key words: allergic rhinitis; allergoid; desensitization; immunologic; immunotherapy; *Parietaria* pollen.

Professor M. G. Tari
Department of Internal Medicine
Center for Study of Allergic Diseases
I-81021 Arienzo
Italy

Accepted for publication 16 July 1996

Chemical modification has played an important role in the production of vaccines, being used to convert toxins into virtually nontoxic toxoid derivatives that can confer protective immunity. The same concept has been applied in the production of allergoids from allergen extracts, and these were first tested in 1972 (1). The efficacy of various formaldehyde-modified allergen preparations for the treatment of allergic rhinitis and asthma has been demonstrated in several studies, including a number of double-blind, placebo-controlled trials that recorded

improvement in symptoms, reductions in the need for medication, and increased allergen tolerance (2–6).

Chemical interactions between formaldehyde and the components of a standardized allergen extract result in comprehensive changes in the net charge of the proteins and in their tertiary structures. The result is that allergenicity/IgE-binding activity is substantially reduced (1, 7) yet immunogenicity is retained (1, 8). An early study with an aqueous ragweed pollen allergoid showed that substantially

more allergoid than allergen could be injected into hay fever patients before symptoms of anaphylaxis were induced (9), reflecting the reduction in IgE-binding activity. The higher doses of allergoid that could be administered, in comparison with allergen, resulted in higher specific IgG concentrations, and a high-dose schedule with an allergoid elicited higher specific-IgG concentrations than were achieved with a low-dose schedule (1).

Adsorption of allergoids onto aluminum hydroxide suspension confers the benefits of a depot preparation, and a double-blind, placebo-controlled study in hay fever patients demonstrated definitive immunologic changes accompanying a significant clinical improvement (6).

Various *Parietaria* species are found throughout central and southern Europe; the USA, particularly California; and Australia. The flowers are wind pollinated, and the pollen is recognized as an important cause of pollinosis, particularly in the Mediterranean zone (10). *Parietaria* pollen contains an aggressive allergen (11), and symptoms ensue once the pollen count reaches approximately 30 grains/m³ of air, as opposed to 50 grains for grass pollen (12). A previous report noted that the maximum tolerated dose of *Parietaria*-pollen extract was approximately 25 times less than that of timothy grass (*Phleum pratense*) pollen (11). These factors heighten the desirability of using therapeutic preparations with minimal IgE-binding activity in order to reduce the risk of side-effects and enable patients to tolerate a higher dose of allergen, thereby increasing the chances of successful specific immunotherapy.

A previous study (13, 14) compared *Parietaria* allergen and allergoid depot preparations during 1 year of therapy and demonstrated their safety and clinical efficacy, together with an enhanced immunogenic response that could be achieved with the allergoid through the use of larger quantities of protein relative to the allergen. The present double-blind, placebo-controlled study was designed to confirm the safety, suitability, and efficacy of an alum-adsorbed *Parietaria judaica*-pollen allergoid, Allergovit®, by applying a more comprehensive objective assessment of patient responses to treatment over a 2-year period.

Material and methods

Study design

The clinical trial was designed and conducted in the Center for the Study of Allergic Diseases, Arienzo, Italy. A total of 40 patients entered the study, which ran for a period of 24 months. A double-blind, placebo-controlled format was adopted during the

first year when patients were assigned at random to the active ($n=20$) or placebo ($n=20$) groups. The study commenced in December 1989, when a clinical evaluation was made together with provocation tests, skin prick tests, and the collection of blood samples. Therapy commenced immediately with weekly injections of increasing doses of the preparation. Patients kept diary cards recording symptom and medication scores throughout the year. Further clinical evaluations were made in March and June, at the beginning and end of the peak of the pollen season, and in November, 1 year after commencement of therapy. Additional blood samples were collected in March, May, June, and November.

In the second year, all patients received the active preparation. The placebo group from the first year was treated in accordance with the protocol established for the first year. Patients who had received active treatment in the first year continued with maintenance injections at intervals of 3–4 weeks. Diary cards were again completed by all patients.

Patient selection

A total of 40 subjects were selected from patients attending the outpatient department by the following criteria:

- 1) clinical history of hypersensitivity to *Parietaria* pollen with symptoms of rhinitis, with or without asthma, for at least 3 consecutive years
- 2) positive skin prick test to *Parietaria*-pollen extract
- 3) positive nasal provocation test with *Parietaria*-pollen extract
- 4) positive *in vitro* specific-IgE test
- 5) no immunotherapy with *Parietaria*-pollen preparations during the previous 3 years
- 6) freedom from acute and chronic infections of the respiratory tract and active immunologic and systemic disease.

All patients gave informed written consent before admission to the study. The patient details are summarized in Table 1.

Therapeutic preparations and dosage

The active preparation consisted of an alum-adsorbed, *Parietaria*-pollen allergoid (Allergovit, Bracco, Milan, Italy, and Allergopharma, Reinbek, Germany), provided in a treatment set of two vials designated A and B, containing 1000 TU/ml and 10 000 TU/ml, respectively. Maintenance therapy was conducted with additional vials of the higher concentration. The *Parietaria*-pollen extracts used in the routine preparation of the allergoid are

Table 1. Patient groups

Number of patients	Allergovit 20	Placebo 20
Age (years)		
Mean	33.65 ± 7.07	31.65 ± 8.96
Range	20–46	13–50
Sex (M:F)	10:10	10:10
Skin prick test		
(Allergen/histamine Wheal area ratio)	3.1 ± 0.23	3.1 ± 0.22
Total IgE (kU/ml)		
Mean	156.6 ± 149.1	228.2 ± 248.5
Median	101.0	144.5
Range	19–580	37–1172
Specific IgE (kU/ml)	(n = 17)	(n = 16)
Mean	24.1 ± 17.5	27.8 ± 24.0
Median	18.8	17.8
Range	3.2–18.8	0.35–100.0
Rhinitis symptoms	(n = 20)	(n = 19)
Duration (years)	4.7 ± 4.1	4.2 ± 3.6
Asthma symptoms	(n = 14)	(n = 10)
Duration (years)	3.1 ± 2.5	3.4 ± 2.9

standardized against a well-characterized, biologically standardized reference extract. The placebo preparation consisted of an alum-suspension colored with caramel NF acid. An initial series of injections of 0.1, 0.2, 0.4, and 0.8 ml of strength A, representing a cumulative dose of 1500 TU, was followed for eight patients, while slight variations in this dosage scheme resulted in one patient receiving 1600 TU and the remaining 11 patients, 1650 TU. Treatment continued with injections of increasing concentrations of strength B up to a maximum of 1.0 ml. All patients achieved the top dose by the beginning of April. This resulted in an initial treatment mean cumulative dose of 24 500 TU. Maintenance injections of half the maximum dose were then administered at intervals of 3–4 weeks, and continued until the end of the first year of the study (November). The dose was then increased up to the maximum dose, while patients in the placebo group commenced treatment with the active preparation. All patients received maintenance treatment through the second pollen season and until the end of the study.

Quantitative skin prick test

Quantitative skin prick tests were performed with four concentrations of *P. judaica*-pollen extract containing 13 500, 4500, 1500, and 500 BU/ml; histamine dihydrochloride solutions at 1% and 0.1%; and a 0.9% sodium chloride control. All solutions contained 50% glycerol and 0.4% phenol as preservative, and they were tested in triplicate on the volar surfaces of the forearms according to

a coded randomization schedule. Wheal areas were recorded by marking the outline of the wheal in ink, copying the impression onto clear adhesive tape (Sellotape®), and measuring the area by digitized planimetry. Tests were performed on admission to the study (December 1989), after the pollen season (November 1990), before the pollen season (February 1991), and after the pollen season (November 1991). For the purposes of analysis, the mean wheal area elicited with 1500 BU/ml was expressed relative to the histamine response.

Specific nasal reactivity

Changes in airways resistance were determined with a nasal resistance meter (Rhinotest MP 500-microprocessor-controlled rhinomanometer, Allergopharma). A lyophilized *P. judaica* extract was reconstituted in physiologic saline and used to prepare a series of threefold dilutions. Nonspecific nasal reactivity was established after administration of physiologic saline, and challenges were then performed with increasing concentrations of the allergen, commencing with 10.0 BU/ml.

A flow reduction of at least 40% by comparison with the solvent curve was considered positive. Provocation tests were performed on admission to the study, after the initial course of seven injections (February 1990), during the pollen season (June 1990), and after the pollen season (November 1990). Further challenges were performed during the second year of the study in February, June, and November 1991.

Nasal inspiratory peak flow (NIPF)

Patients were issued with a nasal peak-flowmeter (Marcos, Monza, Italy) and asked to keep a record in their diary cards of the best of three measurements made twice a day in the morning and the evening.

Symptom scores

Patients maintained daily symptom/medication score cards throughout the study. Separate scores for nasal, conjunctival, and bronchial symptoms were recorded on a four-point scale of severity. The sum of the scores was defined as the symptom score of the patient on a particular day, and data were computed as mean weekly score. Corresponding scores were compiled for medication usage.

Pollen counts

A Burkard recording volumetric spore trap (Burkard, Rickmansworth, UK) placed in Arienzo was used

to collect pollen grains. *Parietaria*-pollen counts were expressed as grains/m³ of air per day, and records were compiled throughout the 2 years of the study (1990–91).

Blood samples

Eight blood samples were collected from each patient at 3-month intervals during the course of the study. Serum samples were stored at –20°C.

Total and specific immunoglobulin measurements

Measurements were performed in duplicate on all serum samples for IgE and *Parietaria*-specific IgE, IgG, IgG1, and IgG4. Total and specific IgE levels were determined with the CAP System (Pharmacia Diagnostics, Uppsala, Sweden).

Parietaria-specific IgG was measured in allergen-coated polyvinyl microtiter plate wells. Dilutions of the serum samples (1:100) were incubated in the plates for 1 h at 45°C. After washing, ¹²⁵I-labeled protein A was added and incubated at 37°C for 2 h. The plates were washed, and the bound radioactivity was measured in a Gamma counter. IgG concentrations were determined by reference to a standard curve.

Specific IgG1 and IgG4 subclasses were determined by solid-phase immunoenzymatic assays (Allerblock IgG1 and IgG4 Medical System, Genoa, Italy). Two 100-μl samples of a 1:300 dilution of each serum were incubated at room temperature for 3 h in microtiter plate wells coated with *P. judaica*-pollen extract. The microtiter plates were washed, and a dilution of either a mouse antihuman IgG1 or IgG4 monoclonal antibody was added to each well. After incubation for 3 h at 37°C, followed by washing of the plate, peroxidase-labeled antimouse IgG (goat) was added to each well, and the plates were incubated for a further 3 h. Unbound enzyme conjugate was removed by washing, and a peroxidase substrate (*o*-phenylenediamine and hydrogen peroxide) was added to each well. Conversion of the substrate was measured in terms of the change in absorbance at 492 nm, and was proportional to concentrations of specific IgG1 and IgG4.

Lymphocyte subpopulations

T-cell subsets were enumerated with FITC-conjugated CD3 and CD8 monoclonal antibodies (OKT3, OKT8; Ortho Diagnostic Systems, Inc, Raritan, New Jersey, USA) in a flow cytometer (Spectrum III). The normal mean value for CD4/CD8 was taken to be 1.74 (range 1.02–2.46) (15).

Side-reactions

The investigating physician recorded all possible side-effects, including immediate and late reactions and systemic responses. Reactions were graded as mild, moderate, or severe. Data for one patient in the placebo group were lost and could not be included in the final analysis. The trial physician could recall no untoward reactions in this patient.

Statistical analysis

Nonparametric tests were applied to the analysis of the data. Comparisons within treatment groups were made with the Wilcoxon signed-rank test, and between-group comparisons with the Mann–Whitney *U*-test. In all cases, a *P* value ≤0.05 was considered to be significant. Mean values are expressed as mean ± standard deviation.

Results

Patient groups

The various baseline characteristics of the two patient groups were well matched, as indicated in Table 1 together with further data for nasal inspiratory peak flow (NIPF) and nasal reactivity in Tables 2 and 4, respectively. There were no statistically significant differences between the groups. One patient from the placebo group was excluded from the evaluations because of incomplete documentation.

Pollen counts and symptom scores

The genus *Parietaria* belongs to the family Urticaceae, and the pollen count for this family was monitored daily throughout the 2 years of the trial.

Table 2. Nasal inspiratory peak flow (NIPF) (l/min)

	Allergovit	Placebo ¹
Mean morning NIPF		
Before therapy	136.6 ± 12.4 ²	131.8 ± 14.1
After 1 year	165.4 ± 22.2	140.1 ± 11.1
	<i>P</i> < 0.05*	
After 2 years	176.4 ± 18.2	162.3 ± 10.2
	<i>P</i> < 0.01*	<i>P</i> < 0.05*
Mean evening NIPF		
Before therapy	155.6 ± 11.1	142.7 ± 12.1
After 1 year	179.9 ± 16.4	150.3 ± 12.1
	<i>P</i> < 0.01*	
After 2 years	190.1 ± 14.2	170.2 ± 10.2
	<i>P</i> < 0.01*	<i>P</i> < 0.01*

¹ Active treatment in second year. ² l/min, mean ± SD. * Comparison with value before therapy.

The mean counts (Fig. 1) show that the main pollen seasons occurred between February and July in each year of the study.

The mean weekly symptom scores coincided with natural exposure to pollen (Fig. 1). Comparison of the active-treatment and placebo groups during the first year of the study showed significant differences between the groups in their response to natural exposure to the allergen as reflected in symptom scores (Fig. 1) and use of medication. Both the morning and evening NIPF rates were significantly increased in the active-treatment group after 12 and 24 months (Table 2). There were no significant increases in the placebo group after 12 months, but after treatment during the second year of the study, this group also recorded a significant improvement. In both groups of patients, the NIPF measurements were lower in the morning than in the evening.

Side-effects

The active-treatment group received a total of 547 injections over 2 years, while the ex-placebo group received 333 injections of the active preparation during the course of the second year of the study. Local immediate skin responses greater than 5 cm in diameter were associated with 13 out of a total of 880 injections of the active preparation (1.5%) in eight patients. These responses were associated with larger doses of the allergoid, but all subjects were subsequently able to tolerate these. No immediate systemic reactions were noted (Table 3). Local late reactions in excess of 5 cm in diameter were recorded in association with 27 injections of the active preparation; 16 (3.3%) in seven patients in the treatment group over 2 years, and 11 (1.5%) in seven patients from the ex-placebo group during treatment in the second year. All local reactions resolved without treatment and caused no problems for the patients.

Moderate systemic responses were seen after 10 injections in eight patients, and these were successfully controlled with either beclomethasone dipropionate (500 mg $\times$ 2 spray) or fenoterol (400 mg $\times$ 2). In one case, a reaction occurred with 0.9 ml strength A, but the patient continued to the next higher dose without problems. The remaining nine reactions occurred with the higher doses of strength B; in all cases, subsequent dose reduction avoided further systemic response.

Specific nasal reactivity

Nasal provocation showed that there was no difference in sensitivity between the two patient groups at the beginning of the trial (Table 4). After

Table 3. Side-effects

	Allergovit group Allergovit		Placebo group Placebo	
First year	382 injections (20)		372 injections (19)	
	Immediate	Late	Immediate	Late
Local reaction > 5 cm	9 (6)	9 (5)	0	0
Moderate systemic reaction	0	3 (2)	0	0
Anaphylactic reaction	0	0	0	0
Second year	Allergovit		Allergovit	
	165 Injections (20)		333 Injections (19)	
	Immediate	Late	Immediate	Late
Local reaction > 5 cm	0	7 (5)	4 (2)	11 (7)
Moderate systemic reaction	0	4 (4)	0	3 (3)
Anaphylactic reaction	0	0	0	0

Table 4. Nasal provocation test

	Allergovit	Placebo
Before therapy	600 ¹ (200–1000)	550 (100–1000)
After 1 year	2000 (1000–2500) $P < 0.01^*$	600 (200–2000) $P < 0.01^*$
After 2 years	5000 (2500–5000) $P < 0.01^*$ $P < 0.01^*$	2000 (1000–2500) $P < 0.01^{**}$ $P < 0.01^{**}$

¹ Median allergen concentration – BU/ml. * Comparison with value before therapy;

** comparison with value after 1 year.

12 months, there was a significant increase in the allergen provocation dose for the active-treatment group from a median value of 600–2000 BU ($P < 0.01$), while there was a substantially smaller yet significant difference for the placebo group ($P < 0.01$). The difference between the groups was also significant ($P < 0.01$). After the second year of treatment, the provocation dose was increased still further to 5000 BU in the active-treatment group ($P < 0.01$) (Table 4), and the ex-placebo group also showed a significant increase in tolerance of the allergen ($P < 0.01$).

Skin prick test

Active treatment was associated with a significant reduction in the allergen/histamine wheal ratio, reflecting reduced skin sensitivity. During the course of the first year of treatment, the ratio decreased from a median value of 3.05 to 1.8 ($P < 0.01$); and by the end of the second year, the

ratio was further reduced to 1.0 ($P < 0.01$). In the placebo group, there was no change during the first year – ratios of 3.1 and 3.05 at 0 and 12 months, respectively. At the end of the second year, after 1 year of therapy, the ratio was decreased significantly to 1.75 ($P < 0.01$) (Fig. 2).

Parietaria-specific IgE

Specific IgE levels in the active-treatment group did not change significantly during the course of the study, although there was a progressive trend

to a lower concentration. There was an increase in the placebo group in association with the pollen season during the first year, but this was not statistically significant (Fig. 3).

Parietaria-specific IgG and IgG subclass

There were no differences in the allergen-specific IgG levels between the two patient groups at the beginning of the study. After 6 months, the IgG antibody levels were significantly increased ($P < 0.01$) in the active-treatment group, and levels

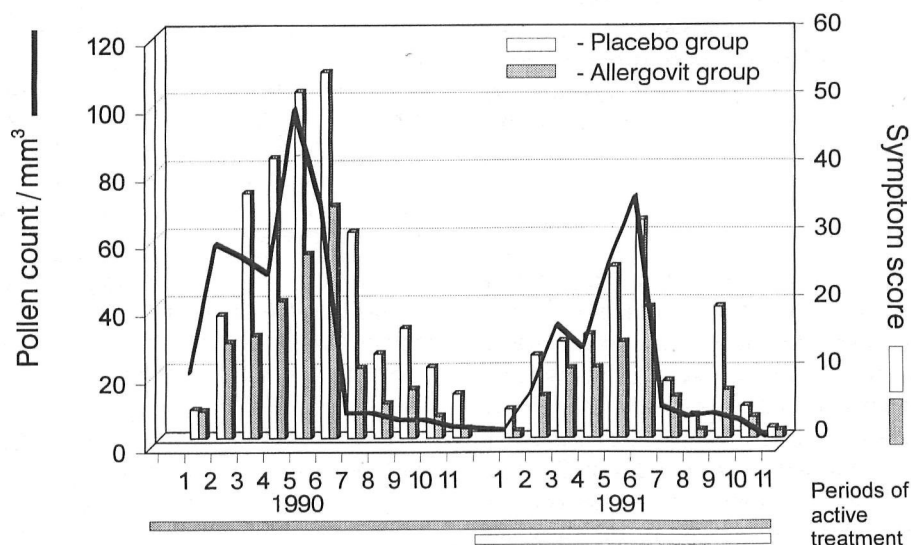


Fig. 1. Pollen counts and symptom scores. Pollen counts were expressed as mean number of pollen grains/mm³ per day. Symptom scores, combining nasal, bronchial, and conjunctival symptoms, were expressed as weekly mean.

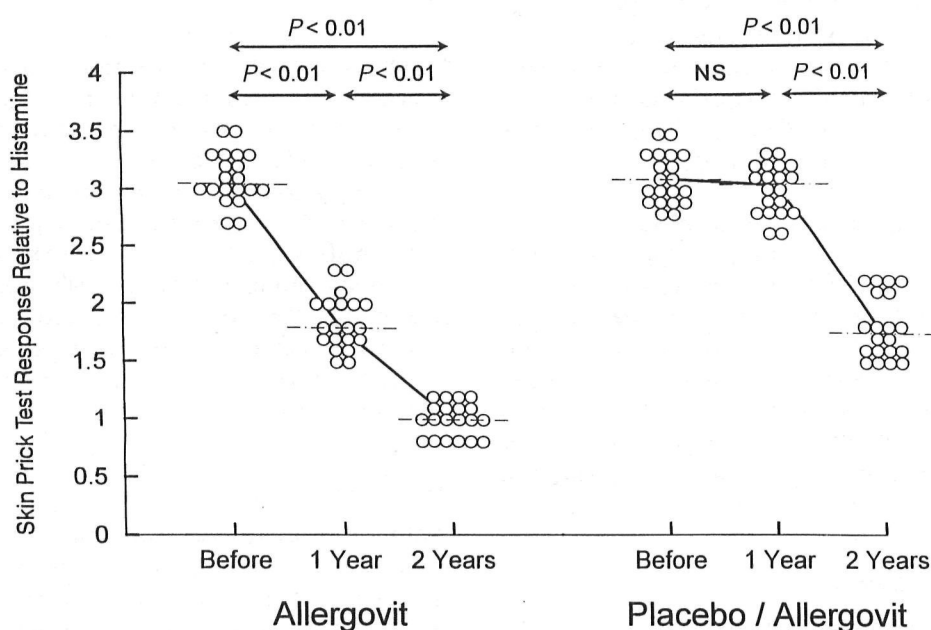


Fig. 2. Changes in skin prick test reactivity. Reactivity was expressed by ratio of mean wheal area of three skin prick tests for *P. judaica*-pollen extract (1500 BU) and histamine dihydrochloride (0.1%).

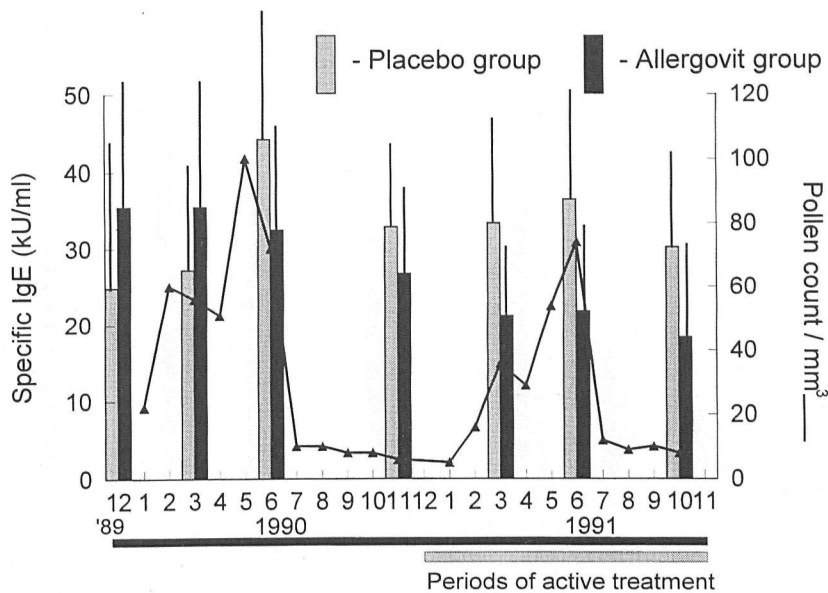


Fig. 3. Changes in *Parietaria* pollen-specific IgE antibodies during course of immunotherapy.

remained significantly greater than the baseline values at later time points in the trial. Antibody levels in the placebo group were unchanged during the first year of the study, but were significantly raised after active treatment commenced (Fig. 4).

Specific IgG1 antibody levels were significantly increased after 6 months of active treatment ($P < 0.01$), but declined thereafter as the treatment continued (Fig. 4). On the other hand, increases in specific IgG4 were not seen until 12 months after commencement of therapy, and levels remained significantly raised during the second year of treatment. There were no significant changes in specific IgG1 and IgG4 in the placebo group.

Lymphocyte populations

At the end of the study, there was a significant reduction ($P < 0.05$) in the CD4+/CD8+ ratio in the active-treatment group. However, while there were increases in the numbers of CD8+ cells, and decreases in CD4+ cells, these absolute changes were not statistically significant (data not shown).

Discussion

This investigation involved a more comprehensive appraisal of clinical parameters than was reported in a previous trial which compared a *Parietaria*-pollen allergoid-depot preparation with an allergen-depot preparation and patients on symptomatic medication alone (13, 14). *Parietaria* Allergovit immunotherapy was well tolerated by all patients, and the incidence of side-effects was low. Treatment resulted in significant reductions in specific

cutaneous reactivity, increased nasal tolerance, and increased nasal inspiratory flow rate. These objective assessments of efficacy endorsed the results from the patients' diary cards which indicated significant improvement in symptoms and reduction in the use of medication. The immunologic activity of the therapeutic preparation was demonstrated by its ability to modulate specific IgG antibody responses. All the changes were clearly associated with the specific immunotherapy.

It is usually recommended that alum-adsorbed pollen allergoids be used for preseasonal immunotherapy, but because of the very protracted *Parietaria*-pollen season in southern Italy, typically 8–9 months between February and October, the allergen is effectively elevated to perennial as opposed to seasonal status. Consequently, it has proved beneficial in normal practice to continue therapy during the pollen season. In view of the fact that the placebo group from the first year converted to active treatment in the second year, it was appropriate to continue maintenance therapy in the active-treatment group between the two pollen seasons while the ex-placebo group were receiving their initial course of preseasonal therapy.

The improvements in symptoms were corroborated by the objective measurements of NIPF, which showed progressive improvement in association with immunotherapy. This may indicate reduced nasal inflammation, as shown by reduced numbers of eosinophils infiltrating the nasal mucosa as a result of immunotherapy with grass-pollen preparations (16), and significant reductions in levels of the proinflammatory cytokines interleukin (IL)-1 β and

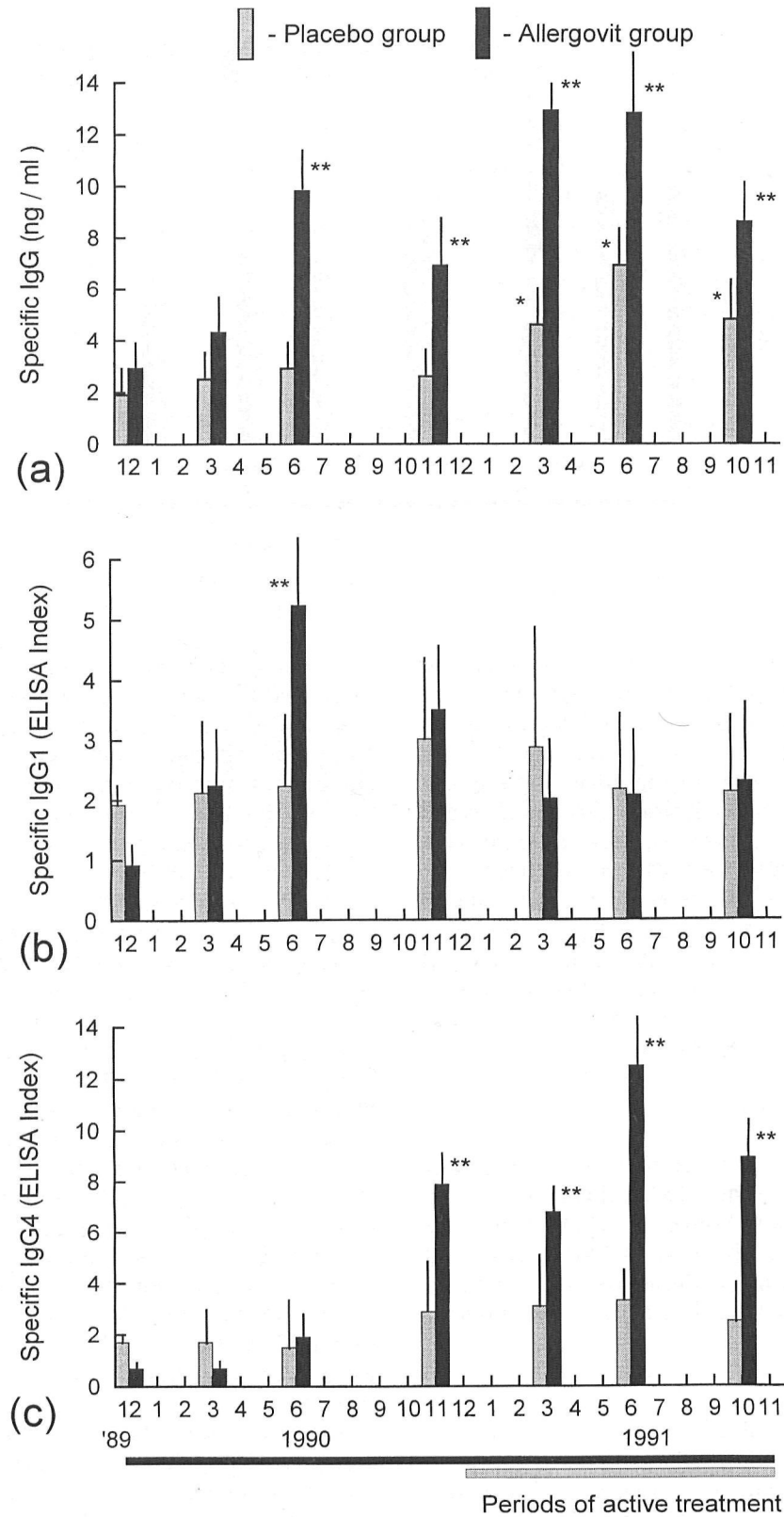


Fig. 4. Changes in *Parietaria*-specific IgG antibodies during course of immunotherapy. a) Specific IgG; b) specific IgG1; c) specific IgG4 (* $P < 0.05$; ** $P < 0.01$).

IL-8 in association with treatment with an alum-adsorbed grass-pollen allergoid (17).

Associations between successful immunotherapy and reductions in the magnitude of late-phase responses in the skin (18–20) and lung (21) are well documented. Furthermore, both early- and late-phase allergen-induced nasal responses are attenuated (22), and reductions in the size of immediate skin responses have been observed in studies with grass-pollen immunotherapy (20), and with *Parietaria*-pollen allergen and allergoid depot preparations (13). The results of the present study are consistent with these reports, showing a significant attenuation of the immediate skin response after 1 year of therapy with a further reduction after 2 years, and increased allergen tolerance in nasal provocation tests.

There were no significant changes in *Parietaria*-specific IgE, although there was a continuing trend to lower concentrations in the active-treatment group over the 2 years of the study, despite natural exposure to pollen during the seasons and in contrast to an increase in the placebo group. These observations are in line with those reported in a previous study (14) which showed that specific IgE increased in the control group during the pollen season, but that changes in the allergoid-treated group were relatively small. Therefore, the allergoid did not appear to stimulate *Parietaria*-specific IgE production, as has often been reported for unmodified allergen preparations (23), and may also have suppressed an IgE response to natural pollen exposure. Lack of IgE-binding activity by the allergoid would prevent IgE-mediated antigen presentation involving either Fc ϵ RI or Fc ϵ RII (CD23) receptors, an action which has been recognized as a potent mechanism of allergen-induced immune response in specific T cells (24, 25). Such a response would lead to expansion of the Th2 population and promote B-cell switching to IgE production. The use of allergoids would avoid such a reaction, and offer an additional advantage for such preparations in specific immunotherapy.

Processing of the allergoid would probably involve cells of the macrophage/monocyte lineage, favoring a Th1 response (26, 27). Levels of total specific IgG were markedly increased during the first and second years of therapy by comparison with concentrations at the outset of the study. While these increases were initially attributable, at least in part, to specific-IgG1 antibodies, specific IgG4 antibodies assumed a more prominent position by the end of the first year of therapy and during the second year. These observations suggest that the specific-IgG subclass responses emerge at the expense of the IgE response.

The reduction in the CD4/CD8 ratio is consistent with previous reports that immunotherapy induces an increase in circulating allergen-specific CD8+ cells (28, 29) and a significant reduction in the CD4+/CD8+ ratio (29). As has already been noted, reductions in the magnitude of late-phase responses in the lung (21) and early- and late-phase response in the nose (22) have been associated with successful allergen immunotherapy. The increased nasal tolerance observed in the present study in association with clinical improvement and reduction of the CD4/CD8 ratio is consistent with these reports, and supports the view that modulation of T-cell subsets is of fundamental importance.

In conclusion, this immunotherapy proved to be both safe and clinically efficacious, as judged by both subjective and objective assessment, and was associated with concomitant changes in specific immunologic parameters. The substantially reduced IgE-binding activity of allergoids in comparison with native allergens not only reduces the potential risk of inducing immediate reactions but also, presumably, prevents their IgE/IgE-receptor-mediated uptake by antigen-presenting cells, an action that would favor a Th2-lymphocyte response and enhanced IgE production.

Acknowledgment

We thank Birgit Joppich for help with the statistical analysis.

References

1. Maasch HJ, Marsh DG. Standardized extracts: modified allergens – allergoids. *Clin Rev Allergy* 1987;5:89-106.
2. Bousquet J, Hejjaoui A, Skassa-Brociek W, et al. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. I. Rush immunotherapy with allergoids and standardized orchard grass-pollen extract. *J Allergy Clin Immunol* 1987;80:591-8.
3. Bousquet J, Hejjaoui A, Soussana M, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. IV. Comparison of the safety and efficacy. *J Allergy Clin Immunol* 1990;85:490-7.
4. Bousquet J, Maasch HJ, Hejjaoui A, et al. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. III. Efficacy and safety of unfractionated and high-molecular-weight preparations in rhinoconjunctivitis and asthma. *J Allergy Clin Immunol* 1989; 84:546-56.
5. Bousquet J, Maasch H, Martinot B, Hejjaoui A, Wahl R, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. II. Comparison between parameters assessing the efficacy of immunotherapy. *J Allergy Clin Immunol* 1988;82:439-46.
6. Pastorello EA, Pravettoni V, Incorvaia C, et al. Clinical and immunological effects of immunotherapy with alum-adsorbed grass allergoid in grass-pollen-induced hay fever. *Allergy* 1992;47:281-90.
7. Marsh DG, Norman PS, Roebber M, Lichtenstein LM. Studies on allergoids from naturally occurring allergens.

- III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. *J Allergy Clin Immunol* 1981;68:449-59.
8. Salgado J, Campama M, Gilabert A, Queralto J. Biological characterization of a formylated allergoid from *Dermatophagoides pteronyssinus*. *Rev Esp Fisiol* 1992;48:245-51.
9. Norman PS, Lichtenstein LM, Kagey-Sobotka A, Marsh DG. Controlled evaluation of allergoid in the immunotherapy of ragweed hay fever. *J Allergy Clin Immunol* 1982;70:248-60.
10. D'Amato G, Ruffilli A, Sacerdoti G, Bonini S. *Parietaria* pollinosis: a review. *Allergy* 1992;47:443-9.
11. Basomba A. The importance of allergen dosage and treatment schedules in immunotherapy. *Proceedings XII International Congress of Allergology and Clinical Immunology*, Washington, 1985:335-40.
12. D'Amato G, Ruffilli A, Ortolani C. Allergenic significance of *Parietaria* (pellitory-of-the-wall) pollen. In: D'Amato G, Spieksma FTM, Bonini S. editors. *Allergenic pollen and pollinosis in Europe*. Oxford: Blackwell Scientific, 1991: 113-18.
13. Bruno G, Mari A, Frank E, Franceschini P, Biocca MM, Balsano F. Untersuchungen mit *Parietaria*-Allergoiden in der Immuntherapie. I. Sicherheit und Wirksamkeit der Immuntherapie mit aluminiumadsorbierten Allergen- und Allergoid-Extrakten aus *Parietaria-judaica*-Pollen. *Allergologie* 1990;13:380-6.
14. Bruno G, Mari A, Frank E, Franceschini P, Biocca MM, Balsano F. Untersuchungen mit *Parietaria*-Allergoiden in der Immuntherapie. II. Immunologische Veränderungen während der Immuntherapie mit aluminiumadsorbierten Allergen- und Allergoid-Extrakten aus *Parietaria-judaica*-Pollen. *Allergologie* 1990;13:424-32.
15. Matsumoto K, Okubo K, Yokoyama MM. Distribution of marker specific lymphocyte in healthy human subjects. *J Clin Lab Immunol* 1985;16:143-7.
16. Hauser U, Bachert C, Frank E. Hemmung des Einstroms von Entzündungszellen in die Nasenschleimhaut durch die allergen-spezifische Immuntherapie. *Allergo J* 1995;4:164-8.
17. Hauser U, Cromwell O, Frank E, Bachert C. Reduction of the cytokine content of nasal secretions as a result of allergen specific-immunotherapy. *Allergy* 1995;50 Suppl 26: 276-7.
18. Fling JA, Ruff ME, Parker WA, et al. Suppression of the late cutaneous response by immunotherapy. *J Allergy Clin Immunol* 1989;83:101-9.
19. Rocklin RE. Immune mechanisms in allergen specific immunotherapy. *Clin Immunol Immunopathol* 1989;53:S119-31.
20. Varney VA, Hamid QA, Gaga M, et al. Influence of grass pollen immunotherapy on cellular infiltration and cytokine messenger RNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993;92:644-51.
21. Warner JO, Price JF, Soothill JF, Hey EN. Controlled trial of hyposensitization to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 1978;2:912-15.
22. Durham SR, Varney VA, Jacobson MR, Sudderick RM, Kay AB. Effect of grass pollen immunotherapy on allergen-induced early and late nasal responses [Abstract]. *J Allergy Clin Immunol* 1993;91:290.
23. van Metre TE, Adkinson NF. Immunotherapy for aero-allergen disease. In: Middleton E, Reed CE, Ellis EF, Adkinson NF, Yunginger JW, Busse WW, editors. *Allergy: principles and practice*. 4th ed. 1993:1489-1509.
24. Maurer D, Ebner C, Reininger B, et al. The high affinity IgE receptor (Fc ϵ RI) mediates IgE-dependent allergen presentation. *J Immunol* 1995;154:6285-90.
25. Mudde G, Bheekha R, Bruijnzeel-Koomen CAFM. Consequences of IgE/CD23-mediated antigen presentation in allergy. *Immunol Today* 1995;16:380-3.
26. Fiebig H. Immunologische Aspekte der spezifischen Immuntherapie (Hyposensibilisierung). I. Die Steuerung der IgE-Synthese. *Allergo J* 1995;4:336-9.
27. Fiebig H. Immunologische Aspekte der spezifischen Immuntherapie (Hyposensibilisierung). II. Die Umorientierung der T-Helferzellreaktion. *Allergo J* 1995;4:377-82.
28. Nagaya H. Induction of antigen-specific suppressor cells in patients with hay fever receiving immunotherapy. *J Allergy Clin Immunol* 1985;75:388-94.
29. Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double blind study. *Allergol Immunopathol (Madr)* 1990;18:277-84.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.