

Local nasal immunotherapy with extract in powder form is effective and safe in grass pollen rhinitis: A double-blind study

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Background: Local nasal immunotherapy has been studied, by means of an extract in powder form, in patients with allergic rhinitis caused by grass pollen.

Methods: Thirty-two patients allergic to grass were studied for 37 weeks in a double-blind controlled trial. Subjects were selected on the basis of a positive history, skin test result, RAST finding, and result of intranasal challenge to grass pollen. Two 16-patient groups were randomly assigned to active or placebo treatment. The treatment lasted 26 weeks (14 for the build-up phase, 12 for the maintenance period).

Results: No significant differences were observed in nasal symptoms during the treatment. During the pollen season the mean weekly symptom and medication scores were significantly lower in the treated group, compared with the control group, even considering each allergic symptom separately. Moreover, only in the treated group was a significant increase of specific nasal threshold to grass pollen observed after treatment.

Conclusions: This study indicates that local nasal immunotherapy with allergen in powder form can be a suitable alternative to the traditional subcutaneous immunotherapy in terms of clinical efficacy and safety in grass-allergic rhinitis. (*J ALLERGY CLIN IMMUNOL* 1996;97:34-41.)

Key words: Hay fever, local nasal immunotherapy, allergic rhinitis, grass pollens, pollenosis

Grass pollens are the most common cause of hay fever in the world. In Northern Italy the pollination period runs from the end of April to the end of June. The highest pollen counts are registered in the Padana Plain, where a percentage of 60% to 80% of patients with hay fever are sensitized.¹ Traditional subcutaneous immunotherapy for grass pollinosis has been shown to be effective in reducing nasal symptoms and medication scores.^{2,3} However, the cost/benefit ratio of subcutaneous

Abbreviations used

AU: Allergenic units
LNIT: Local nasal immunotherapy

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immunotherapy is increased in grass pollen allergic rhinitis because of the short period of pollination, the need for prolonged compliance, and above all the risk, though rare, of systemic side effects.^{2,3} Therefore the use of alternative routes of immunotherapy seems to be justified in this seasonal allergic rhinitis. Conflicting results have been reported with oral immunotherapy, whereas a greater efficacy has been demonstrated with sublingual administration.⁴ However, an anecdotal case of severe anaphylaxis caused by oral immunotherapy must be cited.⁵ In the present study we evaluated in patients with grass pollinosis the efficacy and safety of local immunotherapy with an allergen in powder form. The clinical efficacy and safety of this kind of intranasal immunotherapy has

already been demonstrated in the treatment of allergic rhinitis resulting from other allergens.^{6,7}

METHODS

Subjects

Two 16-subject groups (15 men and 17 women) with a history of allergic rhinitis for at least 2 years were selected. Demographic data of the study population are shown in Table I. Criteria for inclusion in the study were as follows:

1. Typical history of grass-allergic rhinitis (presence of symptoms from April to June)
2. Positive skin prick test result (3+ to 4+) to grass pollen extract in glycerinated solution (15,000 protein nitrogen unit [PNU]/ml; Laboratorio Farmaceutico Lofarma, Milan, Italy) and a negative skin prick test result for the other aeroallergens, such as *Dermatophagoides pteronyssinus* and *farinae*, cat and dog dander, molds (*Alternaria*, *Cladosporium*), birch, *Parietaria*, *Cupressus*, mugwort, and *Olea* pollen (Laboratorio Farmaceutico Lofarma)
3. RAST positivity (at least class 3) and negative for other aeroallergens
4. Positive response to grass pollen-specific nasal provocation test
5. Patient's symptoms had not been controlled in the previous pollen season by the traditional pharmacologic therapy (antihistamines, local steroids, local decongestants, cromolyn)
6. Informed written consent.

Patients were excluded for the following reasons: asthma, long-term use of nasal corticosteroids or decongestants, prior specific immunotherapy, other allergen sensitivity, the state of pregnancy or lactation, and the presence of nasal polyps.

Antigen

The active product (supplied by Laboratorio Farmaceutico Lofarma) is an allergen in powder prepared from a mixture of five grass pollen extracts (*Phleum pratense*, *Dactylis glomerata*, *Lolium italicum*, *Poa pratensis*, and *Festuca elatior*—20% of each pollen) absorbed on lactose and dehydrated.

This product, enclosed in hard gelatin capsules and characterized in dosages by ten different colors, is titrated in allergenic units (AU), by a RAST-inhibition technique with reference to an internal standard.⁸ As regards the *Phleum pratense*, an allergenic unit corresponds to 0.25 ± 0.063 IU of *Phleum pratense*, as determined by a comparative RAST-inhibition analysis between the internal (in-house) standard and the International Union Immunological Society–World Health Organization–First *Phleum pratense* International Standard. The gelatin capsules are permeable to humidity, and for this reason they come in plastic/aluminium blister packages. For particle-size analysis, the powder was suspended in silicone oil, sonicated, and analyzed

TABLE I. Demographic data of study population

	Active treatment group	Placebo treatment group	p Value
No. of patients	16	16	NS
Gender (M/F)	7/9	8/8	NS
Mean age	31.8	31	NS
±SD	10.2	12.1	NS
Duration of allergic rhinitis (mean years)	6.1	5.7	NS
Skin reactivity (mean area of wheals)	76.3 mm ²	68.2 mm ²	NS
Specific nasal sensitivity (mean threshold dose)	11.3 AU	9 AU	NS
Drop out	3	1	NS

NS, Not significant.

with a laser light-powered (wavelength, 632.8) analyzer (model 715; Cilas Alcatel, Marcousses, France): 75% of particles were between 15 and 80 μ m with partition value (50/50%) around the dimension of 45 μ m. The placebo consisted of lactose and in exterior was identical to the active products.

Skin tests

Skin reactivity-threshold assessment was evaluated by means of skin prick tests with grass pollen extracts given in decreasing concentrations, according to "the end-point method."⁹ All patients were tested with the undiluted extract and with the following dilutions: 1:20, 1:50, 1:100, 1:200, 1:1000 wt/vol, using Pricker needles (Dome Hollister Stier, Spokane, Wash.). Extracts used for the end-point titration were dilutions of the same diagnostic extract used for the admittance testing of patients. The same batch was used for the whole trial. The wheals were outlined with a fine-point marker, and the outlines were transferred by means of transparent tape onto permanent records. The areas were measured by means of planimetry and expressed in square millimeters. The end-point is the highest dilution eliciting a wheal larger than 3 mm.^{9,10}

RAST

Sera were collected at the end of September 1991 and early in April 1992. The RAST was performed as described by Wide et al.,¹¹ as regards the general procedure, and following the manufacturer's instructions, concerning particulars of the assay.

Reagents were purchased from a commercial source and consisted of polystyrene beads, 6.4 mm in diameter, as solid-phase, ¹²⁵I-anti-human IgE affinity-purified antibody raised in goat as tracer, *Poa pratensis*-coated beads, and grass-positive sera as reference system (Sferikit; Laboratorio Farmaceutico Lofarma). The final radioactivity was measured (Cobra Auto-gamma; Packard

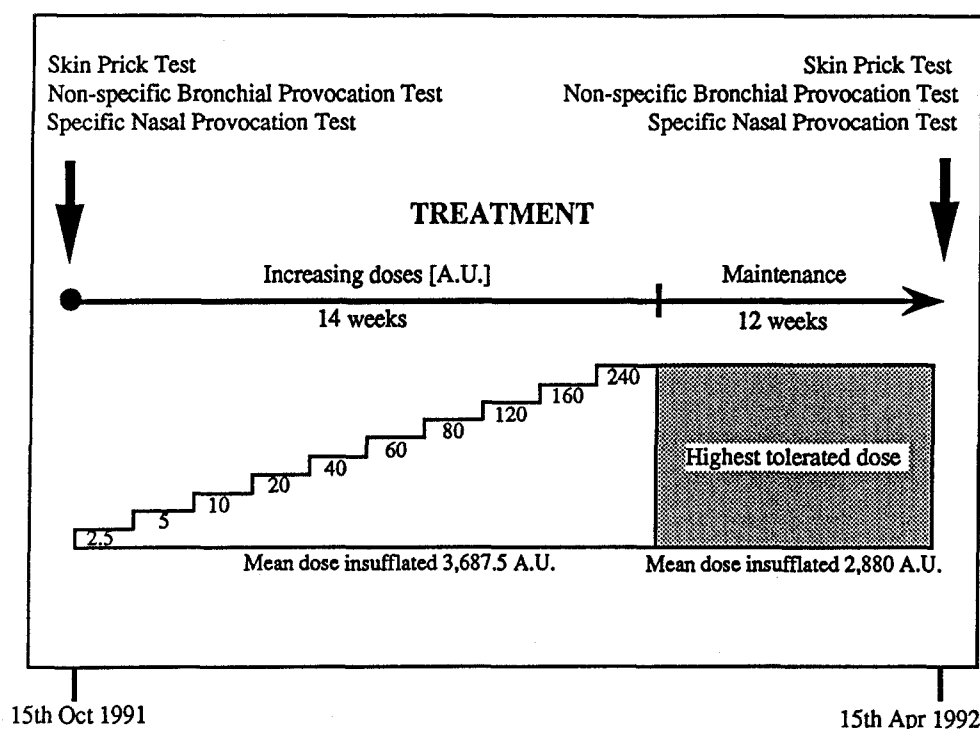


FIG. 1. Schedule of treatment.

Instruments Pero, Milano, Italy) and the bound percentage calculated.

Immunotherapy program

Preseasonal local nasal immunotherapy (LNIT) was carried out by self-administration and was administered for 26 weeks, according to two different schedules (Fig. 1). The first schedule (14 weeks) consisted in the administration of increasing doses of the allergen by proper device, alternate day, alternate nostril. This rhino-insufflator (MIAT, Milan, Italy) consists of a body made up of a nasal adaptor, a capsule holder, two spring-activated pins for perforating the capsule, and a rubber pump containing the air reserve necessary for the distribution of the powder. During administration, the pressure on the rubber pump provokes an air stream that passes through the holes at the ends of the capsules and carries the powder into the nasal adaptor and onto the nasal mucosa. Premedication with cromolyn (10.4 mg) 30 minutes before treatment was carried out for each patient. To avoid progression of the allergen through the bronchial tree, patients were told to vocalize during each administration. The schedule provided for the following doses: 2.5, 5, 10, 20, 40, 60, 80, 120, 160, and 240 AU. Each dose was administered five times before going to the next dose.

The second schedule (maintenance period) was carried out for 12 weeks. Each patient was treated with maximal tolerated doses (240 AU) once weekly in one

nostril. The mean dose administered in the first period was 3.687 AU; in the latter period, 2.880 AU.

Experimental design

The design of the study was a double-blind placebo-controlled trial. Patients were randomly divided in two groups according to randomization tables¹²: group 1 (16 subjects) received placebo; group 2 (16 subjects) received active grass extract. Before and after local immunotherapy, the following tests were performed (Fig. 1): (1) skin reactivity assessment by skin prick test with grass extracts, (2) nonspecific bronchial provocation test with methacholine, to exclude an important degree of non-specific bronchial hyperreactivity ($PD_{20} < 400 \mu g$). Bronchial hyperresponsiveness was assessed by means of a methacholine dose-response curve, using a dosimeter (ME.FAR. MB3; Markos, Monza, Milan, Italy) connected to a De Vilbiss 646 ampulla.¹³ Methacholine (Laboratorio Farmaceutica Lofarma) was used in two concentrations: 0.2% and 1% to obtain the targeted dose to be inhaled by changing the number of puffs (from one to six). After baseline measurement of FEV_1 , patients inhaled five puffs of saline, followed by increasing doses of methacholine. Starting from $0.020 \mu g$ the dose was doubled at each step. FEV_1 was measured 45 seconds after saline as well as after each dose step. The test was ended when FEV_1 fell $>20\%$ from the post-saline FEV_1 value. The highest dose of methacholine used in this protocol amounted to 1.280 mg.¹³ Nasal

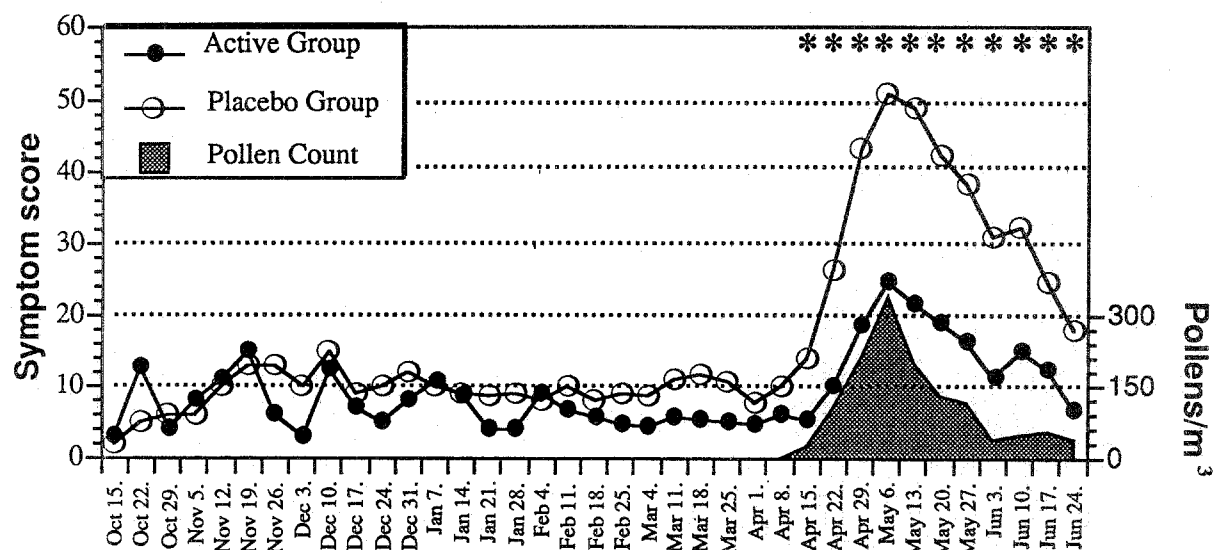


FIG. 2. Mean weekly symptoms score in treated (closed circles) and in placebo (open circles) groups.

sensitivity was determined by the use of quantitative challenges with increasing concentrations of allergen in powder form (2.5, 5, 10, 20, 40, 60, and 80 AU) which were delivered, as during the treatment, by a manual rhino-insufflator. Doses were administered at 20-minute intervals.

Threshold doses of allergen were determined from the amount required: (1) to increase the total nasal resistance to airflow by 50% or more, as compared with the inert control (lactose) using anterior rhinomanometry (RN4 Rhinomanometer; Markos)¹⁴ (2) to elicit at least two of the four symptoms: itching, sneezing, discharge, and obstruction.¹⁵

The reproducibility of this specific nasal provocation test was demonstrated in our previous studies.⁷

Clinical evaluation

Both LNIT-treated and control patients kept a daily report of allergy symptoms during the preseasonal LNIT and during the pollen season (from 10/15 to 6/30). Daily symptom-medication scores were tabulated according to patients' records. Specific symptoms recorded were nasal blockage, nasal itching, sneezing, rhinorrhea, and eye irritation and watering. Once each day patients graded each symptom for severity from "0" to "3" ("0" = none, "1" = mild, "2" = moderate, "3" = severe) according to their quality of life. The amount of antihistamines (terfenadine 120 mg) used was also recorded. Patients were instructed to use this drug when the daily score was 7 or greater or when one symptom was graded as being severe. They stopped use of cromolyn during the pollen season. Patients were told to report any other symptom, even if not included in the diary. The diaries of patients were collected and reviewed monthly by the investigators.

The patient self-evaluation was made at the end of the

study, before the code was broken. Each patient was asked for his or her overall evaluation of this treatment with categories on improvement of this kind: poor, moderate, good, excellent.

Statistical analysis

Statistical analysis was done with nonparametric tests. The Spearman rank-correlation test was used for regression analysis; the Mann-Whitney U test, for paired comparisons. The χ^2 test was used to test the significance of the differences among the overall evaluations expressed by each patient at the end of the study.

RESULTS

Four patients dropped out of the study: three from the active group and one from the placebo group. Two patients in the active group withdrew from the study because of family problems; the other two patients, one for each group, withdrew because of the appearance of exclusion criteria (one became pregnant and one began to use a systemic steroid for a nonrespiratory disease).

During the treatment no systemic or bronchospastic reactions occurred in either group and the mean weekly symptom and medication scores did not differ significantly between the two groups (Figs. 2 and 3).

During the entire pollen season the mean weekly symptom score was lower in the treated group, reaching a statistical significance each week for 11 weeks (Fig. 2). The medication scores were significantly higher in the control group each week for 5 weeks, when the pollen count was highest (Fig. 3). Moreover, the total mean score of each

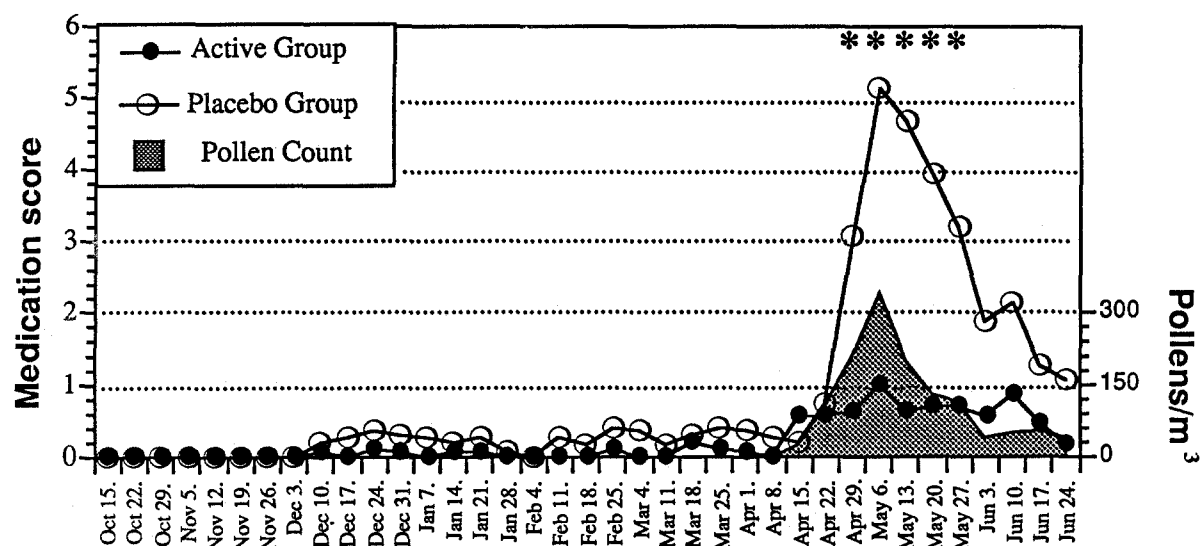


FIG. 3. Mean weekly medication score in treated (closed circles) and in placebo (open circles) groups.

allergic symptom was significantly lower in the treated group, in comparison with the control group (Fig. 4).

The overall self-evaluations from the patients finishing the trial was significantly better in the treated group (Table II). After treatment nasal sensitivity to grass allergen, measured by nasal provocation test, significantly decreased in the actively treated group (Fig. 5), whereas no difference was observed in the control group (Fig. 6). In fact, a decrease of nasal specific sensitivity was observed in 10 subjects belonging to the active group and in only two patients of the placebo group.

There were no significant differences between the two groups regarding skin reactivity, specific serum IgE levels. The results of the nonspecific bronchoprovocation test with methacholine remained negative in all subjects after LNIT as before treatment. No significant fall ($>20\%$ of the baseline value) until the administration of 1.2 mg/ml of methacholine was observed in any patients of both groups. In the active group no difference was registered before or after treatment in skin reactivity as evaluated by means of the mean wheal area at the different grass extract dilutions. Accordingly, the mean IgE-specific serum levels (before treatment, 17.38 ± 14.4 bound percent radioactivity; after treatment, 19.28 ± 15.3 bound percent radioactivity) did not exhibit any difference. There was also no difference in the dose-response curve obtained with methacholine in the two groups before and after treatment. At

the highest dose in the active group the fall in FEV₁ ranged from 0% to 18% before treatment and from 2% to 18% after treatment; the fall in FEV₁ in the placebo group ranged from 0% to 16% before treatment and from 0% to 18% after treatment.

DISCUSSION

Our data showed an impressive clinical efficacy of LNIT in powder form in grass pollinosis. After 26 weeks of treatment, during the whole pollen season, the symptom score was significantly lower in the treated group, compared with the control group, reaching statistical significance for 11 weeks. Moreover, a significant reduction of medication score was observed in the same period for 5 weeks. In agreement with previous studies on LNIT,^{6,7,16} we demonstrated a reduction in specific nasal reactivity, as indicated by a significant increase in the mean threshold dose in the treated group. Although some authors have recently reported an increase of nonspecific bronchial hyperreactivity after nasal challenges with allergen solutions,¹⁷ we did not observe asthma attacks or an increase in nonspecific bronchial hyperresponsiveness in the active group after treatment. The powder particles have a mean diameter of about 45 μm and do not reach the small airways, confirming the safety of this form of extract when used for nasal provocation and for LNIT.^{6,7}

The introduction of lyophilized allergen in powder form appears to be further progress in regard to stability and standardization. The aqueous solu-

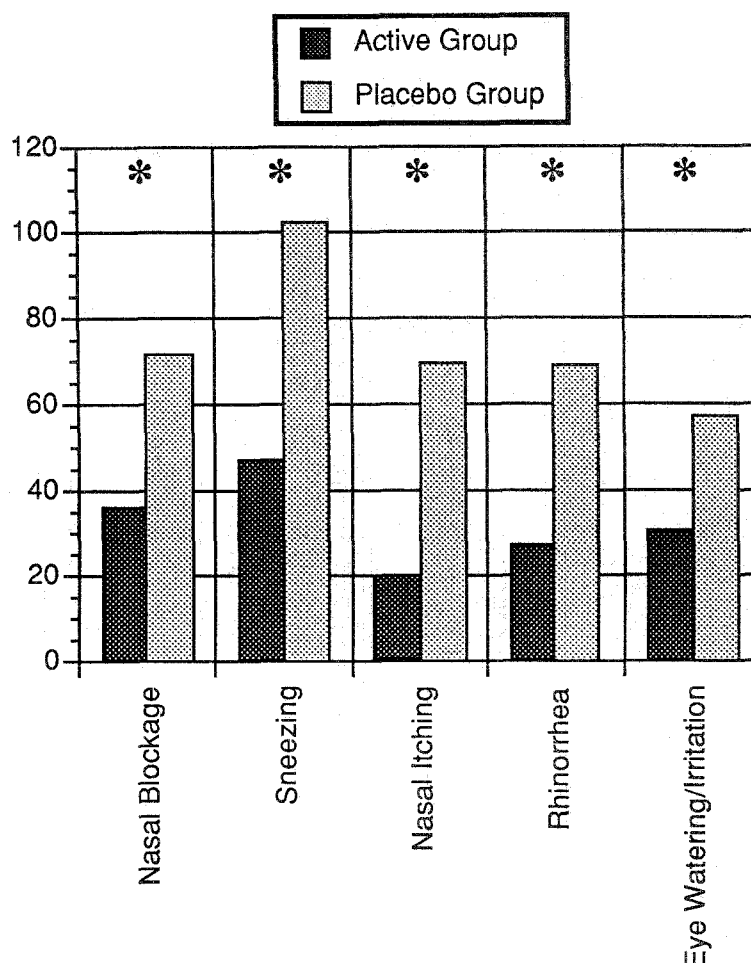


FIG. 4. Comparison of mean total score for each symptom.

tion of allergen may have a poor stability because of the volume effect and self-digestion.^{18,19} As would be predicted, a low dose of aqueous extract used in a study of LNIT was clinically ineffective.²⁰ On the other hand, high doses of aqueous extracts have been clinically effective in grass and ragweed allergic rhinitis, but the local side effects are intolerable.²¹⁻²³ Better results regarding clinical efficacy and tolerability have been reported using chemically modified extracts in ragweed and grass allergic rhinitis,^{24,25} though these results have not been confirmed by other authors.¹⁶

The low dose of cromolyn, used as premedication (10.4 mg, alternate day), is in our opinion negligible as a therapeutic measure, but it is sufficient to reduce local discomfort and, perhaps, possibly explains the lack of priming effect caused by the repeated intranasal administration of allergen.

Although the mechanism of LNIT is not known, four possibilities for its therapeutic action may be

TABLE II. Overall self-evaluation after treatment

Improvement	Active treatment group	Placebo treatment group
Poor	3	11
Moderate	4	3
Good	4	1
Excellent	2	0

$\chi^2 = 8.414$; $0.02 < p \leq 0.05$.

suggested: (1) It may induce increased nasal specific IgA and IgG. However, previous studies have revealed no clinical and immunological correlation.²⁴⁻²⁶ (2) There may be a systemic immunologic action, shown by the increase of serum specific IgE, reported using intranasal aqueous extract,²⁶ as well as by the relevant allergenic activity observed in animal models after intranasal treatment with allergen in powder form.²⁷ In the present study the lack of modifications of skin reactivity and

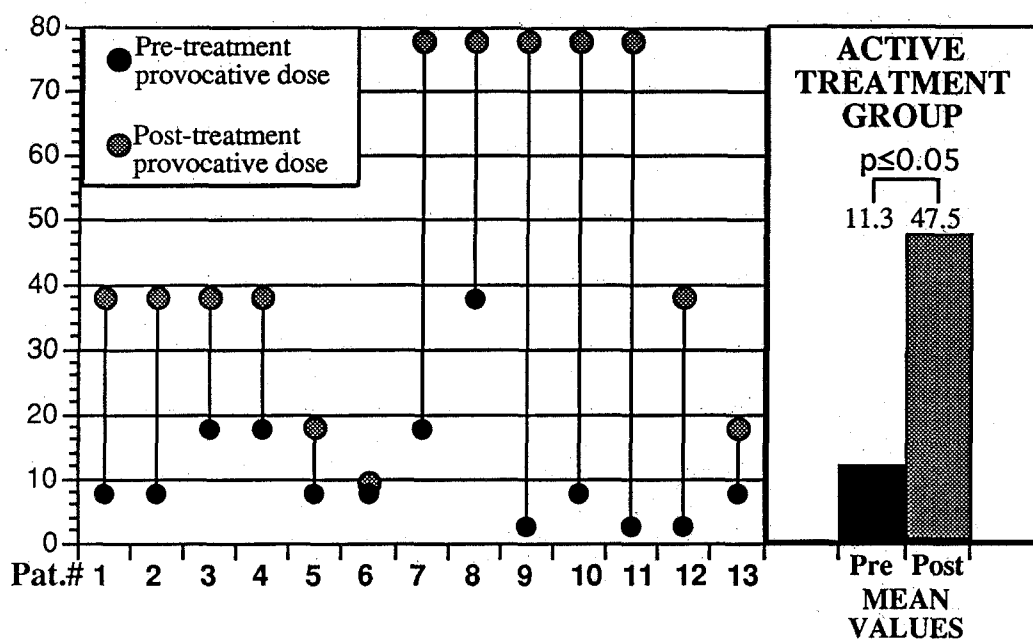


FIG. 5. Nasal specific threshold doses of each patient belonging to the active treatment group and mean specific threshold dose of the active treatment group before and after treatment.

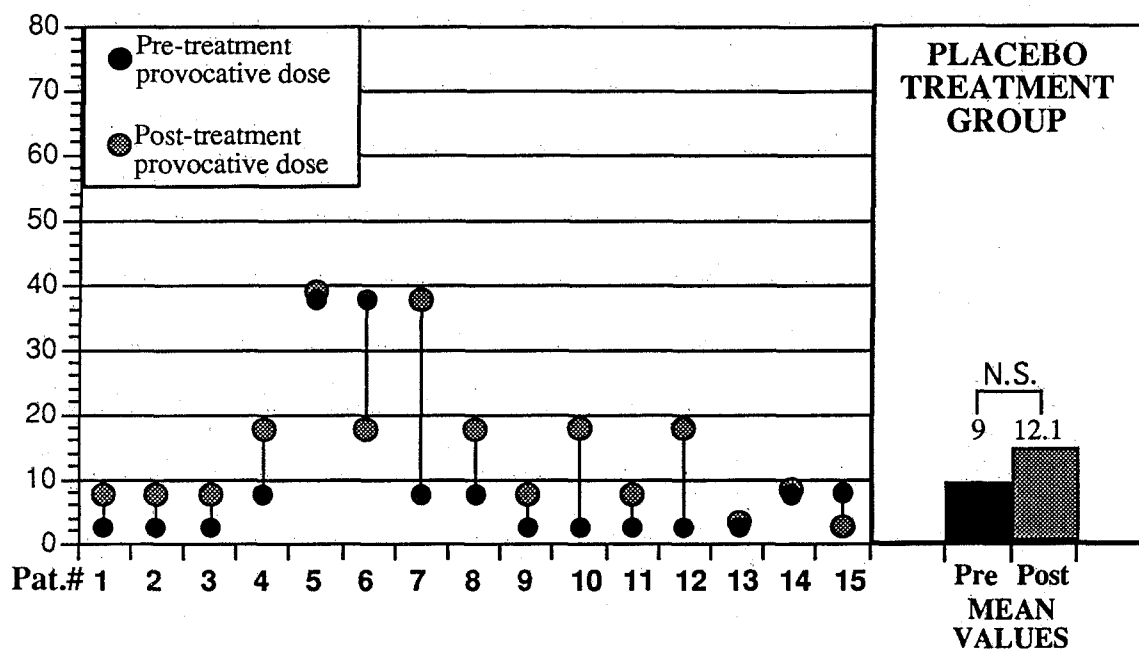


FIG. 6. Nasal specific threshold doses of each patient belonging to the active treatment group and mean specific threshold dose of the placebo treatment group before and after treatment.

specific IgE levels proved little systemic effect of this LNIT. On the other hand, the efficacy of the treatment on eye symptoms does suggest a systemic effect. (3) There may be a decrease of the histamine release as shown by the reduction of the early response to the specific nasal provoca-

tion and a reduction of intercellular adhesion molecule 1 expression on epithelial cells on specific nasal challenge.^{28,29} (4) There may be an induction of a local tolerance caused by repeated nasal administration of high dosages of allergen extracts, as demonstrated in animal models.³⁰

Together with the clinical efficacy, the safety and compliance of this kind of immunotherapy has to be underlined. The lack of systemic and asthmatic reactions in subjects without asthma or bronchial hyperresponsiveness (expressed by a $PD_{20} > 1.2$ mg/ml) allows for its use at home, so avoiding recurrent injections and medical calls.

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