

A double-blind, placebo-controlled trial of local nasal immunotherapy in allergic rhinitis to *Parietaria* pollen

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Summary

We assessed the efficacy and safety of local nasal immunotherapy (LNIT) using an extract in macronized powder form of *Parietaria* pollen, a very important allergenic plant in the Mediterranean and other parts of the world. Twenty-six patients aged 13–37 years, with seasonal allergic rhinitis to this pollen, were enrolled in a double-blind placebo-controlled trial, carried out from autumn 1991 to the end of June 1992. They were selected on the basis of a positive skin-prick test, radioallergosorbent test (RAST) and intranasal challenge to *Parietaria* antigen. Patients were randomly divided into two groups of 13; the first group was given *Parietaria* antigen, and the second placebo. We recorded mean weekly symptom scores and drug consumption for 17 weeks during the pollen season in the year 1992, and specific serum-IgE and IgG levels. Three patients in the active group withdrew from the study because of bronchial symptoms. A significant difference was observed in mean weekly nasal symptom scores, in drug consumption and in specific nasal threshold to *Parietaria* allergenic extract in the treated and control groups. No difference was observed in serum IgE and IgG levels. Serum IgE levels rose significantly only in the control group after the pollen season. This study indicates that LNIT may be a useful alternative to traditional subcutaneous immunotherapy in patients with allergic rhinitis.

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Introduction

The efficacy of specific immunotherapy (SIT) for the treatment of allergic rhinitis pollinosis in patients has been confirmed in controlled clinical trials [1–5]. However, SIT has frequently been criticized and is now recommended in these nasal conditions only if drugs such as local corticosteroids in association with new-generation anti-H1-anti-histamines give no therapeutic gain. This attitude is to some extent justified for classical subcutaneous SIT, considering the multiple injections required over several years, with all their possible side-effects, instead of a practical pharmacological treatment for a nasal disorder that frequently lasts only 30–40 days a year [6].

Drugs, however, can only eliminate or reduce the nasal symptoms, and do not modify the long-term course of allergic disease. SIT, however, is an integral part of their treatment since it usually reduces their severity and improves the patient's quality of life [1,6].

With the aim of improving both the safety of SIT and the patients' compliance, allergens have been investigated by other methods rather than the subcutaneous route.

SIT administered locally into the shock organ has been tried in patients with allergic rhinitis, and local nasal immunotherapy (LNIT) has been proposed for various years as an alternative to traditional subcutaneous SIT. Since the use of aqueous extracts is frequently associated with significant rhinitis symptoms, we preferred to use an allergenic extract in powder form to treat patients with allergic rhinitis to *Parietaria* pollen, a very important allergenic plant in the Mediterranean area, with a very long pollination period.

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Table 1. Demographic data

	Placebo treated	<i>Parietaria</i> treated
Number of patients	13	13
Mean age (years $\pm$ SD)	26.8 $\pm$ 9	24.8 $\pm$ 7.4
Sex (M/F)	5/8	6/7
Years of disease ($\pm$ SD)	3.5 ($\pm$ 1.2)	3.7 ($\pm$ 2.1)

Materials and methods

Patients

The randomized double-blind trial involved 26 patients (15 females and 11 males) aged between 13 and 37 years (Table 1). Inclusion and exclusion criteria are summarized in Table 2. All patients gave informed consent before entry into the study.

Study design

Patients were randomly assigned to two groups (Fig. 1): the first group received placebo, the second group was treated with active *Parietaria* extract.

Before therapy which was started in the autumn of 1991, the following baseline data were collected: skin-prick tests (SPT), specific nasal provocation tests (sNPT), non-specific bronchial provocation test (NsBPT), serum samples (Sm). The same data were collected again at the end of the study in June 1992. Sm, SPT and NPT were also obtained at the beginning of the *Parietaria* pollen season.

From 2 March 1992 to 26 June 1992 (17 weeks) daily

Parietaria pollen count (grains/m³ of air), symptom scores and medication used by patients were recorded. At the end of the study patients were asked to assess the pattern of nasal symptoms.

Allergenic extracts and LNIT

An active product and a placebo (both kindly supplied by Laboratorio Farmaceutico Lofarma, Milan, Italy) were used for the study. The native product was prepared from *Parietaria judaica* — *P. officinalis* (50–50 mix) pollen extract, standardized in allergenic units (AU) by a inhibition — RAST technique in comparison with in-house reference preparations (IHR). Titration in international units (IU) was not possible because no *Parietaria* international reference standard is available. The native product extract was incorporated into an inert excipient mass (lactose) and dehydrated. For particle size analysis, the powder was suspended in silicone oil, sonicated and analysed with a laser light-powered (wave-length 632.8 nm) analyser (Cilas mod. 715, France): 70% of particles were 25–80 μ m (mean value 45 μ m). Hard gelatine capsules were filled with 25 ng of

Table 2. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• A history of rhinitis during <i>Parietaria</i> pollen season for at least 3 years • A 3⁺ or greater positive skin-prick test to <i>Parietaria</i> extract in glycerinated solution (5% p/w) (Lofarma Allergeni Milan Italy) • A RAST positivity to <i>Parietaria</i> of at least class 3 • A positive NPT to <i>Parietaria</i> pollen extract	• Associated bronchial asthma or chronic cough • Presence of perennial nasal symptoms • Previous specific immunotherapy • Chronic use of topical corticosteroids and/or decongestants drugs • Sensitization to other allergens • Pregnancy or lactation • Nasal polyps or other nasal abnormalities

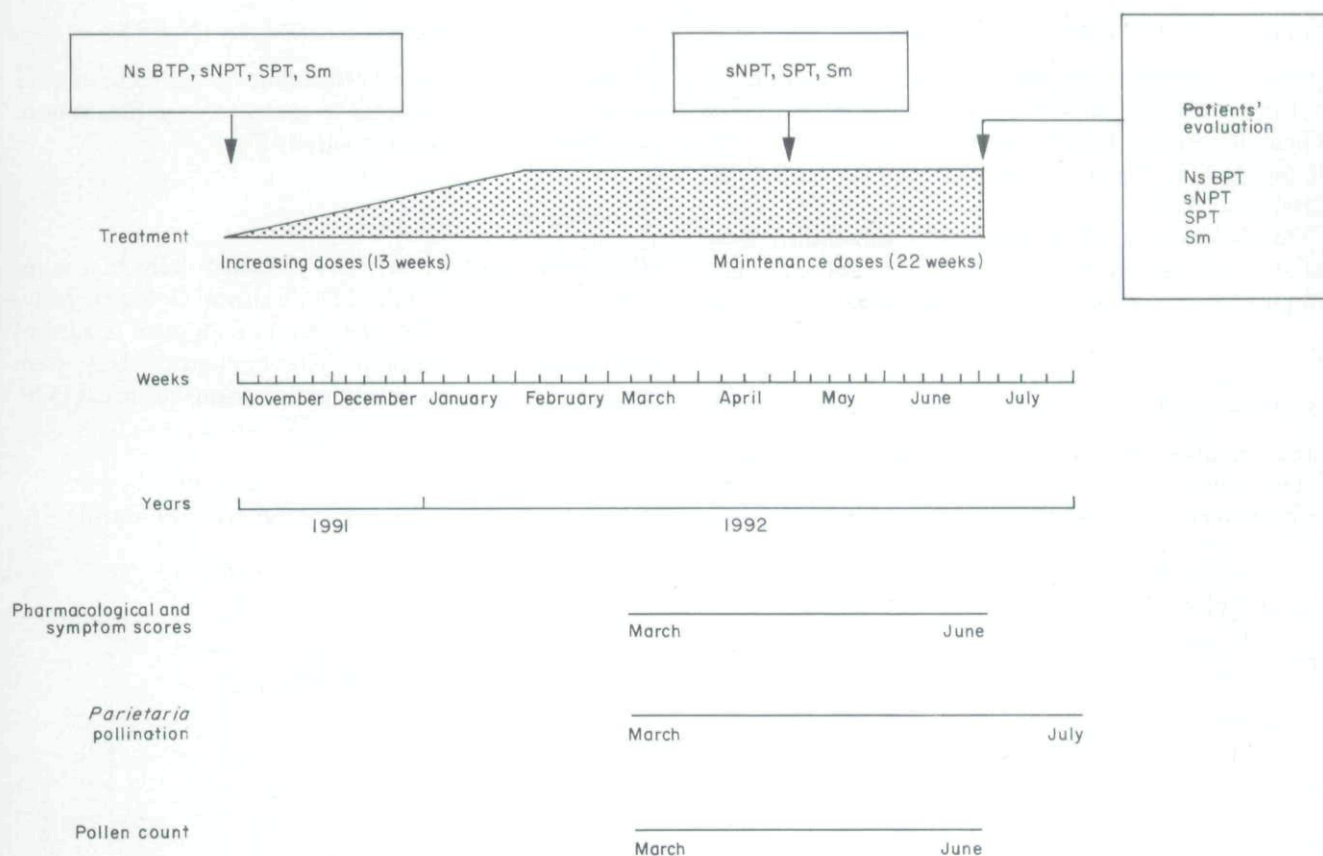


Fig. 1. Study design. Ns BPT. Non-specific bronchial provocation test. sNPT. Specific nasal provocation test. Sm. Serum measurement. SPT. Skin-prick test.

the powder, at increasing doses: 2.5–5–10–20–40–60–80–120–160 and 240 AU. The powder was administered into the nostrils with a nasal insufflator (MIAT, Milan, Italy).

The placebo consisted of lactose and looked identical to the active product.

Placebo and active LNIT were self-administered from November 1991 to July 1992 according to two schedules (Fig. 1):

- 1 The first schedule (13 weeks) consisted of increasing doses of the allergen using the special nasal insufflator, alternate nostrils on alternate days. Each patient received cromolyn (5.2 µg) 15–30 min before treatment. To prevent the allergen-powder entering the bronchial tree, patients were advised to vocalize during administration. Each of the doses listed above was taken four times.

- 2 The second schedule (maintenance period) lasted 22 weeks. Each patient was treated twice weekly with the maximal tolerated doses (240 AU) in eight patients, 160 AU in two and 120 AU in one).

Skin-prick tests

Skin-prick tests (SPT) were done in duplicate with *Parietaria judaica* allergenic extract (Lofarma Laboratories, Milan, Italy) using decreasing concentrations (2%, 1%, 0.5%, 0.1% w/v) and a standardized pricker, according to the 'end-point method'. A positive histamine control (10 mg/ml) was also done. The weals were outlined with a fine-point marker and transferred onto personal case records using transparent tape. The areas were measured by planimetry and expressed in mm, and the mean of two dilutions was calculated. A weal of at least 3 mm or larger was considered the end-point dilution.

Specific nasal provocation test (sNPT)

Specific nasal sensitivity was evaluated by quantitative challenges with increasing concentrations of *Parietaria* pollen allergenic extract in powder form (2.5, 5, 10, 20, 40, 60, 80 AU). We used the same manual nasal insufflator as for LNIT.

Threshold doses of allergen were established from the amount required to elicit at least two of four nasal symptoms: itching, sneezing, discharge and obstruction.

Serum assays (Sm)

Serum samples were collected and *Parietaria* IgE antibodies were assayed (Sferikit RAST, Laboratorio Farmaceutico Lofarma, Milan, Italy).

Non-specific bronchial provocation test (NsBPT)

This test was done with methacoline up to the cumulative dosage of 1200 μ g in order to assess any significant non-specific bronchial hyperreactivity [7].

Pollen counts

Parietaria pollen grains were collected daily in a volumetric spore trap (VPPS 2000, Lanzoni Bologna, Italy) placed 25 m above the street level on an open terrace of 'A. Cardarelli' Hospital. Aerobiological data were expressed as the daily mean pollen grains/m³ of air [8,9].

Clinical symptoms

All patients kept daily symptom diaries during the

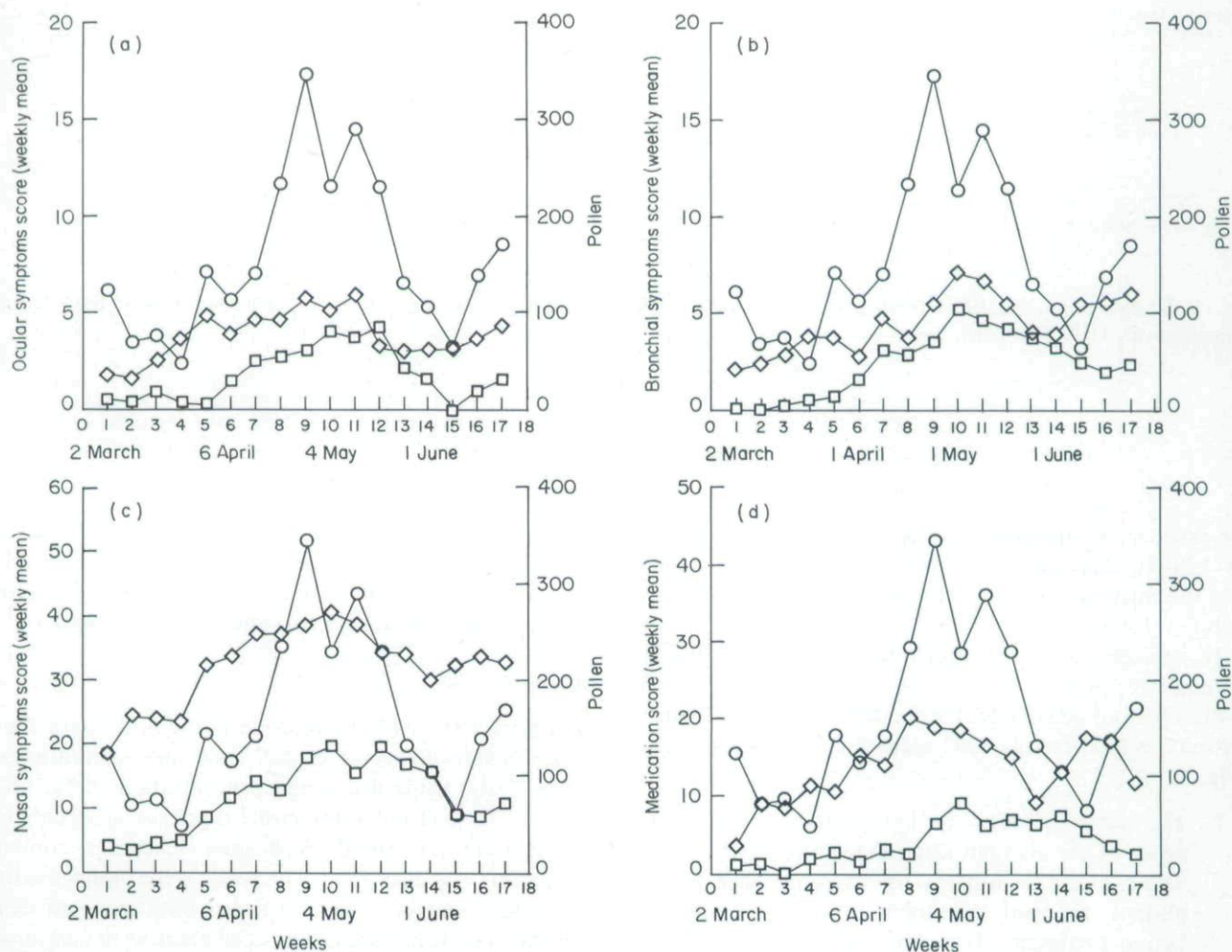


Fig. 2. (a) Ocular symptom scores (weekly mean values) in two patient groups. (b) Nasal symptom scores (weekly mean values) in the same two groups of patients. (c) Bronchial symptom scores (weekly mean values) in the two groups of patients. (d) Weekly mean values of drug consumption in the two groups of patients. □. Active group. ◇. Placebo group. ○. Pollen granules m³.

Parietaria pollen season from 22 March 1992 to 28 June 1992 in which they recorded nasal itching, nasal obstruction, sneezing, runny nose, eye irritation and watering. These symptoms were rated using a semi-quantitative scale from 0 to 4 (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe).

All patients also kept a daily report of the amount of symptomatic medication taken (terfenadine tablets, 60 mg orally when needed).

At the end of the trial each patient was asked for an overall assessment of the seasonal symptomatology as compared with previous seasons, rating it as worsened, not improved, little improved, much improved.

Statistical methods

Symptom and drug consumption scores in the active and placebo-treated groups were analysed by Friedman's test supplemented by Newman Keul's test, and by analysis of variance supplemented by Tukey's test.

Differences between symptom and drug consumption scores for the two groups were compared by the Mann-Whitney test. NPT results were analysed by the analysis of variance supplemented by Tukey's test and differences before and after treatment, or after season were evaluated with the Wilcoxon test. The same was done for serum-IgE and IgG levels.

Results

Three patients in the active group withdrew from the trial

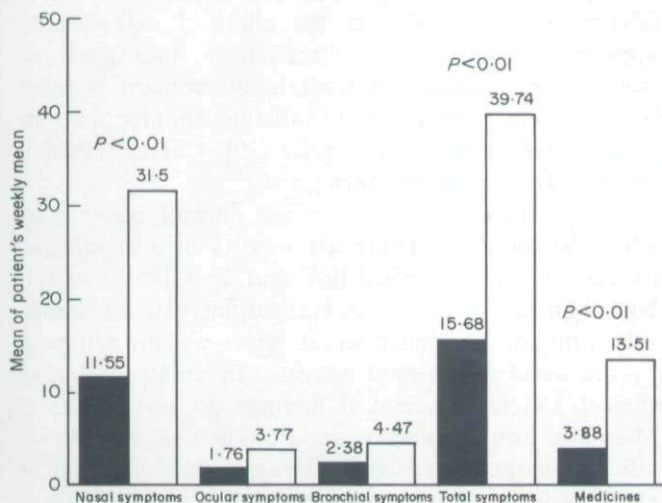


Fig. 3. Weekly mean values of clinical symptoms (nasal, ocular bronchial and total) and drug consumption in the two groups of patients during the pollen season. □. Active group. ■. Placebo group.

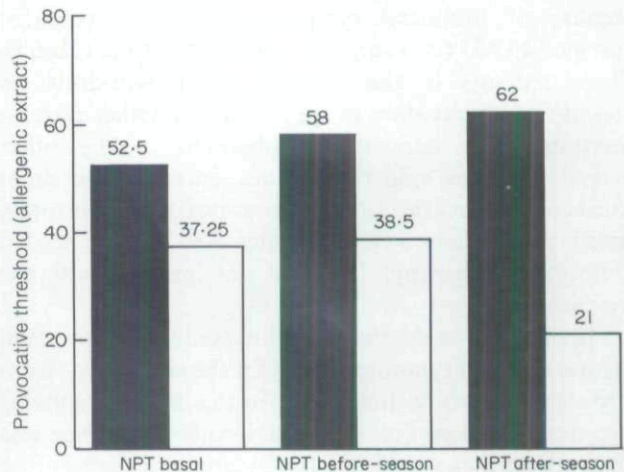


Fig. 4. Specific nasal threshold by using NPT with allergenic extract of *Parietaria* pollen in various times of the study in the two groups of patients. □. Active group. ■. Placebo group.

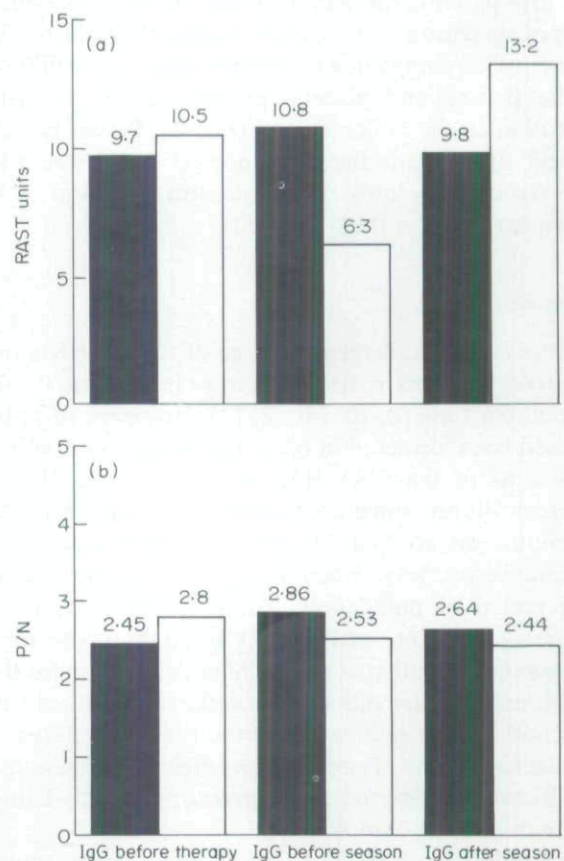


Fig. 5. (a) Serum IgE levels (RAST Units) to *Parietaria* pollen allergens in various times of the study in the two groups of patients. (b) Serum IgG levels in the two groups of patients. ■. Active group. □. Placebo group.

because of bronchial symptoms after two repeated doses of 40 AU (two subjects) and 60 AU (one subject). Three patients in the placebo group withdrew on account of aggravation of all symptoms. Other adverse reactions were occasionally observed in the other patients, such as mild rhinorrhoea, sneezing and slight nasal congestion after LNIT. These local reactions rarely lasted more than a few minutes and rarely needed symptomatic therapy. They did not interfere with the trial schedule.

Figure 2 shows the mean weekly ocular (a), bronchial (b) and nasal (c) symptom scores for the two groups from 2 March 1992 to 28 June 1992. In this figure is showed also drug consumption (d). A significant difference was observed in mean weekly nasal symptom scores during the 17 weeks. Mean weekly bronchial symptom scores were the same during the fourth and fifth week. Drug consumption differed significantly in the two groups, during weeks 4, 6, 7, 8, 9, 10, 11, 15 and 16.

Figure 3 shows the differences between nasal, bronchial, ocular and total symptoms and drug consumption in the two groups with the weekly mean for each patient in the pollen season. The specific nasal threshold to *Parietaria* pollen allergenic extract was significantly different in the treated and placebo groups only at the third control after the pollen season (Fig. 4). Serum IgE and IgG did not differ in the two groups (Fig. 5). Serum IgE rose significantly only in the control group after the pollen season ($P < 0.01$) (Fig. 5a).

Discussion

Parietaria pollen allergens are one of the most common causes of pollinosis in the Mediterranean area and the first in southern Italy (8, 10, 11, 12, 13). However, this plant has also been identified in other European countries [14], as well as in the USA [15] and in Australia [16]. Its allergens induce sometimes severe nasal and bronchial symptoms on account of their very high atmospheric concentrations, very strong allergenic properties and the long period of pollination. The pollen appears at the beginning of spring and persists throughout the spring and summer months, often reaching peaks of more than 500 grains per cubic metre of air in the beginning of June. A second, shorter pollination period runs from the end of August to October. The plant's prodigious pollen output, which ensures its reproductive success, frequently induces severe respiratory symptoms.

In Naples, about 80% of all pollinosis patients present sensitization to *Parietaria*, as an isolated allergen or in association with other aeroallergens [8,12]. *Parietaria* hay fever is therefore often a frustrating experience for both patient and clinician, since the common preventive

and symptomatic drugs are often ineffective in highly sensitized patients, so SIT seems indispensable. There are two studies [17,18] confirming the clinical efficacy of subcutaneous SIT in patients with respiratory allergy to *Parietaria*.

However, the search for an alternative way to administer allergenic extracts is justified by the need to limit the adverse effects of subcutaneous SIT without reducing its clinical efficacy. Before prescribing LNIT an accurate diagnosis of allergic rhinitis is essential in order to exclude associated sensitization of the lower respiratory tract and consequently the possibility of inducing bronchial asthma.

This study demonstrates the clinical effectiveness of LNIT in macronized powder form in the treatment of *Parietaria*-induced allergic rhinitis. The formulation proved safe and well tolerated.

Some of the patients who participated in the trial were followed for 1 year after it ended: nine continued LNIT and seven did not. Those continuing LNIT did not continuously need drugs, whereas patients who suspended LNIT needed extra symptomatic drug therapy during the 1993 spring pollination season.

On the same topic, Andri *et al.* [19] showed that LNIT using an extract in macronized powder form reduced drug consumption in allergic rhinitis to *Parietaria*. LNIT with aqueous ragweed extract was clinically effective, with a dose-relation [20–23]. LNIT was also effective in the treatment of grass-induced allergic rhinitis [24–26]. Nickelsen [27] and Wypych [26] used a polymerized ragweed extract (PRW) in order to avoid local side-effects. High doses of PRW have been clinically effective with few adverse reactions so this extract may offer advantages in LNIT over the allergoid extract that appears to have limited effectiveness. Two previous studies used allergenic extract in macronized powder form to treat grass-induced allergic rhinitis, one in children [28] and one in adults [29]. Clinical efficacy was significant and tolerance good.

The mechanism underlying the clinical benefits of LNIT is not clear. There are reports of a significant increase in specific nasal-IgA and IgG [26,27,30,31]. Nickelsen [30] observed a marked increase of serum IgE during the treatment period, which was not followed by the usual co-seasonal increase. In all these studies clinical and immunological findings do not correlate. Mistrello, using a sensitive modified RAST, found significant allergenic activity in the systemic circulation in animal models after intranasal treatment with *Parietaria* allergen in powder form [32]. Sedwick and Holt [33] demonstrated in animal models that nasal-associated lymphoid tissue (NALT) is the site of immune mechanisms which prevent sensitization to antigens which cross

epithelial barriers. More precisely, these authors demonstrated, by experiments of transfer of splenic lymphocytes from aerosol-exposed to virgin animals, that this immune mechanism consists in IgE-restricted tolerance mediated by T-suppressor cells. These studies confirmed the pivotal role of respiratory tract lymph nodes in controlling the local homeostasis within the IgE isotype. It is worthwhile to note that in LNIT the amount of antigens administered is much higher than in a course of natural pollen inhalation: it is well known in experimental models that a high-dose immunization weakens the IgE response.

Nevertheless, immunological evaluations of local and systemic antibody production after LNIT do not enable us to identify a precise immune mechanism. However, at the present status of our knowledge, the decrease of local (nasal) sensitivity to offending allergen could be evoked as the main mechanism of action of LNIT.

In conclusion, LNIT is an effective treatment for induced *Parietaria* allergic rhinitis. It is safe and can be self-administered by the patients with only periodical allergological controls. LNIT in powder form may be a suitable alternative to traditional subcutaneous immunotherapy in terms of clinical efficacy and safety. Further investigations are needed to clarify the mechanism of LNIT, the optimal length of treatment and the best dose schedule to enhance the efficacy of treatment.

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References

- 1 Bousquet J, Michel FB. Advances in specific immunotherapy (Review). *Clin Exp Allergy* 1992; 22:889-96.
- 2 Norman PS. Immunotherapy for nasal allergy. *J Allergy Clin Immunol* 1988; 81:992.
- 3 Varney VA, Gaga M, Frew AJ et al. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by anti allergic drugs. *Br Med J* 1991; 302: 265-9.
- 4 Brunet CV, Bedard PM, Lavoic A, Jobin M, Hebert J. Allergic rhinitis to ragweed pollen. Reassessment of the effects of immunotherapy on cellular and humoral responses. *J Allergy Clin Immunol* 1992; 89:76-86.
- 5 Ilipoulos O, Proud D, Adkinson NF et al. Effects of immunotherapy on the early, late and rechallenge nasal to provocation with allergen-changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 1991; 87: 855-66.
- 6 EAACI Subcommittee, Steering Committee: Immunotherapy (Position paper). *Allergy* 1993; 48(Suppl.):9-36.
- 7 Gruppo di lavoro 'Asma ed iperreattività' bronchiale' (Coordinatori: D'Amato G., Moscato G.). I test di provocazione bronchiale in allergologia respiratoria. *Giorn It Allergol Immunol Clin* 1992; 2:87-94.
- 8 D'Amato G, Lobefalo G. Allergenic pollens in the southern Mediterranean area. *J Allergy Clin Immunol* 1989; 83:116-22.
- 9 D'Amato G, Gentili M, Russo M et al. Detection of *Parietaria judaica* airborne allergenic activity: comparison between immunochemical and morphological methods including clinical evaluation. *Clin Exp Allergy* 1994; 24: 566-74.
- 10 D'Amato G, Ruffilli A, Ortolani C. Allergenic significance of *Parietaria* (Pellitory-of-the-wall) pollen. In: D'Amato G, Spiekma FThM, Bonini S eds. Allergenic pollen and pollinosis in Europe. Oxford: Blackwell Scientific Publications 1991; 113-8.
- 11 D'Amato G, Ruffilli A, Sacerdoti G, Bonini S. *Parietaria* pollinosis: a review. *Allergy* 1992; 47:443-9.
- 12 D'Amato G, Liccardi G. Pollen-related allergy in the European Mediterranean area. *Clin Exp Allergy* 1994; 24: 210-9.
- 13 D'Amato G, Dal Bo S, Bonini S. Pollen-related allergy in Italy. *Ann Allergy* 1992; 68:433-7.
- 14 Holgate SJ, Jackson L, Watson HK, Gandeerton M. Sensitivity to *Parietaria* pollen in Southampton area as determined by skin prick and RAST tests. *Clin Allergy* 1988; 18:549-56.
- 15 Kaufman HS. *Parietaria*: an unrecognized cause of respiratory allergy in the United States. *Ann Allergy* 1990; 64: 293-6.
- 16 Bass D, Baldo BA. *Parietaria* as a cause of asthma. *Med J Aust* 1984; 14:511-4.
- 17 Ortolani C, Pastorello EA, Incorvaia C et al. A double-blind placebo controlled study of immunotherapy with alginate conjugated extract of *Parietaria judaica* in patients with *Parietaria* hay-fever. *Allergy* 1994; 49:13-24.
- 18 D'Amato G, Kordash TR, Liccardi G et al. Immunotherapy with Alpare in patients with respiratory allergy to *Parietaria* pollen: a two year double-blind placebo-controlled study. *Clin Exp Allergy* 1995; 25:149-59.
- 19 Andri L, Senna GE, Betteli C et al. Local nasal immunotherapy in allergic rhinitis to *Parietaria*. A double-blind controlled study. *Allergy* 1992; 47:318-23.
- 20 Georgitis JW, Nickelsen JA, Wypych JI et al. Local nasal immunotherapy with high doses of polymerized ragweed extract. *Int Arch Allergy Appl Immunol* 1986; 81:170-3.
- 21 Nickelsen JA, Goldstein S, Mueller UF et al. Local nasal immunotherapy for ragweed allergic rhinitis. I. Clinical response. *J Allergy Clin Immunol* 1981; 68:33-40.
- 22 Welsh W, Zimmerman EM, Yunginger JW, Kern EB, Gleich GJ. Preseasonal intranasal immunotherapy with nebulized short ragweed extract. *J Allergy Clin Immunol* 1981; 65:191.

- 23 Welsh PW, Butterfield JH, Yunginger JW, Agarwall MK, Gleich GJ. Allergen-controlled study of intranasal immunotherapy for ragweed hay fever. *J Allergy Clin Immunol* 1983; 71:454-60.
- 24 Georgitis JW, Reisman RE, Clayton WF et al. Local intranasal immunotherapy for grass-allergic rhinitis. *J Allergy Clin Immunol* 1983; 71:71-6.
- 25 Schumacher MJ, Pain MCF. Intranasal immunotherapy with polymerized grass-pollen allergens. *Allergy* 1980; 37: 241-8.
- 26 Wypych JI, Georgitis JW, Tronolone MJ. Local nasal immunotherapy comparing polymerized and aqueous grass extract. *J Allergy Clin Immunol* 1984; 73:140.
- 27 Nickelsen JA, Georgitis JW, Wypych JI et al. Local nasal immunotherapy (LNIT) for allergic rhinitis with polymerized ragweed extract. *J Allergy Clin Immunol* 1983; 71 (suppl):188.
- 28 Bardare M, Zani G, Buschini MP et al. Nasal hyposensitization with a grass pollen extract in children affected with rhinoconjunctivitis. XIIIth. Congress of ICACI. Montreaux, 1988:257.
- 29 Saia B, Martin R, Bizzotto R, Gemignani G, Marcer G. Preseasonal local immunotherapy with macronized grass pollen powder. XIVth Congress of EAACI. Berlin, 1989:114.
- 30 Nickelsen JA, Goldstein S, Mueller UF et al. Local nasal immunotherapy for ragweed allergic rhinitis. II. Immunological response. *J Allergy Clin Immunol* 1981; 68:41-5.
- 31 Nickelsen JA, Goldstein S, Mueller UF et al. Local nasal immunotherapy for ragweed allergic rhinitis. III. A second year of treatment. *Clin Allergy* 1983; 13:509-19.
- 32 Mistrello G, Rapisarda G, Falagiani P. Detection of IgE binding activity in serum after intranasal treatment of normal rabbits with *P. judaica* extract. *Allergy* 1991; 46: 52-8.
- 33 Sedgwick DJ, Holt PG. Induction of IgE isotype specific tolerance by passive antigenic stimulation of respiratory mucosa. *Immunology* 1983; 50:625-30.

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