# 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, expecially 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 μg; 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 hyperthrophy), 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 updosing 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; $PD_{20}$: 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 budeseonide 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; $PD_{20}$: 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 $PD_{20}$ 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 $PD_{20}$ 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 $FEV_1$ showed a statistically significant increase in the three SLIT groups versus baseline, although this increase (less than 10%) could be judged not

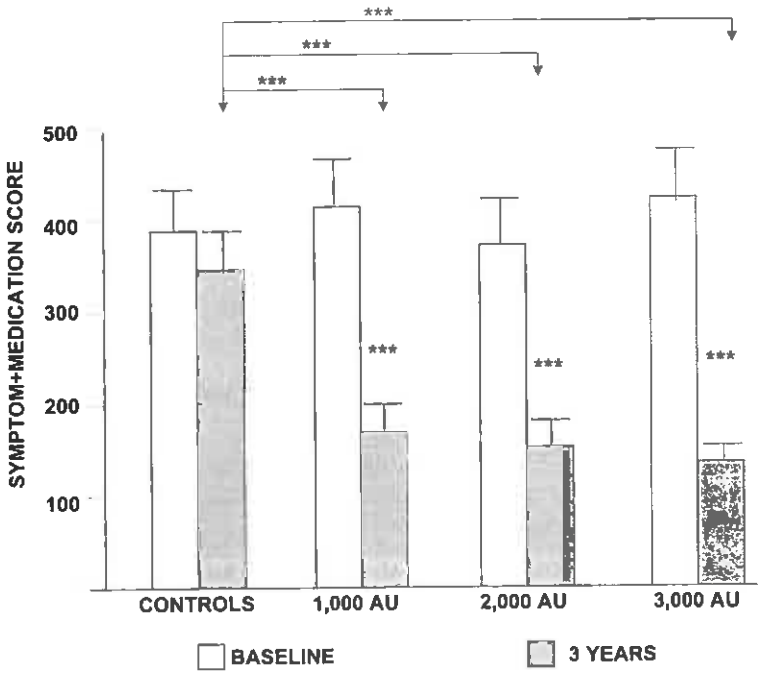


**Fig. 1.** *Mean and SEM of the symptom-medication score in the control and SLIT groups. The significant differences within groups are shown beside the bars, and the significant differences among groups are reported above the bars (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$).*

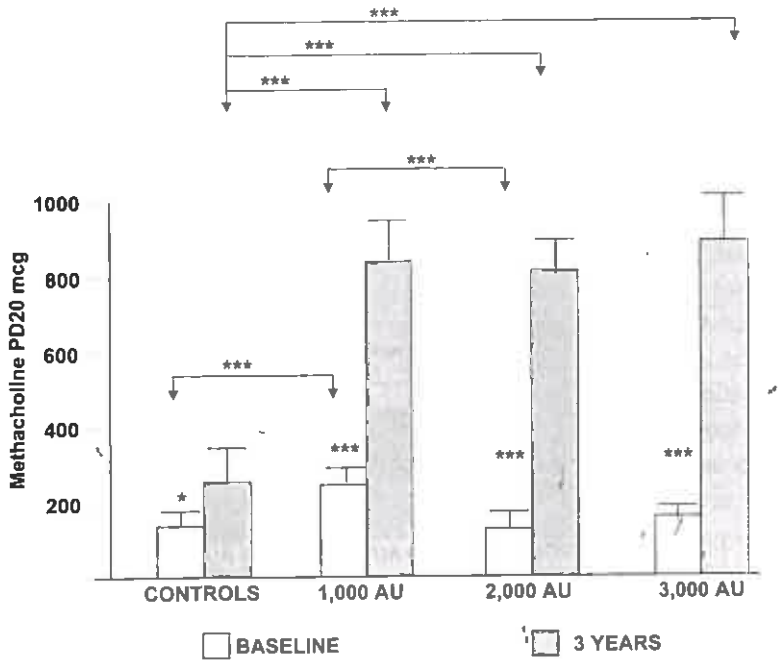


**Fig. 2.** *Mean and SEM of the $PD_{20}$ (mcg) in the control and SLIT groups. The significant differences within groups are shown beside the bars, and the significant differences among groups are reported above the bars (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$).*

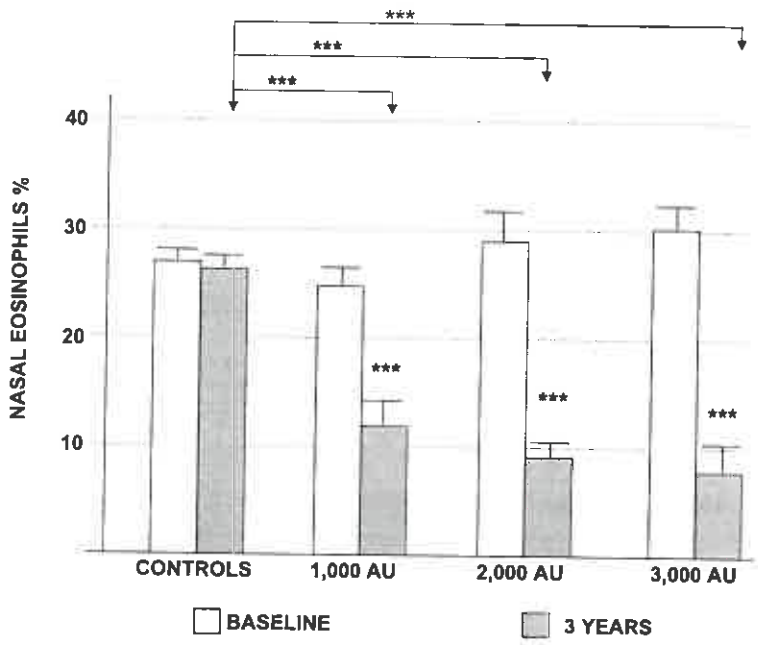


**Fig. 3.** *Mean and SEM of nasal eosinophils (%) in the control and SLIT groups. The significant differences within groups are shown beside the bars, and the significant differences among groups are reported above the bars (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$).*

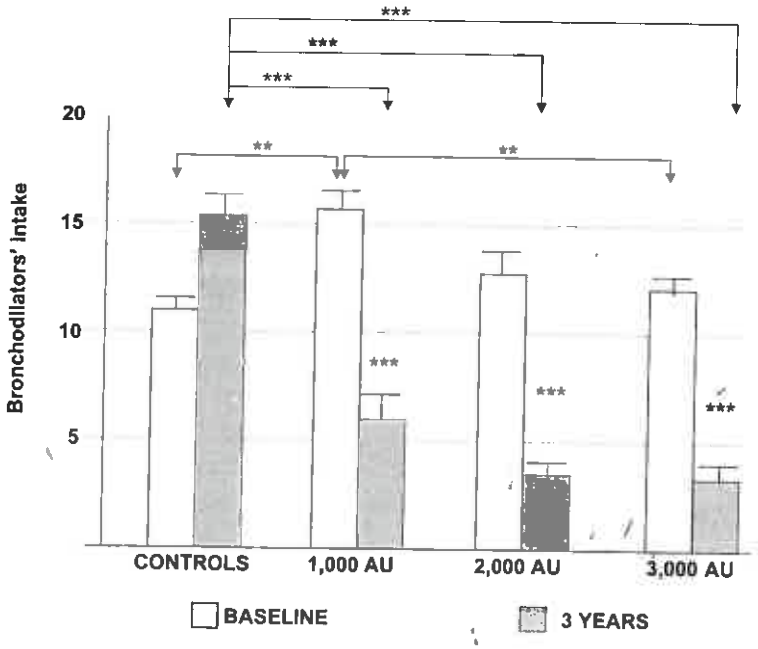


**Fig. 4.** *Mean and SEM of the bronchodilators (monthly doses) use in the controls and SLIT groups. The significant differences within groups are shown beside the bars, and the significant differences among groups are reported above the bars (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$).*

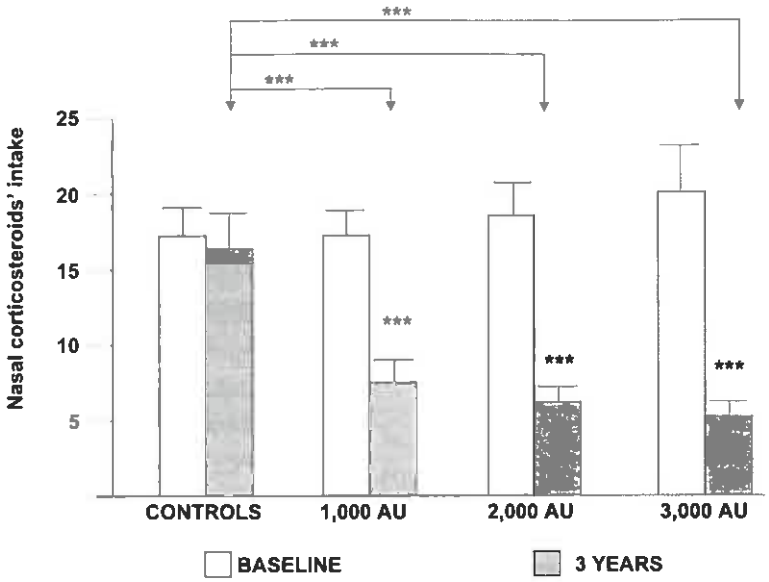


**Fig. 5.** *Mean and SEM of the nasal corticosteroid use (monthly doses) in the controls and SLIT groups. The significant differences within groups are shown beside the bars, and the significant differences among groups are reported above the bars (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$).*

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 form 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 counterbalance 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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## REFERENCES

1. Bousquet J, Lockey R, Malling HJ. WHO Position Paper. Allergen Immunotherapy: therapeutical vaccines for allergic diseases. Allergy 1998; 53:1-42.
2. Committee on Safety in Medicine. CSM update: desensitising vaccines. BMJ 1986; 293:948.
3. Bousquet J, Van Cauwenberge P, Kalthaev N. Allergic rhinitis and its impact on asthma (ARIA). Position Paper endorsed by WHO. J Allergy Clin Immunol 2001; 108(S):147-334.
4. Nanda A, O'Connor M, Anand M, et al. Dose dependence and time course of the immunologic response to administration of standardized cat allergen extract. J Allergy Clin Immunol 2004; 114: 1339-44.
5. Haugaard L, Dahl R, Jacobsen L. A controlled dose-response study of immunotherapy with standardized, partially purified extract of house dust mite: clinical efficacy and side effects. J Allergy Clin Immunol 1993; 91:709-22.
6. Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass-allergen tablets: a randomised controlled trial in seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006; 117:802-9.
7. Didier A, Malling HJ, Worm M, Horak F, Jager S, Montagut A, André C, de Beaumont O, Melac M. Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. J Allergy Clin Immunol 2007; 120:1338-45.
8. Mistrello G, Brenna O, Roncarolo D, Falagiani P. Monomeric chemically modified allergens: immunologic and physicochemical caracterization. Allergy 1996; 51:8-15.
9. Dreborg S, Frew A. Position Paper: allergen standardization and skin tests. Allergy 1993; 48(S): 49-82.
10. Hargreave FE, Ryan G, Thomson NC. Bronchial responsiveness to histamine and methacholine in asthma: measurement and clinical significance. J Allergy Clin Immunol 1981; 68:347-55.
11. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo JL. Airway responsiveness. Standardized challenge test with pharmacological, physical and sensitising stimuli. Eur Resp J 1993; 6:53-58.
12. Siegel S, Castellan NJJ. Nonparametric statistics for the behavioral sciences. Ed. McGraw-Hill, New York, 1988.
13. Sokal RR, Rohlf FJ. Biometry. The principles and practice of statistics in biological research. 3rd Ed.; W.H. Freeman & C., New York, 1995.

14. Good P. Permutation tests: a practical guide to resampling methods for testing hypotheses. 2nd Ed. Springer Verlag, New York, 2000.
15. Creticos PS, Van Metre TE, Mardiney MR. Dose response of IgE and IgG antibodies during ragweed immunotherapy. J Allergy Clin Immunol 1984; 73: 94-9.
16. Alvarez-Cuesta E, Cuesta-Herranz J, Puyana-Ruiz J, Cuesta-Herranz C, Blanco-Quiros A. Monoclonal antibody-standardized cat extract immuno-therapy: risk-benefit effects from a double-blind placebo-controlled study. J Allergy Clin Immunol 1994; 93: 556-66.
17. Canonica GW, Baena Cagnani C, Bousquet J, Bousquet PJ, Passalacqua G, Potter P, Valovirat E. Recommendations for standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy. A statement of a World Allergy Organization (WAO) taskforce. Allergy 2007; 62:317-24.
18. WAO Position Paper on sublingual immunotherapy. Canonica GW, Bousquet J, Lockey R, Casale TB editors. Allergy 2009; 64:3-45.
19. Compalati E, Penagos M, Tarantini F, Passalacqua G, Canonica GW. Specific immunotherapy for respiratory allergy: state of the art according to current meta-analyses. Ann Allergy Asthma Immunol 2009; 102:22-8.
20. Sander I, Fleischer C, Meurer U, Bruning T, Raulf-Heimsoth M. Allergen content of grass pollen preparations for skin prick testing and sublingual immunotherapy. Allergy 2009; 64:1486-92.
21. Marogna M, Bruno M, Massolo A, Falagiani P. Long-lasting effects of sublingual immunotherapy for house dust mites in allergic rhinitis with bronchial hyperreactivity: a long-term (13-year) retrospective study in real life. Int Arch Allergy Immunol 2007; 142:70-8.
22. Passalacqua G, Albano M, Fregonese L, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite induced rhinoconjunctivitis. Lancet 1998; 351:629-32.
23. Passalacqua G, Pasquali M, Ariano R, Lombardi C, Baiardini I, Majani G, et al. Randomized double blind controlled trial with sublingual carbamylated allergoid immunotherapy in mild rhinitis due to mites. Allergy 2006; 61:849-54.
24. Marogna M, Massolo A. Sublingual immunotherapy in the contest of a clinical practice improvement program in the allergological setting: results of a long term observational study. Eur Ann Allergy Clin Immunol 2003; 35:133-40.
25. Marogna M, Tiri A, Riva G. Clinical practice improvement program for immunotherapy of respiratory allergic diseases. Int J Immunopathol Pharmacol 2001; 14:93-101.
26. Cosmi L, Santarlasci V, Angeli R, et al. Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-γ-and interleukin-10-production. Clin Exp Allergy 2006; 36:261-72.
27. Lombardi C, Gargioni S, Venturi S, Zoccali P, Canonica GW, Passalacqua G. Controlled study of preseasonal immunotherapy with grass pollen extract in tablets: effect on bronchial hyperreactivity. J Invest Allergol Clin Immunol 2001; 11:41-5.
28. Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G. Clinical functional and immunological effects of sublingual immunotherapy in birch allergy. J Allergy Clin Immunol 2005; 115: 1184-8.
29. Baroffio M, Barisione G, Crimi E, Brusasco V. Noninflammatory mechanisms of airway hyper-responsiveness in bronchial asthma: an overview. Ther Adv Respir Dis 2009; 3:163-74.