

Short communication

Sublingual immunotherapy abrogates seasonal bronchial hyperresponsiveness in children with *Parietaria*-induced respiratory allergy: a randomized controlled trial†

Background: The use of immunotherapy in asthmatic children is still controversial. Sublingual immunotherapy (SLIT) may represent an advance, due to the good safety profile, but little is known about its effects on lung function and nonspecific bronchial responsiveness.

Objective: The aim of this study was to assess the effects of SLIT on these parameters, in children with *Parietaria* pollen-induced asthma.

Methods: Thirty children with asthma solely due to *Parietaria* who participated in a previous randomized, placebo-controlled trial with SLIT were studied: pulmonary function test and methacholine challenge were carried out at baseline in winter 1999 (out season), during the 1999 season (before randomization), and during the 2001 season.

Results: Before randomization, there was a significant fall in methacholine provocation concentration during the pollen season vs baseline in both groups (SLIT group 9.78 ± 5.95 mg/ml vs 3.37 ± 2.99 mg/ml; placebo 8.70 ± 6.25 mg/ml vs 2.44 ± 2.25 mg/ml; $P = .005$). In the second pollen season, the response to methacholine returned to baseline values in the active group (9.10 ± 7.7 mg/ml; $P = \text{NS}$ vs baseline), whereas in the placebo group a significant increase in reactivity was still present (2.46 ± 2.26 ; $P = 0.008$ vs baseline). No significant difference in FEV_1 and FEF_{25-75} between the two groups was observed at all times.

Conclusions: Our data show that SLIT abrogates the seasonal bronchial hyperreactivity in children with asthma due to *Parietaria*. This may be regarded as an indirect evidence of the effect on bronchial inflammation.

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Although it has been widely used for over 80 years, the role of allergen-specific immunotherapy (SIT) in the treatment of bronchial asthma in children is still a matter of debate (1–3). The British Society of Allergy and Clinical Immunology and the European Academy of Allergology and Clinical Immunology advised against the use of immunotherapy in patients less than 5 years of age, for safety reasons, and also raised some doubts on the usefulness for the treatment of asthma. Moreover the NHLBI/WHO working group recommended that SIT should be considered only when exposure to causal allergens cannot be avoided, or when pharmacological treatments are unable to control the disease (4, 5). However, a meta-analysis of the randomized controlled trials in allergic asthma concluded that SIT in the

paediatric population significantly improves symptoms, reduces drug intake and affects, to some extent, bronchial hyperreactivity (6, 7). Of note, SIT is the only treatment capable of modifying the natural course of respiratory allergy by interfering with the underlying immunological mechanisms.

During the past 15 years the interest in noninjection routes of administration of SIT has progressively increased, and a large number of clinical trials have been published recently (for a review, see 8, 9). Among the noninjection routes, the sublingual immunotherapy (SLIT) has been thoroughly investigated, especially from a clinical point of view, and it is now recognized as a safe and effective treatment for both adults and children (9). Its efficacy has been confirmed in rhinitis in a meta analysis (10), and some studies have also demonstrated its efficacy in asthma (11, 12). The mechanisms of action of SLIT are still not fully clarified, but it is capable of down-regulating the allergen-induced inflammatory response

†During the preparation of the manuscript, our good friend Dr Silvano Parmiani died. This article is dedicated to him.

(13). Bronchial hyperresponsiveness (BHR) is a distinctive feature of asthma and it is partly sustained by inflammation. Thus, the effect of a given treatment on BHR in asthma might be regarded as an objective support to the subjectively evaluated clinical effects. Nevertheless, very few studies have investigated the action of SLIT on nonspecific BHR and lung function. In the present article we report the effects of a 2-year course of SLIT with *Parietaria* pollen extract on BHR and lung function in children with seasonal asthma and severe hayfever. The clinical outcomes of the treatment in the same patients' population have been previously published (14).

Methods

Study design

The study was double-blind and placebo-controlled. During the *Parietaria* pollen seasons 1999 and 2000 (April–June), all participants recorded symptoms and rescue medication use on a diary card and completed a visual analogue scale in order to assess the clinical efficacy. These results have been the subject of a previous publication (14). In Winter 1999 (January–March), out of the pollen season, baseline pulmonary function test (PFT) and BHR assessment were performed. They were repeated during the 1999 and 2001 *Parietaria* pollen seasons. SLIT started in December 1999, after randomization, and lasted until November 2001. The study was approved by the Ethics Committee of the University of Messina, and the parents gave their informed consent. The study plan is outlined in Fig. 1.

Patients

Thirty children (8–14 years) participated in the previously published clinical study (14). Briefly, the inclusion criteria were the following: (a) clinical history of seasonal asthma during spring; (b) single sensitization to *Parietaria judaica*, assessed by skin prick test and radioallergosorbent (RAST) assay; (c) a forced expiratory volume in 1 s (FEV_1) $\geq 75\%$ of the predicted value at baseline assessment;

(d) a methacholine (MCh) concentration causing a 20% fall in FEV_1 (PC_{20}) > 2 mg/ml, measured out of the pollen season.

Patients were excluded if they had previously received immunotherapy. Cardiovascular diseases, systemic immunological disorders, or a history of severe asthma were the other exclusion criteria.

Immunotherapy and concomitant treatments

The SLIT was standardized in Biological Units (BU) by a RAST-inhibition procedure (15) and the content of the major allergen Par j 1 was expressed in micrograms. The extract was prepared in five increasing concentrations (0.0016, 0.08, 0.4, 2 and 10 BU/ml) in glycerinated (50% v/v) and phenolated (0.4% p/v) aqueous solution. The top concentration (10 BU/ml) contained 0.6 µg/ml of Par j 1. The administration of SLIT started with one drop from the lowest concentration vial, then increasing by one drop daily, up to five drops. This was repeated with each of the subsequent vials, up to the maintenance dose of five drops from the top concentration vial that was given three times a week for the remainder of the study.

Drops had to be taken in the morning at least 15 min before eating and kept under the tongue for at least 2 min before swallowing. The cumulative amount of Par j 1 given during the whole treatment was about 40 µg, i.e. around fourfold the dose of the conventional injection route.

During the 2000 (but not the 2001) *Parietaria* pollen season patients received on a regular basis inhaled fluticasone dipropionate (50 µg twice daily). Some rescue drugs were allowed during the pollen seasons: nedocromil sodium eye drops and nasal spray, loratadine (5 mg one tablet a day) and inhaled salbutamol. Nasal corticosteroids were not allowed during the whole study.

Pollen counts

Pollen counts were obtained by means of a Burkard 7-day volumetric spore trap (Burkard Manufacturing Co. Ltd, Rickmansworth, UK) located on the roof of the highest building (18 m) of the hospital. Weekly average counts for *Parietaria* pollen were calculated from daily counts and expressed as grains/m³ of air.

Pulmonary function test and methacholine challenge

Bronchial hyperresponsiveness was measured at three time points: before and during the 1999 and 2001 pollen seasons (Fig. 1). During the 2000 pollen season MCh challenge was not performed because all children were on inhaled fluticasone.

A Multispiro-PC pneumotachograph (Burke and Burke, Würzburg, Germany) was used for PFT. The best of three forced expiratory manoeuvres, starting from vital capacity, was selected. The MCh challenge was carried out according to the method described by Chai et al. (16). Methacholine (Sigma Diagnostics, St Louis, MO) solutions were prepared at different concentrations (0.075, 0.15, 0.31, 0.625, 1.25, 2.5, 10.0, 25.0 mg/ml) in buffered saline (pH 7, 4). A MEFAR-MB3 dosimeter (Markos, Monza, Italy) was used to generate the aerosol from a DeVilbiss 646 nebulizer ampoule (DeVilbiss Health Care, Somerset PA). Subjects inhaled five deep breaths of buffered saline solution, followed by increasing concentrations of methacholine at 5-min intervals. FEV_1 was measured 60 and 90 s after inhalation of each concentration. The procedure was stopped when the FEV_1 value fell by more than 20% from baseline. The results are expressed as the percentage fall in FEV_1 when compared with the baseline value registered with the saline solution alone and was plotted against the log of

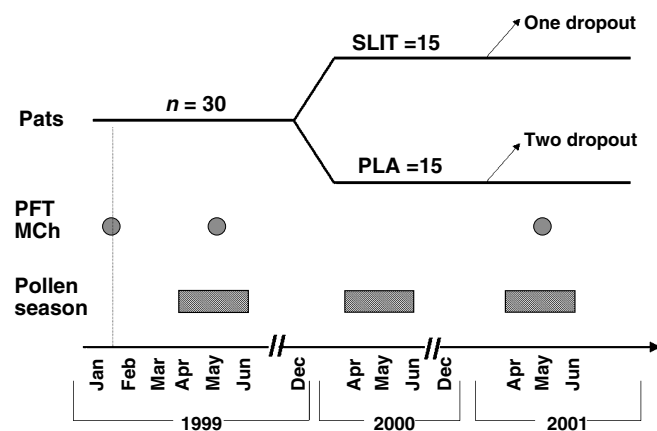


Figure 1. Study design (PFT, pulmonary function test; MCh, methacholine challenge).

concentrations of inhaled methacholine. The dose-response curves were characterized by their position and the maximal response was expressed as PC20-FEV₁.

Statistical analysis

The analysis of nonparametric data was performed by the Mann-Whitney *U*-test for inter-group comparisons or by the Wilcoxon test for intra-group comparison. Student's *t*-test was used to compare parametric data inter-group, whereas the *t*-paired test was used to analyse parametric data for paired intra-group comparisons. $P < 0.05$ was considered significant. A standard statistical software (BMDP Inc., Los Angeles, CA) was used.

Results

Sublingual immunotherapy and placebo-treated patients were well matched at baseline for the considered parameters (Table 1). Twenty-seven of 30 children completed the 2-year study. All 14 patients in the active group achieved the maintenance dose of *Parietaria* SLIT treatment, and 13 in the placebo group reached the maintenance drops of placebo.

Pollen counts

In 1999, 2000 and 2001 weekly *Parietaria* pollen count in Messina peaked between May and June (Fig. 2). The average weekly pollen counts in the 11 weeks considered were 42.6, 45.5, and 45.6 grains/m³ for the years 1999, 2000, and 2001, respectively. In 2001 the average weekly pollen counts increased 7% compared with the baseline year 1999, but this change was not significant ($P = 0.74$, NS).

Pulmonary function test and MCh challenge

The behaviour of BHR at the three time points is shown in Fig. 3. In winter 1999 (baseline), the MCh PC20 was 9.78 ± 5.95 mg/ml in the SLIT group and 8.70 ± 6.25 mg/ml in the placebo group ($P = \text{NS}$). As

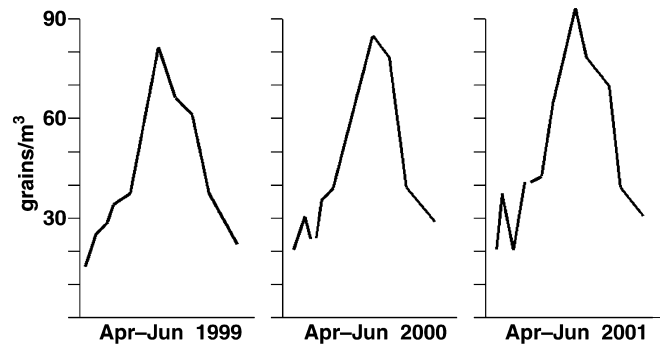


Figure 2. *Parietaria* pollen counts (grains/m³) in the Messina area during the three considered pollen seasons.

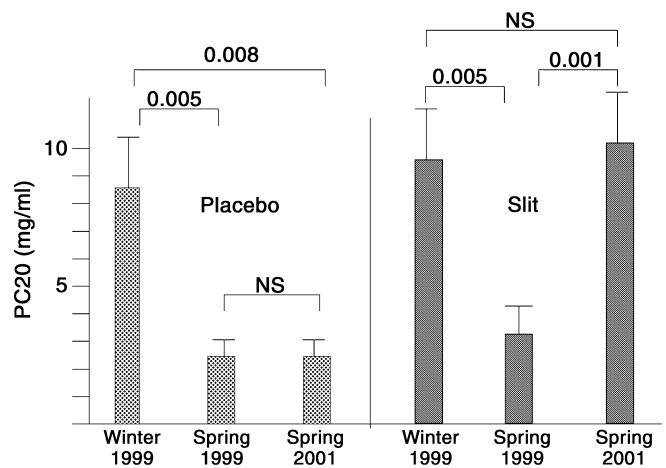


Figure 3. Nonspecific bronchial responsiveness, expressed as methacholine PC20, in the two groups at baseline and during pollen seasons 1999 and 2001.

Table 1. Demographic and clinical characteristics of the patient population at baseline

	SLIT group 15	Placebo group 15	<i>P</i>
Sex (m/f)	7/8	6/9	NS
Mean age (years)	11	10	NS
Age range (years)	8–14	8–13	NS
Lung function parameters*			
FEV ₁ (L)	2.1 ± 0.5	2.3 ± 0.7	NS
FEV ₁ (% pred)	91.7 ± 11.2	94.5 ± 12.6	NS
FEF _{25–75} (L/S)	2.2 ± 0.6	2.1 ± 0.8	NS
FEF _{25–75} (% pred)	84.3 ± 16	82 ± 12.1	NS
MCh PC20 (mg/ml)			
Winter 1999	9.78 ± 5.95	8.70 ± 6.25	NS
Spring 1999	3.37 ± 2.99	2.44 ± 2.25	NS

* Absolute value and percentage of the predicted value.

expected, in spring 1999 the PC20 decreased significantly in both groups: 3.37 ± 2.99 mg/ml in the SLIT group and 2.44 ± 2.25 mg/ml in the placebo group ($P = 0.005$ for both groups vs baseline) without significant difference between the two groups. In spring 2001, in the SLIT group, the PC20 was 9.10 ± 7.7 mg/ml ($P = \text{NS}$ vs baseline and $P = 0.01$ vs Spring 1999). In the placebo group, the PC20 was 2.46 ± 2.26 ($P = 0.008$ vs baseline and $P = \text{NS}$ vs spring 1999). The difference was also significant between the placebo and active group only in spring 2001 ($P = 0.001$). Therefore, all patients had a significantly higher MCh reactivity during the 1999 pollen season when compared with the values obtained out of the pollen season, but after 24 months the in-season PC20 was unchanged for the placebo group whereas it remained at the out-season value in the SLIT group.

Pulmonary function test data are shown in Table 2. Although those children receiving SLIT showed a trend toward improvement during the pollen seasons, no significance was reached.

Table 2. Mean (SD) of FEV_1 and $FEF_{25-75\%}$ as percentage of the predicted value at baseline and during the pollen seasons

	Winter 1999	Spring 1999	Spring 2000	Spring 2001
FEV_1				
Active	88.2 (4.1)	82.0 (5.4)	86.1 (6.3)	88.4 (3.7)
Placebo	92.6 (5.0)	78.9 (5.9)	78.4 (4.6)	75.6 (4.9)
$FEF_{25-75\%}$				
Active	82.1 (5.3)	70.6 (4.2)	74.0 (6.6)	73.4 (6.1)
Placebo	80.0 (4.5)	73.5 (5.4)	67.6 (6.0)	65.5 (5.8)

Discussion

Several studies with SLIT have reported reductions by 30–60% of the use of rescue medications and/or both symptoms and medications scores (for review see 9). Among these trials, four (17–20) were carried out with *Parietaria* pollen in adults and children suffering from seasonal asthma and/or rhinitis, but none of these studies have investigated the effects of SLIT on lung function or BHR.

Indeed, previous trials carried out with the subcutaneous route of IT have shown a significant reduction in BHR in the active treated groups (21–23), but in other trials the reduction of BHR was inconsistent (24–26), despite the positive effect of subcutaneous IT on allergen-specific provocation (27–30). Only an open-controlled trial conducted SLIT using chemically modified grass allergens, observed a reduction of BHR after 3 years of preseasonal treatment (31).

The present study shows that immunotherapy carried out by the sublingual route with *Parietaria* abrogates the seasonal increase in BHR in children with seasonal asthma. The MCh PC20 in the active group, after 18 months of continuous treatment, was similar to that measured out of the pollen season, whereas the placebo patients maintained their seasonal increase in BHR.

Although a trend toward better performances was observed in the SLIT group, no significant improvement in respiratory function (FEV_1 , FEF_{25-75}) at the end of the trial in either the two groups was found. This can be explained considering that, according to the enrolment criteria, none of the asthmatic children in both groups had an impairment of lung function ($FEV_1 \geq 75\%$ and/or $FEF_{25-75} \geq 65\%$) at baseline and during the pollen season. It is well known that pollen-sensitized subjects suffer from asthma attacks during the season (32) but, between attacks, their lung function often remains in the normal limits and inflammation is testified only by BHR.

Allergen immunotherapy 'switches off' the allergic reaction by interrupting the chain of events which characterize the allergic inflammation. Conventional subcutaneous IT may act either by inducing immune deviation of T-cell responses in favour of TH1 or by diminishing TH2/THO T cell responses, for example by induction of T-cell unresponsiveness (33). So far, our knowledge of the mechanisms of action of SLIT is still incomplete, although recent data have suggested that they could be similar to those of subcutaneous IT (34). The immunological effect is probably the reason why immunotherapy can affect BHR that is, at least in part, sustained by inflammation (35–37). Thus, our finding that SLIT abrogates the seasonal increase in BHR is indirect evidence in favour of the anti-inflammatory effect of the treatment, as previously demonstrated in the nose and conjunctiva (13, 21).

In summary, these data are a step in order to understand the mechanisms of action of SLIT and further support its therapeutic use in asthmatic children. Although more convincing findings are needed concerning its immunological mechanisms of action, SLIT can be considered as an effective tool for the treatment of patients with seasonal allergic asthma.

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