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# Long-term treatment with allergoid immunotherapy with *Parietaria*. Clinical and immunologic effects in a randomized, controlled trial

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**Key words:** allergoid; *Parietaria* pollen; specific immunotherapy.

**Background:** Specific immunotherapy (SIT) is a valuable treatment for respiratory allergy, and the use of chemically modified allergens (allergoids) has improved its safety, as testified by several studies. We evaluated the effects of a SIT course with an allergoid extract of *Parietaria* pollen in a double-blind, placebo-controlled trial.

**Methods:** The study was double-blind in the first year; then it was prolonged up to 3 years with all patients on active treatment. Clinical effectiveness, safety, skin reactivity, systemic immunologic parameters, and subjective assessment were evaluated. We also had available a self-evaluation recorded in a follow-up visit 4 years after the discontinuation of SIT.

**Results:** A significant reduction of the symptoms plus drug intake scores during the pollen seasons was observed in the patients receiving active SIT. The placebo patients, after switching to active SIT, also showed significant clinical improvement. The clinical efficacy persisted during years 2 and 3 of treatment. After year 1, the actively treated patients reported a significant subjective improvement (frequency of symptoms,  $P=0.001$ ; duration of symptoms,  $P=0.024$ ; physical performance,  $P=0.043$ ) compared with the placebo group. The self-evaluation by visual analog scale showed that all patients maintained a significant clinical improvement up to 4 years after discontinuing SIT (year 1: active = +31.6%, placebo = -15.7%; year 7: active = +35.8%, placebo = +31.3%). The systemic immunologic changes after active SIT paralleled those described elsewhere (IgE decreased from 22 to 9 and from 21 to 8 IU/ml; IgG4 increased from 43 to 87 and from 18 to 60 IU/ml). A significant decrease in skin reactivity to three different allergen concentrations was observed at year 3 compared with pretreatment values ( $P<0.05$ ).

**Conclusions:** The investigational SIT with *Parietaria* appeared to be effective and safe; a 3-year course of treatment achieved a long-lasting efficacy.

## Date:

Accepted for publication 17 September 1998

## To cite this article:

Ariano R., Kroon A.M., Augeri G., Canonica G.W. & Passalacqua G. Long-term treatment with allergoid immunotherapy with *Parietaria*. Clinical and immunological effects in a randomized, controlled trial. *Allergy* 1999; **54**, 313–319.

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ISSN 0105-4538

Allergen-specific immunotherapy (SIT) is presently regarded as an effective therapeutic tool for treating respiratory allergy. Its clinical effectiveness has been proved for allergic rhinitis and asthma in a large number of controlled studies (for review, see Refs. 1–3), and a recent meta-analysis study has confirmed the clinical observations (4). SIT acts as a biologic response modifier, and it is now the only treatment able specifically to interfere with the immune response and to modify the natural history of the allergic disease; this fact implies that SIT has to be considered a prophylactic desensitizing treatment rather than a merely symptomatic therapy (1).

The use of standardized extracts (5) has improved the reliability of SIT and has permitted a batch-to-batch consistency which has improved the safety of the treatment. Two other approaches can be used to minimize the risk of severe adverse events: the first is the noninjective administration of the extract, which is presently considered a promising route (6); the second is the use of modified extracts. This latter approach involves the chemical modification of the allergens (by formaldehyde or glutaraldehyde) in order to reduce their allergenicity (i.e., reactivity to specific IgE) yet still maintain the capacity of interacting with the immune system and of interfering with the immunologic response. The chemically modified allergens are commonly called allergoids (7, 8), and several clinical trials supporting their efficacy and safety have been so far performed (9–11). In addition, because of their safety, allergoids usually allow the use of simplified schedules convenient for the allergist. The efficacy of SIT has been confirmed, and several results indicate that the beneficial effects of SIT can be maintained for years after discontinuation (12–14); therefore, this specific issue is currently under investigation.

*Parietaria* (wall pellitory) is a member of the Urticaceae family widely diffused in Mediterranean areas; its pollination peaks between March and June, but *Parietaria* can be considered almost a perennial allergen in some regions. Since this plant is a common cause of respiratory allergy in the Mediterranean region, SIT with *Parietaria* has sometimes been investigated over the last 10 years.

In the present study, we investigated the clinical effectiveness and the safety of allergoid immunotherapy with *Parietaria* in patients with respiratory allergy. The first year of the study involved a double-blind, placebo-controlled design; this was followed by 2 years of open study. In addition to the clinical evaluation, some paraclinical parameters (skin reactivity, specific Ig) were also monitored, as detailed below. The patients were also followed for up to 4

years after the treatment discontinuation by means of a self-evaluation scale.

## Material and methods

### Study design

The study started in October 1990. It was designed as randomized, double-blind, and placebo-controlled, with two parallel groups, in the first year. In October 1991, the protocol was changed, and the previously placebo-treated patients were switched to active therapy. All patients therefore received a new buildup phase of SIT in order to keep the treatment blinded to the subjects; the study was prolonged in this manner for two more years, until October 1993, when the SIT was discontinued. The clinical effectiveness and the safety of the investigational treatment were assessed by means of a clinical diary card, in which patients recorded the presence and severity of symptoms and the drug intake amount during pollen seasons. In addition, a subjective assessment of the effectiveness of the treatment was recorded during the study and 4 years after SIT discontinuation. Some paraclinical parameters were assessed as well, including skin reactivity, serum IgG4, and serum IgE.

### Patients

Twenty-five patients, 17 females and eight males aged 13–62 years (mean 32.1 years), suffering from rhinoconjunctivitis (five out of them also from mild intermittent asthma) were enrolled in the study. All the patients had single sensitization to *Parietaria*, as assessed by skin test (class ++ or more) and RAST (class II or higher), and their disease had lasted at least 2 years. None of the patients had anatomic alterations of the upper respiratory tract, or suffered from immunodeficiencies, malignancies, or severe psychologic disorders. Moreover, no patient was receiving chronic steroid or  $\beta$ -blocking treatments, or had received SIT during the previous 5 years. Pregnant or lactating women were not admitted. All patients or their relatives were asked to sign an informed consent before the study, and the trial was approved by the central ethical committee. The patient population was homogeneous at the beginning of the study, as summarized in Table 1.

**Table 1.** Demographic data

|                             | Active | Placebo |
|-----------------------------|--------|---------|
| <i>n</i>                    | 13     | 12      |
| Sex (M/F)                   | 4/9    | 4/8     |
| Age (years)                 |        |         |
| Mean                        | 33.6   | 30.5    |
| Range                       | 13–62  | 17–51   |
| Duration of disease (years) | 12     | 11      |
| Asthma                      | 3/13   | 2/12    |

### SIT and concomitant medications

The investigational SIT (Purethal<sup>®</sup>-*Parietaria*, Haarlem Allergen Labs BV, Haarlem, The Netherlands) is a glutaraldehyde-modified allergoid obtained from equal amounts of *Parietaria judaica* and *P. officinalis* pollens. The modification reduces the allergenicity to 1% or less. The composition of the extract per ml was as follows: allergoid 500 µg, aluminum hydroxide 1.1 mg, sodium chloride 9 mg, phenol 5 mg, and distilled water 1 ml. The molecular weight of the product is in the range 100–2000 kDa with a maximum of about 900 kDa. The extract used as starting material for modification was standardized in allergy units (AU), according to FDA protocols, which are based on intracutaneous tests; 500 µg of the modified material corresponds to about 20 000 AU of unmodified allergen. The potency of Purethal is therefore designated as 20 000 AUeq (equivalent) per ml. The administration schedule involved a buildup phase of increasing doses (1000, 2000, 4000, 6000, 8000, 10 000 AUeq) to be injected each week. The maintenance phase involved the injection of 10 000 AUeq at monthly intervals. In the case of side-effects, the dose was repeated or

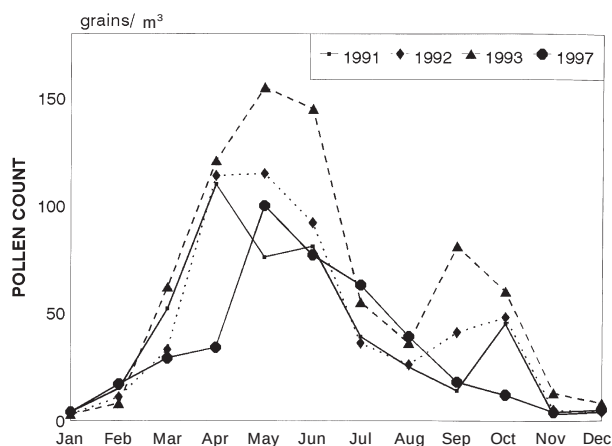


Figure 1. Monthly mean *Parietaria* pollen counts (grains/m<sup>3</sup>) during 4 years of interest.

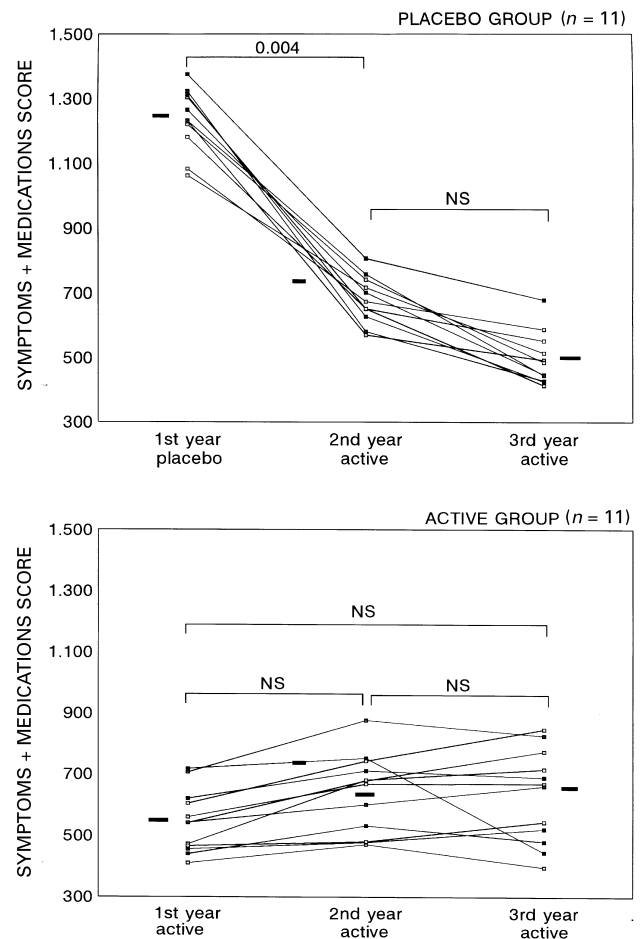


Figure 2. Symptom plus drug-intake scores for each pollen season (March–September), patient per patient. Upper panel: placebo group; lower panel: active group. Median values are plotted as horizontal thick bars. Intragroup comparisons are also shown.

temporarily reduced, according to the international guidelines (2). The total amount of extract administered was 100 000–120 000 AUeq in the first year and 120 000 AUeq in the following years. The placebo treatment had the same composition, except for the allergen, and it was indistinguishable in aspect and color from the active one.

All patients could use, if needed, some symptomatic medications: loratadine or cetirizine (one tablet of 10 mg/day), intranasal beclomethasone (100 µg/puff), and inhaled albuterol (100 µg/puff).

### Clinical parameters: symptoms/drug intake, side-effects, and self-evaluation

All patients were required to record on a standard diary card the presence and the intensity of allergic symptoms during the pollen seasons (March–October). Each symptom (sneezing, rhinorrhea, nasal obstruction, nasal/conjunctival itch-

**Table 2.** Self-evaluation questionnaire after year 1

| Parameter             | Active | Placebo | P value             |
|-----------------------|--------|---------|---------------------|
| Frequency of symptoms |        |         | 0.001               |
| Decreased             | 10     | 1       |                     |
| Unchanged             | 3      | 9       |                     |
| Increased             | 0      | 2       |                     |
| Physical performance  |        |         | 0.043               |
| Improved              | 6      | 1       |                     |
| Unchanged             | 6      | 9       |                     |
| Worsened              | 1      | 2       |                     |
| Duration of symptoms  |        |         | 0.024               |
| Shortened             | 5      | 0       |                     |
| Unchanged             | 8      | 7       |                     |
| Lengthened            | 0      | 5       |                     |
| Satisfaction          |        |         | 0.002               |
| Yes                   | 11     | 1       |                     |
| Indifferent           | 1      | 0       |                     |
| No                    | 1      | 11      | Fisher's exact test |

ing, lacrimation, cough, and wheezing) was graded on an arbitrary scale as follows: 0: no symptom, 1: moderate, 2: severe. Moreover, each dose of each symptomatic drug taken was recorded as score 1. The mean cumulative score for each season was considered.

The side-effects due to SIT injections were classified as follows:

- 1) mild local: wheal/flare <5 cm, granuloma persisting <1 week, slight pain
- 2) moderate local: wheal/flare <10 cm, granuloma persisting <3 weeks

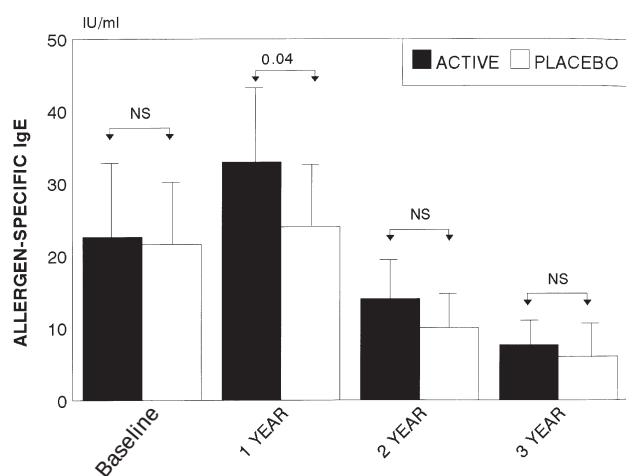


Figure 3. Allergen-specific IgE serum levels (mean, SEM) in two groups of patients.

**Table 3.** Self-evaluation (percent improvement)

|         | 1991        | 1992        | 1993        | 1997        |
|---------|-------------|-------------|-------------|-------------|
| Active  | +31.6 (±10) | +36.2 (±12) | +48.3 (±13) | +35.8 (±15) |
| ↑       |             |             |             |             |
| P       | 0.01        | NS          | 0.05        | NS          |
| ↓       |             |             |             |             |
| Placebo | -15 (±7)    | +29.5 (±9)  | +38.1 (±12) | +31.3 (±11) |

- 3) severe local: wheal/flare >10 cm, granuloma persisting >3 weeks, pain requiring medications
- 4) mild systemic: rhinitis, conjunctivitis, asthma, urticaria not requiring treatment
- 5) severe systemic: like the previous but requiring pharmacologic treatment.

A self-assessment of the efficacy of the treatment as compared to the pretreatment situation was performed after year 1 via a questionnaire on frequency of symptoms, physical performance, duration of complaints, and global satisfaction. Each of these parameters was graded as improved, unchanged, or worsened. Finally, the patients were required to grade the perceived change of their clinical condition as + (improvement) or – (worsening) on a visual analog scale, after 1, 2, and 3 years of therapy and 4 years after its discontinuation.

### Skin reactivity

Skin reactivity was assessed by a standardized skin prick test, with three increasing allergen concentrations (i.e., 3000, 10000, and 30000 AU/ml) plus a negative and a positive control. The skin tests were performed at baseline and during year 3 of study, when all the 23 followed patients were receiving active treatment. The wheal reaction was measured as the sum of the largest diameter plus its orthogonal.

### Systemic immunologic parameters

Blood samples were obtained from each patient at the beginning of the study and after 1, 2, and 3 years of treatment. These samples were assayed at the same time for allergen-specific IgG4 and IgE measurement. The measurement of serum specific IgG4 and IgE was performed through a commercial immunoenzymatic method (FAST Test, 3M Diagnostic Systems, Santa Clara, CA, USA) employing a fluorescent substrate (15). The results were expressed in IU/ml by means of a six-point calibration curve.

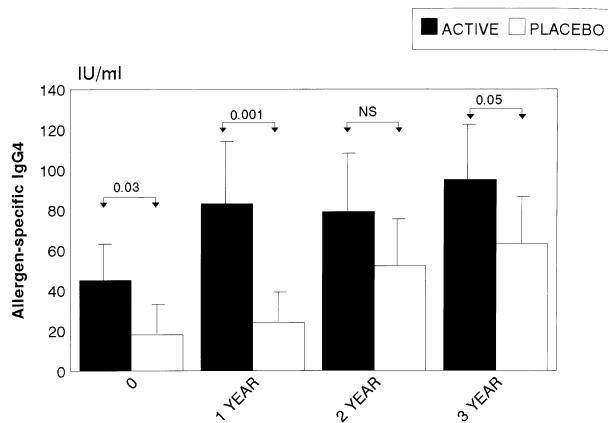


Figure 4. Allergen-specific IgG4 serum levels (mean, SEM) in two groups of patients.

### Pollen counts

The pollen counts for the relevant area were made with a continuous volumetric apparatus (Hirst spore trap VPPS 2000, Lanzoni srl, Italy) placed 20 m above ground level, far from sources of pollution and exposed to winds. The pollen counts were performed by the personnel of the Allergology Department of Bordighera Hospital.

### Statistical analysis

The parameters evaluated by scores (symptoms/drug intake, side-effects, subjective evaluation) may be nonnormally distributed; therefore, the Wilcoxon and Mann-Whitney nonparametric tests were used. On the other hand, Student's *t*-test for parametric values was employed. Fisher's exact probability test was used for the self-evaluation data by questionnaire.

## Results

Two patients dropped out during year 3 of the study: one patient from the original placebo group became pregnant, and one patient from the original active group showed low compliance with the treatment and study design. Therefore, we had available data of 25 patients in year 2 and of 23 patients at year 3 and at the long-term evaluation. All these patients showed good compliance with the treatment schedule. For better evaluation of the investigated parameters, the two groups were kept separated as "placebo" and "active" throughout the whole study, and also when all patients were receiving active SIT. The pollen counts in the regions concerned were superimposable during the 3 years of

the study and in 1997, 4 years after SIT discontinuation (Fig. 1).

### Safety

No systemic or local reaction was observed in the placebo-treated patients. In the actively treated patients, two moderate (asthma) and three mild (rhinitis) systemic reactions were observed during the buildup phase. All these reactions occurred within minutes after the injection, and they were relieved with symptomatic treatment. Five mild, three moderate, and four severe local reactions were also recorded in the active group. All the adverse reactions were easily managed by dosage repeat or reduction. No reaction was observed during the maintenance phase of the treatment, and no anaphylactic reaction occurred.

### Clinical efficacy

The symptom plus drug intake scores of the two groups for each considered pollen season are plotted in Fig. 2. The active group showed a significant improvement already in year 1 of treatment in comparison to the placebo group ( $P=0.02$ ). After switching to the active treatment, the former placebo group also showed a significant improvement from baseline values (see Fig. 2, upper panel), and their clinical scores approximated those of the active group. The global subjective assessment of the efficacy of the treatment, provided by the patients after year 1 of treatment, is summarized in Table 2. There was a significant difference between the treated and placebo group as far as personal judgment is concerned ( $P<0.05$  for each evaluated item). The results of the self-evaluation by analog scale are shown in Table 3. The actively treated patients reported a subjective improvement from year 1, and this improvement remained unchanged during the whole treatment period up to 4 years after the discontinuation of SIT. The former placebo group improved after switching to active treatment, and it maintained this improvement up to 4 years after SIT discontinuation (Table 3).

### Skin reactivity

A decrease in skin reactivity (main wheal diameter plus its orthogonal) was observed in both groups at year 3 of treatment from baseline for the three allergen concentrations used (3 kAU/ml: 17.1 vs 14.2 mm,  $P=0.02$ ; 10 kAU/ml: 22.8 vs 19.2 mm,  $P=0.01$ ; 30 kAU/ml: 27.2 vs 22.6 mm,  $P=0.002$ ).

## Allergen-specific IgE and IgG4

After year 1 of treatment, a significant increase of the specific IgE was observed only in the actively treated group ( $P=0.004$ ). Subsequently, the IgE concentration constantly and significantly decreased (baseline vs year 2:  $P=0.04$ ; baseline vs year 3:  $P=0.003$ ). On the other hand, no change was detectable in the placebo group after year 1. After year 2 of treatment, we observed also in the previously placebo group a significant fall of IgE levels (baseline vs year 2:  $P=0.01$ ; baseline vs year 3:  $P=0.006$ ). The results are plotted in Fig. 3. As far as specific IgG4 is concerned, a significant increase was observed in the active group (but not in the placebo one) after 1 year of treatment, and this effect remained unchanged at years 2 and 3 (baseline vs years 2 and 3:  $P<0.01$ ). After switching to active treatment, the previously placebo group also showed an increase in serum IgG4. The increase was less important than in the active group and reached statistical significance in comparison with baseline only at year 3 ( $P=0.05$ ) (Fig. 4).

## Discussion

SIT is a valuable tool for the prophylactic treatment of respiratory allergies when it is correctly prescribed and administered (1, 2). Improving the safety of the treatment has long been one of the major aims of the clinical research, and the modified allergens (allergoids) represent a rational approach to improving the safety. Allergoids are chemically modified (by glutaraldehyde or formaldehyde) allergens, which maintain the capacity of modulating the immune response without reacting to the specific IgE. Allergoid preparations have been tested and validated in several controlled trials on allergic patients. In the present randomized clinical trial, we evaluated for up to 3 years the clinical efficacy and some paraclinical effects of allergoid immunotherapy (Purethal<sup>®</sup> *Parietaria*, Haarlem Allergen Labs) with *Parietaria* pollen. The study was kept double-blind and placebo-controlled during year 1; in years 2 and 3, the active treatment was administered to all patients. The clinical effectiveness of the investigational SIT was clearcut: the patients receiving the active treatment

showed a significant improvement of the symptom plus drug-intake score. The cumulative score of symptoms plus drug consumption can be considered a reliable global index of clinical improvement, since symptoms and drug intake are strictly dependent on each other.

A significant reduction of the specific IgE levels was observed in all patients after 12 months of active treatment in comparison with the baseline levels. Furthermore, an increase in the allergen-specific IgG4 was also observed, as previously described. In the present experimental conditions, these changes well correlate with the clinical efficacy. Nevertheless, it is not possible to determine whether the Ig modifications are an active part of the mechanism of action of SIT (blocking antibodies theory) or rather represent an epiphenomenon of the allergen administration (16, 17). In any case, the observation confirms that SIT affects the immune response and the specific Ig balance. As far as the skin is concerned, a reduction of the clinical early wheal/flare response has been observed, and it was consistent with the reported detailed observations in the cutaneous model (18).

The subjective self-assessment herein performed by a questionnaire globally confirmed the objective clinical measurements. It is important to underline that the perceived improvement refers to the global physical performance, which can be considered an indirect index of the quality of life. Interestingly, the overall self-evaluation by analogic scale showed that the clinical improvement was maintained for up to 4 years in all patients, whether receiving a 3-year or a 2-year course of treatment. Moreover, taking into consideration the intrinsic limits of such a simple self-evaluation, we may assume that the duration of the treatment is critical to the long-lasting effects.

As far as the safety of the treatment is concerned, some reactions, both systemic and local, occurred, but they were not life-threatening and responded to simple management by the allergist. Furthermore, a temporary dose reduction was sufficient to overcome the inconvenience and to achieve the scheduled dose. In conclusion, the herein investigated allergoid SIT with *Parietaria* pollen was demonstrated to be an effective and safe therapeutic approach for the long-term treatment of respiratory allergy.

## References

1. Allergen immunotherapy: therapeutical vaccines for allergic diseases. Bousquet J, Lockey RF, Malling H-J, editors. In: WHO position paper. *Allergy* 1998;**53 Suppl** 44:1–12.
2. EAACI position paper on allergen immunotherapy. Malling H-J, Weeke B, editors. *Allergy* 1993;**48 Suppl** 14:9–35.
3. British Society of Allergy and Clinical Immunology. Position paper on allergen immunotherapy. *Clin Exp Allergy* 1993;**23**:3–14.

4. Abramson MJ, Puy RM, Weiner JM. Is allergen specific immunotherapy effective in asthma? *Am J Respir Crit Care Med* 1995;**151**:969–974.
5. Norman PS. WHO-IUIS International Standards. Advantages of these extracts. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M* 1994;**87**:59–64.
6. Passalacqua G, Canonica GW. Alternative routes for immunotherapy. *J Invest Allergol Clin Immunol* 1996;**6**:81–86.
7. Grammer LC, Shaughnessy MA, Patterson R. Modified forms of allergen immunotherapy. *J Allergy Clin Immunol* 1985;**76**:397–401.
8. Marsh DG, Norman PS, Roebber M, Lichtenstein LM. Studies of allergoids from naturally occurring allergens. III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. *J Allergy Clin Immunol* 1981;**68**:449–459.
9. Bousquet J, Heijaoi A, Soussana M, Michel FB. Double blind placebo controlled immunotherapy with mixed grass pollen allergoids. IV. Comparison of the efficacy and safety of two dosages of a high molecular weight allergoid. *J Allergy Clin Immunol* 1990;**85**:490–497.
10. Pastorello EA, Pravettoni V, Incorvaia C, Ortolani C. Clinical and immunological effects of immunotherapy with alum absorbed grass allergoid in grass pollen induced hay fever. *Allergy* 1992;**47**:281–290.
11. Meriney DK, Kothari H, Chinoy P, Grieco MH. The clinical and immunological efficacy of immunotherapy with modified ragweed extract (allergoid) for ragweed hay fever. *Ann Allergy* 1986;**56**:34–38.
12. Grammer LC, Shaughnessy SA, Suszko IM, Shaughnessy JJ, Patterson R. Persistence of efficacy after a brief course of polymerized ragweed allergen: a controlled study. *J Allergy Clin Immunol* 1984;**73**:484–489.
13. Hedlin G, Heilborn H, Lilja G, Löwenstein H. Long-term follow up of patients treated with a three-year course of cat or dog immunotherapy. *J Allergy Clin Immunol* 1995;**96**:879–885.
14. Jacobsen L, Nüchel-Petersen B, Wihl JÅ, Löwenstein H, Ipsen H. Immunotherapy with partially purified and standardized tree pollen extracts. IV. Results from long-term (6-year) follow-up. *Allergy* 1997;**52**:914–920.
15. Jones LA, Jones RM, Sun JV, Tsay YG, Rock HS, Nicolson R. IgE FAST inhibition. A rapid technique for the standardization of pollen allergen extract potency. *Ann Allergy* 1986;**56**:222–225.
16. Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on IgE and IgG antibodies to ragweed antigens. A six-year prospective study. *J Allergy Clin Immunol* 1982;**70**:261–271.
17. Wittman AM, Stapel SO, Sjamsoedin DH, Jansen HM, Aalberse RC, van der Zee JS. Fel d 1 specific IgG antibodies induced by natural exposure have blocking activity in skin test. *Int Arch Allergy Immunol* 1996;**109**:369–375.
18. Varney VA, Hamid QA, Gaga M, Durham RS. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late phase cutaneous response. *J Clin Invest* 1993;**92**:644–651.