

Local nasal immunotherapy for *Dermatophagoides*-induced rhinitis: Efficacy of a powder extract

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Background: Local nasal immunotherapy by means of an extract in "macronized" powder form was studied in allergic rhinitis to *Dermatophagoides* species.

Methods: Twenty-four *Dermatophagoides*-sensitive patients were studied for 12 months in a double-blind controlled trial. Subjects were selected on the basis of a positive history, skin test, radioallergosorbent test, and intranasal challenge to *Dermatophagoides* antigen. Two 12-patient groups were selected at random; the first group was given active treatment, and the second received placebo.

Results: After 6 months, the mean weekly symptom and medication scores were significantly lower in the treated group than in the control group. The treated group had a significant increase of specific nasal threshold to *Dermatophagoides* antigen after treatment. Adverse reactions to local nasal immunotherapy, which were limited to the upper respiratory tract, occurred very rarely and did not interfere with dose schedule.

Conclusion: Local nasal immunotherapy in powder form may be a suitable alternative to the traditional subcutaneous immunotherapy in terms of clinical efficacy and safety. (*J ALLERGY CLIN IMMUNOL* 1993;91:987-96.)

Key words: Local immunotherapy, allergic rhinitis, *Dermatophagoides*, double-blind study

Intranasal administration of allergens has been used for several years. One of its applications is as a nasal provocation test for diagnostic purposes in patients affected with allergic rhinitis. The test consists of progressive increasing concentrations of allergen placed into the nostril. A positive reaction is determined by a rhinomanometer or by the development of symptoms such as sneezing, rhinorrhea, and nasal obstruction.¹ The provocative threshold dose is determined by this procedure. The nasal provocation test is useful both to confirm the diagnosis of allergy and to evaluate the efficacy of a specific hyposensitization treatment. In fact, one of the mechanisms of this treatment is the reduction of specific nasal reactivity, that is an increase in the threshold dose.²

The second application for intranasal administration

Abbreviations used

LNIT: Local nasal immunotherapy
Ig: Immunoglobulin
AU: Allergenic unit

of allergen is as a specific local nasal immunotherapy (LNIT). Progressive administration of allergen solutions are sprayed or placed directly into nostrils or inhaled.³⁻⁶ In the case of seasonal allergens (pollens) the administration is usually performed preseasonally. The treatment may be repeated for 2 or 3 years. LNIT has been reported to produce the following favorable modifications:

1. a decrease in nasal reactivity to the offending allergen⁷⁻¹⁰
2. local production of allergen-specific antibodies belonging to IgA and IgG class in nasal secretions^{2, 11, 12, 13, 14}
3. reductions of rhinitis symptoms and medication usage^{4, 7, 11, 15}

Controlled studies of LNIT did not induce every change but rather one change in one study and not in

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TABLE I. Controlled studies of local nasal immunotherapy

Study	Treatment	Amount	Symptom/medication score			Nasal sensitivity
			Nasal	Eye	Med	
Taylor and Shivalkar ¹⁰	Grass	NR	+	+	+	↓
Johansson et al. ²	Grass allergoid	280,000 PNU	+	—	—	↓
Welsh et al. ⁶	Ragweed	NR	+	+	NR	NR
Schumacher and Pain ⁹	Grass allergoid	3.68 mg	—	—	—	↓
McLean et al. ⁴⁰	Ragweed	<7 µg AgE	±	±	NR	NR
Mathews et al. ³⁵	Polymerized ragweed	710 µg AgE	—	NR	—	↑
		63,000 PNU				
Nickelsen et al. ¹⁵	Polymerized ragweed	60 µg AgE	+	+	+	↑
		350 µg AgE	+	—	+	↑
Georgitis et al. ¹¹	Polymerized ragweed	544 mg AgE	+	—	+	NR
Horak et al. ⁸	Grass powder	NR	+	—	+	↓
Andri and Senna ⁷	Parietaria powder	5350 A.U.	+	—	+	↓

+, Treatment benefit; —, no benefit; ±, beneficial but not statistically significant; ↑, increase after treatment; ↓, decrease after treatment; NR, not reported.

TABLE II. Demographic data of study population

	Active extract-treated	Placebo-treated
Patients (n)	12	12
Mean age (yr) (± SD)	19.5 (± 4.6)	19.4 (± 3.8)
Sex (M/F)	7/4	6/4
Months of disease (± SD)	42 (± 30.6)	42 (± 17.2)

another (Table I). In these studies preparations consisted of aqueous allergen solutions and were administered into the nostril by means of squeeze bottles.

Disadvantages of the aqueous preparation are poor stability as a result of container absorption and self-digestion. Allergen molecules may be observed on the inner surface of the container. This phenomenon can cause a considerable loss of allergenicity, especially in diluted solutions.¹⁶ This can be prevented by use of surfactants (e.g., polysorbate preparations) or inert proteins (e.g., albumin), but false reactions may develop.¹⁷ Self-digestion is a phenomenon that is due to the presence of proteolytic enzymes in the extract, which can denature allergens. Partial purification of allergenic extract can prevent this phenomenon, but self-digestion may be unavoidable when the allergen is an enzyme. For example, *Der p* I, the main allergen

in house dust mite, *Dermatophagoides pteronyssinus* is a cysteine protease.¹⁸

Another disadvantage of aqueous extracts is a difficulty in dosing an extract amount of allergen. In fact, metered dose inhalers are not used for allergen products because of chemical incompatibility between the aqueous solution and propellant. The unique way to administer aqueous solutions is as a spray with the use of a squeeze bottle. Unfortunately, there is a poorly reproducible dosage, which varies according to the pressure applied to the squeeze bottle.

Further progress in LNIT may be made by the introduction of a lyophilized "macronized" powder (partition value = 45 µm) resulting in allergen stability and standardization.¹⁹ In a previous study we reported the clinical efficacy and safety of powder nasal immunotherapy in *Parietaria*-induced allergic rhinitis.⁷ The present study evaluated the efficacy of LNIT with allergen in powder form in the perennial rhinitis that is caused by *D. farinae* and *D. pteronyssinus*.

METHODS

Subjects

Twenty-four subjects (15 men and 9 women) who had experienced allergic rhinitis for at least 2 years were selected. The mean age was 19.45 years (standard deviation = 4.161 years) (Table II). Criteria for inclusion in the study were:

1. a history typical for perennial-allergic rhinitis
2. a 3⁺-4⁺ positive skin-test to *Dermatophagoides ex-*

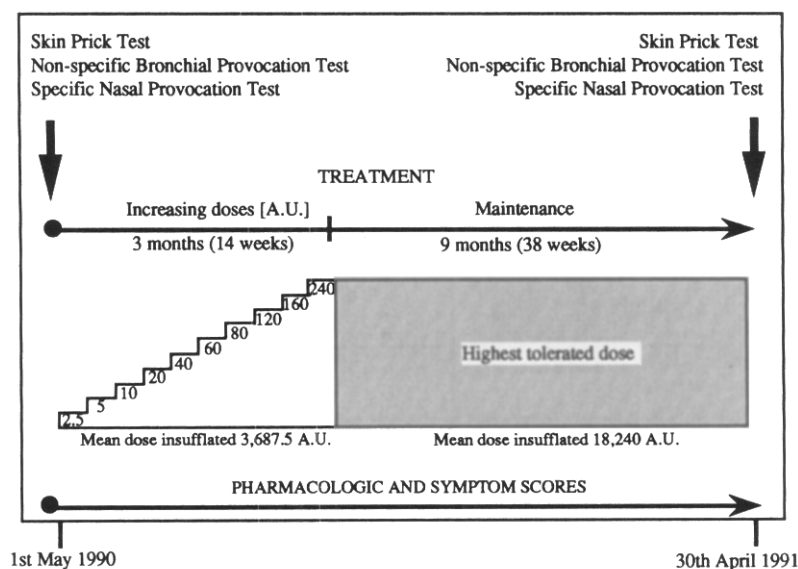


FIG. 1. Study design.

TABLE III. Rating scale for patient assessment of symptoms

Symptom	Rating
Nasal blockage	0 = Breathing through the nose freely and easily 1 = Slight difficulty breathing through the nose 2 = Moderate difficulty breathing through the nose 3 = Breathing through the nose very difficult/impossible
Sneezing	0 = Absent 1 = Occasionally present (less than 5 episodes per day) 2 = Troublesome episodes of sneezing (5-10 episodes/day) 3 = Frequent, troublesome episodes of sneezing (>10 episodes/day)
Nasal itching	0 = Absent 1 = Present, mostly unaware of it 2 = Present, but not a persistent distraction 3 = Present, a persistent distraction
Rhinorrhea	0 = Absent 1 = Present, mostly unaware of it 2 = Present, but not a persistent distraction 3 = Present, a persistent distraction
Eye watering/irritation	0 = Absent 1 = Present, mostly unaware of it 2 = Present, but not a persistent distraction 3 = Present, a persistent distraction

tract in glycerinated solution (5% wt/vol) (Lofarma Allergeni, Milan, Italy); negativity to other inhaled allergens: pollens of grass, *Parietaria officinalis*, mugwort and trees (*Olea europaea*, birch, hazelnut, alder), molds (*Alternaria tenuis*, *Cladosporium* species), cat and dog dander

- a RAST positivity to *Dermatophagoides* of at least class 2
- a positive response to specific nasal provocation test

In addition, each subject gave informed consent before entry into the study. Patients were excluded because of: asthma, long-term use of nasal corticosteroids and/or decongestants, prior specific parenteral immunotherapy, other allergen sensitivity, pregnancy or lactation, or presence of nasal polyps.

Antigen

Active product, which was supplied by Laboratorio Farmaceutico Lofarma (Milan, Italy), is an antigen in powder

		SECOND SESSION			
FIRST SESSION	Provocative threshold	2.5 A.U.	5 A.U.	10 A.U.	20 A.U.
	2.5 A.U.	●	● ●		
	5 A.U.	●	● ●		
	10 A.U.			● ● ● ●	●
	20 A.U.			●	

FIG. 2. Reproducibility of the specific nasal provocation test; correlation between first and second session ($r = 0.843$; $p \leq 0.001$).

prepared from bodies of *D. pteronyssinus* and *D. farinae* incorporated into an excipient mass (lactose) and dehydrated. For particle-size analysis, the powder was suspended in silicone oil, sonicated, and analyzed with a laser-light-powered (wavelength 632.8) analyzer (model 715, Cilas Alcatel, Marcoussis, France): 75% of particles were between 15 and 80 μm with partition value (50/50%) around the dimension of 45 μm . This product, enclosed in hard gelatin capsules characterized in dosages by 10 different colors, is titrated in allergenic units (AU), with reference to an internal standard. One AU corresponds to 7.5 ± 1.5 IU, as determined by a comparative RAST-inhibition analysis between the internal (in house) standard and the International Union Immunological Society–World Health Organization First *Dermatophagoides pteronyssinus* International Standard.²⁰

The placebo consisted of lactose, and in exterior it was similar to the active products.

Immunotherapy program

The design of the study was a double-blind placebo-controlled trial. LNIT was carried out by self-administration and was continued for 12 months (from May 1, 1990 to April 30, 1991) according to two different schedules (Fig. 1).

The first schedule (14 weeks) consisted of administration of increasing allergen doses, on alternate days in alternate nostrils. The powder was administered into the nostrils with a manual nasal insufflator (MIAT, Milan, Italy). Premedication with cromolyn sodium (10.4 mg) 20 to 30 minutes before treatment was carried out in each patient in both groups, to reduce local side effects. In order 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 the next dose was given.

The second schedule (maintenance period) was carried out for 9 months; each patient was treated with maximal tolerated doses (240 AU) twice weekly.

The total dose administered in the first schedule was 3,687 AU (27,656 IU), and the total dose for the second schedule was 18,240 AU (136,800 IU).

Experimental design

Patients were randomly divided in two groups according to randomization tables.²¹ group 1 (12 subjects) received placebo; group 2 (12 subjects) received active *Dermatophagoides* extract. Before and after LNIT, the following tests were performed: skin test reactivity, bronchial provocation, and nasal challenge (Fig. 1).

Skin reactivity was assessed by prick test with *Dermatophagoides* extracts²² (Lofarma Allergeni). Wheals were outlined with a fine-point marker and transferred via transparent membrane onto permanent records. The areas were measured by planimetry and expressed in square millimeters.

Sera were assayed for IgE antibodies to *Dermatophagoides* by Sferikit RAST (Lofarma Allergeni). Results were calculated as percentages of bound iodine-125–labeled anti-IgE. Nonspecific bronchial provocation test was performed with methacholine until the cumulative dosage of 1260 μg was reached, in order to exclude significant nonspecific bronchial hyperreactivity. Nasal 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) delivered by a manual rhinoin insufflator. Doses were administered in 20-minute intervals. Threshold doses of allergen were determined from the amount required: (1) to increase the total nasal resistance to airflow of 50% or more as compared with the inert control with the use of anterior rhinomanometry (RN4 Rhinomanometer, Markos, Monza, Italy)²³; (2) to elicit at least two of the four symptoms: itching, sneezing, discharge, and obstruction.²⁴ To assess the reproducibility of this specific nasal provocation test we compared two specific challenges, which were carried out at a 1-week interval in 12 patients who were randomly chosen from the study group.²⁵ The threshold doses obtained from the two challenge sessions correlated closely ($r = 0.843$; $p \leq 0.001$) (Fig. 2).

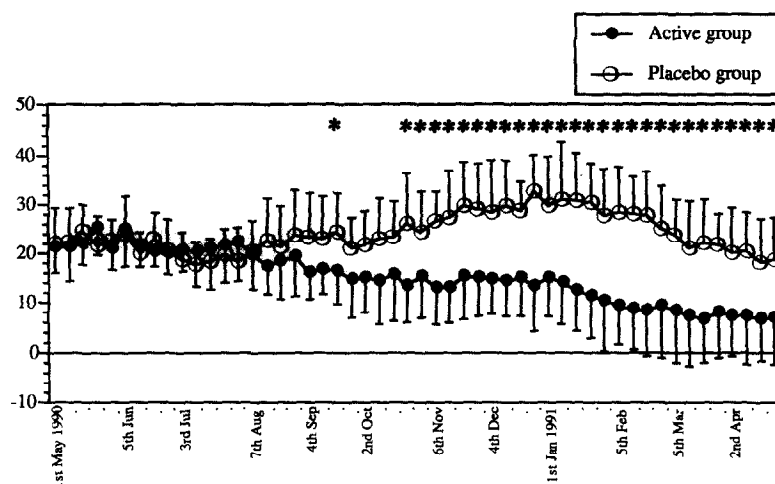


FIG. 3. Mean monthly symptoms score in treated (solid circles) and placebo (open circles) groups.

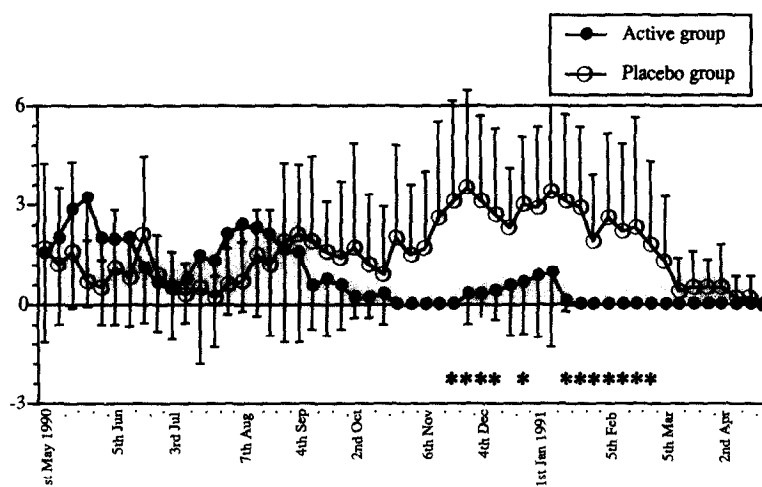


FIG. 4. Mean monthly medication score in treated (solid circles) and placebo (open circles) groups.

Clinical evaluation

The patients were seen monthly to evaluate compliance with the treatment. A clinical assessment was performed at the beginning and at the end of the study, after 12 months of treatment.

Both LNIT-treated and control patients kept a daily report of allergic symptoms during the treatment period. Daily symptoms-medication scores were tabulated according to each patient's records. Specific symptoms recorded were nasal obstruction, sneezing, runny nose, and eye irritation and watering. Patients graded each symptom daily for severity from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe) according to the criteria shown in Table III. The amount of antihistamine (terfenadine 60 mg) used was also recorded. The patients were instructed to use this drug when needed. Dust avoidance measures (bedrooms should

be accurately cleaned with a vacuum cleaner twice weekly, windows should be kept completely open for at least 1 hour per day and slightly opened during the entire day, humidifiers should not be used) were suggested to all patients. Patient self-evaluation was done at the end of the study before opening the blinded code. Each patient was asked for his or her overall evaluation of this treatment with categories of *not improved*, *improved*, or *asymptomatic*.

Statistical analysis

Statistical analysis was done with nonparametric tests. Spearman's rank-correlation test was used for regression analysis, the Mann-Whitney U test was used for impaired comparisons, and the Wilcoxon W test was used for paired data. The chi square test was used to test the significance of differences among the overall evaluations, which were

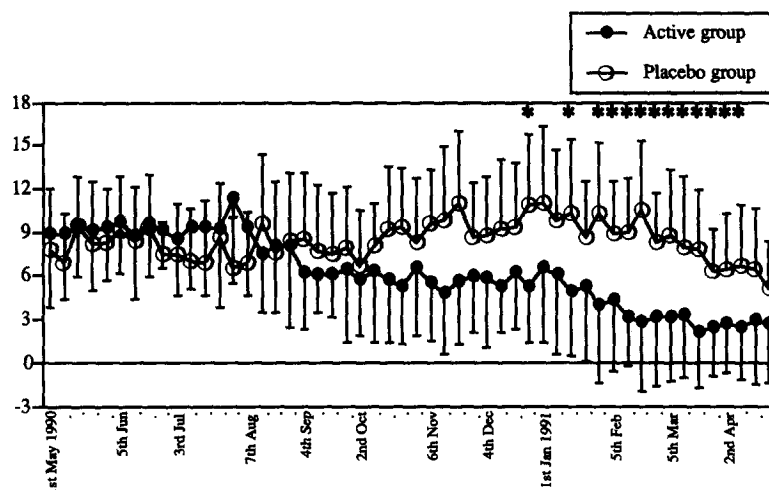


FIG. 5. Mean monthly score of nasal obstruction in treated (solid circles) and placebo (open circles) groups.

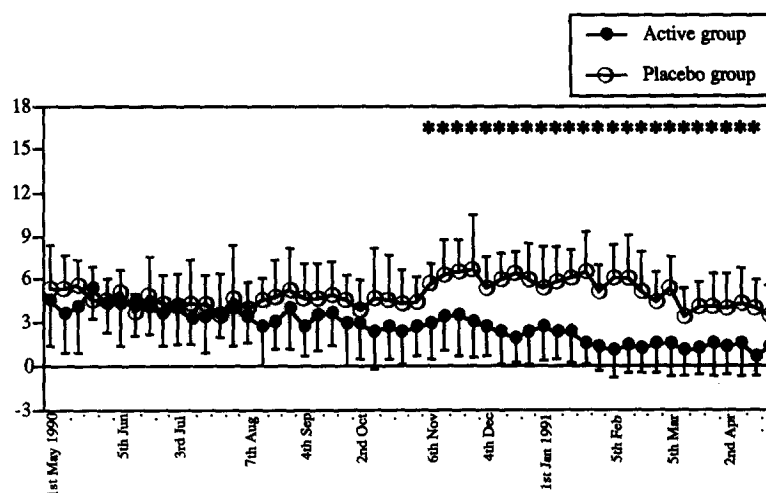


FIG. 6. Mean monthly score of sneezing in treated (solid circles) and placebo (open circles) groups.

TABLE IV. Individual monthly global symptom scores of patients belonging to the active group

Month	Patient No.										
	1	2	3	4	5	6	7	8	9	10	11
May	80	89	97	63	102	74	117	82	67	97	115
Jun	74	96	61	89	82	81	110	87	87	89	110
Jul	121	84	100	85	75	103	157	78	132	90	108
Aug	86	35	85	79	93	98	61	71	96	55	90
Sep	50	47	77	59	61	82	56	71	62	62	119
Oct	73	65	70	11	0	90	101	73	119	90	104
Nov	65	53	59	3	0	70	73	54	77	77	86
Dec	41	59	62	35	0	58	60	57	98	88	88
Jan	40	71	111	33	0	27	77	68	73	107	113
Feb	0	49	87	0	0	15	21	53	34	45	91
Mar	0	38	79	0	0	0	0	50	55	10	113
Apr	0	35	100	0	0	0	7	41	43	8	113

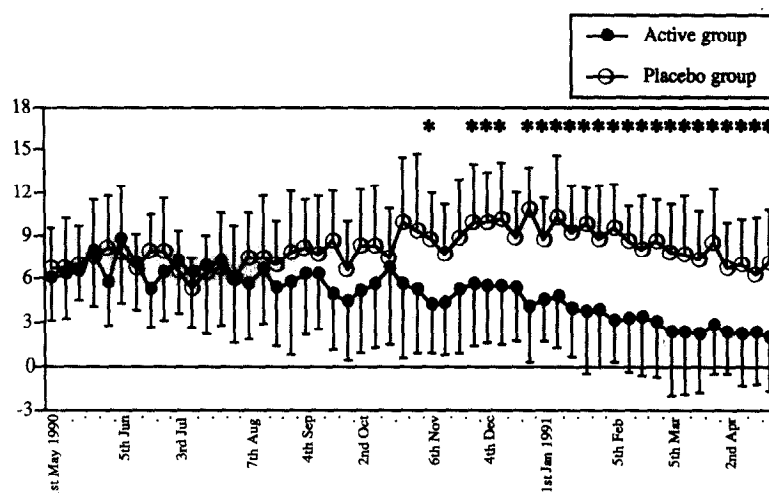


FIG. 7. Mean monthly score of rhinorrhea in treated (*solid circles*) and placebo (*open circles*) groups.

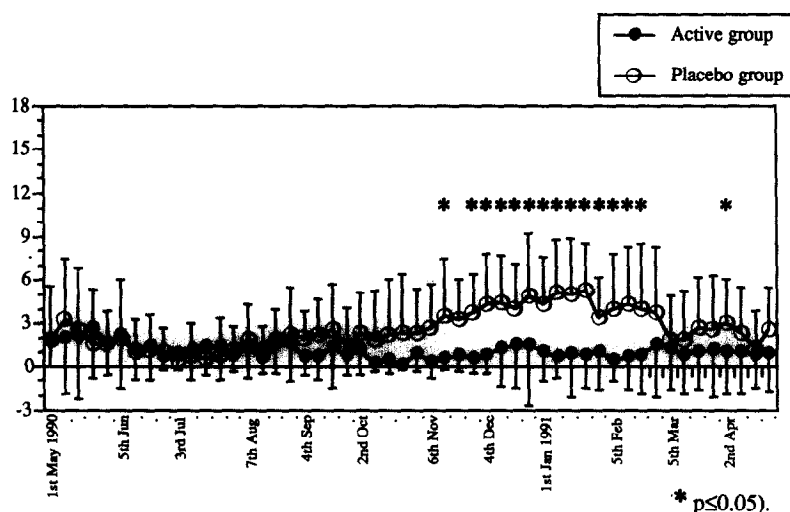


FIG. 8. Mean monthly score of eye irritation and watering in treated (*solid circles*) and placebo (*open circles*) groups.

TABLE V. Individual monthly global symptom scores of patients belonging to the placebo group

Month	Patient No.									
	1	2	3	4	5	6	7	8	9	10
May	85	81	98	55	74	99	97	71	115	129
Jun	80	102	92	61	88	86	109	89	110	78
Jul	102	102	63	68	76	103	115	130	108	79
Aug	100	54	42	59	78	102	131	86	90	81
Sep	116	58	57	76	103	104	139	45	119	122
Oct	107	86	125	176	73	122	174	83	104	94
Nov	75	99	136	142	73	123	154	85	86	105
Dec	70	134	135	155	72	143	142	122	88	90
Jan	61	177	136	201	168	184	182	181	113	133
Feb	54	120	85	152	117	132	128	152	91	82
Mar	18	87	67	126	93	111	99	122	113	79
Apr	17	68	52	110	110	124	148	123	123	106

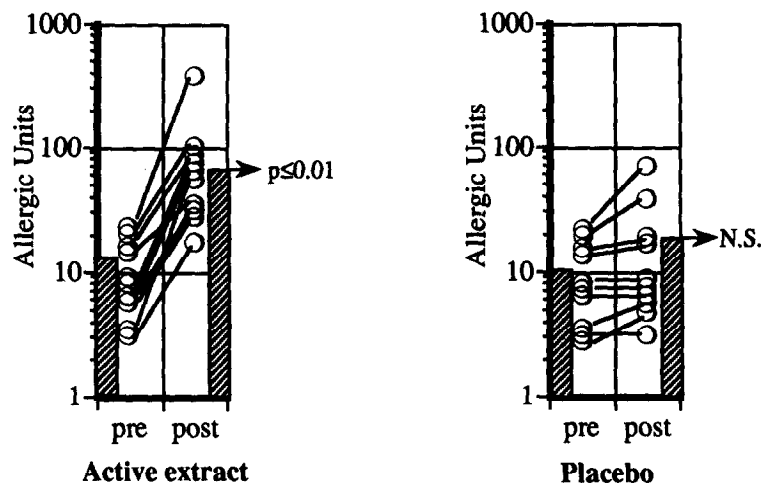


FIG. 9. Threshold doses of each patient (open circles) before and after treatment. Hatched bars indicate mean threshold dose of each group before and after treatment.

TABLE VI. Overall self-evaluation after treatment

Self-evaluation	Active-treated	Placebo-treated
No improvement	0	3
Fair improvement	5	5
Good improvement	4	2
Asymptomatic	2	0

Total $\chi^2 = 5.6$.

$0.1 < p \leq 0.2$.

expressed by each patient at the end of the study. Statistical analysis was performed with StatView program (Brainpower, Inc., Calabasas, Calif.) on a Macintosh microcomputer (Apple Computer, Inc., Cupertino, Calif.).

RESULTS

Two patients in the control group and one in the treated group withdrew from the study because of logistic problems. There were no systemic reactions during the treatment. Minimal local adverse reactions immediately after powder administration were reported by four patients in the treated group and by one patient in the control group. These mild side effects did not interfere with dose schedule. No capsules were returned during the treatment by the patients who completed the trial. The mean monthly symptom and medication scores for each group were calculated by summing the individual symptom scores for each patient for each month and dividing by the number of patients within each group. After 6 months a significant difference was observed in mean monthly total symptoms and medication score in comparison with the control group (Figs. 3 and 4). Mean symptom scores were also lower in the treated group when each

nasal symptom was considered separately: rhinorrhea, obstruction, sneezing, eye irritation and watering (Figs. 5 to 8). Individual monthly global scores of the patients from both active and placebo-treated groups are shown in Tables IV and V. We observed a decrease in global monthly score in nine patients in the treated group and in two patients in the control group. Eight patients in the active group and two patients in the placebo group reduced the use of antihistamines during the study.

Overall evaluations from the patients who completed the trial were better but not statistically significant ($0.1 < p < 0.2$) in the treated group (Table VI). After treatment nasal sensitivity to *Dermatophagoides* allergens measured by nasal provocation test significantly decreased in the actively treated group, whereas no difference was observed in the control group (Fig. 9). There were no significant differences between the two groups with regard to skin reactivity, specific IgE, and aspecific bronchial hyperresponsiveness before and after treatment. Results of the bronchial provocation test with methacholine were negative in all patients before and after treatment. No significant difference was observed in the active group in mean area of *Dermatophagoides*-induced wheal before ($34.2 \pm 2.51 \text{ mm}^2$) and after treatment (active = $33.96 \pm 3.15 \text{ mm}^2$) and in *Dermatophagoides*-IgE-specific levels (before treatment: 17.43 ± 10.8 bound percent radioactivity; after treatment: 16.58 ± 9.2 bound percent radioactivity).

DISCUSSION

For several years LNIT has been used as an experimental treatment program mostly for seasonal allergic rhinitis. Intranasal spraying of high doses of unmo-

dified aqueous extracts has caused immediate rhinitis symptoms with definitive clinical efficacy.^{26, 27} On the other hand, low doses of unmodified aqueous extract have been well tolerated but without clinical efficacy.²⁸ More recently, chemically modified allergens with a reduced allergenicity (while still retaining immunogenicity) have been used in LNIT studies. These extracts have shown good clinical results in a dose-dependent manner, and local adverse effect have been rare.^{11, 27, 29} Recently preseasonal LNIT by means of freeze-dried allergen in powder form has been studied in allergic seasonal rhinitis. Controlled studies in adults^{7, 8, 30} and children³¹ have shown good clinical results and tolerability in grass-pollen- and *Parietaria*-induced rhinitis.

This study investigated LNIT with allergen extract in powder form for perennial allergic rhinitis caused by *Dermatophagoides* species. There are few studies on LNIT in perennial allergic rhinitis. After 26 weeks of treatment, we reported statistically significant lower total symptom scores in the treated group compared with the placebo group. A similar trend has been demonstrated if we consider each symptom one by one, especially the symptoms of sneezing and nasal secretions. The amount of antihistamine used in the same period was significantly lower in the treated group than in the control group. The small decrease in symptom score in the placebo group may be due to the suggested dust avoidance measures. A significant decrease of nasal reactivity to allergen was observed only in the treated group, which is similar to results of other studies.^{2, 7, 9, 10} An increase in bronchial specific threshold dose was observed in allergic bronchial asthma that was due to dust mite after bronchial local immunotherapy with allergen in powder form.³ There were no asthma attacks during the treatment, which confirms that macronized *Dermatophagoides* extract does not induce bronchoconstriction in allergic rhinitis.³² However, other authors have recently reported an increase in nonspecific bronchoreactivity after nasal challenges with solutions that contain allergen.³³ Low-dose cromolyn sodium (10.4 mg) premedication must be considered negligible as a therapeutic measure.

The mechanisms underlying the clinical benefits of LNIT are not clearly defined. Local immunotherapy has been shown to induce local immunologic responses. Some authors noted a significant increase in nasal specific IgA and IgG^{2, 11-13, 34}; however, clinical and immunologic findings do not correlate.^{11, 35} Alternatively, patients who were treated locally with aqueous extract showed a measurable systemic response, by a marked increase in serum IgE during the treatment period, which was not followed by the ex-

pected coseasonal increase.^{4, 15} Welsh et al.,⁶ on the other hand, noted no change in serum IgE and IgG when aqueous extract was used.^{6, 26} The reduction of ocular symptoms in the treated group may suggest an effect of LNIT mediated by a systemic absorption. In a recent study³⁶ clinically relevant allergenic activity in the systemic circulation was revealed by a sensitive modified RAST (immunocapture RAST) in animal models after intranasal treatment with allergen in powder form. On the other hand, the absence of modification induced by LNIT on skin reactivity is not in favor of this hypothesis, and the quantity of allergen that can possibly reach the systemic circulation is lower than that obtained by means of subcutaneous immunotherapy. In animals passive antigenic stimulation of the respiratory mucosa may have a protective function. It has been shown that repeated inhalation of aerosols that contain ovalbumin or ragweed antigen induced a transient IgE response in rats, which ceased spontaneously despite continuing aerosol exposure. Subsequent challenge of the animals revealed that aerosol exposure had selectively suppressed responsiveness to these antigens in the IgE class without affecting corresponding IgG responses.³⁷⁻³⁹

We propose LNIT as an alternative to the traditional subcutaneous treatment. The advantage of this hyposensitizing therapy compared with the traditional treatments by subcutaneous injection seems to be significant safety, since there were no severe or systemic side effects noted. In addition, LNIT results in a rapid and specific clinical response. Furthermore, patients report a better compliance because of the avoidance of injections and fewer physician visits. Further investigations are needed to clarify the mechanism, the optimal length of treatment, the duration of therapeutic effect after stopping treatment, and the clinical efficacy in direct comparison with traditional parenteral immunotherapy.

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