

Original article

Dose dependence of immunological response to sublingual immunotherapy

Background: Sublingual-swallow immunotherapy (SLIT) is an accepted treatment for allergic rhinitis but its optimal dosage is scantily investigated. We studied the dose dependence of clinical efficacy and immunological response to SLIT by administering two different dosages of the same allergen in rhinitic children monosensitized to grass pollen.

Methods: Seventy-one patients with comparable age and symptoms were randomized to receive SLIT by the same grass pollen extract from Stallergènes (Antony, France), 40 of them with the 100 IR and 31 with the 300 IR extract. All patients recorded diary cards for symptoms, medications and side-effects of the treatment, and had measurements of specific IgE and IgG4 in serum by the CAP System FEIA (Pharmacia, Uppsala, Sweden) and in nasal secretion by an *in situ* incubation method with the same reagents of CAP System FEIA.

Results: Symptom/medication scores during the pollen season were significantly higher in patients treated with the lower dosage compared with those treated with the 300 IR dosage. Side-effects occurred with a comparable rate (25.8% vs 27.5%) in the two groups. Serum-specific IgE and IgG4 had no significant changes after 3 months of SLIT in both groups, while a significant seasonal increase of nasal IgE ($P = 0.015$) and IgG4 ($P = 0.019$) was found only in patients treated with the lower dosage.

Conclusions: A rise of specific IgG4 and a blunting of seasonal increase of specific IgE in serum was repeatedly reported during subcutaneous immunotherapy (SCIT) with pollen extracts. Our findings show such blunting of specific nasal IgE along with a low symptom/medication score in patients treated with SLIT with the higher dosage, but not a concomitant rise of specific nasal IgG4. This suggests a local immunological effect of SLIT, different from systemic mechanisms of SCIT.

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Allergen-specific immunotherapy is an accepted treatment of rhinitis and asthma caused by sensitization to inhalant allergens (1). Sublingual-swallow immunotherapy (SLIT) was introduced as a solution to the problem of systemic reactions to classical subcutaneous route, relatively infrequent but potentially severe and rarely fatal, which in some countries led to a marginal use of this treatment (2).

Early studies were conducted with low doses, but it became subsequently apparent that cumulative dosages much higher than those received during subcutaneous immunotherapy (SCIT) were needed to ensure clinical efficacy and the ARIA consensus document stated that doses at least 50–100 times higher than those of SCIT should be administered (3).

Such doses, which peaked 500 times the dosage of SCIT (4), are definitely safe as showed by a recent meta-analysis (5). However, the dose dependence of the

immunological response, clearly defined with conventional immunotherapy (1), is scantily investigated as to sublingual route. Several studies on SCIT, performed with inhalant allergens or hymenoptera venom, showed an increase of specific IgG, particularly IgG4, in serum and, in case of pollen allergy, a blunting of the seasonal rise of specific IgE antibodies (6–11). The fact that quantitative changes in specific IgG do not always correlate with clinical benefit (7, 9, 11) has questioned the role of such antibodies in immunotherapy, but the importance of modifications of allergen-specific antibodies induced by this treatment was instead focused on the production of IgE and IgG against distinct and not overlapping epitopes (12, 13).

We designed a randomized study to evaluate the relationship between the cumulative dosage received with SLIT and the immunological changes, namely the local and systemic allergen-specific IgE and IgG4 production,

by administering the same allergen extract with two different dosages in children with grass pollen-induced rhinitis.

Material and methods

Study design and patients

From patients referring to the Pediatric Allergy Centre of the University of Perugia during autumn and winter 2002, 90 children suffering from rhinoconjunctivitis, classified as intermittent, mild or moderate persistent according to ARIA document (3), were included in the study and randomized to undergo SLIT with two dosages, respectively, with a ratio to SCIT higher than 300 times (group A, 45 subjects) or equal to 80 times (group B, 45 subjects). All enrolled patients were previously evaluated by standard prick tests with extracts from Stallergenes (Antony, France) for inhalant allergens and serum-specific IgE with the CAP System-specific IgE FEIA (Pharmacia, Uppsala, Sweden).

The standard panel of allergens included *Phleum pratensis* (G6), *Parietaria judaica* (W19), *Olea europaea* (T9), *Dermatophagoides pteronyssinus* (D1), *Alternaria tenuis* (M6), *Felis domesticus* (E1), and *Canis fidelis* (E5), plus a positive (histamine dihydrochloride, 10 mg/ml) and a negative (normal saline) control. All patients who showed a sensitization to allergens different from grass pollen were excluded from the study.

All patients had to record in diary cards during the pollen season their allergic symptoms, classified in a four-scale grading – 0 = absent, 1 = slight, 2 = bothering but no interfering with daily activities and 3 = severe, interfering with daily activities. Patients were provided with levocabastine 0.5 mg/ml eye drops (Janssen-Cilag, Birkerød, Denmark) and levocabastine 50 mcg/dose nasal spray (Janssen-Cilag) for self-medication. Oral antihistamines, oral or nasal corticosteroids or other antiallergic drugs were not allowed. The consumption of levocabastine had also to be reported in the diary, scoring rescue medications as follows: two eye drops – one point, two puffs of nasal spray – one point. Adverse reactions to SLIT were registered in the same diary cards.

The study was approved by the ethical committee of the University of Perugia and all children's parents gave their written informed consent.

Treatment

SLIT was done with a three-grass extract at different concentrations in the two groups: Staloral300® (Stallergenes) for group A and Staloral100® for group B, according to the build-up phase suggested by the manufacturer of, respectively, 11 and 28 days, with a subsequent maintenance phase of 24 drops per week of 300 IR in group A and 40 drops per week of 100 IR in group B. The treatment was started in January 2003 and stopped at the end of the grass pollen season in June 2003.

Serum-specific IgE and IgG4 antibodies

Samples from peripheral blood were obtained by venipuncture before starting SLIT (January), before the pollen season (March), and at the peak of the grass pollen count (June). Specific IgE and IgG4 for *Phleum pratensis* (G6) in serum were measured by the CAP System-specific IgE FEIA and CAP System-specific IgG4 FEIA method (Pharmacia). Values equal or greater than 0.35 kU/l were considered positive.

Nasal-specific IgE and IgG4 antibodies

Nasal-specific IgE and IgG4 for *Phleum pratensis* were detected with the same reagents from Pharmacia for CAP System-specific IgE and IgG4 FEIA with a method of direct *in vivo* incubation as previously described (14, 15). Briefly, the same type of substrates used for the serum assay, i.e. sponges covalently coupled with *Phleum pratensis* extract, were used. The two substrates, covered in the nasal applicator by a permeable membrane, were allowed to incubate in the nasal mucosa at the level of the anterior part of the lower turbinate for 5 min. After nasal incubation, the substrates were washed in Tween 20 NaCl 0.9% (w/v) solution, put in test tubes with 1 ml NaCl 0.9% solution containing sodium azide 0.02% (w/v), and stored at –20°C until tested. Specific IgE and IgG4 were measured with the same fluoroimmunoassay used for serum. All six determinations of nasal and serum samples were run together.

Statistical analysis

Student's *t*-test for paired data, and 95% confidence intervals (95% CI) were used to analyse symptom-medication scores. The Wilcoxon signed rank test for paired data was used to detect differences between values of nasal and serum-specific IgE and IgG4 in the three different exposure periods. Reproducibility of nasal and serum IgE and IgG4 was evaluated by one-way analysis of variance which separates between-subjects and within-subjects components of variation in the measurements.

Results

Of the 90 patients selected for the study, 16 (13 allocated in group A and three in group B) did not start the treatment in January 2003, mostly for intercurrent respiratory infections and also for reasons unspecified by their parents. Following initiation of SLIT, one patient in group A and two in group B dropped out before the first control in March 2003 for reasons unrelated to treatment. Thus, an overall number of 71 children (33 males, 38 females, mean age 9.65 years, range 6–14 years) formed the study population. Of them, 31 (15 males, 16 females, mean age 9.4 years) were included in group A, and 40 (18 males, 22 females, mean age 9.8 years) were included in group B.

The mean cumulative dose administered to group A, treated with the 300 IR dosage of SLIT, was 18031 IR, corresponding to 375 times the cumulative dose received with a standard SCIT of the same duration, while the cumulative dose administered to group B, treated with the 100 IR dosage, was 4068 IR, corresponding to 85 times the SCIT dosage.

Figure 1 shows the symptom-medication scores during the grass pollen season, with a significantly higher mean overall score ($P = 0.024$) in patients of group B, and Table 1 reports the scores during the month of maximal seasonal exposure (June), both significantly higher (symptoms, $P = 0.03$, and medication, $P = 0.04$) in patients of group B as compared with patients of group A. Side-effects occurred with a comparable frequency in the two groups, namely 25.8% in group A and 27.5% in group B.

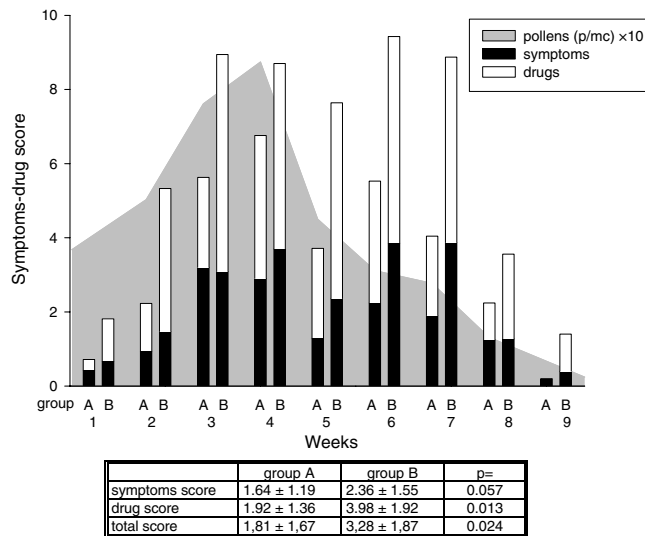


Figure 1. Pollen counts, symptom and drug scores in the two groups of patients during the grass pollen season.

Table 1. Efficacy parameters: symptoms score and medication score (daily mean over the maximal exposure period of June)

Symptoms	Group A (mean ± SD)	Group B (mean ± SD)	P
Sneezing	0.47 ± 0.36	0.78 ± 0.56	0.01
Rhinorrhoea	0.39 ± 0.24	0.67 ± 0.38	0.02
Nasal blockage	0.61 ± 0.78	0.67 ± 0.65	0.17
Nasal pruritus	0.44 ± 0.15	0.77 ± 0.41	0.01
Total rhinitis score	1.47 ± 0.98	2.47 ± 1.75	0.03
Total medication score	1.76 ± 0.87	3.97 ± 2.14	0.04

Table 2 reports the kind and the relative rate of the various side-effects.

Serum-specific IgE and IgG4 did not show significant changes after 3 months of SLIT (from January to March) and showed a significant rise in June after the maximal exposure period in both groups (Table 3). Nasal-specific IgE and IgG4 did not show significant differences after 3 months of SLIT (Table 3), while in June a significant increase of nasal IgE ($P = 0.015$) and IgG4 ($P = 0.019$) was found in patients of group B treated with 100 IR, but not in group A treated with 300 IR (Table 3).

Table 2. Adverse events reported during the study

Description	Group A (n = 31)	Group B (n = 40)
Buccal burning/pruritus	4	5
Bucco-lingual oedema	2	3
Gastrointestinal event	1	2
Labial oedema	1	1
Other	0	0
Total	8 (25.8%)	11 (27.5%)

Table 3. Nasal and serum IgE and IgG4 (mean ± SD) (out of season, before and at the end of the pollen peak) in both groups of patients

	January	March	June	P (January– March)	P (March– June)
Group A					
Serum IgE	31.44 ± 33.57	34.67 ± 35.03	48.64 ± 36.94	NS	0.009
Serum IgG4	6.34 ± 6.99	7.64 ± 6.99	14.12 ± 13.10	NS	0.012
Nasal IgE	24.51 ± 29.77	29.71 ± 32.32	31.05 ± 35.09	NS	NS
Nasal IgG4	6.12 ± 5.95	6.54 ± 6.48	6.98 ± 8.60	NS	NS
Group B					
Serum IgE	37.93 ± 34.43	39.57 ± 35.75	54.81 ± 39.46	NS	0.034
Serum IgG4	7.65 ± 6.99	8.85 ± 6.59	17.22 ± 13.12	NS	0.045
Nasal IgE	23 ± 25.43	26.03 ± 28.91	48.43 ± 36.49	NS	0.015
Nasal IgG4	3.98 ± 4.12	4.81 ± 4.62	10.08 ± 10.33	NS	0.019

Discussion

The dose dependence of efficacy of allergen immunotherapy is well established but it is also known that high doses of allergen extracts are related as well to the occurrence of systemic reactions (16, 17), and this stimulated the search for the optimal dose, defined as ‘the dose of allergen vaccine inducing a clinically relevant effect in most patients without causing unacceptable side-effects’ (1).

The introduction of noninjective routes of IT was a major step in increasing the safety and facilitated this kind of investigation (18). The sublingual route, particularly with the sublingual-swallow method, was investigated thus far in more than 20 placebo-controlled study, and a recent meta-analysis demonstrated its effectiveness in allergic rhinitis, although data on children were insufficient to be conclusive (5). Because of the different methods to measure the allergen content in the extracts from different manufacturers, no recognized standard to which refer the doses administered with SLIT other than the ratio with the cumulative dosage generally received with SCIT is available (19). By this method the ARIA document established that doses at least 50–100 times higher than SCIT must be administered with SLIT to provide clinical efficacy (3). A recent controlled study conducted on patients with ragweed-induced rhinoconjunctivitis suggested a relationship between the cumulative dosage of SLIT and the clinical efficacy, with patients receiving a monthly dose of 3600 IR showing a significantly higher therapeutic response, assessed by scores of nasal and ocular symptoms, than patients receiving lower doses (20).

In the present study, performed in patients allergic to grass pollen, we found that SLIT with a cumulative dose 375 times higher than the standard received with SCIT was more effective than a cumulative dosage 85 times higher than SCIT. Still, the major goal of the study was to investigate the relationship between the cumulative dosage of SLIT and the production of allergen-specific IgE and IgG4 antibodies. Conflicting results have been

obtained for serum-specific IgE and IgG4 production in patients treated with pollen SLIT. Some studies reported significant changes in allergen-specific antibodies (20, 21), while others found no changes (22–25). In our study no change in serum-specific IgE and IgG4 were observed during preseasonal SLIT while a significant increase of these antibodies after the seasonal exposure was detected in both groups of patients. Similarly to results in serum, also a significant rise of nasal IgE and IgG4 was apparent, after the seasonal exposure, in patients of group B but not in patients of group A. In these subjects, treated with higher doses of SLIT, a blunting of both IgE and IgG4 after the seasonal exposure was in fact observed.

In previous studies on pollen SCIT, a significant increase of serum-specific IgG4 accompanied by a blunting of serum-specific IgE was observed after the pollen season (6, 10), but the significance of such immunological response never achieved a clear-cut evidence. However, a recent editorial article on this issue stated that is simplistic to refuse the role of serum-specific IgG on the basis of the lack of correlation with clinical response (13). In fact, some authors suggested that SCIT may act by inducing specific IgE and IgG4 against distinct and not overlapping epitopes and not simply by increasing IgG concentration (12). Other authors, using recombinant Phl p1, Phl p2, and Phl p5 from timothy pollen, showed a strong induction of Phl p5-specific IgG1 and IgG4 and a reduction of the seasonal increase in allergen-specific

IgE correlating with clinical improvement (26). The findings reported in the aforementioned editorial confirm that SCIT may have a protective effect by reducing cross-linking of IgE in mast cells (27) and by inhibiting IgE-mediated facilitated allergen presentation by B cells to T cells (6).

In the patients of our study treated with high-dose SLIT a blunting of the seasonal increase of IgE in nasal secretions similar to that observed in serum from patients treated with pollen SCIT was found, but with no associated increase of specific IgG4. The concept of a local production of IgE antibodies is supported by convincing data (28) and a bioptic study showed that 90% of specific IgE present in nasal mucosa was located on the surface of mast cells (29). Among the mechanisms of SLIT, still undefined, cannot be excluded that, following the allergen capture, sublingual dendritic epithelial cells may migrate and differentiate in the regional lymph nodes and through the contact with local T lymphocytes may induce the inhibition of cross-linking antibodies on nasal mast cell surface resulting in a condition of anergy of allergen-specific T cells (30).

In conclusion, the findings of this study show a seasonal blunting of nasal-specific IgE and IgG4, along with a reduction of symptoms and drug consumption, only in patients treated with higher doses of SLIT. This response, not observed in serum, suggests a locoregional immunological effect of SLIT, different from the systemic mechanism of SCIT.

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