

Grass pollen sublingual immunotherapy for seasonal rhinoconjunctivitis: a randomized controlled trial

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Summary

Background Previous studies suggest that sublingual immunotherapy (SLIT) represents a safer alternative to injection immunotherapy but equivalent efficacy is yet to be confirmed.

Objective To evaluate the efficacy and safety of SLIT in grass pollen-induced seasonal rhinoconjunctivitis.

Methods A randomized, placebo-controlled trial in 56 adults over 18 months. Outcome measures included diary scores of seasonal symptoms and medication use, overall assessments, conjunctival and intradermal provocation tests and serum antibody measurements. To investigate possible mechanisms, sublingual biopsies were taken for measurement of local T cells, antigen-presenting cells and IL-12 mRNA expression.

Results There were no significant differences between the immunotherapy (IT) and placebo groups for diary symptom scores ($P = 0.48$) or rescue medication ($P = 0.19$). The patients' overall assessment of hayfever severity compared with previous years showed a highly significant improvement in favour of the IT group ($P < 0.02$). After treatment the late skin response was smaller ($P = 0.003$) and the ratio of serum allergen-specific IgG4/IgE was higher ($P = 0.05$) in the IT group. Both of these variables correlated with the clinical response to SLIT. There were no differences between groups in either the sublingual epithelium or lamina propria for numbers of CD3⁺ cells (epithelium: $P = 0.9$, lamina propria: $P = 0.2$), CD1a⁺ cells ($P = 0.3$, $P = 0.25$), CD68⁺ cells ($P = 0.9$, $P = 1.0$) or IL-12 mRNA⁺ cells ($P = 0.6$, $P = 0.4$). Local side-effects were minor and there were no serious treatment-related adverse events.

Conclusion Grass pollen sublingual immunotherapy was well tolerated. Although there was no significant change in diary scores, the improvement in overall assessments, which correlated with inhibition of the late skin response and increases in serum IgG4:IgE ratio, indicates the need for larger, dose-ranging studies.

Keywords antibodies, cytokines, hayfever, immunohistochemistry, immunotherapy, rhinoconjunctivitis, sublingual

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Introduction

The efficacy of grass pollen immunotherapy via the subcutaneous injection route is well-documented [1–4], with treatment for 3 to 4 years resulting in prolonged remission for at least 3 years following discontinuation [5,6]. Although highly effective, in view of the possibility of systemic reactions, subcutaneous immunotherapy should be performed only in specialist centres with access to resuscitative measures.

Sublingual administration of allergen may represent a safer and more acceptable route for immunotherapy. The efficacy of sublingual immunotherapy has, however, been questioned

[7, 8]. Four placebo-controlled studies suggesting clinical efficacy [9–12], formed the basis of a World Health Organization report on sublingual allergen immunotherapy [4], following which interest in this alternative route, particularly in Europe, increased and a further eight randomized trials were performed. Some [13–17], but not all [18–20], have provided evidence for efficacy in both seasonal and perennial rhinitis and all studies have confirmed the safety of the sublingual route.

The mechanism of sublingual immunotherapy is unknown, although changes following therapy have been observed in serum antibodies and peripheral blood T cell responses [11, 13, 16, 18, 19]. In studies of patients sensitive to both mite and *Parietaria*, clinical improvement was associated with a decrease in eosinophils and a reduction in the expression of the vascular adhesion molecule ICAM-1 in the conjunctiva [21] and the nose [17].

In this placebo-controlled study in patients suffering from seasonal rhinoconjunctivitis, we have assessed the clinical

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efficacy and safety of sublingual immunotherapy using high doses of grass pollen extract. To test the hypothesis that sublingual immunotherapy may act by altering local dendritic cell numbers [22], or their production of IL-12 [23], events that might favour immune deviation of allergen-specific T lymphocyte responses in favour of a Th1 phenotype [24], we measured T cells, antigen presenting cells and IL-12 mRNA expression in sublingual biopsies taken from a subset of study participants.

Materials and methods

Patients

Fifty-six patients were recruited either from the Allergy Clinic, Royal Brompton Hospital, London, UK, or following an advertisement in the local newspaper. The study was performed with the approval of the local ethics committee and with the written informed consent of all participants.

All patients gave a history of seasonal allergic rhinoconjunctivitis of at least 2 years duration and a positive skin-prick test (weal diameter > 5 mm) to timothy grass pollen extract (*Phleum pratense*, Soluprick[®], ALK Abelló, Hørsholm, Denmark). Patients were excluded if they gave a clinical history of other allergies, had received immunotherapy in the previous 5 years or had any other significant medical illness. Allergy to cat and/or dog was an exclusion criteria only if patients had daily contact with pets. Patients with chronic asthma were excluded. Patients with mild seasonal asthma were included, provided their symptoms were controlled by inhaled β_2 -agonist alone.

The immunotherapy ($n=28$) and placebo ($n=28$) groups were matched at baseline for age (mean, range) (34, 21 to 53) (34, 21 to 55) and gender (15 female, 13 male) (9 female, 19 male), respectively. The immunotherapy and placebo groups were also matched for the dose of grass pollen extract (BU/mL) that caused a positive conjunctival response (median (interquartile range)) (1000 (320, 3200)) (1000 (320, 3200)), the immediate skin prick test size (mm²) (84 (62, 132)) (75 (62, 102)) and the size of the late (6 h) response to intradermal allergen (mm²) (2403 (1841, 3975)) (2463 (2008, 3677)). The active and placebo-treated patients were also matched for their serum concentrations of allergen-specific IgE (KU/L), mean ($\pm$ SD): 250 (257), 189 (251), respectively).

A subgroup of 24 patients (14 on active treatment, 10 on placebo) consented to undergo a sublingual biopsy.

Study design

This was a double-blind, placebo-controlled parallel group study of 18 months duration. One hundred and forty subjects were screened, of whom 56 fulfilled the entry criteria and were randomized. Treatment was commenced in March 1998 for 29 of 56 patients and in August, following the pollen season, for the remaining 27 patients. Randomization to active or placebo treatment was performed by the manufacturer of the grass pollen vaccine using a system of computer-generated random numbers in blocks of 12. The treatment schedule and assessments were performed double-blind, with treatment allocations kept in sealed envelopes by the principal investigator.

Immunotherapy protocol

The immunotherapy treatment was Soluprick SQ (ALK Abelló), a standardized extract of *Phleum pratense* (timothy

grass pollen) diluted in 50% glycerol. The concentration of grass pollen allergen extract was 1 mg/mL. The placebo preparation was identical to the extract in appearance, presentation, taste and colour. The duration of treatment was 12–18 months. The treatment schedule comprised a 6-week up-dosing phase followed by daily maintenance treatment. In view of the high doses of allergen extract employed, up-dosing was supervised in hospital, in the presence of a physician, with availability of resuscitative measures. Maintenance doses were taken at home.

Up-dosing involved three visits per week, with one drop (25 μ g of *Phleum pratense* extract) administered per visit in the first week, two drops in week 2 then four, seven, ten and fourteen drops in weeks 3, 4, 5 and 6, respectively. The maintenance dose was six drops every day (4500 μ g of *Phleum pratense* extract per month), giving a cumulative monthly dose of major allergen (Phl p 5) of approximately 900 μ g, equivalent to 40 to 50 times the amount contained in the usual monthly maintenance injections via the subcutaneous route. Drops were kept under the tongue for 3 min before swallowing.

Symptoms and medication scores

Patients recorded seasonal symptom and medication scores in diaries from May through to August 1999 as previously described [3]. During the peak pollen season (June 1999) and at the end of the trial (November 1999) patients made an overall assessment of their hay fever in response to the question 'How has your hay fever been this year, compared with previous years before treatment?', on a scale of +3 (a lot better) to -3 (a lot worse).

Adverse event recording

During the up-dosing phase any local or systemic symptoms occurring within 1 h were recorded under supervision and documented at the study site. Patients reported any delayed (within 48 h) local or generalized symptoms and any symptoms occurring during the maintenance phase of treatment to the study investigators.

Skin tests

Intradermal allergen tests were performed as previously described [3] with an injection of 10 SQ-U (in 0.02 mL of diluent) of grass pollen extract (*Phleum pratense*, Aquagen, ALK Abelló). Diluent solution was used as a negative control. The areas of the early (15 min) and late (6 h) skin responses were measured and recorded in mm².

Conjunctival provocation tests

Conjunctival provocation tests were performed as previously described [3]. A positive reaction was defined when two of four symptoms (itching, redness, tears or swelling) occurred.

Immunological parameters

Venous blood was taken from an antecubital vein at baseline, after the induction phase, during the peak pollen season and at the end of the study. Blood was analysed for allergen-specific IgE to *Phleum pratense* extract and for Phl p 5-specific IgG4 (Magic Lite, ALK Abelló) [25].

Sublingual biopsies

Biopsies were taken following infiltration of the postero-lateral sublingual region of the floor of the mouth with lidocaine 1%

with epinephrine 1 : 200,000. Cup and ring forceps (Explorent GmbH, Tuttlingen, Germany) were used. Biopsies were divided into two equal halves and processed for immunohistochemistry [26] and *in situ* hybridization [27] studies. Immunohistochemistry was performed using the APAAP method [26]. Monoclonal antibodies used were CD1a, CD3 and CD68 (all from DAKO, Ltd, Cambridge, UK). *In situ* hybridization was performed using an ^{35}S labelled riboprobe specific for IL-12p40 mRNA, by a previously described method [27]. Slides were counted blind in coded random order using an Olympus BH2 microscope (Olympus Optical Company Ltd, Tokyo, Japan) at magnification $\times 200$. The cross-sectional areas of the sublingual epithelium and lamina propria were quantified by use of a computer-assisted graphics tablet. Counts were expressed as the numbers of cells/mm ^{2} . The mean intraobserver coefficient of variation for repeat counts was less than 10%.

Statistical analysis

Results were analysed using a statistical package (Minitab[®] Inc, State College, PA, USA). Symptoms and medication scores were expressed as the area under curve for the 9-week period that corresponded to the peak pollen counts. Between-group differences were compared using the two-tailed Mann–Whitney *U*-test. Spearman's rank test (r_s) was used for correlations. Categorical data were analysed using the chi-square test. *P*-values less than 0.05 were considered to be statistically significant.

From a previous study of subcutaneous immunotherapy [3] it was calculated that the inclusion of 48 patients would give a 90% power to detect a 50% difference in seasonal symptoms between the groups ($\alpha = 0.05$).

Results

Clinical parameters

Forty-nine of the 56 patients completed the study. Seven patients withdrew (two active, five placebo). Six withdrawals were for social reasons and were not related to the clinical protocol. One patient (active treatment) suffered troublesome local side-effects. Weekly pollen counts in London peaked in June 1999 at levels higher than the average range. Greater than 99% of symptom and medication diaries were returned from the 49 subjects who completed the study. For both groups the presence of symptoms and use of drugs closely paralleled the average weekly grass pollen count (Fig. 1). Median symptom scores were 28% lower and rescue medication scores were 45% lower in the immunotherapy group compared with the placebo group, although there was wide variability within groups. The differences did not achieve significance (Table 1, Fig. 1). Similarly, median values for individual nose, eye, chest, mouth and throat symptoms were higher in the placebo group, but did not achieve significant difference between groups (Table 1). When asked, at the peak of the pollen season, how the severity of their hayfever symptoms compared with previous years, 20 of 26 (77%) immunotherapy-treated subjects reported improvements compared with nine of 23 (39%) placebo-treated patients ($\chi^2 = 8.86$, d.f. = 2, $P < 0.02$) (Fig. 2). Similarly, the between-group differences for overall assessments remained significant ($P < 0.05$) when repeated at the end of the trial.

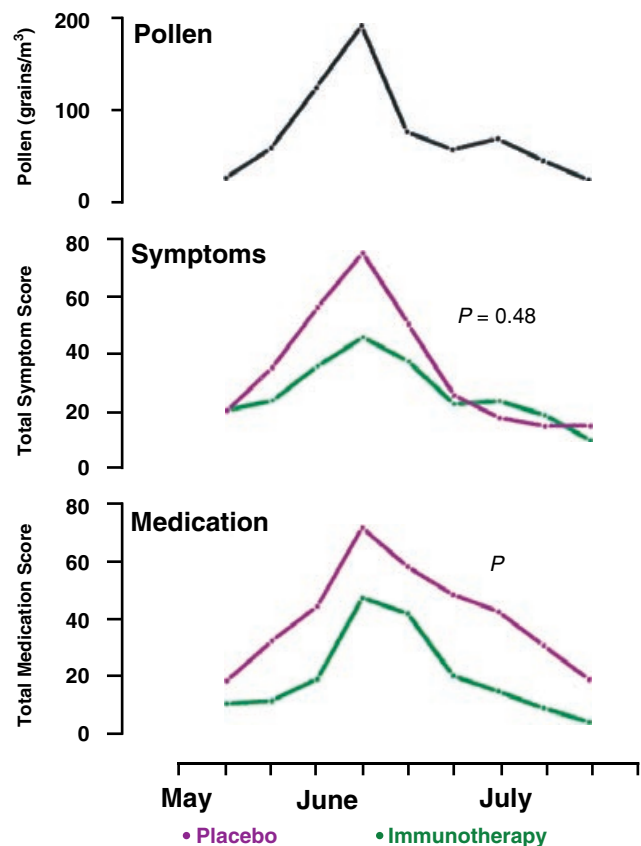


Fig. 1. Grass pollen counts (weekly means obtained using a Burkard spore trap, at an elevation of 18m, in North London), symptom and medication scores (weekly median) for 9 weeks during the 1999 pollen season. *P*-values refer to comparisons (Mann–Whitney *U*-test) for area under curve (AUC) values between immunotherapy and placebo-treated patients.

No immediate (within 1 h) or delayed systemic reactions nor severe local reactions were observed during either the induction phase or maintenance phase of treatment. In total, 475 adverse events were reported in 28 patients in the immunotherapy group and 90 in the 28 patients in the placebo group, an average of 17 and 3.2 adverse events per patient, respectively. In the immunotherapy-treated group 33% of the events were observed in three patients. The majority of reactions (380, 80%) reported in the immunotherapy-treated group were very mild local reactions, consisting of itching and swelling in the floor of mouth following the sublingual drops, almost always during the up-dosing phase. These were not troublesome for the patients and none required treatment. Moderate reactions, which included infection, malaise and rhinitis, occurred in the immunotherapy-treated group (93 of 475:19.6%) and placebo group (64 of 90:71%) and were unrelated temporally to the taking of the sublingual drops. Three serious adverse events were reported in the study, two in the active group (0.4%) (motorbike accident and one episode of epiglottitis which was unrelated to vaccine administration and was resolved with continued vaccine administration) and one in the placebo group (1%) (motorbike accident). All three events were not related to the study treatment.

Table 1. Summary of results

Clinical outcomes*	Immunotherapy 1999 (n = 26) median (interquartile range)	Placebo 1999 (n = 23) median (interquartile range)	Point estimate† median (95%CI)	P-value‡
Total symptoms	1694 (750, 3821)	2349 (1015, 3903)	– 273 (– 1316, 700)	0.48
Nose	742 (436, 1772)	1288 (469, 1522)	– 191 (– 676, 322)	0.37
Eyes	462 (81, 1285)	550 (231, 1036)	– 18 (– 326, 312)	0.86
Mouth/throat	65 (0, 353)	277 (7, 525)	– 103 (– 277, 46)	0.25
Chest	117 (0, 474)	32 (0, 214)	0 (– 28, 158)	0.64
Rescue medication*	1418 (224, 3110)	2569 (1134, 4736)	– 803 (– 2051, 392)	0.19
CPT§	3200 (1000, 26500)	3200 (1000, 100000)	680 (0, 6800)	0.18
Skin responses¶				
EPR (mm ²)	394 (354, 526)	454 (333, 605)	– 24 (– 110, 67)	0.55
LPR (mm ²)	2754 (1640, 3961)	4138 (2926, 5163)	– 1375 (– 2457, – 531)	0.003

*Results represent median values (interquartiles ranges) for area under curve (AUC) analysis for 9 weeks during the grass pollen season. †Point estimates and 95% confidence intervals (CIs) were calculated with Minitab software. ‡Two-tailed Mann–Whitney U-test was used for between-group comparisons. §CPT, conjunctival provocation test. ¶Intradermal tests were performed outside the pollen season. EPR = early phase response (20 min); LPR = late phase response (6 h).

Objective tests

At the end of the study the size of the late skin response at 6 h after intradermal allergen was 33% smaller in the immunotherapy group than in the placebo group ($P = 0.003$) (Table 1). There was no significant difference between the groups in the size of the immediate (15 min) skin response nor in immediate allergen sensitivity in the conjunctiva (Table 1).

Serum levels of specific IgE and IgG4 antibodies to Phl p 5 were similar in both groups at baseline. In the placebo group there were no significant changes in the levels of either antibody throughout the study. In the immunotherapy group IgE increased after up-dosing then tended to decrease with time, while IgG4 increased steadily and remained raised at the end of the study. After 12 to 18 months of treatment the specific IgG4 level was markedly raised in the immunotherapy group ($P < 0.005$), whereas the specific IgE levels had returned towards baseline ($P = 0.03$) and the IgG4:IgE ratio was greater in the immunotherapy group compared with the placebo group ($P = 0.06$) (Fig. 3).

The clinical response to immunotherapy (symptom scores, rescue medication and overall assessments) was compared with changes in objectives measures (late skin response and IgG4). The size of the late skin response correlated with rescue medication scores ($r_s = 0.44$, $P < 0.05$), symptom scores ($r_s = 0.39$, $P < 0.1$) and the patients' overall assessments of hay fever severity ($r_s = -0.47$, $P < 0.05$). Similarly, the patients overall improvement in hay fever severity correlated with the level of serum allergen-specific IgG4 ($r_s = 0.46$, $P < 0.05$) and the ratio of serum IgG4:IgE ($r_s = 0.40$, $P < 0.05$).

Immunohistology of the sublingual mucosa

The sublingual biopsy technique that we developed proved to be both acceptable and safe. There were no immediate or long-term complications following the procedure. Biopsies were painless and any slight discomfort after the local anaesthetic wore off was relieved by paracetamol and resolved within 24 h. The median time to complete healing of biopsy sites was 3 days. Comparison of cell counts in sublingual mucosal biopsies taken from the 14 patients who received immunotherapy and the 10

who received placebo did not reveal significant differences in either the epithelium or the lamina propria for the number of CD1a⁺, CD68⁺ or CD3⁺ cells. IL-12 mRNA⁺ cells were present in low numbers in both the epithelium and the submucosa. No significant differences for IL-12 mRNA⁺ cells were evident between the groups (Table 2, Fig. 4).

Discussion

In this randomized controlled trial, sublingual immunotherapy with high-dose *Phleum pratense* extract did not significantly reduce seasonal symptom and medication scores when compared with placebo treatment. There was, however, a significant improvement in the overall assessment in the immunotherapy group compared with the placebo group when patients were asked to compare their symptoms with previous pollen seasons. This was accompanied by significant differences in the late skin response and increases in the serum IgG4 and the allergen-specific IgG4:IgE ratio, which correlated with the clinical response to treatment.

Symptom and medication scores paralleled pollen counts and were reduced by 28% and 45%, respectively, in the immunotherapy group. There are several reasons why these differences may not have achieved statistical significance. First, our power calculation was based on a previous study that, with the benefit of hindsight, included patients with more severe disease who had failed to respond to conventional anti-allergic drugs. In view of the documented safety of sublingual immunotherapy, the target group in this study included patients with a much broader disease spectrum. The resulting increased between-patient variability in symptom and medication scores may have reduced the power to detect a difference between the groups.

Secondly, it is possible that the inclusion of an initial observation year may have allowed matching for severity between the groups at baseline, based on seasonal symptoms and medication use. This approach also allows an assessment of the change in symptoms between the two pollen seasons, which may reduce the effect of inter-individual variation in symptom reporting, making a false negative result less likely. It is notable, however,

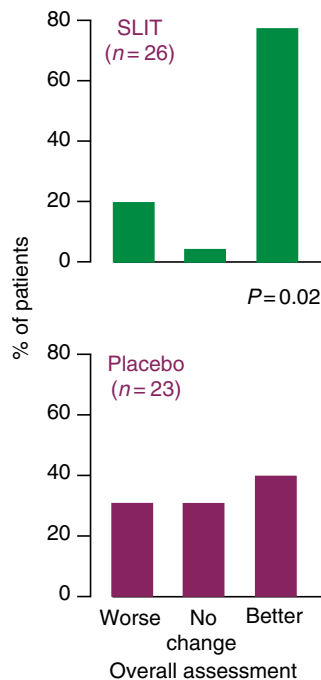


Fig. 2. Overall assessments (response to question 'How was your hayfever this year compared with previous years?': -3 (much worse) to +3 (much better)). *P*-value refers to between-group comparison by χ^2 test.

that none of the previous studies of SLIT in seasonal allergic rhinitis (in which baseline assessment is more problematic than in perennial allergic rhinitis) have employed this methodology. Therefore, the equally likely possibility of false-positive results due to baseline mismatching in many of the previous, often smaller, studies must be acknowledged.

The inclusion of a baseline year also raises a number of other potential problems. These include intra-individual variation in symptom reporting from year to year, the high variability in pollen counts between years and the inevitable prolongation of the study by at least 1 year, possibly resulting in higher drop-out rates and the inevitable loss of paired data that are vital to draw valid conclusions. Although the addition of a baseline year is clearly favoured by some [28], this view is not based on consensus. From a previous study involving a depot pollen extract (Alutard SQ) in birch-sensitive patients it has been calculated that baseline symptom assessment reduces sample sizes by only 16% (Arne Haahr Andreassen, personal communication). It is therefore clear that a baseline observation year is not the answer to all of the difficulties with studies such as these.

In contrast to the diary scores, the overall assessments, which were based on the individuals' global view of their hayfever compared with previous years, have an in-built baseline correction that may reduce the impact of within-group variability in symptom severity. Significant differences in this variable were observed between the treatment groups on two occasions and improvement correlated with objective tests. In previous studies of injection immunotherapy [3] we have shown improvements of two points in overall assessments alongside 60% and 80% reductions in symptom and medication scores, respectively. The magnitude of the improvement in diary scores in this study is approximately half of these values (28% and 45%) and the overall assessment shows a mean improvement of 1.5 points in

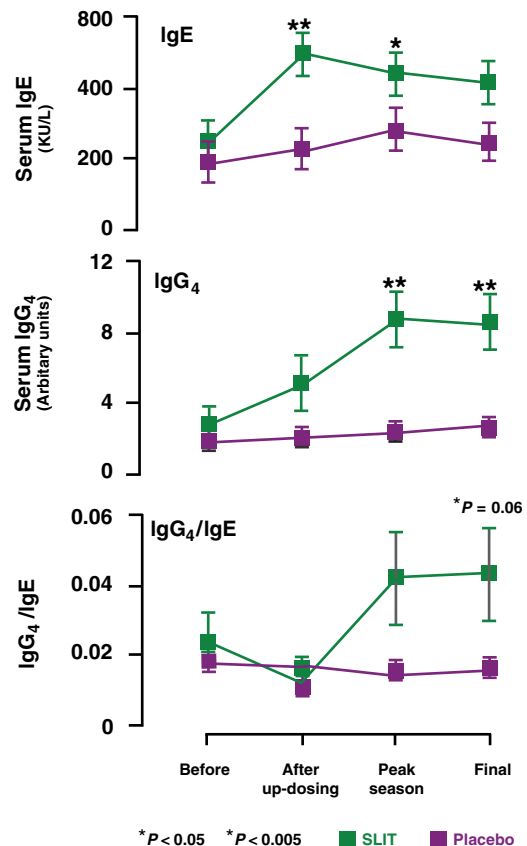


Fig. 3. Allergen-specific IgE, IgG₄ and IgE : IgG₄ ratio (mean $\pm$ SEM) at baseline, after up-dosing, peak pollen season 1999 and final (after 18 months treatment) assessments. *P*-values refer to comparisons between values for immunotherapy and placebo groups (Mann-Whitney *U*-test).

the SLIT-treated group, again around half of that observed with injection immunotherapy.

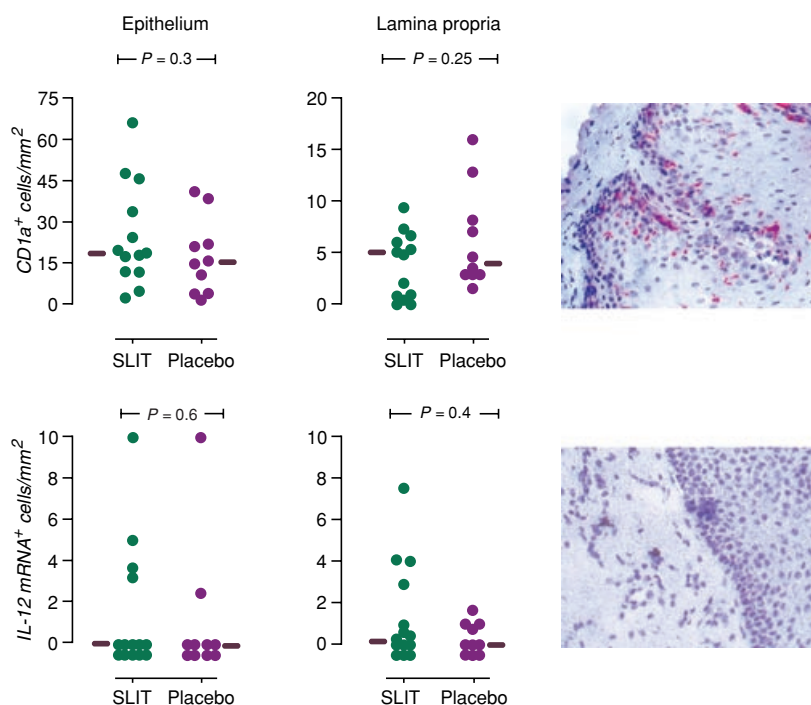
Several studies of sublingual immunotherapy have reported reductions of 30 to 60% in the need for rescue medication [9–11, 13,15,21], or reductions in both symptoms and medication scores [9, 15, 21]. These studies differ in duration of treatment, dose of allergen and allergen used. Perhaps surprisingly, four studies that employed low doses of *Parietaria* [11,15,21], or grass pollen extract [9] showed better efficacy than four studies using much higher doses of *Parietaria* [16,17] or grass pollen extract [10,13]. Similarly our study, using extremely high doses of allergen, did not prove efficacy. It therefore appears that there is not a clear dose-response and the optimal dosage for SLIT remains to be determined.

An analysis of eight double-blind, placebo-controlled studies of sublingual-swallow immunotherapy in children and adults [29] showed that the majority of adverse events were mild local reactions. Our study confirms the good safety profile of sublingual-swallow immunotherapy with no serious treatment-related adverse events. Mild local reactions were common in the immunotherapy group, but were not bothersome for the patients and required no treatment.

Reduction in the size of the late skin response is a consistent finding in studies of subcutaneous immunotherapy [3,30,31]. In the current study, due either to between-batch variation in

Table 2. Immunohistochemistry and *in situ* hybridization of the sublingual mucosa after treatment. All data are cells per mm²

	Immunotherapy Median (interquartile range)	Placebo Median (interquartile range)	Point estimate* Median (95%CI)	P-value*
Epithelium				
CD1a	18.4 (11.8,39.6)	15.2 (3.7,25.9)	5.7 (– 6.0,20.5)	0.3
CD68	0 (0,4.7)	0 (0,4.9)	0 (– 3.1,4.4)	0.9
CD3	14.9 (4.9,90.4)	29.6 (4.8,55.8)	1.1 (– 28.7,38.0)	0.9
IL-12p40 mRNA	0 (0,3.4)	0 (0,0.6)	0 (0, 08)	0.6
Lamina propria				
CD1a	4.8 (0.6,6.3)	4.0 (2.9,9.3)	– 2.1 (– 6.1,2.1)	0.25
CD68	28.6 (11.3,45.4)	28.0 (16.8,40.2)	– 2.2 (– 24.1,20.6)	1.0
CD3	43.7 (7.6, 121.4)	68.2 (56.6, 125.4)	– 30.7 (– 87.5,44.0)	0.2
IL-12p40 mRNA	0.37 (0.3,18)	0 (0,1.0)	0 (– 0.1,2.2)	0.4

*Median values were compared using the Mann–Whitney *U*-test.**Fig. 4.** (a): Numbers of positive cells in sublingual biopsies immunostained for CD1a, with example of staining ($\times 400$). (b): Numbers of positive cells in sublingual biopsies hybridized for IL-12p40 mRNA with example of staining ($\times 400$). Data shown are for individual subjects after 12 to 18 months of sublingual immunotherapy or placebo. Horizontal bars represent median values. P-values refer to between group comparisons by Mann–Whitney *U*-test.

allergen or to an unavoidable change in operator, we observed an apparent increase in the late response in the placebo group, compared with no change in the SLIT group. Despite this there is a clear treatment effect, with no difference between the groups at baseline and a clear difference at the conclusion of the study, with the response being 33% smaller in the SLIT group. Furthermore, the size of the late response correlated with the clinical response to treatment. We also showed that the peak seasonal allergen-specific IgG4:IgE ratio was significantly higher in the immunotherapy group than in the placebo group. It is therefore apparent that, despite the inconclusive result for seasonal symptoms and medication use, allergen is being absorbed from the sublingual region and is inducing changes in humoral and cellular immune responses that correlate with the clinical response.

Conventional subcutaneous immunotherapy may act either by inducing immune deviation of T cell responses in favour of Th1 [32] or by diminishing Th2/Th0 T cell responses, for example by induction of T cell unresponsiveness [33]. Two studies of the mechanism of sublingual immunotherapy [17, 21], have confirmed decreases in effector cells (eosinophils) in the target organ (eye/nose) and a decrease in the expression of the adhesion molecule ICAM-1, and one study has shown a decrease in allergen-specific T cell proliferation in cultures of peripheral blood mononuclear cells [34].

The sublingual epithelium contains high numbers of CD1a-positive dendritic cells [35]. We hypothesized that following repeated exposure to high doses of allergen during sublingual immunotherapy, epithelial dendritic cells might migrate to the submucosa and differentiate into so-called DC1-type dendritic

cells [22,24], in the lamina propria, with increased IL-12 production [23]. T cells, CD1a-positive dendritic cells and IL-12 mRNA expressing cells were detectable in both the epithelium and the lamina propria. However, there were no treatment-related differences in either total T cells, dendritic cells or the number of IL-12 mRNA⁺ cells. These observations do not, however, exclude the possibility that, following antigen capture, dendritic cells migrate and differentiate to DC1 (IL-12) producing cells in the draining cervical glands that would, in turn, bias allergen-specific T cells in favour of development of a Th1 rather than Th2 phenotype. Our findings of a systemic switch in serum allergen-specific antibodies from IgE to IgG4 would be consistent with this.

In summary, our findings demonstrated that grass pollen sublingual immunotherapy was well tolerated. Although there was no change in diary scores, there was an improvement in the patients' overall assessments compared with previous years that correlated with suppression of the late response and immunological changes. We conclude that there is a need for a large, dose-ranging study, with careful consideration of the possible value of an initial year of symptom monitoring, to better define the clinical efficacy and the optimal therapeutic dose for sublingual grass pollen immunotherapy.

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