

THE CLINICAL EFFICACY OF A SUBLINGUAL MONOMERIC ALLERGOID AT DIFFERENT MAINTENANCE DOSES: A RANDOMIZED CONTROLLED TRIAL

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Sublingual immunotherapy is widely recognized as a viable treatment for allergic rhinitis and asthma, but the optimal dosage is still under debate, especially with modified allergens. We assessed the clinical effects of a monomeric allergoid across 3 different maintenance doses in mite-monosensitized patients with rhinitis and intermittent asthma. Eighty-nine patients allergic to HDM were randomized to 3 maintenance doses of monomeric allergoid (Lais®, Lofarma) or medications only. All the patients recorded their symptoms and rescue drug consumption in a diary card from November to February. Additionally, nasal eosinophil count, spirometry and methacholine bronchial challenge were performed at the beginning of the study and after 3 years. The symptom scores showed a clear improvement in all the three active arms versus baseline and versus the controls, irrespective of the dose. Likewise, a similar improvement versus baseline was seen for nasal inflammation and bronchial hyperreactivity. The SLIT with monomeric allergoids produces clinically significant results across a wide range of doses. The absence of significant side effects, even at high doses, is probably due to their low level of allergenicity.

Allergen-specific immunotherapy (SIT) is nowadays regarded as a viable treatment for asthma and allergic rhinitis due to environmental inhalant allergens (1). The main rationale of Specific Sublingual Immunotherapy (SLIT), proposed about 20 years ago, is to improve the safety of the treatment. In fact, the risk of systemic reactions with subcutaneous therapy (SCIT) is always present. These reactions, though rare, can be severe or even fatal, and somewhat limit the use of immunotherapy (2). The initial studies with SLIT were conducted with relatively low doses, but it soon became evident

that cumulative doses higher than those used with SCIT were required to obtain appreciable clinical effects. In fact, SLIT is usually named “high-dose” SLIT, and the ARIA document suggested that SLIT should be administered at dosages 50-100 times higher than SCIT (3).

The dose-dependency of the response has been reasonably established for SCIT (1, 4-5), and some data are also available for SLIT with grass extracts (6-7). The above-mentioned considerations apply to traditional allergenic extracts, containing the native allergens, but there is currently no data in

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literature about the dose-related response with allergoids. Allergoids are chemically modified allergens, with the rationale of reducing the IgE reactivity, yet maintaining the immunogenicity. The chemical modifications are commonly carried out with aldehydes (formaldehyde, glutaraldehyde), which produce polymerized allergoids with a large molecular size, which are suitable for injection but not sublingual administration. On the other hand, the reaction with potassium cyanate produces monomeric allergoids (8), which maintain their original molecular size and weight and are therefore suitable for sublingual administration.

The aim of the present study is to assess whether the clinical efficacy of a monomeric allergoid for SLIT is dose-dependent. Thus, in a randomized controlled trial, the effects of three different long-term maintenance doses were studied in patients with respiratory allergy due to house dust mites.

MATERIALS AND METHODS

Study design

This is a prospective, randomized, four parallel group, controlled open trial involving subjects with allergic rhinitis and/or asthma due to mites. After a run-in period to assess the baseline conditions, eighty-nine patients were randomly allocated to four groups receiving medications only, or medications plus SLIT at three different maintenance doses (1,000, 2,000 or 3,000 Allergy Units AU per week). The randomization was made according to a computer-generated list. Symptoms and drug intake scores were recorded from November to February at baseline and after 3 years of study. Pulmonary function, bronchial hyperresponsiveness to methacholine, and nasal eosinophil count were also evaluated at the same time-points. The study was approved by the inner ethics committee, and all patients signed an informed consent.

Patients and diagnostic procedures

Subjects with monosensitization to house dust mites and suffering from rhinitis and intermittent asthma were enrolled at the Cuasso al Monte Hospital. Inclusion criteria were: a) persistent rhinitis and intermittent asthma ($FEV_1 > 80\%$ predicted) for at least 2 years; b) positive MCh challenge with a methacholine provocation dose ($PD_{20} < 400 \mu g$); c) nasal eosinophils $> 10\%$ of the total cells; d) CAP-RAST for *Dermatophagoides pteronissynus* and *Dermatophagoides Farinae* greater than class I. Main exclusion criteria were persistent asthma, mechanical alterations of the nose (polyposis, turbinate hypertrophy),

malignancies, systemic autoimmune diseases and/or chronic use of systemic steroids.

Skin prick tests were performed according to international guidelines (9), with standardized commercial extracts (Alk Abello, Lainate, Milan, Italy) for the following allergens: *Dermatophagoides pteronyssinus* and *farinae*, *Graminaceae*, artemisia, ragweed, pellitory, dog and cat dander, birch, olive, *alternaria* and *cladosporium*. The respiratory function tests were performed by a plethysmographic box to study specific conductance and resistance (Masterlab Jaeger, Wurtzburg, Germany). The MCh challenge (10-11) was conducted using a dosimeter (Jaeger) activated by the inhalatory effort with administration of increasing doses of MCh: 30-60-120-240-390-690-1290 μg . The dose of methacholine causing a 20% fall in FEV_1 (PD_{20}) was then calculated. Before the test, the patients underwent a wash-out period of 48 hours for bronchodilators.

The nasal scraping was performed with a nasal cotton tip. The sample was smeared onto glass and dried, stained using the May Grundwald-Giemsa method, and read under immersion. The eosinophil count was expressed as percentage of the total white cells count per 10 fields. Patients were advised to interrupt any medication excluding salbutamol at least 7 days before the nasal scraping.

SLIT and concomitant treatments

The mite-SLIT containing the carbamylated monomeric allergoid was given as soluble tablets (Lais®, Lofarma S.p.A., Milan, Italy) at three different maintenance doses continuously for 3 years, using the therapeutic protocol recommended by the manufacturer. The tablets contain a 50/50 mixture of *Dermatophagoides Pteronyssinus* and *Dermatophagoides Farinae*, standardized in AU. The treatment involved a 14-week up dosing phase until the 1,000 AU dose was reached. Subsequently, the maintenance dose of 1,000, 2000 or 3000 AU (1, 2 or 3 tablets per week) was given.

All the patients received a continuous treatment with cetirizine 10 mg daily. Additional medications permitted were: salbutamol (100 mcg 1-2 puffs as needed) and nasal steroids (budesonide 100 μg , 1 puff per nostril). The latter was prescribed on medical advice.

Clinical evaluation

The patients were required to fill in the diary cards during the run-in period and after 3 years of treatment, from November to February. The following clinical symptoms were recorded: cough, wheezing, dyspnea, nasal obstruction, nasal itching, rhinorrhea, sneezing, conjunctival itching, redness, watery eyes. Each symptom was scored from 0 (= absent) to 3 (= severe), so that the

Table I. Statistics of demographic and clinical parameters at baseline (BAS), and after 3 years of treatment (3Y).

	CONTROLS (N=20)		1,000 AU (N= 22)		2,000 AU (N= 24)		3,000 AU (N= 23)				
									Chi-square		p (Exact)
SEX (M/F)	11/9		12/10		13/11		12/11			0.312	0.949
									GLM ANOVA		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	df2	F	p
AGE	21.55	0.690	24.27	2.469	22.25	0.623	22.57	1.716	85	0.518789	0.670
SMS BAS	393.60	17.062	410.91	18.642	381.67	15.883	418.48	13.806	85	1.076129	0.364
SMS 3Y	350.83	16.876	182.95	14.016	162.48	10.562	145.10	10.307	85	49.77201	0.000
FEV ₁ BAS	88.15	1.381	87.09	0.627	88.67	1.206	88.13	0.763	85	0.419066	0.740
FEV ₁ 3Y	85.06	1.752	96.52	1.330	98.19	1.050	96.52	1.585	85	16.54275	0.000
PD ₂₀ BAS	149.80	19.330	248.50	20.692	144.71	16.137	173.83	19.027	85	6.466042	0.001
PD ₂₀ 3Y	264.78	45.905	835.52	81.490	805.29	62.037	840.14	52.945	85	18.29965	0.000
EOS BAS	27.05	1.410	24.82	1.705	29.38	1.550	30.04	1.669	85	2.235367	0.090
EOS 3Y	26.17	1.327	12.24	1.692	9.33	1.430	8.14	1.168	85	31.47022	0.000
B2 BAS	11.30	0.811	15.64	0.670	13.21	0.915	12.30	0.574	85	5.764715	0.001
B2 3Y	15.39	1.895	6.00	1.095	4.14	0.513	4.14	0.924	85	20.18897	0.000
NCS BAS	17.45	1.208	17.05	1.268	18.29	1.064	20.04	1.055	85	1.353424	0.263
NCS 3Y	16.17	1.424	7.00	1.257	6.00	0.822	5.10	0.938	85	19.54698	0.000

The significant differences are in bold. SMS: symptom-medication score; PD₂₀: methacholine provocation dose in mcg; EOS: nasal eosinophils in %; B2: bronchodilator use; NCS: nasal corticosteroid use

maximum possible daily score was 30 points. Each dose of salbutamol, or nasal budesonide was scored 1 point. The mean monthly score (symptoms+medications) was calculated and used for statistical analysis. The drug intake score (mean monthly doses of nasal budesonide and salbutamol) was also analyzed separately.

Statistical Analysis

The equality of the gender ratio in the different treatment groups at baseline was tested by Pearson Chi-Square (12), while differences in age and clinical parameters were assessed by a modified ANOVA (GLM) (13). A GLM procedure was also used to compare the differences between groups after 3 years of treatment. The multiple comparisons were performed using the Tamhane's T2 test (conservative pair-wise comparison test based on a *t* test). This test is robust against violations of homogeneity of variance assumptions. The differences within groups at baseline and after 3 years of treatment

were tested using a *t* test for paired samples. The probability levels for Pearson Chi-Square were computed using a complete randomization method (permutation or exact test; P_{Exact}), or by a Monte Carlo simulation (14) when it was not possible to use the permutation method. The Statistical Package for Social Sciences ver. 15.01 was used for calculations (SPSS®).

RESULTS

Eighty-nine patients fulfilling the inclusion criteria were enrolled in the study. After the November 2004 – February 2005 baseline period, they were randomized to the three maintenance regimens of 1,000 AU (N= 22), 2,000 AU (N= 24), 3,000 AU (N= 23) or to drugs only (N= 20). The median cumulative dose taken by the patients per year in the three groups was approximately 60,000

Table II. Paired comparisons of the clinical parameters at baseline and after 3 years of treatment.

	Paired Differences				95%CI difference			
		Mean	SD	SEM	lower	upper	t	p (2-tailed)
CONTROL	SMS BAS - SMS 3Y	38.50	97.29	22.93	-9.88	86.88	1.68	0.1115
	FEV ₁ BAS - FEV ₁ 3Y	3.39	10.94	2.58	-2.05	8.83	1.31	0.2062
	MCH BAS - MCH 3Y	-107.39	201.45	47.48	-207.57	-7.21	-2.26	0.0371
	EOS BAS - EOS 3Y	0.17	6.05	1.43	-2.84	3.18	0.12	0.9083
	B2 BAS - B2 3Y	-4.22	10.09	2.38	-9.24	0.79	-1.78	0.0936
	NCS BAS - NCS 3Y	0.89	6.22	1.47	-2.20	3.98	0.61	0.5523
	1,000 AU SMS BAS - SMS 3Y	228.24	97.98	21.38	183.64	272.84	10.67	0.0000
	FEV ₁ BAS - FEV ₁ 3Y	-9.38	7.88	1.72	-12.97	-5.79	-5.45	0.0000
	MCH BAS - MCH 3Y	-592.19	375.12	81.86	-762.94	-421.44	-7.23	0.0000
	EOS BAS - EOS 3Y	12.62	12.33	2.69	7.01	18.23	4.69	0.0001
	B2 BAS - B2 3Y	9.67	6.09	1.33	6.89	12.44	7.27	0.0000
	NCS BAS - NCS 3Y	10.10	8.88	1.94	6.05	14.14	5.21	0.0000
	2,000 AU SMS BAS - SMS 3Y	231.81	63.91	13.95	202.72	260.90	16.62	0.0000
	FEV ₁ BAS - FEV ₁ 3Y	-10.00	7.21	1.57	-13.28	-6.72	-6.35	0.0000
	MCH BAS - MCH 3Y	-679.05	285.97	62.40	-809.22	-548.88	-10.88	0.0000
	EOS BAS - EOS 3Y	20.57	7.78	1.70	17.03	24.11	12.12	0.0000
	B2 BAS - B2 3Y	8.62	4.10	0.90	6.75	10.49	9.62	0.0000
	NCS BAS - NCS 3Y	12.86	4.44	0.97	10.84	14.88	13.26	0.0000
	3,000 AU SMS BAS - SMS 3Y	286.00	52.84	11.53	261.95	310.05	24.81	0.0000
	FEV ₁ BAS - FEV ₁ 3Y	-8.33	6.48	1.41	-11.28	-5.39	-5.90	0.0000
	MCH BAS - MCH 3Y	-671.24	239.34	52.23	-780.18	-562.29	-12.85	0.0000
	EOS BAS - EOS 3Y	22.43	10.26	2.24	17.76	27.10	10.01	0.0000
	B2 BAS - B2 3Y	8.14	5.42	1.18	5.68	10.61	6.89	0.0000
	NCS BAS - NCS 3Y	15.33	7.26	1.58	12.03	18.64	9.68	0.0000

The results of the *t* test for paired samples are shown in bold. SMS: symptom-medication score; PD₂₀: methacholine provocation dose in mcg; EOS: nasal eosinophils in %; B2: bronchodilator use; NCS: nasal corticosteroid use

AU, 120,000 AU and 180,000 UA, respectively. There were 8 dropouts during the study (2 in the control group, 1 in the 1,000 AU, 3 in the 2,000 AU and 2 in the 3,000 AU group). None of the dropouts was related to the treatment, but due to non-adherence to the protocol. The 4 groups were demographically homogeneous at baseline as summarized in Table I. There was no significant difference in the clinical scores, nasal eosinophils and use of nasal corticosteroid (Figs. 1-5). The use of bronchodilators was significantly greater at baseline in the 1,000 AU group versus the controls and the 2,000 AU group ($p < 0.01$) (Fig. 4). Furthermore, a

significant difference in the PD₂₀ was detected at baseline between the controls and the 1,000 AU and between the 1,000 and 2,000 AU groups (Fig. 2).

All the considered parameters displayed a significant difference between baseline and after 3 years (Table II; Figs. 1-5) in the three SLIT groups, whereas there was no difference in the control group. The only exception was the PD₂₀ value, which was increased in the control group at the 3rd year ($t = -2.26$, $df = 17$, $p = 0.037$) versus baseline (Fig. 2). The FEV₁ showed a statistically significant increase in the three SLIT groups versus baseline, although this increase (less than 10%) could be judged not

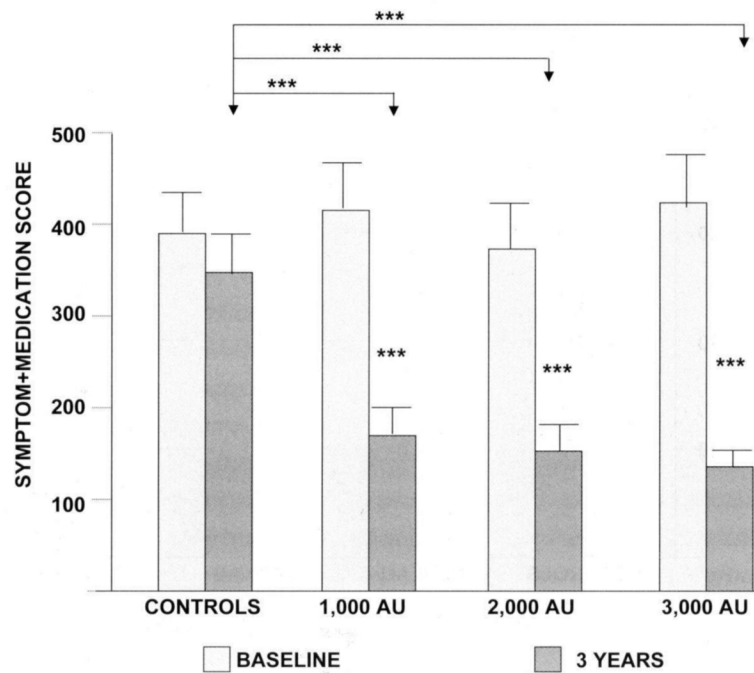


Fig. 1. Mean and SEM of the symptom-medication score in the control and SLIT groups. The significant differences within groups are shown beside the bars, and the significant differences among groups are reported above the bars (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$).

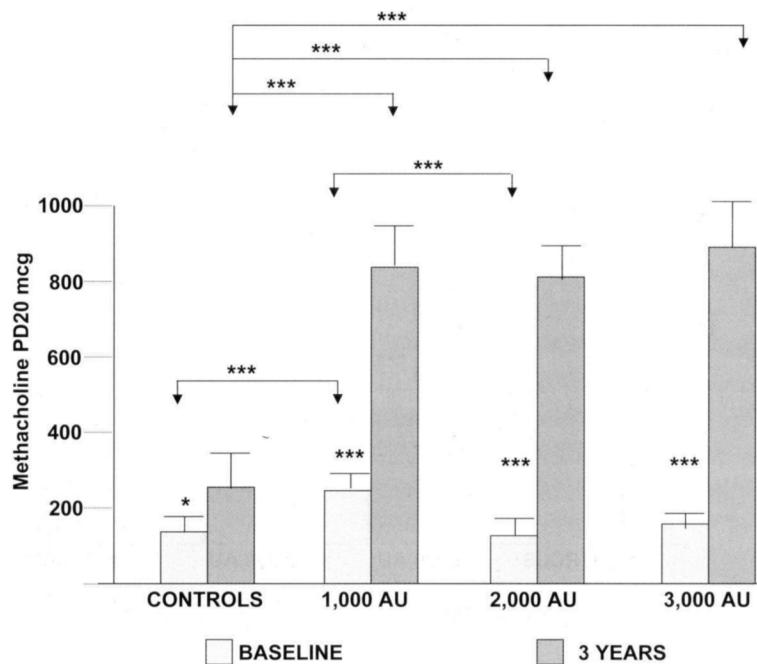


Fig. 2. Mean and SEM of the PD_{20} (mcg) in the control and SLIT groups. The significant differences within groups are shown beside the bars, and the significant differences among groups are reported above the bars (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$).

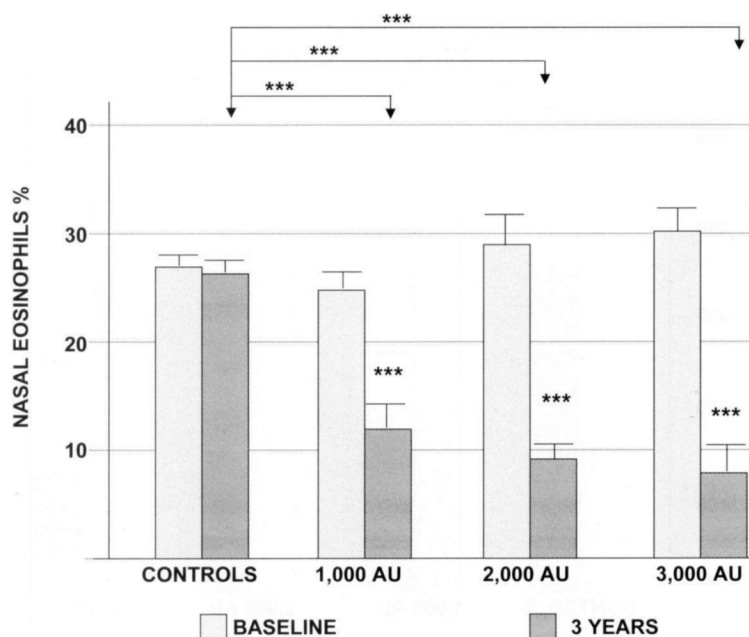


Fig. 3. Mean and SEM of nasal eosinophils (%) in the control and SLIT groups. The significant differences within groups are shown beside the bars, and the significant differences among groups are reported above the bars (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$).

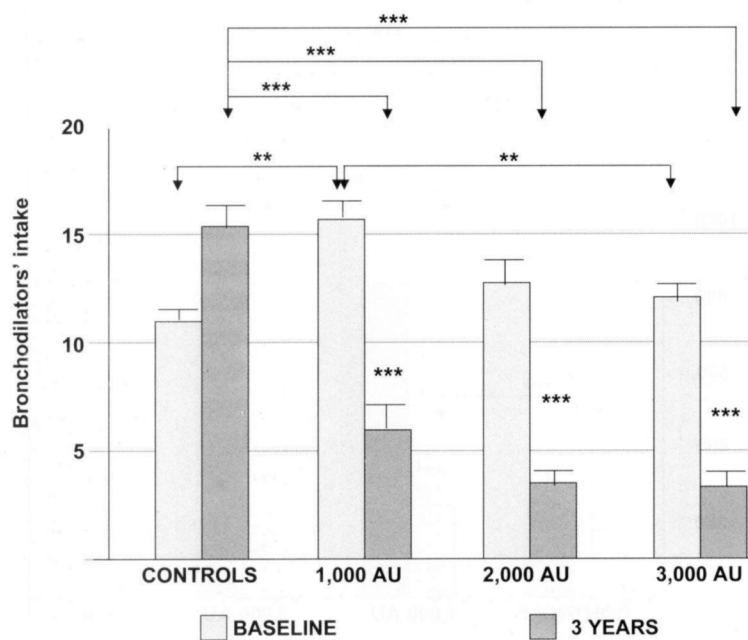


Fig. 4. Mean and SEM of the bronchodilators (monthly doses) use in the controls and SLIT groups. The significant differences within groups are shown beside the bars, and the significant differences among groups are reported above the bars (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$).

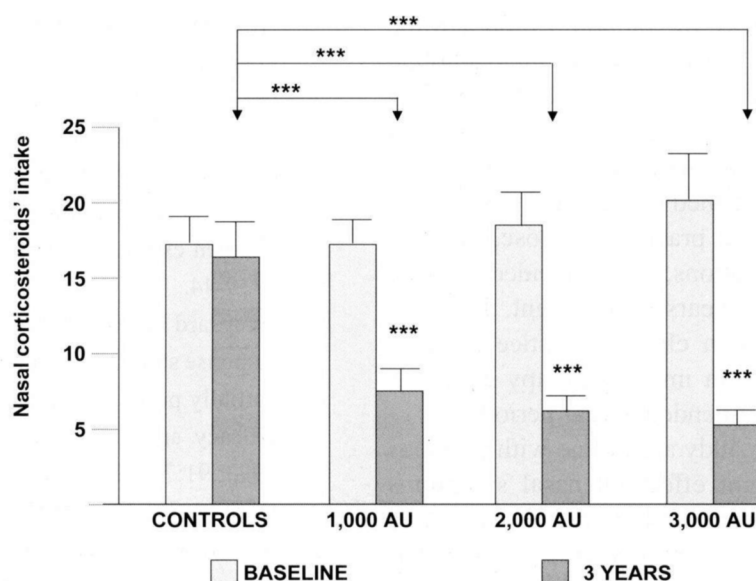


Fig. 5. Mean and SEM of the nasal corticosteroid use (monthly doses) in the controls and SLIT groups. The significant differences within groups are shown beside the bars, and the significant differences among groups are reported above the bars (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$).

clinically relevant (Table II). No local or systemic side effect was reported, except a case of generalized itching without urticaria in one patient from the 2,000 AU group. This event was judged as being probably related to the treatment, and resolved after 5 days of oral antihistamine.

DISCUSSION

It has been suggested that the efficacy of immunotherapy is to a certain extent dependent on the dose administered during the maintenance phase. In parallel, it has been shown that increasing the dose of allergenic extract increases the rate of adverse events (5, 15-16). In fact, with SCIT, the maintenance dose is usually identified as "the vaccine dose that can induce a significant clinical effect without causing appreciable side effects" (1). The introduction of non-injection routes of administration, namely the SLIT, has increased the safety of specific immunotherapy, and has made dose-ranging trials more realistic and feasible (17).

The sublingual route has been evaluated in more than 60 double-blind randomized trials (18), and recent meta-analyses have confirmed its efficacy in

allergic rhinitis and asthma (19). On the other hand, the largely variable standardization methods used by the different manufacturers makes the comparison among extracts difficult, and each producer compares the SLIT dose to the cumulative dose used during an analogous SCIT course. In this sense, it has been established that the effective SLIT dose should be 50-100 times greater than in a correspondent SCIT (1). It is clear that each product differs from the others, also in terms of protein and allergen content (20), thus dose-ranging studies for each individual product should be performed. Based on this background we carried out a clinical trial to compare the effects of three different maintenance doses of a monomeric allergoid in mite allergic patients. The main result of this trial was that the three doses used did not differ in terms of clinical efficacy, safety and effects on functional and immunological parameters, and all the three doses performed significantly better than the pharmacotherapy alone. There was a difference of note, although not significant in many parameters between the 1,000 and the 2,000 AU doses, with a slightly better outcome for the latter. The study could not be placebo-blinded, since the ethical committee raised concerns about the long duration

of the trial. Nonetheless, the randomization and the use of objective parameters such as the methacholine challenge and nasal eosinophils should counter-balance the lack of a placebo group. In addition, this study was not aimed at demonstrating the clinical efficacy, already ascertained (21-23), but to compare different dosages. For a practical purpose, in order to avoid protocol deviations, it was decided to make the evaluations after 3 years of treatment. This also reflects what happens in clinical practice (24-25), where the efficacy of an immunotherapy course is judged after the recommended 3-year period.

The results of our study are in line with previous trials. The concomitant effect on nasal symptoms and nasal inflammation indirectly testify for a systemic effect of this immunotherapy, which has been demonstrated capable of evoking a T regulatory response (26). The effect on non-specific bronchial reactivity was particularly noticeable and similar to that described elsewhere (27-28). Although bronchial hyperreactivity is multi-factorial (29), it is partly determined by bronchial inflammation and its reduction indirectly suggests that SLIT reduces or modulates the bronchial inflammatory events.

As a general conclusion, the clinical effects obtained with the "low" dose of monomeric allergoid are comparable to those shown with "higher" doses and the 2,000 AU dosage could be reasonably regarded as the best choice. The costs of this type of therapy can thus be contained, with a consistent improvement in pharmacoeconomy parameters, which nowadays are assuming continually increasing importance.

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