
Long-term comparison of sublingual immunotherapy vs inhaled budesonide in patients with mild persistent asthma due to grass pollen

Maurizio Marogna, MD*; Igino Spadolini, MD†; Alessandro Massolo, BS‡; Daniele Berra, MD§; Pietro Zanon, MD§; Elena Chiodini, BS||; Giorgio Walter Canonica, MD¶; and Giovanni Passalacqua, MD¶

Background: Few studies have compared the effects of immunotherapy and inhaled steroids. The main limitation of such studies is the long duration required to fully appreciate the effects of immunotherapy.

Objective: To compare the effects of inhaled budesonide and sublingual immunotherapy (SLIT) in mild persistent asthma for up to 5 years.

Methods: Patients with mild persistent asthma and rhinitis due to grass pollen were enrolled in an open randomized controlled trial. After a run-in season, they were randomized to either budesonide, 800 $\mu\text{g}/\text{d}$, in the pollen season or continuous grass SLIT for 5 years. All patients received rescue medications. Symptoms were evaluated by diary cards filled out from May to July at baseline and after 3 and 5 years. In-season nasal eosinophils and bronchial hyperresponsiveness were also assessed.

Results: Fifty-one patients were enrolled and 46 completed the study. The bronchial symptom scores and the use of bronchodilators decreased significantly in both groups, but the improvement was greater in the SLIT patients at 3 and 5 years. The nasal symptom score and the intake of nasal steroids decreased only in the SLIT group, and the difference vs the budesonide group was always significant. In the SLIT group vs the budesonide group, a statistically significant decrease of nasal eosinophils was found at 3 and 5 years ($P < .01$). The bronchial hyperresponsiveness improved significantly only in the SLIT group.

Conclusion: In patients with grass pollen-induced asthma, in the long term SLIT was equally effective as inhaled budesonide in treating bronchial symptoms and provided an additional benefit in treating rhinitis symptoms and bronchial hyperresponsiveness.

Ann Allergy Asthma Immunol. 2009;102:69–75.

INTRODUCTION

The currently accepted definition of asthma, which is characterized by episodes of reversible bronchial obstruction, underlines the crucial role of the inflammatory process.^{1,2} Although remodeling and bronchial hyperresponsiveness (BHR) are partially independent of the inflammation,³ the inflammatory process plays a central role in the pathogenesis of the disease, and for this reason, inhaled corticosteroids are the mainstay of asthma treatment.¹ On the other hand, specific immunotherapy (SIT) is the only treatment capable of modifying the response to allergens at the early steps and restoring the imbalance between T_H1 and T_H2 lymphocyte subsets.⁴ In the last decade, sublingual immunotherapy (SLIT) has gained more credibility, and it is now included in

guidelines⁵ and used in many European countries. The main advantages of SLIT are self-administration at home and favorable safety profile.⁶

It has been consistently shown that SIT, including SLIT, can downregulate the inflammatory phenomena at the target organs during exposure to allergens.^{7–10} In addition, specific immunotherapy is effective in reducing asthma symptoms^{11,12} and can reduce the degree of BHR,^{13,14} which is indirectly related to bronchial inflammation. Nonetheless, few studies have compared the effects of inhaled corticosteroids and SIT in allergic asthma, mainly because long periods of observation are needed to fully appreciate the effects of SIT. This aspect is still a matter of debate. Therefore, we planned a randomized study to compare the effects of SLIT and inhaled budesonide in patients with grass-induced asthma by assessing several parameters during a 5-year period.

METHODS

Study Design

This is an open, 2-parallel group randomized controlled trial involving patients with rhinitis and mild asthma solely due to

Affiliations: * Pneumology Unit, Macchi Hospital Foundation, Cuasso al Monte, Varese, Italy; † Medical Department, Anallergo SpA, Florence, Italy; ‡ Department of Ecology, University of Calabria, Calabria, Italy; § Pneumology Unit, Busto Arsizio Hospital, Busto Arsizio, Italy; || Nuclear Medicine Unit, Busto Arsizio Hospital, Busto Arsizio, Italy; ¶ Allergy and Respiratory Diseases, DIMI, University of Genoa, Genoa, Italy.

Disclosures: Authors have nothing to disclose.

Received for publication July 14, 2008; Accepted for publication October 6, 2008.

grass pollen. Patients with an incomplete response to inhaled budesonide, 200 μg twice daily, in the previous seasons were evaluated for baseline parameters during the 2001 pollen season (run-in) and then randomized to receive either budesonide, 400 μg twice daily, or SLIT in addition to rescue medications. The randomization was made according to a computer-generated allocation list. The evaluated parameters (seasonal symptoms and drug intake score, pulmonary function, BHR, and nasal eosinophils) were evaluated at the run-in and after 3 and 5 years of treatment (years 2004 and 2006). The study design is summarized in Figure 1. Because of the planned duration of the study, the Ethical Committee of the Cuasso al Monte Hospital denied permission to blind the treatments and to use a placebo arm. All the patients signed informed consent forms.

Patients and Diagnosis

Outpatients referred to the Allergy Unit, Cuasso al Monte Hospital (Varese, Italia), who had mild persistent asthma and rhinitis due to grass pollen were enrolled in the study. The inclusion criteria were as follows: (1) age between 18 and 65 years, (2) clinical history of mild asthma¹ and rhinitis only during the local grass season in the last 2 years, (3) forced expiratory volume in 1 second (FEV_1) more than 79% of predicted, and (4) single sensitization to grass pollen as evaluated by skin prick tests and CAP-radioallergosorbent test (RAST) assay. Exclusion criteria were as follows: (1) moderate to severe asthma; (2) skin sensitizations other than grass pollen; (3) asthma or rhinitis symptoms out of the grass pollen season; (4) previous courses of immunotherapy; (5) systemic immunologic diseases, malignancies, and long-term treatment with systemic steroids; and (6) major anatomical abnormalities of the nose, including polyps, septal deviation, or turbinate hypertrophy.

Asthma and rhinitis were diagnosed according to current guidelines.^{1,5} The response to inhaled budesonide was con-

sidered incomplete if the patients maintained their symptoms of mild asthma despite the treatment with 200 μg twice daily. Skin prick tests were performed, according to recommendations, with a standard panel (Alk Abello, Milan, Italy), including mites, grass, *Parietaria*, birch, olive, mugwort, ragweed, cat, dog, *Alternaria*, and *Cladosporium*. Positive (1% histamine) and negative (diluent) controls were also applied. The result was considered positive for a wheal diameter 5 mm greater than the negative control. CAP-RAST assays (Unicap; Pharmacia, Uppsala, Sweden) were performed for the same allergens and considered positive for a class 2 or greater response.

Interventions

SLIT was given as a glycerinated solution, containing grass allergens standardized in RAST units (RU) per milliliter (Anallergo, Firenze, Italia),¹⁵ and prepared in vials at different concentrations (100, 300–1,000, and 3,000–10,000 RU/mL). The buildup lasted 40 days with daily increasing doses from each vial, until a concentration of 10,000 RU/mL was reached. The maintenance dose was 5 drops from the 10,000-RU/mL vial 3 times a week. The treatment was given continuously starting September 1, 2001, until the end of July 2006. A one-third dose reduction was applied during the pollen season. The cumulative dose per year was on average 70 μg of Phl p 1, which is approximately 10 times greater than the corresponding SIT. All patients receiving SLIT were carefully instructed about the administration technique and the possible adverse effects, and a physician was always available for telephone contact. Any troublesome effect related to SLIT had to be recorded. Adherence was evaluated by measuring the remaining volume of the extract in returned vials and, by subtraction, the actual consumed volume. Adherence was expressed as the percentage of the actual vs expected consumption. Concerning inhaled budesonide, after verifying the inspiratory flow (optimum inspiratory flow; HS Clement Clarke International, Essex, England), the device was chosen according to the patient's preference. The correct use of the device was reviewed at each control visit.

All patients received oral cetirizine, 10 mg/d, during the pollen seasons plus the following as rescue medications: inhaled salbutamol (100 μg per puff, 1 to 2 puffs on demand) for lower airways symptoms and nasal budesonide (200 $\mu\text{g}/\text{d}$) for rhinitis symptoms, according to physician prescription.

Symptom and Drug Intake Scores

The patients were instructed to fill out a diary card from May to July, recording their symptoms and the drugs used. Symptoms were subdivided into (1) upper airways symptoms (nasal itching, discharge, sneezing, and obstruction) and (2) lower airways symptoms (cough, wheezing, chest tightness, and nocturnal symptoms). Each symptom was scored from 0 (absent) to 3 (severe). The possible maximum monthly score was therefore 360 for both. Each dose of the rescue medications (inhaled salbutamol and nasal budesonide) was scored

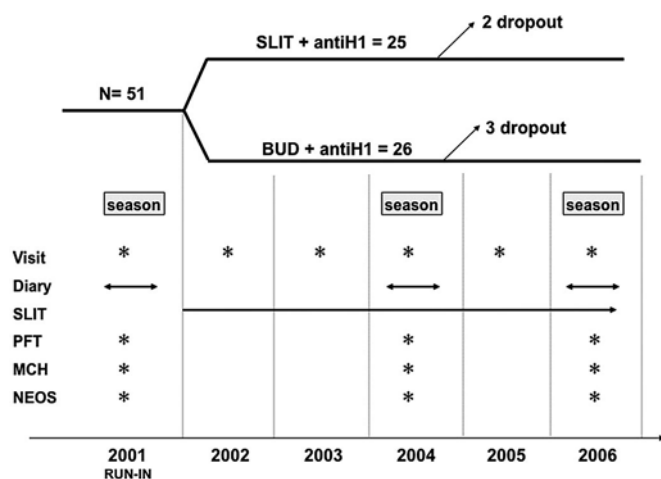


Figure 1. Study design. BUD indicates budesonide; MCH, methacholine test; NEOS, nasal eosinophils in scraping; PFT, pulmonary function test; SLIT, sublingual immunotherapy.

as 1. A mean monthly score for bronchial symptoms (lower airways score [LAS]) and nasal symptoms (upper airways score [UAS]) was then calculated for the 3-month period and used for the statistical analysis. The intake of a nasal corticosteroid (NCS) and bronchodilator in the same period was calculated as well.

Pulmonary Function and Methacholine Challenge

The pulmonary function tests, including forced vital capacity and FEV₁, were performed with a computerized spirometer (Masterlab Yaeger, Wurtzburg, Germany) The methacholine challenge was performed during the pollen seasons with an inspiration-activated dosimeter (Masterlab Yaeger), delivering methacholine from 30 to 1,200 μg in refracted doses. A computerized dose-response curve identified the provocation dose causing a 20% decrease in FEV₁ (PD₂₀). The test result was considered negative if no response was obtained at 1,200 μg of methacholine.¹⁶

Nasal Eosinophils

Nasal smears were collected with a cotton swab from the anterior third of the inferior turbinate. The smears were transferred on a glass slide, air dried, stained with May-Grünwald-Giemsa, and read in optical microscopy.¹⁷ Eosinophils were expressed as the percentage of the total cells per 10 fields. Smears were collected during grass pollen season, with the patient advised to discontinue use of intranasal steroids (if any taken) at least 10 days before.

Statistical Analysis

Equality of sex ratios in different treatment groups at baseline was tested by the Fisher exact test, whereas differences in the baseline level of clinical parameters were tested with the Mann-Whitney test. The different effects of treatments on clinical parameters in each year were tested with the Mann-Whitney test,^{18,19} whereas the changes from baseline were evaluated with the Wilcoxon test for paired comparisons.

To achieve the same statistical power and corresponding parametric statistics, when all the assumptions are met, the probability levels for the Pearson χ^2 , Mann-Whitney, and Wilcoxon tests were computed using a complete randomization method (permutation or exact test; P_{exact}). A Monte Carlo simulation based on a 100,000 sampled tables (P_{MC})^{20,21} was used when the permutation method was not applicable. All the statistical analyses were computed using SPSS statistical software, version 12.01 (SPSS Inc, Chicago, Illinois).

RESULTS

Fifty-one patients fulfilled the inclusion criteria, underwent a baseline evaluation, and were randomized to SLIT (25 patients) or budesonide, 800 μg (26 patients). Fifteen patients preferred to use a metered dose inhaler and 11 choose the Turbohaler (AstraZeneca, Basiglio, Italy) device. The demographic and clinical characteristics at baseline were homogeneous (Table 1). At baseline (Table 2), the UAS was slightly higher in the SLIT group vs the budesonide group ($P_{\text{exact}} = .02$), whereas the opposite happened for NCS intake ($P_{\text{exact}} = .04$). There were 5 dropouts during the first year of the study. In the SLIT group, 1 patient spontaneously stopped SLIT after 2 months and 1 refused to continue the study for personal reasons. In the budesonide group, 2 patients did not follow the prescribed therapy and 1 was lost to follow-up. The complete data set was available for 46 patients at the end of the study. The pollen counts were comparable in the 3 considered years, with an overall 10% decrease in 2006 (Figure 2). The adherence to budesonide treatment, as derived by diary cards, was more than 80% in all patients. The adherence to SLIT during the study period was more than 80% in 17 of 23 patients, more than 60% in 4 of 23 patients, and less than 60% in 2 patients only. The summary of all parameters (including UAS, LAS, NCSs, bronchodilator, nasal eosinophils, and PD₂₀) is reported in detail in Table 2.

The main finding was that in 2004 and 2006 all the comparisons detected a statistically significant improvement in the SLIT group compared with the budesonide group (Table 1). No difference was found in UAS from baseline in patients taking budesonide in 2004 ($P_{\text{exact}} = .58$) and 2006 ($P_{\text{exact}} = .23$). Conversely, statistically significant differences from baseline were found in 2004 ($P_{\text{exact}} < .001$) and 2006 ($P_{\text{exact}} < .001$) in the SLIT group. In addition, the difference between groups was significant in 2004 and 2006 (Figure 3A). The intake of NCSs was significantly lower in the SLIT group at the 3 considered time points (Table 2). The LAS values significantly differed from baseline in both groups in 2004 ($P_{\text{exact}} < .001$ for the budesonide group; $P_{\text{exact}} < .001$ for the SLIT group) and in 2006 ($P_{\text{exact}} < .001$ for the budesonide group; $P_{\text{exact}} < .001$ for the SLIT group). The difference between groups became significant only in 2006, as shown in Figure 3B. The bronchodilator intake was similar in the 2 groups at baseline but decreased significantly in the SLIT groups in 2004 and 2006 (Table 1). Nasal eosinophils signif-

Table 1. Baseline Characteristics of the 2 Groups

Characteristics	SLIT (n = 25)	Budesonide (n = 26)	P value
Age, mean (SD), y	26.7 (1.4)	27.2 (1.3)	.72
Age range, y	17–41	19–43	
M/F	11/14	12/14	>.99
Duration of asthma, mean (SD), y	7.5 (2.5)	7.0 (2.5)	.52
FEV ₁ , mean (SD), %	87 (0.7)	84 (0.7)	.31

Abbreviations: FEV₁, forced expiratory volume in 1 second; SLIT, sublingual immunotherapy.

Table 2. Summary of the Evaluated Parameters at Baseline (Year 2001) and in the Subsequent Pollen Seasons^a

Parameter	Budesonide (n = 23)	SLIT (n = 23)	<i>P</i> _{exact}
2001			
UAS	89.8 (10.4)	116.2 (12.3)	.02
LAS	162.2 (4.9)	176.0 (6.2)	.06
NCSs	24.8 (3.1)	19.1 (2.2)	.04
Bronchodilator	22.4 (0.9)	23.0 (1.5)	.83
Nasal eosinophils	14.8 (1.9)	17.2 (1.7)	.40
PD ₂₀	354.2 (33.7)	297.7 (28.6)	.24
2004			
UAS	95.6 (7.9)	54.4 (7.4)	<.001
LAS	130.9 (6.1)	80.3 (9.4)	<.001
NCSs	26.0 (2.6)	11.8 (1.5)	<.001
Bronchodilator	18.5 (1.4)	8.0 (1.3)	<.001
Nasal eosinophils	15.2 (1.6)	9.0 (1.1)	.002
PD ₂₀	486.2 (47.8)	806.6 (50.7)	.002
2006			
UAS	108.3 (11.4)	33.0 (5.2)	<.001
LAS	110.2 (5.3)	52.1 (12.5)	<.001
NCSs	26.0 (2.3)	6.0 (0.9)	<.001
Bronchodilator	13.0 (1.2)	5.1 (1.4)	<.001
Nasal eosinophils	17.4 (1.6)	5.4 (1.2)	<.001
PD ₂₀	684.1 (51.7)	900.4 (58.7)	.005

Abbreviations: LAS, lower airways score; NCSs, nasal corticosteroids; PD₂₀, methacholine provocation dose causing a 20% decrease in forced expiratory volume in 1 second; UAS, upper airways score.

^a Data are presented as mean (SEM).

icantly decreased vs baseline only in the SLIT group in 2004 (*P*_{exact} <.001) and 2006 (*P*_{exact} <.001). The difference between groups was also significant (Figure 4). The PD₂₀ significantly increased vs baseline in both groups in 2004 (*P*_{exact} = .04 for the budesonide group; *P*_{exact} <.001 for the SLIT group) and 2006 (*P*_{exact} <.001 for the budesonide group; *P*_{exact} <.001 for the SLIT group), but a significant difference was found between groups in favor of SLIT at both time points (Figure 5). The FEV₁ of the patients remained more than 80%

during the whole study. The treatments were equally well tolerated, without reported adverse events for both SLIT and budesonide. None of the dropout was related to possible adverse effects of the treatments.

DISCUSSION

Specific immunotherapy has a complex mechanism of action, essentially affecting the early steps of the immunologic response to allergens. This mechanism involves the selective downregulation of T_H2 cytokine and cell responses, presumably mediated by the T-regulatory cells.^{4,22} The final result is a broad spectrum of anti-inflammatory actions in the target organs of the allergic reaction. The clinical effects of immunotherapy are not immediate, such those of traditional drugs (ie, bronchodilators or antihistamines), but the immunomodulation is profound and long-lasting. On the basis of this background, it is currently stated that SIT does not replace drugs but must be used in addition to them to achieve the maximum benefit.⁵ Nevertheless, the direct comparison of the effects of drugs and SIT is still a matter of debate and a source of controversy. The main problem in comparison studies is that the clinical benefits of SIT can be appreciated only in the long term, whereas pharmacotherapy has a prompt action that can be measured within days. Another problem is that a rigorous head-to-head comparison would

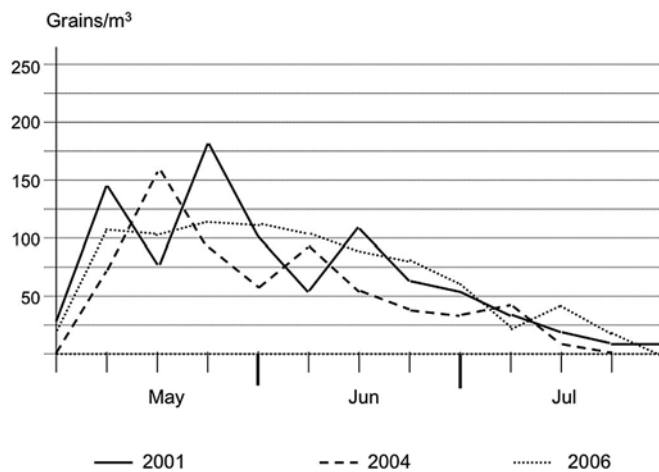


Figure 2. The pollen counts in years 2001, 2004, and 2006.

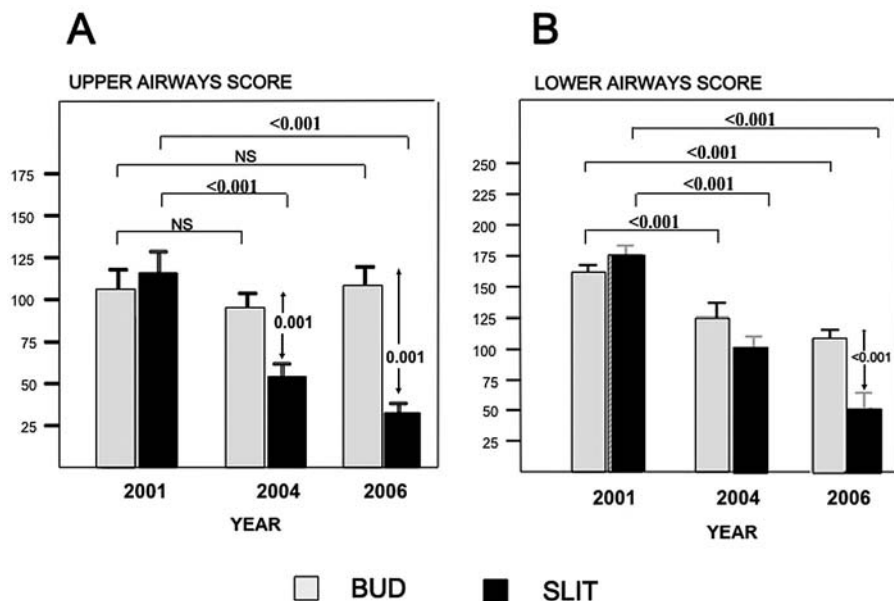


Figure 3. Airways scores. A, Upper airways scores (mean and SEM). The significant between-group comparisons are shown near the bars. The significant difference vs baseline are reported above the bars. B, Lower airways scores (mean and SEM). The significant between-group comparisons are shown near the bars. The significant difference vs baseline are reported above the bars. BUD indicates budesonide; NS, nonsignificant; SLIT, sublingual immunotherapy.

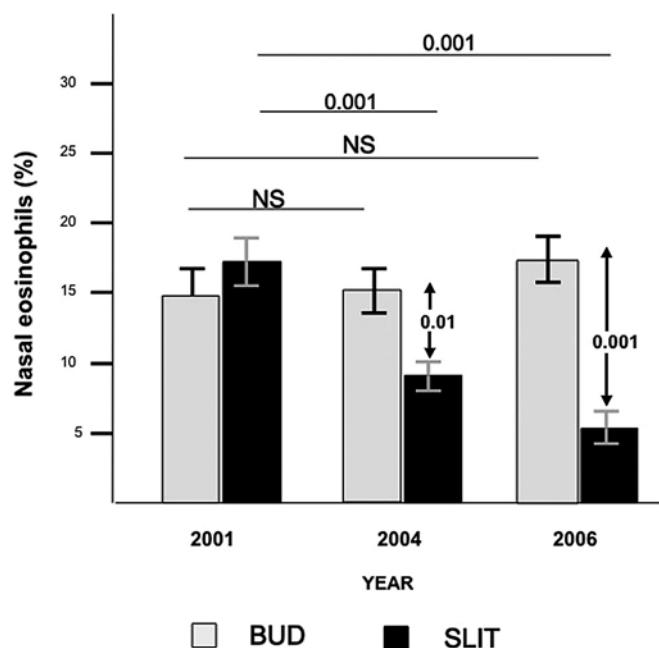


Figure 4. Nasal eosinophils in percentage of total cells (mean and SEM). The significant between-group comparisons are shown near the bars. The significant difference vs baseline are reported above the bars. BUD indicates budesonide; NS, nonsignificant; SLIT, sublingual immunotherapy.

require a double-blind and double-dummy design, which is difficult to do for long periods.

Few studies have compared immunotherapy and pharmacologic treatments. Rak et al,²³ in a double-blind study, showed that nasal steroids were more effective than injection immunotherapy in controlling rhinitis in the short term, although immunotherapy decreased the seasonal BHR in asthmatic patients. Similarly, Pajno et al²⁴ in a placebo-controlled trial demonstrated that the clinical efficacy of SLIT plus fluticasone is equal to that of fluticasone alone, but the addition of SLIT also improves non-bronchial symptoms. Shaikh²⁵ compared SIT and inhaled budesonide in an open study and found that the inhaled steroids produced a more rapid and relevant benefit, but specific immunotherapy maintained its effects after discontinuation. These studies, conducted in the short term, overall failed to demonstrate a clear advantage of immunotherapy over drugs. In the present study, we compared SLIT and inhaled budesonide in patients with seasonal asthma, taking into account different parameters and evaluating at the same time the effects on bronchial and nasal symptoms. Of note, this was done for a long period. Bronchial scores and BHR significantly improved vs baseline in both groups, but the magnitude of the benefit in the long term was greater in SLIT patients. Furthermore, SLIT patients experienced an additional clinical benefit on their nasal symptoms that were not achieved, as expected, by inhaled budesonide, which acts only locally. It can be speculated that the improvement in nasal symptoms would have contributed to the control of lower airways symp-

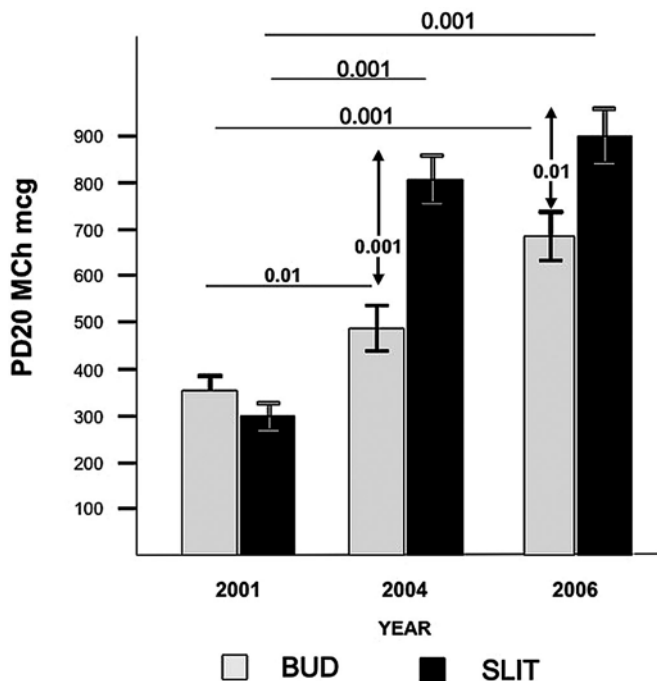


Figure 5. Methacholine (MCh) provocation dose causing a 20% decrease in forced expiratory volume in 1 second (PD₂₀) in micrograms (mean and SEM). The significant between-group comparisons are shown near the bars. The significant difference vs baseline are reported above the bars. BUD indicates budesonide; SLIT, sublingual immunotherapy.

toms, in agreement with the theory of the united airways disease.²⁶ Concerning the economic aspect, based on the cost of inhaled budesonide and SLIT and on the consumption of nasal budesonide and inhaled salbutamol, the average annual expense was approximately €320 in the SLIT group and €120 in the budesonide group. The difference is mainly attributable to the fact that SLIT was given continuously throughout the year, whereas budesonide was taken only during the pollen season. With a preseasonal course, the costs would have been comparable. In addition, this is a only a rough analysis and does not take into account other direct and indirect expenditures.

The main problem with this study is the absence of a placebo, which was not allowed by the ethical committee, because of the long duration of the study. In addition, a double-dummy design was not feasible for practical reasons. To keep the patients adherent to the study protocol and to fully appreciate the slow-onset benefits, the investigational parameters were evaluated after 3 and 5 years of treatment. With this design some information is missing, but this is counterbalanced by the “clean” model (ie, monosensitized patients who had symptoms only during the pollen season). In conclusion, SLIT produces overall a greater benefit compared with inhaled steroids in patients with asthma and rhinitis due to grass pollens and down-

regulates the nasal local inflammation and the nonspecific bronchial responsiveness.

REFERENCES

1. Global Initiative for Asthma (GINA). Update 2006. www.ginasthma.org. Accessed August 2006.
2. Larchè M. Regulatory T cells in allergy and asthma. *Chest*. 2007;132:1007–1014.
3. Jeffery PK. Remodelling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med*. 2001;164(10, pt 2):S28.
4. Akdis M, Akdis C. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol*. 2007;119(4):780–91.
5. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. ARIA: WHO Initiative. *J Allergy Clin Immunol*. 2001;108:S147–S334.
6. Passalacqua G, Durham SR. Allergic rhinitis and its impact on asthma update: allergen immunotherapy. *J Allergy Clin Immunol*. 2007;119(4):881–91.
7. Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med*. 1999;341(7):468–75.
8. Ippoliti F, De Santis W, Volterrani A, et al. Immunomodulation during sublingual therapy in allergic children. *Pediatr Allergy Immunol*. 2003;14(3):216–21.
9. Passalacqua G, Albano M, Fregonese L, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet*. 1998;351(9103):629–32.
10. Ciprandi G, Fenoglio D, Cirillo I, et al. Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol*. 2005;95(1):38–44.
11. Puy R, Abramson A, Weiner JM. Allergen immunotherapy for asthma. *Cochrane Database Syst Rev*. 2003;(4):CD001186.
12. Penagos M, Passalacqua G, Compalati E, et al. Meta-analysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. *Chest*. 2008;133(3):599–609.
13. Grembale RD, Camporota R, Djukanovic R, Marsico S. Effects of specific immunotherapy in allergic rhinitic individuals with bronchial hyperresponsiveness. *Am J Respir Crit Care Med*. 2000;162(6):2048–52.
14. Pajno GB, Passalacqua G, Vita D, Caminiti L, Parmiani S, Barberio G. Sublingual immunotherapy abrogates seasonal bronchial hyperresponsiveness in children with Parietaria-induced respiratory allergy: a randomized controlled trial. *Allergy*. 2004;59(8):883–7.
15. Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G. Clinical, functional, and immunologic effects of sublingual immunotherapy in birch pollinosis: a 3-year randomized controlled study. *J Allergy Clin Immunol*. 2005;115(6):1184–8.
16. Sterk PJ, Fabbri LM, Quanjer PH, et al. Airway responsiveness standardized challenge testing with pharmacological, physical and sensitising stimuli in adults: report of the Working Party on Standardization of Lung Function Tests, European Coal and Steel Community. Official Statement of the European Respiratory Society. *Eur Respir J*. 1993;16(suppl):53–83.
17. Ciprandi G, Ricca V, Landi M, Passalacqua G, Bagnasco M, Canonica GW. Allergen-specific nasal challenge: response kinetics of clinical and inflammatory events to rechallenge. *Int Arch Allergy Immunol*. 1998;115:157–161.
18. Sokal RR, Rohlf FJ. *Biometry. The Principles and Practice of Statistics in Biological Research*. 3rd ed. New York, NY: WH Freeman & Co; 1995.
19. Siegel S, Castellan NJJ. *Nonparametric Statistics for the Behavioral Sciences*. New York, NY: McGraw-Hill; 1988.
20. Greenhouse SW, Geisser S. On methods in the analysis of profile data. *Psychometrika*. 1959;24(2):95–111.
21. Good P. *Permutation Tests: A Practical Guide to Resampling Methods for Testing Hypotheses*. 2nd Ed. New York, NY: Springer Verlag; 2000.

-
22. Moingeon P. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy*. 2006;61(2):151–65.
23. Rak S, Heinrich C, Jacobsen L, Scheynius A, Venge P. A double-blinded, comparative study of the effects of short pre-season specific immunotherapy and topical steroids in patients with allergic rhinoconjunctivitis and asthma. *J Allergy Clin Immunol*. 2001;108:921–928.
24. Pajno GB, Vita D, Parmiani S, Caminiti L, La Grutta S, Barberio G. Impact of sublingual immunotherapy on seasonal asthma and skin reactivity in children allergic to *Parietaria* pollen treated with inhaled fluticasone propionate. *Clin Exp Allergy*. 2003;33:1641–1647.
25. Shaikh WA. Immunotherapy vs inhaled budesonide in bronchial asthma: an open, parallel, comparative trial. *Clin Exp Allergy*. 1997;27:1279–1284.
26. Passalacqua G, Ciprandi G, Canonica GW. United airways disease: therapeutic aspects. *Thorax*. 2000;55(suppl 2):S26–S27.

Requests for reprints should be addressed to:
Giovanni Passalacqua, MD
Allergy & Respiratory Diseases
Department of Internal Medicine
Padiglione Maragliano
L.go R. Benzi 10
16132 Genoa, Italy
E-mail: passalacqua@unige.it