sThe efficacy of sublingual immunotherapy for respiratory allergy is not affected by different dosage regimens in the induction phase

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ABSTRACT

Background: Sublingual administration of allergens is a safe and effective alternative to subcutaneous immunotherapy in patients with respiratory allergies. A drawback to this therapeutic approach is the relatively long and complex management of the induction phase.

Aim of the study: To determine whether different induction regimens affect the outcome of sublingual immunotherapy.

Methods and results: Adult and pediatric patients with allergic rhinoconjunctivitis and/or asthma were included in the study. Ten subjects served as controls and received symptomatic treatments. Forty-three subjects were allocated to sublingual immunotherapy, with three different induction protocols (8-, 15- and 20-day, respectively). Symptom and medication scores, skin test results and (in asthmatic patients) FEV_1 values were monitored for two years. Adverse effects were recorded.

All induction regimens produced a significant improvement in symptom and medication usage ($p < 0.0001$); skin test scores decreased ($p < 0.0001$) and FEV_1 improved ($p < 0.05$). In contrast, symptom and skin test scores did not significantly change in controls. No relevant adverse effects were observed with any of the induction regimens.

Conclusions: For patients with respiratory allergies, sublingual immunotherapy with an 8-day induction protocol is safe and effective. Our results encourage the usage of shorter induction regimens, which produce better compliance with this therapy.

Key words: Allergy. Sublingual immunotherapy. Induction protocol. Effectiveness. Safety.

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RESUMEN

Antecedentes: La administración sublingual de alergenos es una alternativa segura y eficaz a la inmunoterapia subcutánea en pacientes con alergia respiratoria. Una desventaja de este enfoque terapéutico es la gestión relativamente larga y compleja de la fase de inducción.

Objetivo del estudio: Nos cuestionamos si las variaciones en los regímenes de inducción podían afectar el resultado de la inmunoterapia sublingual.

Métodos y resultados: El estudio se realizó sobre pacientes adultos y pediátricos con rinoconjuntivitis alérgica y/o asma. Se utilizaron como controles 10 sujetos que recibieron tratamiento sintomático. Se aplicó una inmunoterapia sublingual a 43 sujetos, con 3 protocolos de inducción diferentes (de 8, 15 y 20 días, respectivamente). Se controlaron durante 2 años los valores de los síntomas y la medicación, los resultados de las pruebas cutáneas y, en los pa-

cientes asmáticos, los valores del volumen espiratorio forzado (FEV_1). Asimismo, se registraron los efectos secundarios.

Con todos los regímenes de inducción se observó una mejoría significativa en los síntomas y el uso de la medicación ($p < 0,0001$); las puntuaciones obtenidas en las pruebas cutáneas descendieron ($p < 0,0001$) y el FEV_1 mejoró ($p < 0,05$). En cambio, las puntuaciones de los síntomas y las pruebas cutáneas no variaron significativamente en los controles. No se observaron efectos secundarios relevantes con ninguno de los regímenes de inducción.

Conclusiones: La inmunoterapia sublingual con un protocolo de inducción de 8 días es eficaz y segura en pacientes con alergias respiratorias. Nuestros resultados aconsejan el uso de regímenes de introducción más breves, que permitan un mejor seguimiento de este tratamiento.

Palabras clave: Alergia. Inmunoterapia sublingual. Protocolo de inducción. Eficacia. Seguridad.

INTRODUCTION

The efficacy of subcutaneous immunotherapy in individuals allergic to inhalant allergens is well documented. However, systemic reactions are possible, and the medical management of each single injective procedure over the few years duration of this treatment is quite complex and requires access to resuscitative measures. Sublingual immunotherapy (SLIT) has been indicated as a safer and simpler approach, and several studies have showed remarkable clinical efficacy[4-11]. On this basis, the European Academy of Allergy and Clinical Immunology (EAACI) as well as the World Health Organization (WHO) published position papers on sublingual allergen immunotherapy[3,12] where this therapeutical approach was suggested as a valid alternative to the traditional subcutaneous route. In the following years several trials were performed involving patients suffering both seasonal and perennial rhinitis. Most studies provided clear-cut evidence of efficacy[6-10,13-20] while a few others only provided evidence for a favorable trend in several clinical parameters[21-24]. In all cases the safety of the sublingual route was confirmed.

Patients' compliance to SLIT is a relevant aspect for this therapy, which has to be continued for a few years. In particular, reaching the maintenance dose with daily administration of increasing doses in adult and pediatric patients might be particularly challenging. In fact, the pre-seasonal induction schedules of SLIT in patients allergic to pollens are usually performed during the late winter. In the non-infrequent cases a cold, a flue, or a minor upper airways disease might occur, this therapy is often interrupted and started over. On this basis rush induction regimens have been used, which cut the conservative 20 to 30 day induction phase down to a week or less[9,14,17,18,21]. Direct comparison between different rush schedules needs dedicated studies. Recently, a report compared the safety of three dosage regimen and indicated very few and minor side effects with any protocol[25]. Here, we evaluated in parallel the efficacy and the safety of sublingual immunotherapy initiated with an 8-day, 15-day or 20-day induction protocol, respectively, in patients with sensitization to seasonal and perennial inhalant allergens, over a 2-year follow up period.

MATERIAL AND METHODS

Patients

Fifty-three patients were recruited at the same outpatient clinic. They all gave a history of seasonal allergic rhino conjunctivitis of at least 2 years duration and positive skin-prick test to one or more of the following allergen extracts: house dust mites mixture *(Dermatophagoides pteronyssinus and farinae), Ambrosia artemifolia*, grass mixture *(Dactlylis glomerata, Festuca pratensis, Lolium perenne, Phleum pratense, Poa pratensis)*, *Parietaria judaica* and *Betulaceae* mixture (*Betula verucosa, Corylus avellana, Alnus glutinosa*) (ALK-Abellò S.p.A., Milan, Italy). Each allergen was purified and biologically standardized, as previously described[7]. The skin prick test was performed according to the recommendations of the European Academy of Allergy and Clinical Immunology[26] and positive results were expressed with a score ranging from 1 to 4, accordingly. Patients with mild asthma were included whose baseline Forced Expiratory Volume in one second (FEV_1) at the first visit was above 75 percent of the predicted. Asthmatic patients fulfilled the criteria for classification of intermittent or mild persistent asthma, according to the International Guidelines for the Diagnosis and Treatment of Asthma (GINA) (National Institutes of Health, NIH publication number 02-3659, revised 2002).

Study design

This was an open study, where consecutive patients were randomly assigned to any of the following 4 groups: controls (untreated), 8-day induction,

Table I

Patient characteristics

Group	N	Age (mean, range)	Sex (M/F)	Rhin. N =	Cong. N =	Asth. N =	SLIT
8-d	14	19,3 (4-43)	10/8	10	10	4	4G, 7HD, 3P
15-d	17	25.9 (5-42)	7/11	12	12	5	3G, 10HD, 1 P, 3 B
20-d	12	16.5 (6-41)	7/5	9	9	3	6 G, 6 HD
CTRL	10	22.7 (10-37)	6/4	10	10	0	4G, 7HD, 3P

The duration of each single induction protocols was used to label the cohorts of patients who were treated with sublingual immunotherapy (8-d, 15-d and 20-d indicates the 8, 15 and 20 day duration of the induction protocol, respectively). Patients of the group labeled "CTRL" did not receive any immunotherapy and served as controls. *Rhin. Cong.* and *Asth.* indicate rhinitis, conjunctivitis, and asthma, respectively. Allergen extracts used for sublingual iposensitizing therapy (SLIT) are indicated with *G* (grass), *HD* (house dust mite), *P (Parietaria)* and *B (Betulaceae)*.

Table II

Number of sensitizations observed per patient at skin test analysis

Group	T = 0	T = 1 year	T = 2 years
8-d induction	1.28	1.28	1.28
15-d induction	1.17	1.17	1.23
20-d induction	1.33	1.25	1.25
All treated	1.25	1.23	1.25
Controls	1.10	1.10	1.70

Numbers of positive skin test per patient at different visits are listed for each group of individuals. No significant differences were observed within groups and between groups.

15-day induction, and 20-day induction. Patients assigned to each group were matched for age, sex, and number of sensitizations (tables I and II).

Immunotherapy protocols

The treatments used in the study were glycerin/phenol solutions prepared from biologically standardized aqueous extracts[27], whose allergen content in major allergens was expressed in Standard Treatment Units (STU) per ml (ALK-Abellò).

The duration of the treatment was 2 years. Four different concentrations of each extract were sequentially used, namely 8 STU, 40 STU, 200 STU, 1000 STU per ml. This allowed for each induction protocol a daily or twice-a-day administration schedule. In table III the amount of allergen which was self-administered per dose and per day in each group of patients is shown; for the 20-day induction protocol this corresponded to a progressive daily increment from 1 to 5 drop for each of the 4 concentrations; for the

Table III

Induction schedules

Day	Induction schedule		
	20-day	15-day	8-day
1	0.32	1.6	1.6
			3.2
2	1.6	3.2	4.8
			6.4
3	0.36	4.8	8
			8
4	1.28	6.4	16
			24
5	1.6	8	32
			40
6	1.6	8	40
			80
7	3.2	16	120
			160
8	4.8	24	200
9	6.4	32	
10	8	40	
11	8	40	
12	16	80	
13	24	120	
14	32	160	
15	40	200	
16	40		
17	80		
18	120		
19	160		
20	200		
Total	749.76	744.0	744.0
Doses per patient	20	15	15

Numbers indicate the dose of allergen extract (in Standard Treatment Unit, STU, per ml) self-administered each day at 8 AM, as sublingual drops. When 2 doses are present in the same day (8-day induction protocol) the extract was taken at 8 AM and at 4 PM.

Table IV

Read outs for symptom and medication usage

Symptoms
The question was:
How have your allergic symptoms been in the observation period (see *materials and methods* for details), compared with previous years (before treatment)?

The score was extrapolated as follows:

If the patient's answer was	The baseline value was added
No symptoms	–3
Much better	–2
Better	–1
Non modified	0
Worse	+1
Much worse	+2

Medications
The question was:
How was the amount of medications you took to control allergic symptoms in the observation period (see *materials and methods* for details), as compared with previous years (before treatment)?

If the patient's answer was	The baseline value was added
No medication	–2
Less	–1
Non modified	0
More medication	+1

15-day protocol the same schedule was followed, but starting with the 40 STU/ml concentration; for the 8-day induction protocol the same progression schedule as in the 15-day protocol was applied, but with a twice-a-day administration. At maintenance, 1000 STU of each extract were taken daily. For each allergen immunotherapy, the treatment was initiated at January 1, 1999, and continued for 2 years. This corresponded to the administration of the following cumulative amounts of major allergens: 115.2 μg Der p 1 plus 57.6 μg Der p 2 (house dust mite mixture), 72 μg group V grass allergens (grass mixture), 648 μg Bet v 1 (*Betulaceae*), 16.8 μg Par J 1 (*Parietaria*), per year, respectively. Cumulative doses were administered which were roughly three times higher than those administered with corresponding protocols of subcutaneous immunotherapy.

Symptom and medication scores

An arbitrary value of 5 was attributed to the baseline symptom and medication usage cumulative score (i.e., before SLIT). This was modified according to the answers that patients gave to questions separately concerning symptom and medication usage, as indicated in table IV.

In the case of sensitization to the perennial allergen, patients were asked to make an overall symptom and medication assessment referring to the previous 3 months, at each visit. The mean value of the first and of the second group of 4 trimesters was used as the read-out for the 1-year and 2-year symptom score, respectively.

In the case of sensitization to grass, *Betulaceae*, and *Parietaria*, the evaluation referred to the overall symptom assessment during the season when pollen counts were peaking in the Northern Italy area where patients were living. This corresponded to February through May for birch, March through June for grass and April through September for *Parietaria*, respectively. The first and second year evaluation were done at the first visit following the corresponding pollination season, respectively.

Spirometric evaluation

All patients included in the study performed spirometry at baseline (t = 0) and yearly thereafter. FEV_1 was measured using a Spirolab II (Med Electronics, Inc., Baltimore, MD) and expressed as percent of predicted value according to Quanjer[28].

Adverse event recording

During the induction phase, any local or systemic symptom occurring within 1 hour were recorded under supervision and documented at study site. Patients were asked to report any delayed (i.e., within 48 hours) local or generalized symptom occurring during the maintenance phase of treatment to the study investigators.

Statistical analysis

Symptom and medication scores and skin-prick test scores were compared using the two-tailed Mann-Whitney U-test for non-parametric data. FEV_1 values were compared using the two-tailed t-test for

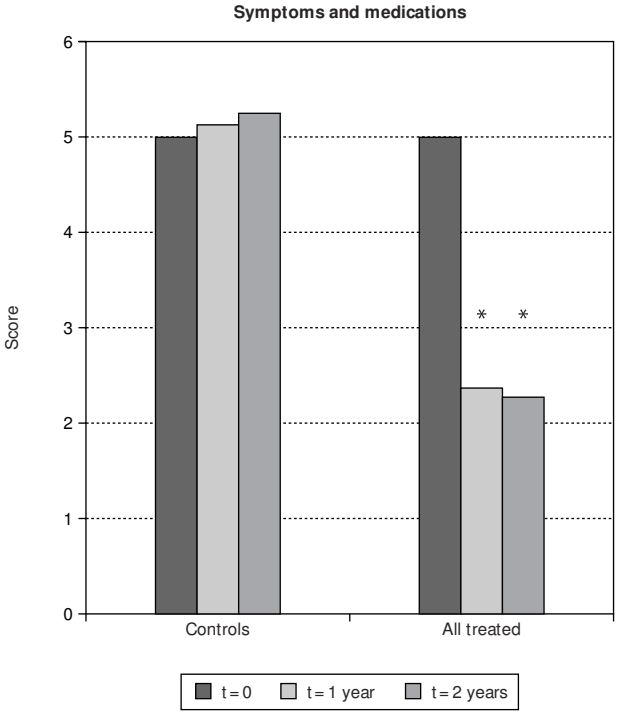

Figure 1.—Symptom and medication usage scores are shown on the *y*-axis. The baseline value was arbitrarily set to 5, and modified at subsequent clinical controls as indicated in Table IV. Scores obtained from control patients *(controls)* and from SLIT treated patients, cumulatively considered *(all treated)*, are shown. Scores were measured at baseline (t = 0) at the times indicated in the legend. The asterisk indicate significant p values for the indicated data versus time 0 and correspond to $p < 0.0001$.

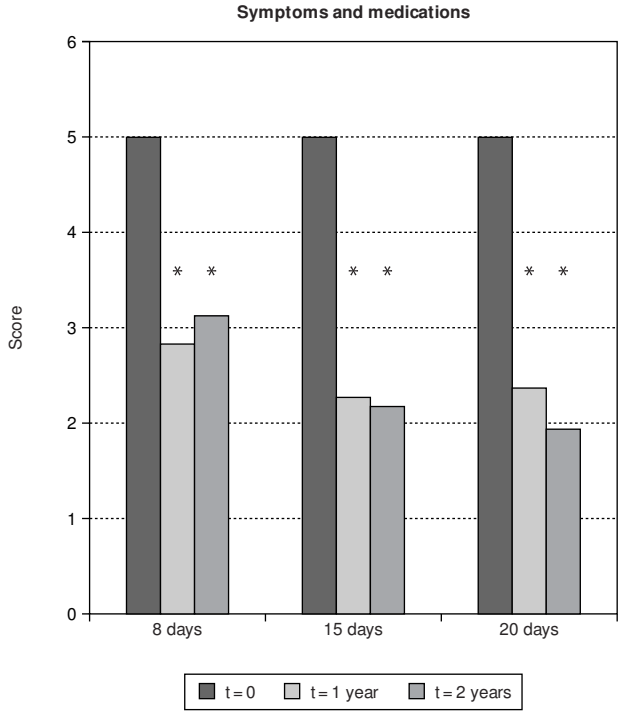

Figure 2.—Mean values of symptom and medication usage scores from SLIT treated patients are shown on the *y*-axis. Patients are grouped according to the induction protocol, which they were assigned to (shown on the χ axis). The times at which the results were obtained are indicated in the legend. The asterisk indicate significant p values for the indicated data versus time 0 and correspond to $p < 0.0001$.

paired data. Calculations were performed using the InStat-3 Software (Graphpad Software Inc, San Diego, CA). P-values less than 0.05 were considered to be statistically significant.

RESULTS

Clinical parameters

Symptom and medication usage

All patients completed the study. In the case of seasonal sensitizations, both for patients receiving SLIT and for untreated controls the presence of symptoms and the usage of medications closely paralleled the counts for the corresponding pollens in Northern Italy (not shown).

Symptom and medication usage scores in the immunotherapy groups, when cumulatively considered, were 48 and 50 % reduced, at 1 and 2 years since beginning of SLIT, respectively ($p < 0.0001$ in both cases) (fig. 1). At difference, values of symptom and medication usage were unchanged in the control, untreated group (fig. 1).

Symptom and medication usage reduction was significant also when comparing time-matched scores of SLIT treated patients with untreated controls at 1 and 2 years (51 % and 55 % less symptoms; $p < 0.0001$ in both cases) (fig. 1). Symptom and medication usage reduction at 1 and 2 years was significant also when patients treated with each induction protocol were separately considered ($p \leq 0.0005$) (fig. 2). No significant difference was observed when comparing time-matched scores observed with the different induction protocols (fig. 2).

Side effects

No immediate (within 1 hour) or delayed systemic reactions, or severe local reactions were observed during either the induction phase or the maintenance phase of the treatment. During the induction phase, 705 doses were cumulatively self-administered by the 43 patients in the 3 protocols. One patient belonging

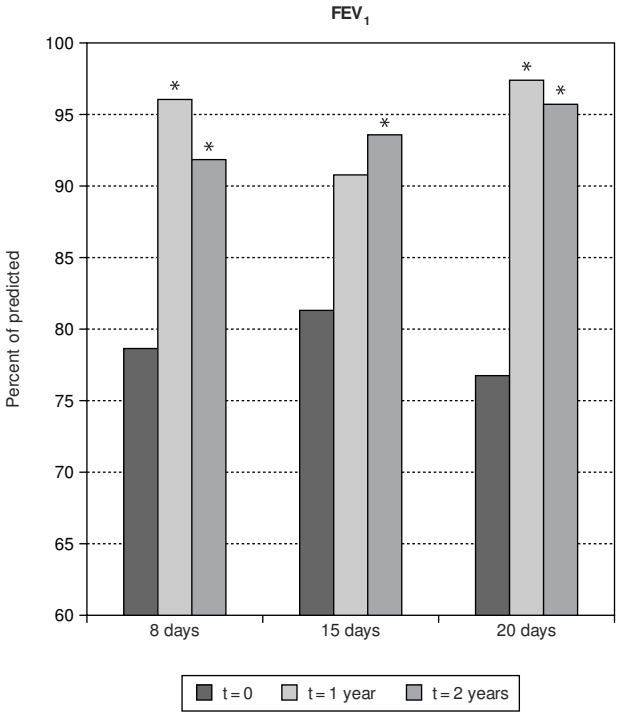

Figure 3.—The Forced Expiratory Volume in one second (FEV_1) is shown on the *y*-axis. Values are expressed as percent of predicted. Patients are grouped according to the induction protocol, which they were assigned to (shown on the *x* axis). The times at which the results were obtained are indicated in the legend. The asterisk indicate significant p values for the indicated data versus time 0 and correspond to $p < 0.05$.

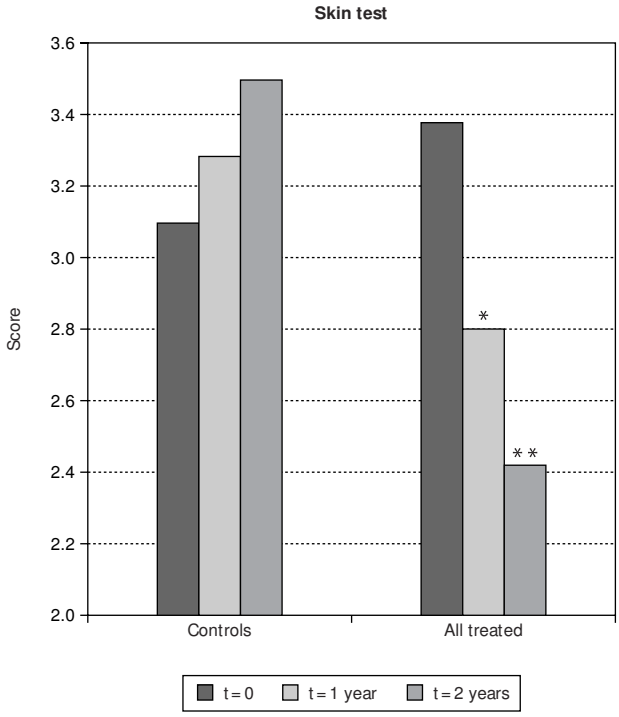

Figure 4.—Mean values of skin test results are shown on the *y*-axis. They were calculated as indicated in *Material and Methods*. Mean values obtained from control patients *(controls)* and from SLIT treated patients, cumulatively considered *(all treated)*, are shown. The times at which skin test were performed are indicated in the legend. The asterisks indicate significant p values for the indicated data versus time 0 and correspond to $p = 0.0123$ (*) and $p < 0.0001$ (**).

to the 15-day induction group suffered nose itching and sneezing as an early local effect after each of the first 15 doses (2.1%). He did not require treatment or discontinuation of SLIT. No side effects were reported in the maintenance phase of any treated group, when a total of 2480 doses were self-administered.

Objective test

FEV_1 values

Asthmatic patients who underwent SLIT significantly improved FEV_1 values after 2 years of therapy with any induction protocol (fig. 3). In particular the predicted value of FEV_1 was 14.8, 12.9 and 18.8% improved at 2 years in the 8-day, 15-day and 20 day induction protocol, respectively. Results were significant also when comparing FEV_1 absolute values (not shown). In the 8-day and 20-day induction protocol a significant FEV_1 improvement was already observed after 1 year of treatment (fig. 3).

Skin tests

At baseline no difference was observed in skin test scores for the major sensitizing allergens when comparing control patients with those who were selected for treatment with immunotherapy (fig. 4). At 1 and 2 years since the beginning of the study, the scores of the early skin response to allergens used for SLIT in treated patients were 18% and 24% reduced respectively ($p < 0.0001$ in both cases) (fig. 4). At difference, no significant changes were observed in the size of the early skin response to major sensitizing allergens in control, untreated patients (fig. 4). Reduction of skin-test scores was significant also when comparing time-matched scores of SLIT treated patients with untreated controls at 1 and 2 years (14% and 30%; $p = 0.0123$ and $p < 0.0001$, respectively) (fig. 4).

Reduction of the skin test scores was significant both at 1 and at 2 year also when considering singularly the groups treated with the different induction protocols. Namely, in the 8-day treated group, reduction of skin scores was 18% and 24% at 1 and 2 years, respectively. In the 15-day treated group, reduc-

tion of skin test scores was 15% and 31% at 1 and 2 years, respectively. In the 20-day treated group, reduction of skin test scores was 15% and 22% at 1 and 2 years, respectively (fig. 5). No significant difference was observed when comparing skin test results observed in the 8-day group at 2 versus 1 year, while a further 19% and 13% reduction of skin test scores ($p < 0.0001$ and $p = 0.0041$) was observed at 2, as compared to 1 year in the 15-day and in the 20-day induction group, respectively (fig. 5).

No significant differences were observed when comparing skin test scores at time matched visits since the beginning of study enter, in patients belonging to the different induction protocols. The only exception were patients belonging to the 8-day induction group, whose skin test scores at 1 years were 12% lower than corresponding skin test values of patients belonging to the 15-day induction group ($p = 0.0044$).

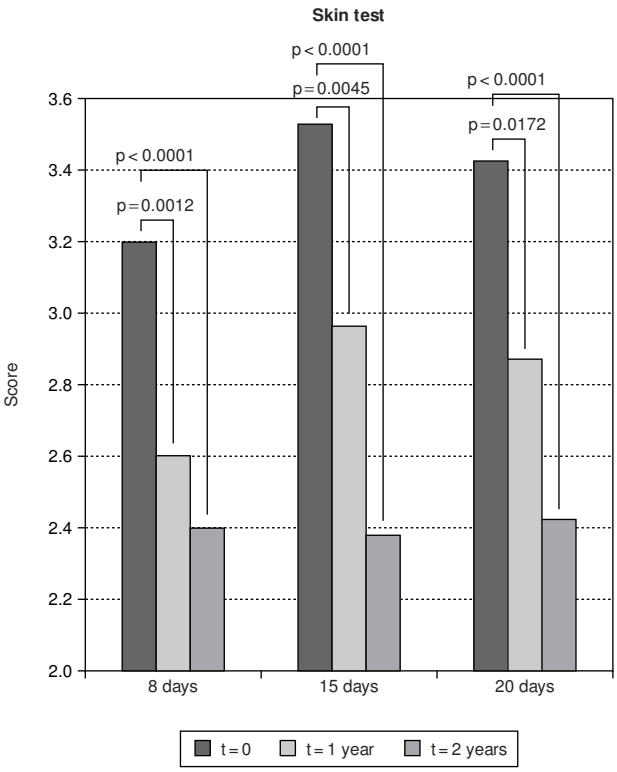

Figure 5.—Mean values of skin test results are shown on the *y*-axis. They were calculated as indicated in *Material and Methods*. Patients are grouped according to the induction protocol, which they were assigned to (shown on the *x* axis). The times at which skin test were performed are indicated in the legend; p values for compared data are shown.

DISCUSSION

Sublingual immunotherapy (SLIT) of allergic diseases has been indicated as a valid alternative to subcutaneous immunotherapy[3,12]. SLIT is easy to handle for patients, since it can be self-administered at home, and requires only periodical supervision by the allergologist. However, approved protocols require a quite complex induction regimen, which is pre-seasonal in the case of pollen allergies. During induction, different doses are to be taken daily for 20 to 30 days, thus requiring a certain degree of compliance until maintenance is reached and doses become constant. Would an upper respiratory disease or a flue occur, the protocol is stopped and restarted later on. This requires a further effort to complete the treatment. On this basis, rush induction protocols have been used, particularly in pediatric patients, which demonstrated clinical efficacy and good safety[9,14,17,18,21]. Recently, a direct comparison of different induction protocols was published, which showed the safety of shorter induction regimens[25].

Here, we made an open study on SLIT in patients suffering rhinitis, conjunctivitis, and asthma and sensitized to common inhalant allergens. We compared efficacy of three induction protocols (8-day, 15-day and 20-day), which had different duration and/or different starting doses. The evaluation of side effects was included in the clinical follow up of these patients. We found that SLIT induced a highly significant reduction of symptom and of medication usage at 1 and 2 years. Untreated control patients remained clinically stable. No differences were found when comparing the improvement of these clinical parameters obtained with each induction protocols. Patients included in this study were virtually monosensitized, and this could have had a role in the remarkable clinical improvement we observed. Non systemic side effects were recorded, and only mild disturbances occurred in a single patient during the 15-day induction regimen. The observed frequency of side effects, and the kind of adverse reactions were in accordance with published data[13,29]. Taken together these clinical results indicate that SLIT with the 8-day induction regimen is as safe and as efficacious as SLIT with longer induction protocols.

The oral cavity is an immunologically privileged site. In fact, it has been long known that in animal models the outcome of the exposure to antigen via the gastro enteric route is different if ingestion rather than sublingual exposure is used[30]. The underling immunological mechanisms likely involve dendritic-like cells (Langheran cells), which have been identified in the oral mucosa[31]. These cells may produce lymphokines, such as IL-12 or TGF-β, which drive the T cell response towards a Th1 or a regulatory phenotype, respectively. Indeed, it has been recently demonstrated in a

mouse model that lipopeptides can be taken up preferentially by dendritic cells within the oral mucosa, and promote an immune response characterized by high level of IFN-γ and IgG2a production[32]. In principle, on this basis it might be explained how the administration of allergen *via* the sublingual (but not the oral)[12] route can result in an effective treatment of respiratory allergy, and possibly of atopic dermatitis[33]. In particular, the recirculation of allergen specific T lymphocytes at peripheral lymphoid and non-lymphoid organs[34], including the oral mucosa, might put SLIT in action, e.g. via the modulation of chemochine receptors[36]. Experimental evidence for these mechanisms awaits dedicated studies.

Here, we show that the baseline levels of skin sensitization to sensitizing allergens were significantly reduced at 1 and 2 year from beginning of SLIT, in accordance with previous reports[6,15,19,29]. In control patients skin reactivity remained unchanged. Moreover, FEV_1 values were significantly increased at 1 and 2 years as compared to baseline in asthmatic patients. This result may be explained with the reduction in the lower respiratory tract of the allergic inflammation, which is casually associated with broncho constriction in asthmatic patients[37]. No differences were observed when comparing reduction of skin sensitization and improvement of FEV_1 obtained with the different induction protocols. Taken together, these objective results indicate that an active allergen specific immunomodulation took place following SLIT, which affected both the immediate skin reaction to allergen as well as the inflammation at the target organ in allergic asthmatic patients.

In conclusion, SLIT is a clinically effective and safe immunomodulating therapy for allergic patients affected by respiratory allergies. Compliance to SLIT may be improved by reducing the induction regimen to 8-day, with no losses in efficacy and safety.

REFERENCES

1. Dolz I, Martinez-Cocera C, Bartolome JM, Cimarra M. A double-blind, placebo-controlled study of immunotherapy with grass-pollen extract Alutard SQ during a 3-year period with initial rush immunotherapy. Allergy 1996;51:489-500.
2. Grammer LC, Shaughnessy MA, Suszko IM, Shaughnessy JJ, Patterson R. A double-blind histamine placebo-controlled trial of polymerized whole grass for immunotherapy of grass allergy. J Allergy Clin Immunol 1983;72:448-53.
3. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. J Allergy Clin Immunol 1998;102:558-62.
4. Tari MG, Mancino M, Madonna F, Buzzoni L, Parmiani S. Immunologic evaluation of 24 month course of sublingual immunotherapy. Allergol Immunopathol (Madr) 1994;22:209-16.
5. Troise C, Voltolini S, Canessa A, Pecora S, Negrini AC. Sublingual immunotherapy in Parietaria pollen-induced rhinitis: a double-blind study. J Investig Allergol Clin Immunol 1995;5: 25-30.
6. Bernardis P, Agnoletto M, Puccinelli P, Parmiani S, Pozzan M. Injective versus sublingual immunotherapy in Alternaria tenuis allergic patients. J Investig Allergol Clin Immunol 1996;6: 55-62.
7. Quirino T, Iemoli E, Siciliani E, Parmiani S, Milazzo F. Sublingual versus injective immunotherapy in grass pollen allergic patients: a double blind (double dummy) study. Clin Exp Allergy1996;26:1253-61.
8. Gozalo F, Martin S, Rico P, Alvarez E, Cortes C. Clinical efficacy and tolerance of two year Lolium perenne sublingual immunotherapy. Allergol Immunopathol (Madr) 1997;25:219-27.
9. Feliziani V, Lattuada G, Parmiani S, Dall'Aglio PP. Safety and efficacy of sublingual rush immunotherapy with grass allergen extracts. A double blind study. Allergol Immunopathol (Madr) 1995;23:224-30.
10. Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double-blind study. Allergol Immunopathol (Madr) 1990;18:277-84.
11. Sabbah A, Hassoun S, Le Sellin J, Andre C, Sicard H. A double-blind, placebo-controlled trial by the sublingual route of immunotherapy with a standardized grass pollen extract. Allergy 1994;49:309-13.
12. Malling HJ, Abreu-Nogueira J, Alvarez-Cuesta E, et al. Local immunotherapy. Allergy 1998;53:933-44.
13. Di Rienzo V, Pagani A, Parmiani S, Passalacqua G, Canonica GW. Post-marketing surveillance study on the safety of sublingual immunotherapy in pediatric patients. Allergy 1999;54: 1110-3.
14. Passalacqua G, Albano M, Riccio A, et al. Clinical and immunologic effects of a rush sublingual immunotherapy to Parietaria species: A double-blind, placebo-controlled trial. J Allergy Clin Immunol 1999;104:964-8.
15. Valle C, Bazzi S, Berra D, Sillano V, Puccinelli P, Parmiani S. Effects of sublingual immunotherapy in patients sensitised to Ambrosia. An open controlled study. Allergol Immunopathol (Madr) 2000;28:311-7.
16. Pajno GB, Morabito L, Barberio G, Parmiani S. Clinical and immunologic effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, placebo- controlled study. Allergy 2000;55:842-9.
17. Feliziani V, Marfisi RM, Parmiani S. Rush immunotherapy with sublingual administration of grass allergen extract. Allergol Immunopathol (Madr) 1993;21:173-8.
18. D'Ambrosio FP, Ricciardi L, Isola S, et al. Rush sublingual immunotherapy in Parietaria allergic patients. Allergol Immunopathol (Madr) 1996;24:146-51.
19. Horak F, Stubner P, Berger UE, Marks B, Toth J, Jager S. Immunotherapy with sublingual birch pollen extract. A short-term double- blind placebo study. J Investig Allergol Clin Immunol 1998;8:165-71.
20. Purello D'Ambrosio F, Gangemi S, Isola S, et al. Sublingual immunotherapy: a double-blind, placebo-controlled trial with Parietaria judaica extract standardized in mass units in patients with rhinoconjunctivitis, asthma, or both. Allergy 1999; 54:968-73.
21. Voltolini S, Modena P, Minale P, et al. Sublingual immunotherapy in tree pollen allergy. Double-blind, placebo- controlled study with a biologically standardised extract of three pollens (alder, birch and hazel) administered by a rush schedule. Allergol Immunopathol (Madr) 2001;29:103-10.
22. Bousquet J, Scheinmann P, Guinnepain MT, et al. Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. Allergy 1999;54:249-60.
23. Pradalier A, Basset D, Claudel A, et al. Sublingual-swallow immunotherapy (SLIT) with a standardized five-grass- pollen ex-

tract (drops and sublingual tablets) versus placebo in seasonal rhinitis. Allergy 1999;54:819-28.
24. Guez S, Vatrinet C, Fadel R, Andre C. House-dust-mite sublingual-swallow immunotherapy (SLIT) in perennial rhinitis: a double-blind, placebo-controlled study. Allergy 2000;55:369-75.
25. Grosclaude M, Bouillot P, Alt R, et al. Safety of Various Dosage Regimens during Induction of Sublingual Immunotherapy. a preliminary study. Int Arch Allergy Immunol 2002;129:248-53.
26. Dreborg S, Frew A. Allergen standardization and skin tests. Position paper. Allergy 1993;48:49-82.
27. Carreira J, Rubio M, Herrero T, et al. Collaborative clinical assay of the biological potency of allergen skin- test extracts. Clin Allergy 1984;14:503-7.
28. Quanjer PH. Standardised Lung Function Testing. Report on working party on standardisation of lung function testing. European Community for Coal and Steel. Bull Eur Physiopathol Respir 1983;19:1-94.
29. Vourdas D, Syrigou E, Potamianou P, et al. Double-blind, placebo-controlled evaluation of sublingual immunotherapy with standardized olive pollen extract in pediatric patients with allergic rhinoconjunctivitis and mild asthma due to olive pollen sensitization. Allergy 1998;53:662-72.
30. Holt PG, Vines J, Britten D. Sublingual allergen administration. I. Selective suppression of IgE production in rats by high allergen doses. Clin Allergy 1988;18:229-34.
31. Lima MT, Wilson D, Pitkin L, et al. Grass pollen sublingual immunotherapy for seasonal rhinoconjunctivitis: a randomized controlled trial. Clin Exp Allergy 2002;32:507-14.
32. BenMohamed L, Belkaid Y, Loing E, Brahimi K, Gras-Masse H, Druilhe P. Systemic immune responses induced by mucosal administration of lipopeptides without adjuvant. Eur J Immunol 2002;32:2274-81.
33. Mastrandrea F, Serio G, Minelli M, et al. Specific sublingual immunotherapy in atopic dermatitis. Results of a 6-year follow-up of 35 consecutive patients. Allergol Immunopathol (Madr) 2000;28:54-62.
34. Crimi E, Gaffi D, Frittoli E, Borgonovo B, Burastero SE. Depletion of circulating allergen-specific TH2 T lymphocytes after allergen exposure in asthma. J Allergy Clin Immunol 1997;99: 788-97.
35. Borgonovo B, Casorati G, Frittoli E, Gaffi D, Crimi E, Burastero SE. Recruitment of circulating allergen-specific T lymphocytes to the lung on allergen challenge in asthma. J Allergy Clin Immunol 1997;100:669-78.
36. D'Ambrosio D, Mariani M, Panina-Bordignon P, Sinigaglia F. Chemokines and their receptors guiding T lymphocyte recruitment in lung inflammation. Am J Respir Crit Care Med 2001; 164:1266-75.
37. Busse WW, Lemanske RF, Jr. Asthma. N Engl J Med 2001; 344:350-62.