

Clinical, functional, and immunologic effects of sublingual immunotherapy in birch pollinosis: A 3-year randomized controlled study

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Background: Sublingual immunotherapy (SLIT) has been proved effective in allergic rhinitis, but there are few studies assessing its effects on inflammation and on the lower airways. **Objective:** We sought to evaluate at the same time the effects of SLIT on rhinitis symptoms, nasal inflammation, and lower airways function in patients with birch pollinosis.

Methods: Adult patients with rhinitis and asthma monosensitized to birch were evaluated during a run-in pollen season and then randomized to receive openly either drugs alone or drugs plus SLIT and reevaluated in the subsequent 4 pollen seasons. Rhinitis symptoms and consumption of bronchodilators were assessed by means of diary card. A nasal smear for eosinophil count was carried out in and out of pollen seasons, and pulmonary function tests with methacholine challenge were performed at each season.

Results: Of 79 enrolled patients, 27 dropped out, with a significantly higher rate of dropouts in the control group. There was a decrease in symptoms and bronchodilator use in the SLIT group versus the control group, becoming significant at the second and third pollen seasons, respectively ($P < .01$ at all times). Nasal eosinophils decreased significantly in the active group, starting from the third pollen season ($P < .01$). In the SLIT group a significant increase in FEV₁, specific airways conductance, and maximal expiratory flow at 25% of forced vital capacity was seen starting from the second year and was associated with an increase in the methacholine threshold dose ($P < .01$). The differences were significant also at the intragroup comparison over time.

Conclusion: SLIT achieved a significant clinical benefit in birch pollinosis, reduced the eosinophil infiltration in nasal mucosa, and significantly improved pulmonary function during the pollen seasons. (J Allergy Clin Immunol 2005;115:1184-8.)

Key words: Sublingual immunotherapy, birch, rhinitis, asthma, pulmonary function, eosinophil

Abbreviations used

MEF25: Maximal expiratory flow at 25% of forced vital capacity
PFT: Pulmonary function test
Sgaw: Specific airways conductance
SLIT: Sublingual immunotherapy

Allergen immunotherapy, along with allergen avoidance and drug therapy, is a cornerstone in the management of respiratory allergy.¹ Immunotherapy is capable of modifying the immunologic response to the offending allergen at the earliest stages. This results in decreased inflammatory phenomena and, in parallel, in symptomatic improvement during the exposure to allergens themselves. The subcutaneous route of administration has been well established for several decades. Its efficacy has been confirmed in numerous trials,^{1,2} and its mechanisms of action have been extensively investigated.³ The risk of severe adverse events with the subcutaneous route⁴ has favored the development of safer noninjection approaches.⁵ Since its introduction in 1986,⁶ sublingual immunotherapy (SLIT) immediately appeared as a good candidate for clinical use. At present, there are many controlled trials showing its efficacy in respiratory allergy,^{7,8} and some postmarketing surveillance studies confirmed that the safety profile in both adults and children is satisfactory.^{9,10} On the basis of these results, the most recent official documents² have validated the routine use of SLIT.

The clinical use of SLIT is recent, and therefore some aspects have not been fully investigated yet. For instance, there are few data on the mechanisms of action as far as allergic inflammation is concerned.¹¹ Also, little information is available on the possible effect of SLIT on the lower respiratory airways in patients with rhinitis, especially in the long term. Thus we studied, in a randomized controlled fashion, a large population of patients with respiratory allergy (rhinitis, asthma, or both) solely caused by birch pollen and evaluated the effects of the treatment on symptoms, respiratory function, and nasal allergic inflammation, as measured by eosinophil count.

METHODS

Overall design

We performed a randomized, open, parallel-group, controlled study. The ethical committee did not approve the double-blind design

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because of the long-term duration of the study. The 2 groups of patients received either SLIT plus drugs or drugs only for 3 years. After a 1-year baseline assessment (pollen season 2000), there was a randomized phase of 3 years (2001-2003). SLIT was stopped in November 2003, and patients were followed up in the subsequent pollen season (2004). In total, 5 pollen seasons were evaluated. The patients recorded their clinical scores (symptoms and medication intake) during the birch pollen season (February-April). Pulmonary function tests (PFTs) and methacholine challenges were performed at baseline and during each pollen season. Nasal eosinophil counts and skin tests were carried out every year both in and out of the pollen season. The onset of new sensitization and the presence of nasal eosinophils out of the pollen season were reasons for dropout. The study design is summarized in Fig 1.

Patients and diagnosis

Subjects were enrolled at the Allergy Unit, Cuasso al Monte Hospital, Varese, Italy, from a pool of outpatients with respiratory allergy. Mandatory inclusion criteria were as follows: (1) clinical history of rhinitis with or without mild intermittent or persistent asthma due to birch pollen in the past 2 years; (2) positive skin prick test response (>5 mm) and positive CAP-RAST assay result (class III or greater) for birch pollen only; (3) age between 18 and 65 years; and (4) FEV₁ within normal limits ($>79\%$ of predicted value). The diagnosis of rhinitis was made on the basis of clinical criteria, including sneezing, itching, rhinorrhea, and nasal congestion with or without eye symptoms. The symptoms had to be present only during allergen exposure (from February through April). Asthma was diagnosed on the basis of the presence of one or more of the following: episodes of wheezing, cough, or chest tightness (diurnal or nocturnal) during the pollen season. Patients sensitized to other common inhalant allergens, having moderate persistent asthma,¹² or with anatomic abnormalities of the upper respiratory tract were not admitted. Patients undergoing chronic treatment with systemic steroids or having malignancies or systemic immunologic disorders were excluded as well. During the study, those patients with onset of nasal eosinophilia, bronchial hyperreactivity out of the pollen season, or new sensitizations were excluded. Skin prick tests were carried out according to previously published guidelines¹³ with a panel of biologically standardized allergens (ALK-Abellø, Milan, Italy), including mite, grasses, olive, birch, cat and dog dander, ragweed, mugwort, parietaria, *Alternaria* species, and *Cladosporium* species.

SLIT and concomitant treatments

The prescribed SLIT (Anallergo, Florence, Italy) was prepared as a glycerinated solution to be administered as sublingual drops in the morning, with the patient fasted. Patients were carefully instructed about the self-administration, and detailed written instructions were also provided. The extract was standardized through a combined RAST inhibition and bioequivalence method: the potency was expressed as RAST units per milliliter. The build-up phase (about 50 days) involved the administration of the extract at progressively increasing concentrations (100-3000 RAST units/mL). In the maintenance phase SLIT was administered continuously for approximately 3.5 years at the dose of about 102 μ g of Bet v 1 per year. The dose was reduced by one third during the pollen season. SLIT was started approximately in November 2000 and stopped in November 2003. The mean cumulative yearly dose was about 12 times greater than the dose administered subcutaneously.

All patients received the following continuous pharmacologic treatment during pollen seasons: cetirizine or loratadine (10 mg once daily) and nasal cromolyn (10 mg/d). Inhaled salbutamol (2 puffs) on demand was used for asthma attacks. Intranasal beclomethasone dipropionate, 2 puffs per nostril twice daily (400 μ g/d), was

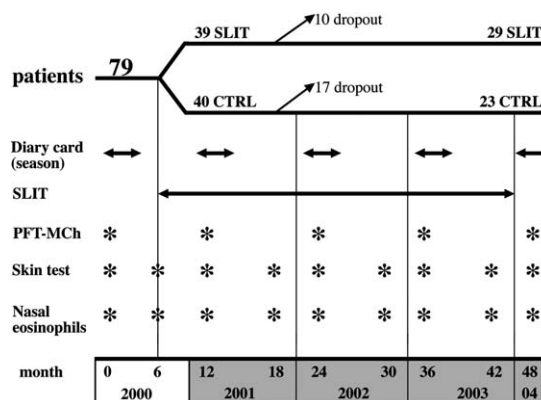


FIG 1. Study design. MCh, Methacholine challenge.

prescribed by the physician only in the case of poor response to antihistamines and cromolyn.

Clinical evaluation

All patients were followed up with regular clinic visits during the whole study, and a referring physician was always available for telephone contact. Patients completed a diary card from February through April each year. The following symptoms were considered: nasal itching, sneezing, rhinorrhea, nasal obstruction, cough, wheezing, and eye itching-redness. Each symptom was scored as follows: 0, absent; 1, mild; 2, moderate; and 3, severe. The use of medications was scored, with 1 point scored for each dose of salbutamol or each dose of nasal steroid. A total monthly score for clinical symptoms and symptomatic drug consumption was then calculated for each patient and used for subsequent statistical analysis.

PFT and methacholine challenge

PFTs were performed by means of plethysmography and computerized spirometry (Masterlab Yaeger, Wurtzburg, Germany). FEV₁, specific airways conductance (Sgaw) for large airways, and maximal expiratory flow at 25% of forced vital capacity (MEF25) for small airways were also measured. The methacholine challenge was carried out each year during birch pollen exposure. A dosimeter (Yaeger) activated by inhalatory effort was used to administer increasing doses of methacholine (30-1290 μ g). The dose of methacholine causing a decrease of 20% in FEV₁ was defined as the PD₂₀. Hyperreactivity was considered severe for a PD₂₀ value of less than 400 μ g, moderate for a PD₂₀ value of 401 to 799 μ g, and mild for PD₂₀ value up to 1290 μ g.^{14,15}

Nasal eosinophils

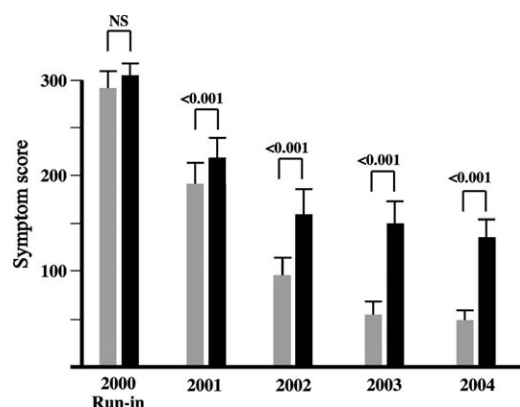
Nasal smears were collected from the anterior third of the inferior turbinate. The smears were transferred onto a glass slide, air-dried, stained with May-Grunwald-Giemsa, and read in optical microscopy. Eosinophils were expressed as the percentage of total white cells per 10 fields. Smears were performed during and out of the birch pollen season. In the birch season patients were advised to discontinue use of intranasal nasal steroids (if any) at least 10 days before the nasal scraping.

Statistical analysis

Comparisons among frequencies were tested by using the Pearson χ^2 test, whereas the Mann-Whitney *U* test was performed to verify differences in age and clinical and functional parameters between the

TABLE I. Demographic and functional data at baseline

	SLIT	Control subjects	<i>P</i> _{Monte Carlo}
Patients	29	23	—
Mean age (y)	27.76	28.96	.243
Range	18-43	19-45	—
Sex (M/F)	16/13	13/10	1.000
Intermittent asthma	26/29	21/23	1.000
Mild persistent asthma	3/29	2/23	1.000
Sgaw (% predicted $\pm$ SEM)	78.5 $\pm$ 2.3	82.6 $\pm$ 2.7	.402
FEV ₁ (% predicted $\pm$ SEM)	94.1 $\pm$ 1.6	96.2 $\pm$ 1.9	.8
Methacholine PD ₂₀ (μ g)	491 $\pm$ 61	475 $\pm$ 58	.8
MEF25 (% predicted $\pm$ SEM)	65.6 $\pm$ 2.8	67.4 $\pm$ 4.3	.8
Nasal eosinophils <10%	1/29	2/23	
Nasal eosinophils 11%-30%	15/29	11/23	.891
Nasal eosinophils 30%-50%	11/29	9/23	
Nasal eosinophils >50%	2/29	1/23	
Symptoms (mean $\pm$ SEM)	297.8 $\pm$ 6.8	304.7 $\pm$ 5.9	.921
β_2 consumption (mean $\pm$ SEM)	9.3 $\pm$ 0.7	10.3 $\pm$ 0.6	.89

FIG 2. Monthly symptom scores (mean $\pm$ SEM) of the pollen seasons in the SLIT (gray bars) and control (black bars) groups.

2 groups.^{16,17} After having recoded methacholine response levels into 4 classes (0, no response to methacholine >1290 μ g; 1, response to methacholine >800 and <1290 μ g; 2, response to methacholine >400 and <800 μ g; 3, response to methacholine >30 and <400 μ g), differences were tested with the Friedman test for paired samples¹⁷ for both control subjects and patients receiving SLIT. The Friedman test was also used to test for significance of the trends in the other clinical and functional parameters in both patients receiving SLIT and control subjects. The probability level has been computed by using a complete randomization method (permutation or exact test; *P*_{exact}) or by using a Monte Carlo simulation on the basis of a 10,000 sampled tables (*P*_{Monte Carlo})^{18,19} when computation was not possible. The type I error (α) has been set at the .01 level. The means (eg, age and symptom scores) have been reported with the standard error of the mean ($\pm$ SE). Comparisons of clinical and functional parameters between patients receiving SLIT and control subjects (fixed factor) were modeled into a unique linear model by using the general linear models for repeated-measures procedure, with sex as a random factor. All the statistical analyses have been computed with the Statistical Package for Social Sciences version 12.01 (SPSS, Inc, Chicago, Ill).

RESULTS

Of 79 patients enrolled, 27 dropped out during the study, and the rate of dropouts was significantly higher in the control group (17 vs 10, $\chi^2 = 6.435$, *df* = 1, *P*_{Monte Carlo} = 0.019). Dropouts were due to consent withdrawal (1 SLIT and 1 control patient) and protocol deviation (6 SLIT and 6 control patients). There were 13 dropouts (3 [8%] in the SLIT group and 10 [25%] in the control group) because of onset of new sensitization, with the difference being significant (*P* < .05). Fifty-two per-protocol patients were analyzed at the end of the study. All patients had at least intermittent asthma, and only 5 had persistent mild disease. The groups did not differ at baseline for demography and clinical and functional parameters (*P*_{Monte Carlo} > .05, Mann-Whitney *U* tests and Pearson χ^2), as shown in Table I. None of the enrolled patients had nasal eosinophils out of the pollen season. Only 4 (14%) patients reported oral itching in the induction phase. This side effect was mild and required no treatment or dosage adjustment.

There was a significant improvement, as demonstrated by the progressive decrease in symptom scores during the pollen seasons seen in Fig 2, and the difference between groups became significant since the first season after beginning SLIT. There was, in parallel, a significant decrease in the need for bronchodilators since the second year of treatment, as reported in Fig 3. Nasal eosinophils during pollen seasons were clearly affected as well (Fig 4), with the difference between groups being significant (*P* < .05) in the third pollen season and highly significant (*P* < .001) in the subsequent seasons. Interestingly, in the fourth and fifth pollen seasons, more than 50% of the patients receiving SLIT had no more eosinophils in the nasal smears (Table II). Considering the trend in the whole study period, the reduction of symptoms ($\chi^2 = 110.207$, *df* = 4, *P* < .001) and nasal eosinophils ($\chi^2 = 88.191$, *df* = 4, *P* < .001) in the SLIT group was also confirmed, although an

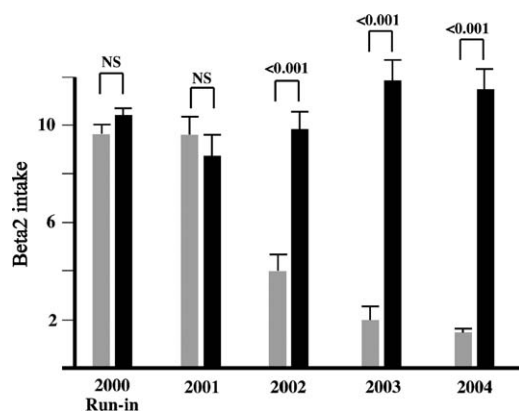


FIG 3. Doses of salbutamol used per month (mean $\pm$ SEM) during the pollen seasons in the SLIT (gray bars) and control (black bars) groups.

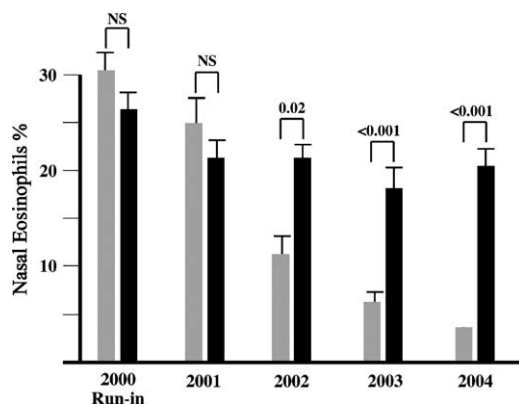


FIG 4. Nasal eosinophils (expressed as percentage of white cells in nasal smears, mean $\pm$ SEM) in the pollens seasons of 2000 through 2004. Gray bars represent the SLIT group, and black bars represent control subjects.

overall reduction in symptoms ($\chi^2 = 110.207$, $df = 4$, $P < .001$) and nasal eosinophils ($\chi^2 = 10.965$, $df = 4$, $P < .05$) was detectable also in the control subjects.

All the functional parameters showed significant changes during the study period, as shown in Fig 5, with the differences between groups becoming significant since the third pollen season. In particular, in the SLIT group there was an increase in FEV₁ ($\chi^2 = 43.951$, $df = 4$, $P < .001$), Sgaw ($\chi^2 = 61.470$, $df = 4$, $P < .001$), and MEF25 ($\chi^2 = 59.882$, $df = 4$, $P < .001$) and a decrease in salbutamol consumption ($\chi^2 = 94.225$, $df = 4$, $P < .001$). A significant opposite trend in FEV₁ ($\chi^2 = 27.035$, $df = 4$, $P < .001$), Sgaw ($\chi^2 = 28.491$, $df = 4$, $P < .001$), MEF25 ($\chi^2 = 12.905$, $df = 4$, $P < .05$), and salbutamol use ($\chi^2 = 34.080$, $df = 4$, $P < .001$) was seen in the control subjects. Methacholine response doses were significantly different in the 5 pollen seasons for the patients receiving SLIT ($n = 29$, $\chi^2 = 69.358$, $df = 4$, $P_{\text{Monte Carlo}} < .001$) but not for the control subjects ($n = 23$, $\chi^2 = 3.263$, $df = 4$, $P_{\text{Monte Carlo}} = .511$), as shown in Fig 5.

The general linear models analysis confirmed these findings in an overall model. SLIT significantly increased

TABLE II. Percentage of patients with no eosinophils in the nasal smear at the different times

	SLIT	Control subjects	P value
Baseline (2000)	0	0	NS
Second season (2001)	5	10	NS
Third season (2002)	33	13	.05
Fourth season (2003)	51	12	.01
Fifth season (2004)	61	14	<.01

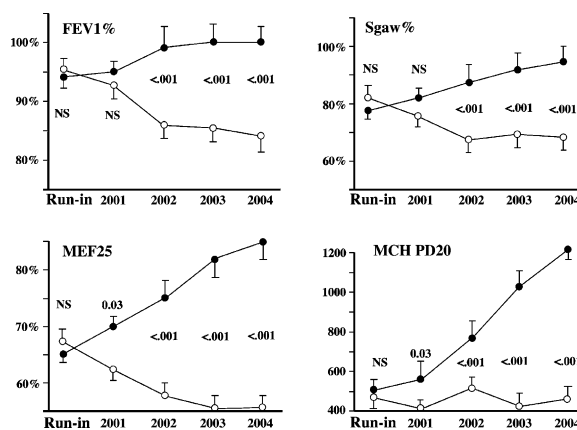


FIG 5. FEV₁ (upper left), Sgaw (upper right), and MEF25 (lower left) during the pollen seasons expressed as a percentage of the predicted value (mean $\pm$ SEM). The PD₂₀ (provocative dose of methacholine in micrograms causing an FEV₁ decrease of 20% from baseline during the pollen seasons) is shown at lower right (mean $\pm$ SEM). Filled circles, SLIT group; open circles, control group.

FEV₁ ($F = 26.562$, $df = 1$, $P < .001$), Sgaw ($F = 38.6022$, $df = 1$, $P < .001$), and MEF25 ($F = 53.790$, $df = 1$, $P < .001$) and decreased symptoms ($F = 114.170$, $df = 1$, $P < .001$) and bronchodilator intake ($F = 189.251$, $df = 1$, $P < .001$).

DISCUSSION

There are now numerous controlled studies that confirm the clinical efficacy and safety of SLIT,⁸ and in addition, a preventative effect similar to the injection route has been recently documented.²⁰ Nevertheless, of course some aspects need to be better addressed, including the demonstration of immunologic effects and of a systemic effect (ie, lower airways). Because immunotherapy in general is considered a biologic response modifier,²¹ the knowledge of the effects on the long term (years) would be of primary relevance. In the present study we aimed at assessing the effects of SLIT on parameters other than rhinitis symptoms, namely pulmonary function and nasal eosinophils, by following the patients for several years. In this regard a double-blind and placebo-controlled design would have enhanced the power of the trial, but it was not allowed for a 4-year study on the basis of ethical considerations. On the other hand, the open design allowed us to enlarge the number of patients enrolled that was great in comparison

with that seen in most SLIT trials involving monosensitized patients. In addition, objective parameters, such as pulmonary function and eosinophil count, cannot be influenced by patients' or investigators' expectations. We choose birch pollen allergy and monosensitized subjects because this represents a very clean experimental model to assess the effects on nasal inflammation. In fact, in our patients the confounding effect of concomitant sensitizations could be ruled out, and the clinical and functional parameters could be evaluated under the limited natural exposure to the allergen. Finally, the study was performed in a real-life situation, with all patients receiving symptomatic medications, and this fact clearly demonstrated the add-on effect of SLIT.

There was a significant improvement in nasal symptoms in the SLIT group compared with in the control group, which have become significant since the pollen season subsequent to the beginning of SLIT. It must be remembered that all patients were receiving regular antihistamine therapy, and thus SLIT really provided a significant additional clinical benefit. Interestingly, there was a significant improvement in the respiratory function. Despite the fact that all patients had normal parameters at the time of enrolment, we documented an increase in airway conductance and in MEF25 and a reduction in the bronchial hyperreactivity to methacholine. This might suggest that SLIT acts also on bronchial allergic inflammation, although it is well known that bronchial hyperreactivity is only in part attributable to inflammation itself.²² The effect is consistent with that described in previous clinical trials with SLIT^{23,24} and the injection route.²⁵ Concerning the effect on local allergic inflammation, this study offered the opportunity to follow up the effects on nasal eosinophils under the natural exposure to pollen and confirmed that the effect was consistent with the time. It is true that antihistamines are able to reduce nasal eosinophilia,²⁶ but it is also true that in the present study all patients received them, and thus the reduction in eosinophil numbers can be attributed only to SLIT. An additional finding was that the clinical, functional, and immunologic effects were maintained in the pollen season of 2004 (ie, after SLIT discontinuation). It was not the aim of our study to assess the basic immunologic mechanisms of action, and therefore a sequential evaluation of allergen-specific IgG4 and IgE was not performed. On the other hand, such an effect was previously demonstrated in a double-blind trial.²⁷ Nevertheless, the prevention of the onset of new sensitization can to some extent support systemic immunologic regulation.

In conclusion, this randomized and controlled clinical trial, performed in real-life in monosensitized patients, confirms that SLIT exerts a significant effect on symptoms and acts at both the nasal and bronchial level.

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