*Allergology International* (2002) **51**: 233–240

Original Article

# Evaluation of two grass pollen extracts for immunotherapy by serum determinations of specific IgE and IgG4 antibodies towards purified Timothy grass pollen allergens (Phl p 1, 2, 4, 5, 6, 7, 11, 12) in patients undergoing hyposensitization treatment

Renato Enzo Rossi,[1] Giorgio Monasterolo,[2] Antonio Diana,[3] Serena Monasterolo[2] and Maurizio Delucchi[3]

[1]Allergy Unit, National Health Service, Regione Piemonte, [2]Laboratorio Analisi, Ospedale S.S. Trinità, Fossano and [3]UOA di Medicina Generale Ospedale S.S. Annunziata, Savigliano, Italy

## Abstract

**Background:** The diagnosis of allergic diseases with recombinant allergens allows us to detect antibodies specific for single allergens in extracts. The aim of the present study was to assess the early effect of grass pollen immunotherapy on IgE and IgG4 responses to eight purified grass pollen allergens in patients undergoing hyposensitization treatment.

**Methods:** The sera of 22 consecutive atopic individuals undergoing cluster regimen grass pollen immunotherapy were analyzed for IgE and IgG4 antibodies specific for grass pollen allergens (Phl p 1, 2, 4, 5, 6, 7, 11, 12). Two serum samples were taken, one before the start of therapy and one between 12 and 15 weeks after the first immunization. Immunotherapy was performed with two allergy vaccines comprising a standardized extract aluminum-adsorbed grass pollen mix and a standardized extract of grass pollen mix adsorbed onto calcium phosphate.

**Results:** One treated patient showed a specific IgE conversion from negative (< 0.35 kUA/L) to positive in the capsulated hydrophilic carrier polymer (CAP) test for Phl p 2, 1 and 4 (1.89, 0.84 and 0.68 kUA/L, respectively). The sera of 10 of 11 patients treated with alluminum-adsorbed grass pollen extract showed a significant increase in specific IgG4 towards natural Timothy grass pollen extract and purified allergens, as well as significant IgG4 levels towards Phl p 1 ($P = 0.000238$) Phl p 2 ($P = 0.000289$), Phl p 4 ($P = 0.000585$), Phl p 5 ($P = 0.000364$), Phl p 6 ($P = 0.000346$) and Phl p 11 ($P = 0.039623$; Mann–Whitney *U*-test) 12 weeks after the onset of immunotherapy. The sera of seven of 11 subjects treated with calcium phospate-adsorbed grass pollen extract had significant IgG4 levels against Timothy pollen allergens, as well as significant IgG4 titers against Phl p 1 ($P = 0.004703$), Phl p 4 ($P = 0.000282$), Phl p 5 ($P = 0.015480$), Phl p 6 ($P = 0.013012$) and Phl p 11 ($P = 0.005178$). Patients treated with aluminum-adsorbed grass pollen extract had higher levels of IgG4 towards Phl p 2, 4 and 6 and natural Timothy grass extract compared with patients treated with calcium phosphate-adsorbed grass pollen extract. Both the alluminum-adsorbed and calcium phosphate-adsorbed grass pollen extract allergy vaccines induced significant titers of specific IgG4 towards *Phleum pratense* pollen extract ($P = 0.008376$ and 0.01148, respectively).

**Conclusions:** These results indicate that grass pollen immunotherapy elicits an array of antibody specificities that reflect the allergen content and the potency of allergen extracts; this could be of pivotal importance to define optimal allergen extract doses.

Correspondence: RE Rossi, Unità di Immunoallergologia, Via Ospedali 14, 12038 Savigliano, Italy.
Email: immunoway@libero.it

Received 18 December 2001. Accepted for publication 17 June 2002.

**Key words:** allergen immunotherapy, IgE grass pollen allergy, IgG4 subclass, recombinant allergens.

## INTRODUCTION

The usefulness of specific immunotherapy (SIT) has been highlighted in a recent World Health Organization report that advocates its use in patients with specific IgE antibodies to clinically relevant allergens.[1] In patients allergic to grass pollen, treatment is usually performed with mixtures of different grass species. The rationale behind this approach is to cover the spectrum of grass pollen allergens as completely as possible.[2] There is evidence that SIT is effective in the prevention of allergic asthma in children.[3] Recently, it was reported that grass pollen SIT improves quality of life in seasonal allergic rhinitis and reduces seasonal asthma symptoms and bronchial hyperresponsiveness.[4]

It is well established that hyposensitization treatment induces allergen-specific IgG4 antibodies.[5,6] It was suggested that blocking antibodies belonging to the IgG4 subclass may be induced by specific immunotherapy.[5] However, several studies failed to correlate the increase of allergen-specific IgG4 levels with successful SIT.[7] In contrast, previous studies on the role of allergen-specific IgE and IgG4 responses were hampered by the lack of well-defined allergen extracts. Recombinant allergens (RA) have now become available for antibody measurements with defined components.

The aim of the present study was to analyze data obtained by measuring the recognition of defined (i.e. recombinant) allergens by serum IgE and IgG4 antibodies in the early phase of SIT in patients undergoing cluster regimen hyposensitization therapy.

## METHODS

### Patients

Twenty-two consecutive patients, age range 16–40 years (A1–11) and 5–56 years (P1–11), who were allergic to grass pollen, as confirmed by a wheal size of at least 4 mm in the skin prick test, were evaluated. All patients had typical symptoms of rhinoconjunctivitis and/or asthma in the grass pollen season from April to July and specific IgE against Timothy pollen extract (Table 1). Patients were excluded from the study if they had received SIT in the preceding 5 years.

**Table 1** Demographic and clinical data of the patients

| | Patients | |
|---|---|---|
| | A1–11 | P1–11 |
| Sex (M/F) | 5/6 | 7/4 |
| Age (years) | | |
| Mean | 28 | 26 |
| Range | 16–40 | 5–56 |
| Diagnosis | | |
| Rhinitis (*n*) | 2 | 3 |
| Rhinitis and asthma (*n*) | 9 | 8 |

Patients A1–11 received Alutard® Grassmix-5 SQ (ALK-Abello, Hørsholm, Denmark), whereas patients P1–11 received Phostal® Grassmix-3 (Stallergenes, Antony, France).

### Study design

Treatment of patients started in November 2000 and observations were continued until November 2001. Two serum samples were taken at the start of therapy (November/December) and preseasonally (March) in the first year of therapy.

All patients were symptomless before and 2 weeks after the last injection of allergen extract. Two different groups were included in the study. Both groups contained sera from 11 patients.

Treatment was performed with Alutard® Grassmix-5 SQ (ALK-Abello, Hørsholm, Denmark; patients A1–11) and Phostal® Grassmix-3 (Stallergenes, Antony, France; patients P1–11).

### Immunotherapy protocol

Active treatment involved either Alutard SQ®, a standardized aluminum-adsorbed extract of grass pollen mix (*Dactilis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense* and *Poa Pratensis*) or Phostal®, a standardized extract of grass pollen mix (*D. glomerata*, *P. pratense* and *P. pratensis*) adsorbed onto calcium phosphate.

A modified 'cluster' regimen of injections of Alutard SQ®, with once weekly visits for 5 weeks, was performed (Table 2), followed by 3 weeks of maintenance injections. Each 1 mL maintenance injection of 100 000 standard quality units (SQU). Was equivalent to 10 000 biologic units[8] and contained approximately 2.4 μg Dag g 5, 3.7 μg Fes p 5, 2.50 μg Lol p 5, 4.02 μg Phl p 5 and 3.04 μg Poa p 5 per 100 000 SQU. A similar 'cluster' regimen of injections of Phostal® (approximately 8.5 μg/mL group 5 allergen) was performed (Table 3).

**Table 2** Alutard® cluster immunotherapy updosing and maintenance schedule

| Visit no. | Week | Injection | Concentration (SQU/mL) | Volume (mL) |
|---|---|---|---|---|
| 1 | 1 | 1 | 100 | 0.1 |
| | | 2 | 1000 | 0.1 |
| | | 3 | 10 000 | 0.05 |
| 2 | 2 | 4 | 10 000 | 0.2 |
| | | 5 | 10 000 | 0.2 |
| 3 | 3 | 6 | 10 000 | 0.5 |
| | | 7 | 100 000 | 0.2 |
| 4 | 4 | 8 | 100 000 | 0.4 |
| | | 9 | 100 000 | 0.4 |
| 5 | 5 | 10 | 100 000 | 0.5 |
| | | 11 | 100 000 | 0.5 |
| 6 | 6 | 12 | 100 000 | 1.00 |
| 7 | 9 | 13 | 100 000 | 1.00 |

Injections were given at 30 min intervals and patients were observed after the last injection of each cluster. Blood samples were taken before treatment and 2 weeks after the 12th injection.

SQU, standard quality units.

**Table 3** Phostal® cluster immunotherapy updosing and maintenance schedule

| Visit no. | Week | Injection | Concentration (IR/mL) | Volume (mL) |
|---|---|---|---|---|
| 1 | 1 | 1 | 0.1 | 0.1 |
| | | 2 | 0.1 | 0.2 |
| | | 3 | 0.1 | 0.2 |
| 2 | 2 | 4 | 1 | 0.1 |
| | | 5 | 1 | 0.1 |
| 3 | 3 | 6 | 1 | 0.2 |
| | | 7 | 1 | 0.2 |
| 4 | 4 | 8 | 10 | 0.2 |
| 5 | | 9 | 10 | 0.2 |
| 6 | 5 | 10 | 10 | 0.4 |
| | | 11 | 10 | 0.4 |
| 7 | 6 | 12 | 10 | 1.00 |
| 8 | 9 | 13 | 10 | 1.00 |
| 9 | 13 | 14 | 10 | 1.00 |

Injections were given at 30 min intervals and patients were observed after the last injection of each cluster. Blood samples were taken before treatment and 2 weeks after 14th injection.

IR, index of reactivity.

Immunotherapy was performed by the same allergist (RER), with full resuscitation facilities available. Local cutaneous reactions at the injection site and any other symptoms occurring within 5 h were documented.

### *In vitro* assays

Serum-specific IgE and IgG4 were analyzed by the Pharmacia CAP System (Pharmacia-Upjohn, Uppsala, Sweden) according to the manufacturer's instructions. The sera were characterized in detail by determination of IgE and IgG4 antibodies towards Phl p 1, 2, 4, 5, 6, 7, 11 and 12. Specific IgE/IgG against Timothy grass was also measured.

### Statistical analysis

Results are expressed as the median and 25th–75th percentile. The non-parametric Mann–Whitney *U*-test and Wilcoxon test were used for statistical comparisons.

Patients were also evaluated with a mixed design analysis of variance (ANOVA). $P < 0.05$ was considered significant.

## RESULTS

### Systemic reactions to SIT

Two mild systemic reactions were seen during the induction period in the Alutard SQ®-treated group (one sneezing, one mild wheezing with mild urticaria and itching). During maintenance treatment, no delayed systemic reactions were observed.

One mild systemic reaction was observed during the induction period in one patient treated with Phostal® (urticaria, itching). All patients continued with therapy without further incident.

### Clinical efficacy of SIT

Patients were asked to give their impressions of the degree of improvement after 10–12 months of treatment, on a scale of 0–3 (0, worsening; 3, remarkable improvement). All patients, except for one treated with Phostal®, declared an improvement in symptoms during the pollen season. However, a double-blind placebo-controlled study was not performed on these patients.

### Timothy and Bermuda grass pollen-specific IgE antibodies and specific IgE towards purified allergens

All pre- and post-SIT sera ($n = 44$) were tested by the CAP system for RA, native Phl p 4 and natural Timothy grass-specific IgE (Fig. 1). The mean of the time interval between the collection of the two samples was 13 weeks. Before the onset of SIT, the sera of patients A1–11 and patients P1–11 were homogeneous with respect to specific IgE levels, as established by ANOVA (data not shown) and the Mann–Whitney *U*-test (Fig. 1).

One treated patient showed a conversion from negative (< 0.35 kUA/L) to positive in the CAP test for Phl p 2, 1 and 4 (1.89, 0.84 and 0.68 kUA/L, respectively).

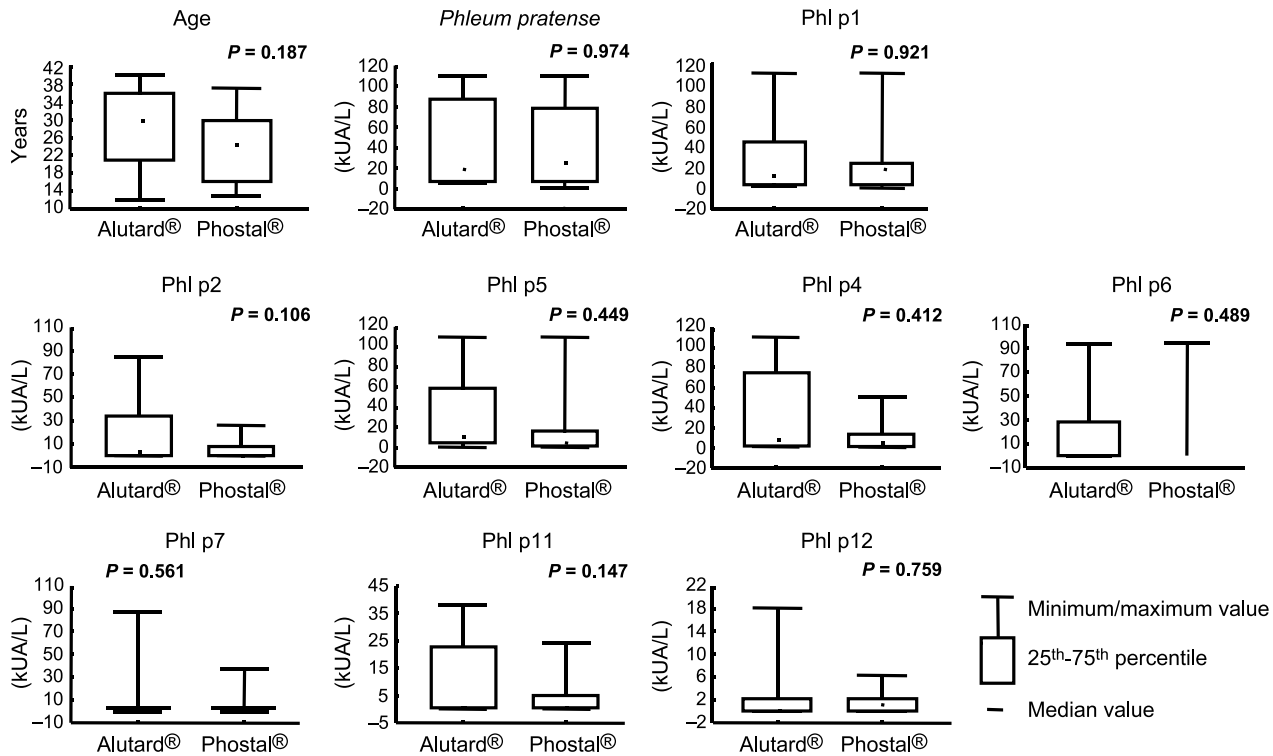


**Fig. 1** IgE levels towards purified allergens and natural extract before the onset of immunotherapy in 11 patients treated with aluminum-adsorbed grass pollen extract (Alutard® Grassmix-5 SQ; ALK-Abello, Hørsholm, Denmark) and calcium phosphate-adsorbed grass pollen extract (Phostal® Grassmix-3; Stallergenes, Antony, France; Mann-Whitney *U*-test).

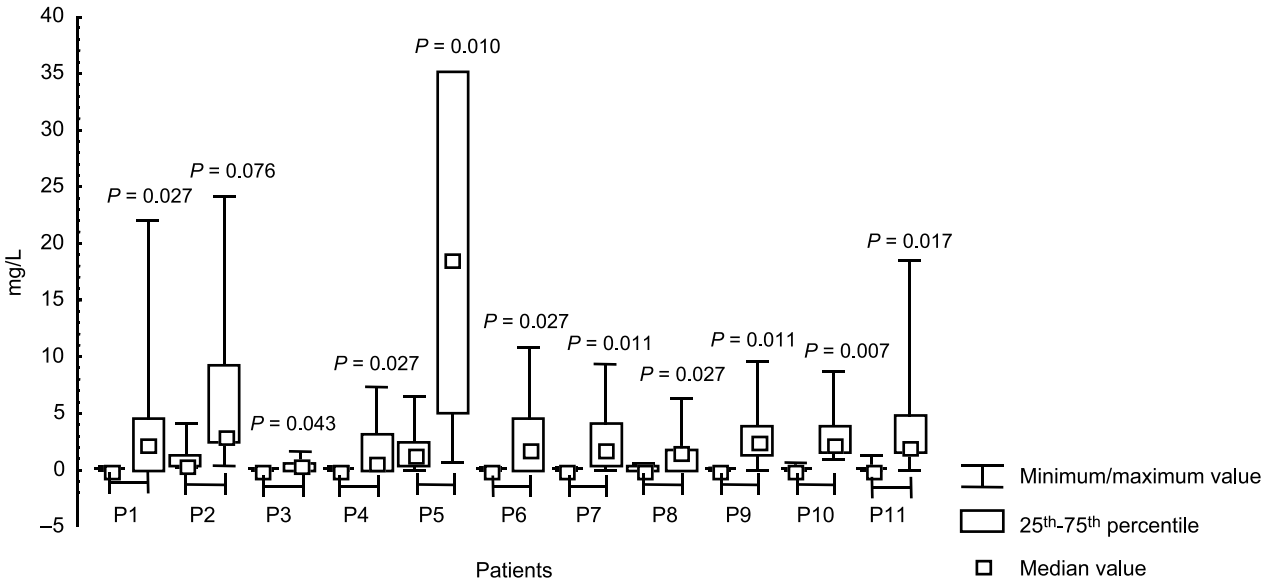


**Fig. 2** Comparison of IgG4 responses at the onset of and 12 weeks after immunotherapy with aluminum-adsorbed grass pollen extract in 11 patients allergic to grass pollen (Wilcoxon test). The amount of allergen administered during the 12 weeks was approximately 65 μg group 5 allergen. A1–A11, patients 1–11.

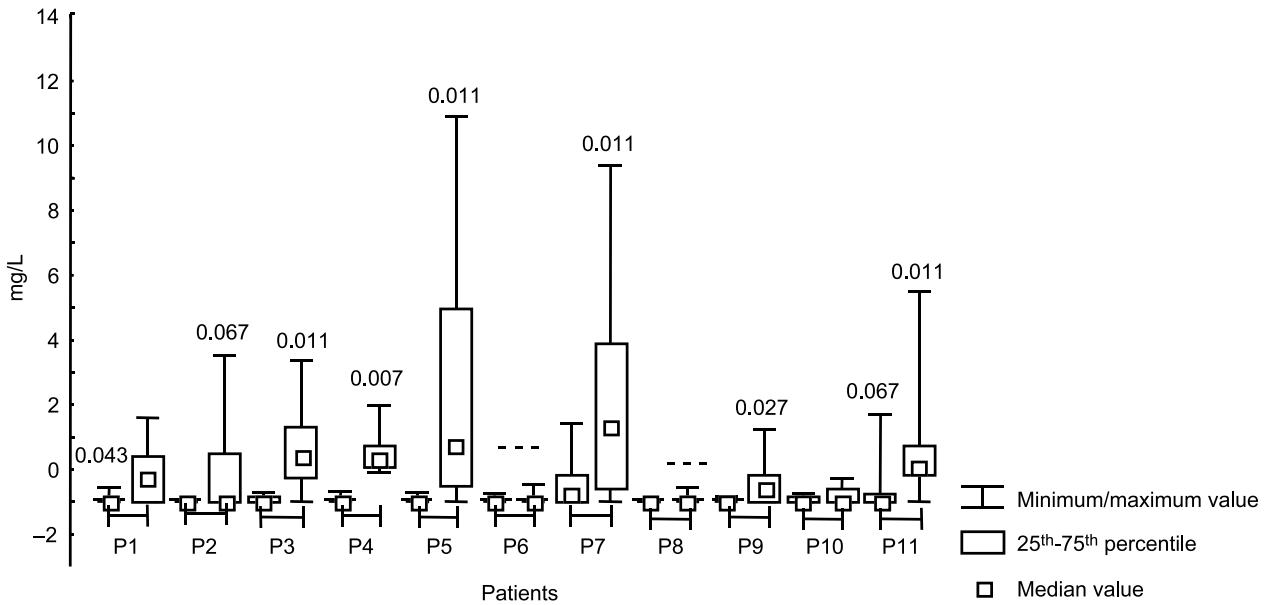


**Fig. 3** Comparison of IgG4 responses at the onset of and 15 weeks after immunotherapy with calcium phosphate-adsorbed grass pollen extract in 11 patients allergic to grass pollen (Wilcoxon test). The amount of allergen administered during the 15 weeks was approximately 40 μg group 5 allergen. P1–P11, patients 1–11.

## Timothy grass, Bermuda grass pollen and purified allergen-specific IgG4 measured by the CAP test

The titers of IgG4 directed against natural extracts and RA (and native Phl p 4) were raised significantly in treated patients compared with values measured before the beginning of SIT (Fig. 2). We documented an increase in IgG4 towards certain allergens in the sera that did not seem to bind specific IgE before and after SIT (14/52; 26.9%). In these cases, post-SIT IgG4 levels did not exceed 1.42 mg/L (value referred to Phl p 5). Alutard SQ® induced significant titers of IgG4 towards Phl p 1 ($P = 0.000238$), Phl p 2 ($P = 0.000289$), Phl p 4 ($P = 0.000585$), Phl p 5 ($P = 0.000364$), Phl p 6 ($P = 0.000346$) and Phl p 11 ($P = 0.039623$), but not Phl p 12 ($P = 0.044310$) and Phl p 7 ($P = 0.288002$). Phostal® induced significant levels of IgG4 against Phl p 1 ($P = 0.0004703$), Phl p 4 ($P = 0.000282$), Phl p 5 ($P = 0.015480$), Phl p 6 ($P = 0.013012$) and Phl p 11 ($P = 0.005175$), but not to the remaining purified allergens. Both allergen extracts for SIT induced significant titers of specific IgG4 towards *Phleum pratense* (Alutard SQ® $P = 0.008376$; Phostal® $P = 0.001148$; Mann–Whitney *U*-test).

The sera of 10 of 11 patients (A1–11) showed a significant increase in specific IgG4 towards natural Timothy grass pollen extract and purified allergens (Fig. 2) after 12 weeks. The sera of seven of 11 subjects (P1–11) had significant IgG4 levels against Timothy grass pollen allergens 15 weeks after the first immunization (Fig. 3). At least in the first phase of SIT, patients treated with aluminum-adsorbed grass pollen extract had higher levels of IgG4 towards Phl p 2, 4 and 6 and natural Timothy grass extract than patients treated with calcium phosphate-adsorbed grass pollen extract (Fig. 4)

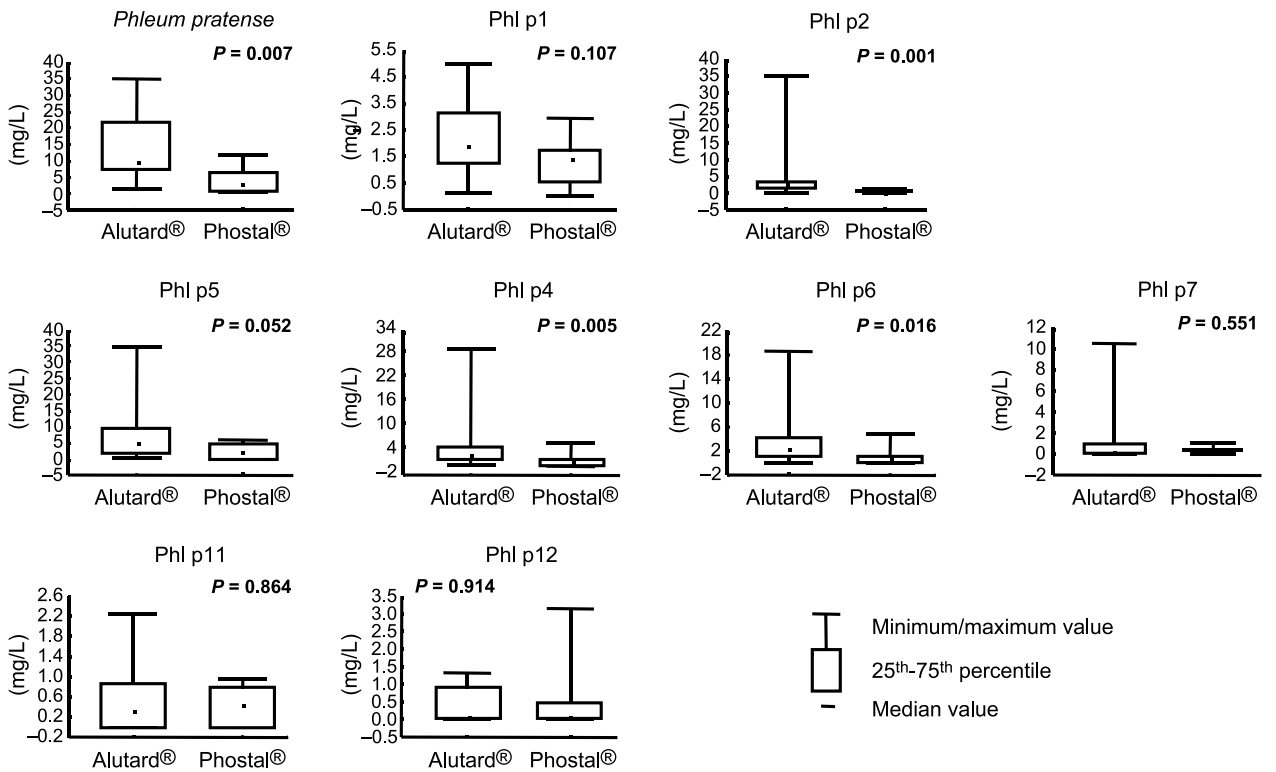


**Fig. 4** Median IgG4 levels of serum IgG4 towards purified allergens and natural Timothy grass pollen extract induced by with aluminum-adsorbed grass pollen extract (Alutard® Grassmix-5 SQ; ALK-Abello, Hørsholm, Denmark) and calcium phosphate-adsorbed grass pollen extract (Phostal® Grassmix-3; Stallergenes, Antony, France) in two groups of allergic patients ($n = 11$ each).

## Discussion

In the present study, the safety profile of the two SIT extracts administered in a modified 'cluster' regimen of injections was good. Local swelling and some mild systemic reactions, which were greater for Alutard SQ®-treated subjects, were seen but were considered by the allergist to be no worse than normally observed with other allergy vaccine regimens. Previously published results demonstrated a marked increase in allergen-specific IgG and IgG4 antibodies in patients treated with conventional subcutaneous SIT, whereas no significant changes in the levels of allergen-specific IgE could be observed.[7,9] In agreement with Gehlhar *et al.*,[10] we did not find any influence of SIT on mean IgE levels, at least in the earlier phase of immunization treatment. However, it is to be noted that other studies have shown an increase in IgE at the beginning of SIT, followed by a decline.[11] In the present study, there was only one grass pollen-allergic subject who had no detectable IgE towards RA and native Phl p 4 at the beginning, but this situation changed 15 weeks later. Although active induction of new IgE specificities by SIT is not really demonstrated, van Ree *et al.*[12] have reported the appearance of IgE with specificities not detected before SIT in approximately 8% of patients. We observed this phenomenon in a serum sample taken 15 weeks after the start of therapy with *Aspergillus* extract (ALK-Abello, Lainate, Italy) and 4000 nitrogen treatment units (NTU) allergen administered. In this case, newly induced IgE antibodies towards rAsp f 3 from negative (< 0.35 kUA/L) to positive (15.1 kUA/L) were detected (RE Rossi *et al.*, unpubl. obs., 2000).

In the present study, 'new' IgE antibodies were directed to major allergens Phl p 2, 1 and 4. This certainly represents an undesiderable effect of SIT. However, IgE production may be partially blocked by the induction of specific IgG4 antibodies. Furthermore, allergen-specific IgG antibodies may bind either to different epitopes or with lower affinity compared with IgE antibodies.[12,13] However, the induction of IgG antibodies by hyposensitization treatment was already noted early[6,14] and the blocking activity of such antibodies has been shown previously.[15] In the present study, we did not investigate the inhibitory effect of these antibodies towards IgE-mediated reactions (i.e. reaction to purified allergen *in vivo* or histamine release using purified allergens *in vitro*).

However, Vrtala *et al.* have shown that a Phl p 6 fragment induces IgG antibodies, which bind to complete Phl p 6 and inhibit the IgE of allergic patients binding to Phl p 6.[16]

Until now, the measurement of IgG4 towards natural allergen extracts has served to ascertain that the immune system is responding to treatment. In a previous study,[17] antibody titers directed against a crude allergen extract, which contains many antigenic proteins besides the relevant allergens, were measured. More recently, tests were performed using purified allergens and these tests showed that IgG antibodies that are allergen-specific are relevant for the observed clinical changes. In fact, Gehlhar *et al.*[10] observed that the ratio of IgG4 to IgG1 could serve as a valuable parameter to assess the success of immunotherapy already 1 year after the onset of immunotherapy. Moreover, sera containing therapy induced allergen-specific IgG antibodies were found to suppress IgE-mediated presentations of allergens to T cells and, thus, to reduce T cell proliferation and cytokine release.[18] Finally, blocking antibodies induced by vaccination with Phl p 1 peptide: (i) reduces Phl p 1-induced effector cell activation; (ii) prevents the production of Phl p 1-specific IgE synthesis; and (iii) inhibits IgE-mediated T cell activation.[19]

The cumulative dose administered with Alutard SQ® and Phostal® (approximately 65 and 40 μg major group 5 allergen, respectively) was relatively high during the 14 weeks of therapy. This may explain the positive effect of SIT on IgG4 production, particularly in patients treated with Alutard SQ®. Moreover, the main implication from the present study is that the allergen extracts for immunotherapy contain, as expected, relevant allergens recognized by the immune system (i.e. Phl p 1, 2, 4, 6 and 11) in addition to the group 5 allergen declared by the manufacturers. Therefore, IgG4 measurements against purified allergens allow us to monitor indirectly the presence of relevant allergens of a particular SIT extract. Given that a patient's allergogram may be highly variable,[20,21] it may be useful to evaluate IgE and IgG antibodies directed towards defined components in order to adjust the dose of the vaccine administered during a course of treatment.

Thus, IgE/IgG4 antibodies to well-definited allergens can be followed up during the course of the disease and the occurrence of IgE antibodies with new specificity revealed and, possibly, antagonized with blocking antibodies, which could be induced by a well-definited grass pollen extract. In conclusion, for the sake of the best definition of immunotherapeutics, the application of extracts standardized by the content of the major allergen in

μg/mL may be desiderable. It may be possible to improve the quality of treatment by achieving individual doses of RA mixture, through the use of RA variants with reduced IgE reactivity, in the near future.

## ACKNOWLEDGMENTS

The authors thank Dr Domenica Condorelli and Daniela Operti (Laboratorio Analisi Ospedale S.S. Trinità di Fossano) for performing the CAP tests.

## REFERENCES

1 Bousquet J, Lockey RF, Malling H-J (eds). WHO position paper. Allergen immunotherapy: Therapeutic vaccines for allergic diseases. *Allergy* 1998; **53** (Suppl.): 20–2.
2 Joint Task Force on Practice Parameters (AAAAI, ACAAI JCAAI). Practice parameters for allergen immunotherapy: Therapeutic vaccines for allergic diseases. A WHO position paper. *J. Allergy Clin. Immunol.* 1996; **98**: 1001–11.
3 Valovirta E. Capacity of specific immunotherapy in prevention of allergic asthma in children: The Preventive Allergy Treatment study (PAT). *J. Invest. Allergol. Clin. Immunol.* 1999; **7**: 369–70.
4 Walker SM, Pajno GB, Torres Lima M, Wilson DR, Durham SR. Grass pollen immunotherapy for seasonal rhinitis and asthma: A randomized, control trial. *J. Allergy Clin. Immunol.* 2001; **107**: 87–93.
5 Aalberse RC, Gaag R, Leeuwen J. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4 restricted response. *J. Immunol.* 1983; **130**: 722–6.
6 Cooke RA, Bernhard JH, Hebeld S, Stull A. Serological evidence of immunity with co-existing sensitization in hay type of human allergy. *J. Exp. Med.* 1935; **62**: 733–50.
7 Birkner T, Rumpold H, Jarolim E *et al*. Evaluation of ummunotherapy-induced changes in specific IgE, IgG and IgG subclasses in birch pollen allergic patients by means of immunoblotting. Correlation with clinical response. *Allergy* 1990; **45**: 418–26.
8 Dreborg S, Basomba A, Belin L. Biological equilibration of allergen preparations. Methodological aspects and reproducibility. *Clin. Allergy* 1987; **17**: 537–50.
9 Ebner C, Siemann U, Bohle B *et al*. Immunological changes during specific immunotherapy of grass pollen allergy: Reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T cell clones specific to Phl p 1, a major grass pollen allergen. *Clin. Exp. Allergy* 1997; **27**: 1007–15.
10 Gehlhar K, Schlaak M, Becker WM, Bufe A. Monitoring allergen immunotherapy of allergic patients: The ratio of allergen specific IgG4 to IgG1 correlates with clinical outcome. *Clin. Exp. Allergy* 1999; **29**: 497–506.
11 Søndergaard I, Poulsen LK, Østerballe O, Weeke B. Evidence of common regulation of IgE and IgG-subclass antibodies in human during immunotherapy. *Allergy* 1992; **47**: 467–70.
12 Van Ree R, Van Leeuwen WA, Dieges PH *et al*. Measurement of IgE antibodies against purified grass pollen allergens (Lol p1, 2, 3 and 5) during immunotherapy. *Clin. Exp. Allergy* 1997; **27**: 68–74.
13 Kobayashi I, Sakiyama Y, Tame A, Kobayashi K, Matsumoto S. IgE and IgG4 antibodies from patients with mite allergy recognize different epitopes of *Dermatophagoides pteronissinus* group II antigen (Der p 2). *J. Allergy Clin. Immunol.* 1996; **97**: 638–45.
14 Loveless MH. Immunological studies of pollinosis. The presence of 2 antibodies related to the same pollen-antigen in the serum of treated hay fever patients. *J. Immunol.* 1940; **38**: 25–50.
15 Vrtala S, Ball T, Spitzauer S *et al*. Immunization with purified natural and recombinant allergens induces mouse IgG1 antibodies that recognize similar epitopes as human IgE–allergen interaction and allergen-induced basophil degranulation. *J. Immunol.* 1998; **160**: 6137–44.
16 Vrtala S, Focke M, Sperr W, Valent P, Kraft D, Valenta R. Recombinant hypoallergenic fragments of the major timothy grass pollen allergen, Phl p 6. for immunotherapy. *J. Allergy Clin. Immunol.* 2001; **107**: S257 (Abstract).
17 Djurup R, Østerballe O. IgE subclass antibody response in grass pollen-allergic patients undergoing specific immunotherapy. Prognostic value of serum IgG subclass antibody levels early in immunotherapy. *Allergy* 1984; **39**: 433–41.
18 van-Neerven RJJ, Wikborg T, Lund G *et al*. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ cells by inhibiting serum-IgE facilitaded allergen presentation. *J. Immunol.* 1999; **163**: 2944–52.
19 Focke M, Mahler V, Ball T *et al*. Nonanaphylactic synthetic peptides derived from B cell epitopes of the major grass pollen allergen, Phl p1, for allergy vaccination. *FASEB J.* 2001; on-line www.asl17.it
20 Valenta R, Vrtala S, Laffer S, Spitzauer S, Kraft D. Recombinant allergens. *Allergy* 1998; **53**: 552–61.
21 Rossi RE, Monasterolo G, Monasterolo S. Measurement of IgE antibodies against purified grass pollen allergens (Phl p 1, 2, 4, 5, 6, 7, 11, 12) in sera of patients allergic to grass pollen. *Allergy* 2001; **56**: 1180–5.