

Original article

Clinical and immunologic effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, placebo-controlled study

Background: Immunotherapy through local routes is thought to be a valuable therapeutic option for respiratory allergy. We investigated the clinical efficacy and immunologic effects of sublingual immunotherapy (SLIT) in asthmatic children with mite-induced respiratory allergy.

Methods: Twenty-four patients (age range 8–15 years), suffering from mild to moderate asthma, with single sensitization to mite allergen, were enrolled. After a 1-year observation phase, patients were randomly allocated to one of two groups, and were given SLIT (sublingual-spit) as drops for 2 years according to a double-blind, placebo-controlled (DBPC) design. Symptoms/medication scores (diary card), visual analog scale, and immunologic parameters (house-dust-mite [HDM]-specific IgE, and total HDM-specific IgG and IgG4) were determined during the observation phase and during the DBPC treatment period.

Results: Twenty-one patients completed the study. At the beginning of the treatment, no difference in environmental allergenic pressure could be shown between the groups. After 2 years of therapy, there was a significant decrease in asthmatic symptoms ($P=0.0001$) and medication use ($P=0.0001$) in the active group compared to the placebo group. The visual analog score on overall asthma symptoms improved in the SLIT group ($P=0.0001$), but not in the placebo group. Nevertheless, the immunologic results did not show significant differences in HDM-specific IgE and total HDM-specific IgG or IgG4 between the active and placebo groups ($P=NS$). No relevant side-effects were recorded throughout the study.

Conclusions: Our results suggest that treatment for 2 years with SLIT is clinically safe and effective in significantly decreasing respiratory symptoms in children with mild to moderate asthma sensitized to HDM. On the other hand, the lack of changes of the immunologic parameters calls for further investigations with special reference to kinetics and mechanism(s) of action of this mode of treatment.

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Asthma is currently defined as a chronic inflammatory disease in which allergens are often implicated as causative and triggering factors of respiratory attacks (1, 2). In children, the presence of allergy influences the persistence and severity of asthma (3), and perennial allergy such as that to house-dust mites (HDM) causes long-term inflammation with a variable degree of unspecific bronchial hyperreactivity (4).

Specific subcutaneous immunotherapy (SIT) with mite extracts has been shown to be effective (5, 6), with a greater improvement in children allergic to *Dermatophagoides pteronyssinus* than in adults (7). However, special attention should be paid to the safety of injective immunotherapy for mite allergy in

patients with asthma triggered by this allergen, because of the risk of important side-effects such as irreversible bronchial obstruction (8, 9). In particular, children under 5 years of age present a significantly higher risk if systemic reactions occur (10, 11). According to the WHO position paper on immunotherapy, age under 5 years is a relative contraindication for allergen-specific immuno-therapy in children.

Some double-blind, placebo-controlled (DBPC) studies strongly support the clinical effectiveness of sublingual immunotherapy (SLIT) with grass, mite, and *Parietaria* extracts (12–20). The most frequent side-effect was the onset of oral/sublingual itching, but it was always described as mild and self-resolving. Headache,

Table 1. Demographic data

	SLIT – active	SLIT – placebo
Total number of patients	12	12
Sex		
F	5	6
M	7	6
Mean age and range (years)	11 (8–14)	12 (8–15)
Mean duration of allergy (years)	4.8	5.1
Dropouts	0	3
Allergen	<i>D. pteronyssinus</i>	<i>D. pteronyssinus</i>

rhinorrhea, nasal obstruction, or urticaria was described only sporadically, and no severe adverse event was reported in these studies.

We recently performed a DBPC study in children suffering from mild to moderate asthma with a biologically standardized (BU) extract quantified in mass units (MU) (ALK-Abelló S.p.A.).

Material and methods

Patients

Twenty-four children aged 8–15 years were selected according to the following criteria:

- 1) a history of mild to moderate asthma with a methacholine PC₂₀ (the concentration of inhaled methacholine that causes a 20% decrease in 1-s forced respiratory volume FEV₁) not below 2 mg/ml (21)
- 2) a positive skin prick test (wheal diameter > 5 mm) to HDM (*D. pteronyssinus* – Soluprick ALK, Denmark)
- 3) specific IgE to HDM of at least class 3 (RAST-CAP System EIA method, Pharmacia, Uppsala, Sweden).

Patients were excluded if they presented one or more of the following conditions:

- 1) a positive skin response to at least one other inhalant allergen of the standard panel for southern Italy
- 2) a clinical history of other allergies, such as seasonal asthma due to pollens
- 3) a history of immunotherapy in previous years
- 4) a history of cardiovascular or other medical or immunologic diseases
- 5) severe asthma (22).

The demographic data of the children studied are shown in Table 1.

Study design

The study was performed with the approval of the

University of Messina Polyclinic Hospital ethics committee, and the children's parents were asked to give their informed consent in writing.

Patients were randomized separately to the active or to the placebo group according to a keyed code. The coordinator, who was blinded as to the group each child was assigned to, was in charge of patient supervision and adjusting rescue treatment according to symptoms. He was also responsible for reporting any reactions and/or side-effects.

Immunotherapy protocol

The active treatments used throughout the study were glycerin/phenol solutions prepared from biologically standardized aqueous mite extracts (23). Therapy consisted of five 3-ml vials at increasing concentrations (0.016, 0.08, 0.4, 2.0, and 10 BU/ml). In accordance with the standardization procedure followed, 10 BU/ml corresponded to 4 and 2 µg/ml, respectively, of the two major mite allergens, Der p 1 and Der p 2 (24).

The drops had to be taken daily, starting with 1 drop from the first vial and increasing by 1 drop every day up to 5 drops. The procedure was then repeated with each of the following vials until the maximum dose (5 drops from vial 4, 10 BU/ml) was reached.

Maintenance therapy (5 drops from vial 4, 10 BU/ml, corresponding to 2.4 µg Der p 1 and 1.2 µg Der p 2 per week) was then administered three times a week until the end of the trial. The placebo was indistinguishable from the active treatment in flavor and appearance.

To exclude therapeutic or side-effects related to absorption by the intestine, the drops of the allergen were to be kept in the mouth for at least 2 min without swallowing, and any liquid remaining in the mouth was to be spat out. Parents were instructed to pay particular attention to the correct administration of the treatment.

Skin prick test

Skin tests were done on the forearm with the inhalant allergen panel for southern Italy: *D. pteronyssinus*, *Parietaria judaica*, *Phleum pratense* and grass mix, *Artemisia*, *Olea*, dog, cat, *Cladosporium*, and *Alternaria* (ALK-Abelló S.p.A., Milan, Italy). The size of the immediate-phase cutaneous responses was assessed 15 min later, in accordance with the recommendations of the EAACI (25). Wheal size was expressed by the formula $(D + d)/2$, where D is the maximum diameter and d the perpendicular diameter at the midpoint. A mean diameter of > 5 mm, was considered a positive result.

Assessments

All patients were asked to record drug requirements (if any) daily, and to indicate their overall asthma symptoms weekly on a visual analog scale (VAS)

(range 0–10) throughout the entire trial (1 January 1993 to 31 December 1995). For each patient, the average score on the VAS was calculated at 4-month intervals.

Only rescue drugs (β_2 -agonist and oral steroid) were allowed during the study, while disodium cromoglycate was allowed during the observation period (1 January 1993 to 31 December 1993) in patients already receiving treatment with this drug.

Patients (or their parents) were instructed to inhale salbutamol (100 μ g, scored 1) for symptoms such as breathlessness, coughing, wheezing, and chest tightness. If symptoms developed that were not controlled by salbutamol, inhaled steroids (beclomethasone, 250 μ g per puff, scored 2 for each administration) and/or a 7-day course of oral prednisone (25 mg per tablet, scored 4 for every 7-day course of treatment) was prescribed.

Asthmatic and other allergic symptoms were recorded daily, divided into night and day symptoms, during the observation period according to a 0–3 grading (0=no symptoms, 3=severe symptoms), and again during the second year of treatment (1 January 1995 to 31 December 1995).

In vitro tests

Sera samples were taken at T0 (before the beginning of SLIT), T1 (12 months later), and T2 (24 months later), and stored at -20°C . At the end of the trial, specific IgE (Magic Lite method, ALK Laboratories, Hørsholm, Denmark), and total specific IgG and IgG4 to *D. pteronyssinus* (Pharmacia Diagnostic, Uppsala, Sweden) were determined according to the supplier's instructions.

Side-effects

Patients were instructed to keep a diary for the evaluation of local and systemic reactions due to SLIT. Symptoms were evaluated on a 0–3 scale (0=no symptoms, 3=severe symptoms), covering nasal symptoms (itching, sneezing, nasal discharge, and nasal obstruction), eye symptoms (itching and redness), mouth and/or throat swelling or itching, swelling of lips/face, coughing, bronchial obstruction, and wheezing.

Environmental analysis

After enrollment and before the beginning of the observation period, all patients' parents were instructed to take standard environmental measures to decrease exposure to mites (i.e., frequent vacuum-cleaning, washing sheets with water at $>55^\circ\text{C}$ at least once a week, use of encasings for both mattresses and pillows, removal of plants and soft toys from the patient's bedroom, and no use of humidifiers).

At the end of the observation period and before the

beginning of the DBPC study, samples of house dust were collected with a vacuum cleaner from the child's bedroom (mattress, pillow, and carpet or moquette, if any) and from the living room (sofas, carpets, or moquette). Samples were analyzed in duplicate in the same experiment for content of Der p 1, Der f 1, and Der 2 by immunoassay using specific mAbs (26) to assess the allergenic pressure in the patient's environment.

Statistical methods

All data was gathered in a double-blind fashion in accordance with the clinical protocol and analyzed with a statistical package (BMDP, Inc., Los Angeles, CA, USA). The results for the placebo and active treatment groups were compared with the Mann–Whitney U-test for nonparametric data and Student's *t*-test or the *t*-test for paired data, when appropriate, for parametric data.

Levels of mite-specific IgE, total mite-specific IgG, and specific IgG4 were statistically analyzed with parametric tests (ANOVA) after normal distribution of the data was confirmed. Values of *P* lower than 0.05 were considered statistically significant.

Results

Demographic data

The two groups were well matched for age and duration of the disease (Table 1). A total of 21 patients (87%) completed the study. The three patients who dropped out were from the placebo group. In two cases, this was due to family problems; in the third case, a serious asthma attack (not related to SLIT) convinced the child's family to withdraw from the study. Therefore, these withdrawals were unlikely to have biased the statistical analysis. All nine patients in the placebo group and 12 in the active treatment group achieved the maintenance dose.

Clinical efficacy

The use of medications and the appearance of symptoms (as recorded on the diary cards) were checked by a physician every 4 weeks throughout the 3-year duration of the trial. During each check visit, parents were also specifically asked about side-effects of the treatment.

Use of medication. Data for the use of medication are shown in Fig. 1. During the observation phase, no difference was seen between the two groups ($P=\text{NS}$). After the first year of treatment, both the placebo group and active treatment group showed a significant reduction in use of medication in comparison to the 1-year observation period before SLIT was started ($P=0.0039$ for placebo, $P=0.0005$ for active

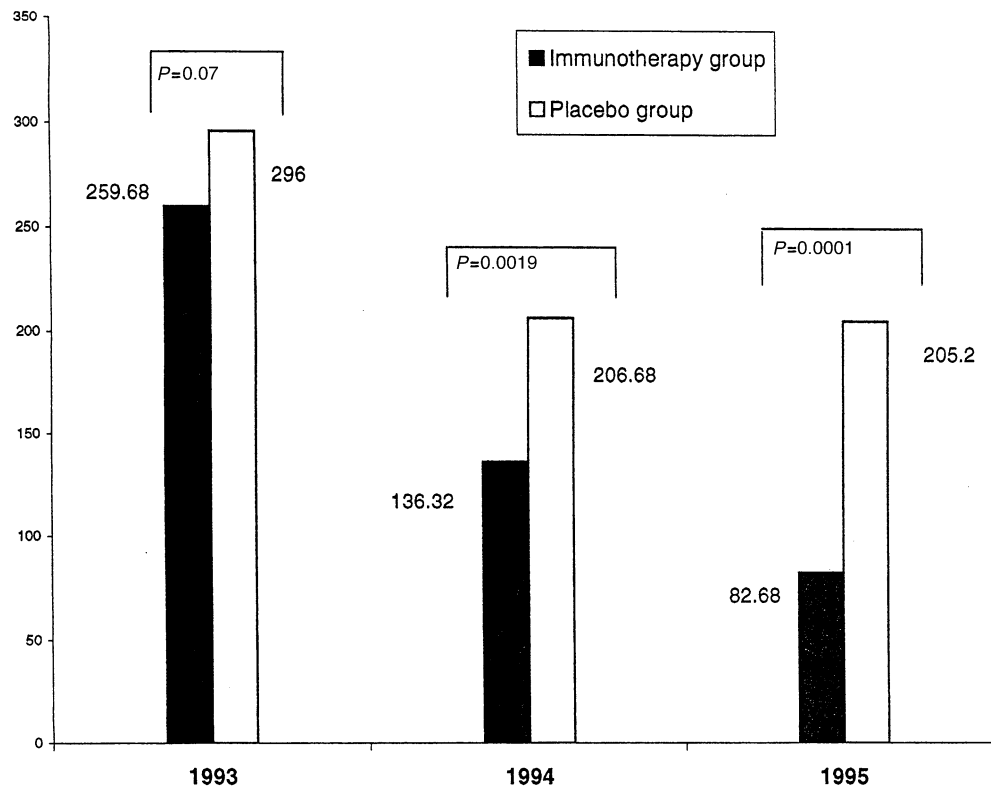


Figure 1. Mean medication scores for each year before (1 Jan–31 Dec 1993) and during immunotherapy (1 Jan 1994–31 Dec 1995). Each drug was scored as follows: score 1: bronchodilators; score 2: inhaled corticosteroids (cromoglycate only during 1993); score 4: oral steroids (7-day course).

treatment), with quantitative reductions of 30.18% and 47.51%, respectively. A further improvement, corresponding to a 68.16% reduction in comparison to the observation period, was seen after the second year

of treatment in the active treatment ($P=0.0066$, in comparison to the first year of treatment), but not in the placebo group ($P=0.6875$). The use of medication in the active group was significantly lower than in the

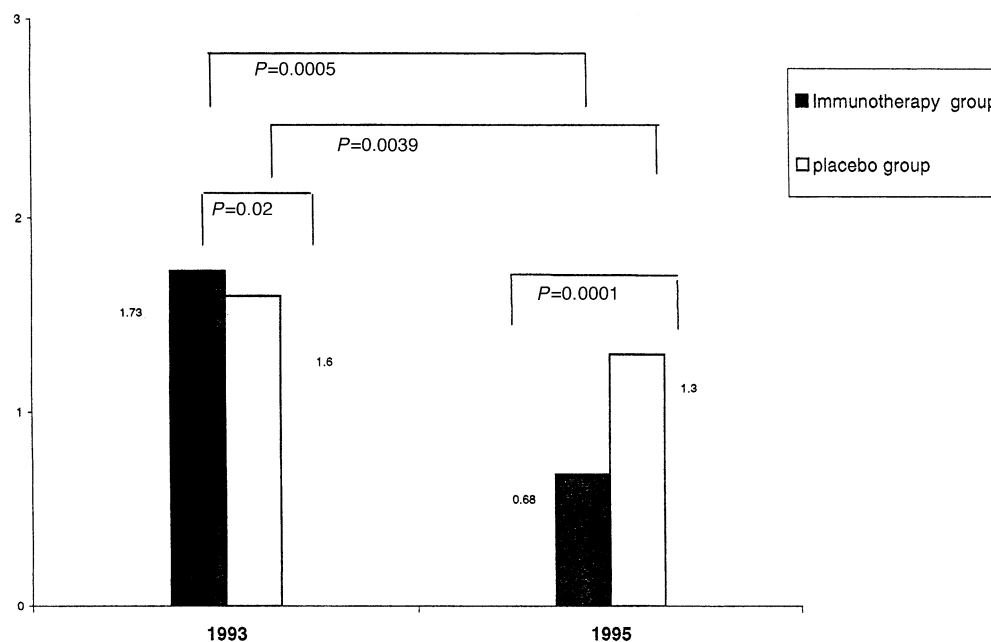


Figure 2. Mean number of asthma episodes per month before (1 Jan–31 Dec 1993) and during second year (1 Jan–31 Dec 1995) of treatment in patients given active treatment or placebo.

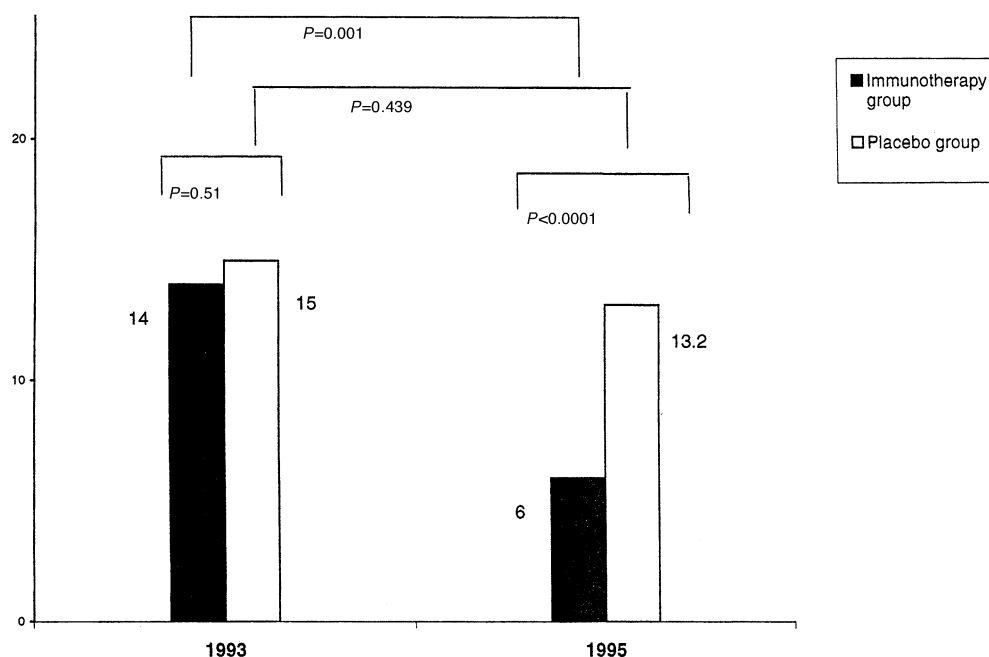


Figure 3. Mean score for nighttime symptoms per month (coughing, wheezing, breathlessness, chest tightness) in patients given active treatment or placebo, before (1 Jan–31 Dec 1993) and during second year of treatment (1 Jan–31 Dec 1995).

placebo group after 1 year ($P=0.0019$), with a further reduction after the second year of treatment ($P=0.0001$).

Episodes of asthma. During the observation period (1993), both groups had more than 1.5 asthma episodes per month, with a significantly higher ($P=0.02$) number of episodes in the group to be treated with active SLIT (Fig. 2). Both the placebo

and the active treatment groups showed significant improvement during the second year of treatment in comparison to the observation period ($P=0.0039$ for placebo, $P=0.0005$ for active treatment). However, the improvement in the active group (60.7%) was significantly greater than the improvement in the placebo group (18.75%, $P=0.0001$).

Nighttime symptoms. With reference to nighttime

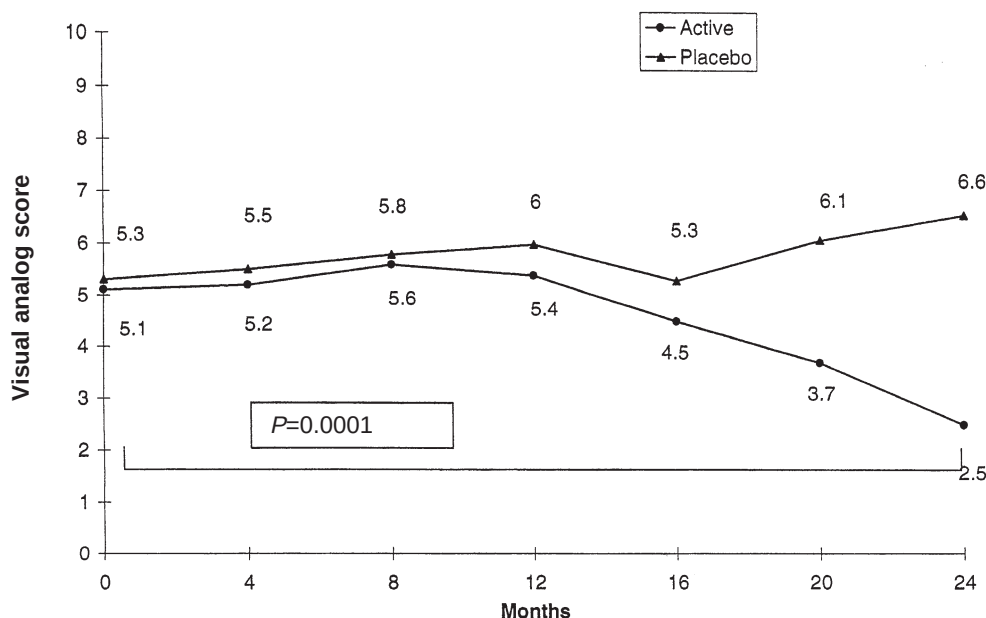


Figure 4. Visual analog score (VAS) on overall asthma symptoms for active SLIT (circle) and placebo (triangle) groups. Data points represent average 4-month VAS.

Table 2. Mean value ($\pm$ SD) of immunoglobulins at times T0 (before treatment), T1 (after 12 months of treatment), and T2 (after 24 months of treatment). Significance was tested with ANOVA

Immunoglobulins	T0	T1	T2	T0-T1-T2
SLIT				<i>P</i>
IgE (SU/ml)	45.4 $\pm$ 12.6	42 $\pm$ 10.4	52.6 $\pm$ 9.7	NS
Total IgG (%)	33 $\pm$ 19	26.7 $\pm$ 13.9	31.3 $\pm$ 16.7	NS
IgG4 (%)	2.85 $\pm$ 1.79	2.46 $\pm$ 1.98	2.53 $\pm$ 1.45	NS
Placebo				
IgE (SU/ml)	52.2 $\pm$ 11.2	48 $\pm$ 7.2	65.3 $\pm$ 9.69	NS
Total IgG (%)	26 $\pm$ 10	29.6 $\pm$ 12	31.9 $\pm$ 14.2	NS
IgG4 (%)	2.7 $\pm$ 1.15	2.44 $\pm$ 1.04	2.66 $\pm$ 0.84	NS

symptoms during the observation year, the groups were well balanced ($P=0.518$) (Fig. 3). During the second year of treatment, there was a statistically nonsignificant ($P=0.439$) decrease in the placebo group, and a highly significant decrease in the active treatment group ($P=0.001$). The difference between the placebo and the active treatment groups was highly significant during the second year of treatment, in favor of the latter ($P<0.0001$). The mean score for nighttime symptoms decreased by 11.7% in the placebo group and by 57.14% in the active treatment group.

VAS. The VAS showed a parallel trend during the first 16 months of therapy, but a clearly divergent trend during the last 8 months, with significantly lower values in the active treatment group (Fig. 4). In this group, the mean score from January 1994 to December 1995 decreased by 50.98% ($P=0.0001$, Mann-Whitney test), while the score in the placebo group increased by 24.53%.

Laboratory tests

No significant changes in *D. pteronyssinus*-specific IgE, total IgG, or IgG4 concentration were detected in either group (Table 2).

Side-effects

Tiredness was reported by four patients in the active group and by one patient in the placebo group. Two local delayed reactions were observed in two patients under active treatment: one case of swelling of the mouth, lips, and face (at 2 h) and one case of itching of the mouth (at 3 h). These side-effects resolved spontaneously without drugs, and were reported during the induction phase with the first administrations of the most diluted vial.

Allergenic pressure

The allergenic pressure due to mite allergens, expressed as micrograms of allergen per gram of house dust, was, on average, similar in the active

treatment and placebo groups. The average amount of group 1 allergens (Der p 1+Der f 1) was 5.4 (0.24–18.8) in the active treatment group and 3.49 (0.39–9.57) in the placebo group; for group 2 allergens, the average values were 2.033 (0.2–5.1) and 2.166 (0.4–5.7), respectively.

None of these differences were statistically significant (data not shown).

Discussion

In this DBPC study, SLIT with a standardized extract of *D. pteronyssinus* was effective in reducing symptoms and use of medication in selected children with mild to moderate asthma. The dose of allergen administered on a yearly basis was around 3.25 times higher than the amount administered with the corresponding injective immunotherapy.

The concept of treating allergic diseases by noninjectable routes of immunotherapy dates back to the beginning of the 1900s (27), but documentation of the clinical efficacy of SLIT or sublingual/oral immunotherapy began to appear in scientific journals only in the 1980s. The evidence in support of SLIT, including some published DBPC studies (12–20) and many other controlled studies available as abstracts or full papers, led the WHO to recognize this approach as a possible therapeutic option (10). Most clinical trials have been conducted mainly in adults, while only three have been done in children (12, 19, 28). A total of 88 children allergic to HDM were studied in two DBPC studies (12, 28), while 64 children allergic to *Olea* pollen were studied in the third (19). One study with mites (12) and the study with *Olea* pollen (19) were able to show a statistically significant positive outcome with actively treated children.

Some of these trials evaluated efficacy after less than 1 year of treatment, in spite of the possibility that a longer duration of treatment might improve the clinical efficacy (14–17, 29, 30). The amount of allergenic extract administered also varied widely from the homeopathic dosages used in the study by Scadding & Brostoff (31) to the high dosages used in other studies, which in some cases were up to 200 times the dosage used for conventional injective immunotherapy (14–16, 19, 28, 29).

The present DBPC study shows that a significant placebo effect (or a spontaneous improvement of patients) takes place during the first year of specific immunotherapy, but not during the following year. This could partly explain the contrasting results obtained in short-duration studies with mite allergens. In spite of this confusing situation, we have shown a significantly greater improvement in children given active treatment than those given placebo SLIT

treatment, with a decrease in the use of medication, the average number of asthma episodes, and night-time symptoms. These changes were confirmed by the improvement in the VAS.

It must be emphasized that these results were obtained with a cumulative dose only around 3.25 times higher than the dose normally administered as injection therapy, and in spite of a significantly worse basal condition (in terms of average number of asthma episodes) in the actively treated group. In our opinion, the long (1 year) observation period before treatment was started and the prolonged treatment period (2 years) in our trial allowed us to achieve these results, which confirm the positive findings of other long-term studies (12, 18, 20) and contradict some negative short-term studies (28, 29).

From a quantitative point of view, the placebo effect was limited to a reduction of around 30% in the use of medication during the first year of treatment, with no further improvement during the second year. In contrast, active therapy achieved significant improvement during the first year and further improvement during the second year, so that at the end of the trial the cumulative decrease in use of medication in children who were given active SLIT was around 68%, clearly a larger reduction than in the placebo group.

This difference between the placebo and the active groups was not only significant from the statistical point of view, but was also clearly perceived by the actively treated subjects, as shown by the VAS.

The improvement of the VAS in the active group compared to the placebo group and to the baseline value took place only after the first 16 months of treatment. This observation may be explained by the fact that all patients started the treatment from a relatively good situation due to at least 1 year of pharmacologic treatment and environmental measures taken during the observation phase. In this situation, the major changes of asthmatic symptoms needed for significant self-assessed improvement of the VAS took place only 16 months after the beginning of the treatment. Of course, a long-lasting treatment needs good patient compliance, especially when the treatment is performed at home, and the clinical benefit becomes evident only after more than 1 year. In any event, our 3-year-long study, with only three dropouts from the placebo group, shows that if patients undergoing SLIT are properly selected, instructed, and followed-up, good compliance can be reached.

Contrasting results have been obtained for specific IgE and IgG. Some studies have reported evidence of a change in allergen-specific antibodies (12, 13, 15, 20), whereas others, as in our trial, found no change (16, 19, 28), although this lack of objective change was not related to the patients' clinical outcome. These contradictory results may reflect the fact that

IgG is not directly involved in reducing the release of allergic mediators, and can therefore be regarded as a "bystander" or epiphenomenon of the systemic administration of a significant amount of allergen (32, 33).

In our study, no patients showed systemic reactions, and local reactions (slight swelling and itching of mouth and lips) occurred in only two patients. These findings are in good agreement with the other published studies and with a large postmarketing surveillance study of SLIT (34).

Most published studies have used the sublingual-swallow technique. In our study, we used the sublingual-spit technique, but we cannot rule out the possibility that some of the allergen administered could have been swallowed. On the other hand, preliminary data on the kinetics of SLIT in healthy persons, as studied with radiolabeled allergen, show that no direct sublingual absorption occurs, and there is no risk that absorption through the oral mucosa may occur too rapidly, while a significant amount of the administered allergen persists in the mouth for several hours (35). These studies support the hypothesis that the locally retained allergen is slowly absorbed and processed through the local oral immune system.

Our data, combined with the available evidence, show that SLIT is efficacious, safe, and well tolerated, and permits significant socioeconomic savings (15, 18) because it is a self-administered treatment. In our opinion, however, SLIT must be prescribed by a specialist, and patients must be instructed to follow the schedule of administration carefully and to attend clinical follow-up visits at least every 3 months.

Some aspects of SLIT which need further investigation and development are as follows:

- 1) the kinetics of the extracts
- 2) the duration of treatment
- 3) the mechanism of action
- 4) the role of the sublingual lymph nodes, which may be crucial in the absorption of allergens in the oral cavity.

We conclude that, despite some problems which need further study, SLIT with a biologically standardized extract of *D. pteronyssinus* in children with mild to moderate asthma can be considered effective in carefully selected patients with proper follow-up carried out at a specialized center.

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