

# Clinical and immunologic effects of a rush sublingual immunotherapy to *Parietaria* species: A double-blind, placebo-controlled trial

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**Background:** The local (noninjection) routes of immunotherapy are presently regarded as viable therapeutic options for respiratory allergy, and their mechanisms of action are currently undergoing investigation.

**Objective:** We evaluated the clinical efficacy of a preseasonal rush sublingual-swallow immunotherapy and its effects on allergic inflammation in patients with seasonal rhinoconjunctivitis caused by *Parietaria* species.

**Methods:** Thirty patients with *Parietaria* species-induced rhinoconjunctivitis (13 with mild intermittent asthma) were randomly assigned sublingual-swallow immunotherapy or placebo in a rush preseasonal course. We assessed the seasonal symptom-drug intake score by diary card and the inflammatory infiltration and the intercellular adhesion molecule 1 expression on nasal epithelium after specific allergenic challenge before and after treatment.

**Results:** The investigated immunotherapy was well tolerated, and no side effects were recorded. A significant reduction of the symptom score ( $P = .016$ ) and drug intake score ( $P = .008$ ) after immunotherapy was observed only in the active group. A decrease of the cumulative score was observed also in the placebo group ( $P = .046$ ), but the significance was clearly higher ( $P = .006$ ) in the active group. In the active group a reduction of neutrophils ( $P = .001$ ), eosinophils ( $P = .01$ ), and intercellular adhesion molecule 1 expression ( $P = .04$ ) after specific nasal challenge was also detected.

**Conclusion:** The present results suggest that this sublingual-swallow immunotherapy administered through a rush schedule is clinically effective and safe and that it decreases the immune-mediated inflammatory responses to the allergen. (*J Allergy Clin Immunol* 1999;96:4-8.)

**Key words:** Sublingual-swallow immunotherapy, allergic inflammation, allergen specific nasal challenge, intercellular adhesion molecule-1

## Abbreviations used

|         |                                               |
|---------|-----------------------------------------------|
| APAAP:  | Alkaline-phosphatase-antialkaline phosphatase |
| ASNC:   | Allergen-specific nasal challenge             |
| BU:     | Biologic unit                                 |
| ICAM-1: | Intercellular adhesion molecule 1             |
| sECP:   | Soluble eosinophil cationic protein           |
| SLIT:   | Sublingual-swallow immunotherapy              |

During the last 15 years, interest has increased, especially in Europe, in local (or noninjection) routes for allergen-specific immunotherapy. These routes (oral, sublingual, local nasal, and local bronchial) have the overall aim of improving both safety and compliance.<sup>1</sup> On the basis of the available experimental data, the World Health Organization<sup>2</sup> and the European Academy of Allergology and Clinical Immunology-European Society of Pediatric Allergology and Clinical Immunology<sup>3</sup> have recently accepted nasal and sublingual immunotherapy as viable alternatives to injection immunotherapy. Several controlled trials<sup>4-12</sup> have demonstrated the clinical effectiveness of the sublingual-swallow immunotherapy (SLIT) in rhinitis and asthma. The rationale for administering an allergen orally is supported by a number of experimental observations concerning the *tolerogenicity* of the oral administration of antigens.<sup>13</sup> In addition, we demonstrated, in human subjects, the possible involvement of the local mucosal immunity with sublingual immunotherapy. Although we saw no direct mucosal absorption of the extract, the allergen persisted for a long time in the mouth after swallowing.<sup>14</sup> Therefore optimal administration should involve mucosal contact and subsequent ingestion of the vaccine (sublingual-swallow).

Concerning the mechanisms of action of local immunotherapy, little is presently known. Nevertheless, evidence exists that nasal and sublingual administration can modulate the allergen-induced inflammatory phenomena<sup>9,15</sup> and T-lymphocyte response.<sup>16</sup> The allergen-induced inflammation, and its possible modulation by immunotherapy, can be easily studied by means of nasal and conjunctival allergen-specific challenge, as previously described.<sup>9,15,17</sup> The events after challenge involve a

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**TABLE I.** Demographic data of the enrolled patients

|                          | Active            | Placebo           |
|--------------------------|-------------------|-------------------|
| No. of patients          | 15 (14 completed) | 15 (13 completed) |
| Sex (M/F)                | 10/5              | 3/6               |
| Mean age (y)             | 33.2              | 30.4              |
| Age range (y)            | 22–47             | 19–36             |
| Mild intermittent asthma | 6/15              | 7/15              |
| Mean duration of disease | 3.1               | 3.6               |
| Smokers                  | 2/15              | 3/15              |

clinical response, the influx of inflammatory cells (mainly eosinophils and neutrophils) in the mucosa, and the epithelial expression of intercellular adhesion molecule 1 (ICAM-1).

This randomized controlled trial was aimed at assessing the clinical efficacy of rush preseasonal SLIT to *Parietaria* species (wall pellitory) pollen and its effects on allergic inflammation. This latter aspect was investigated by means of allergen-specific nasal challenge (ASNC). *Parietaria* extract was chosen because it is widely diffused in the Mediterranean area and it represents the causative agent in about 40% of allergic patients.

## METHODS

### Experimental design

The study was designed as a double-blind, placebo-controlled, 2 parallel group trial, involving the administration of a rush preseasonal SLIT with *Parietaria* extract or placebo. All patients underwent a run-in period (April–June 1995), during which the pretreatment diary card was recorded. The treatment started in October 1995 and was continued up to about March 15, 1996. The diary card was recorded again during the 1996 pollen season. ASNC was performed before and after the immunotherapy course. Some immunologic parameters were monitored, as detailed below. The study was approved by the Ethical Committee of Genoa University and notification was given to the Italian Ministry of Health. The study design is plotted in Fig 1.

### Patients

Thirty adult outpatients (19 men and 11 women) were enrolled. All patients had seasonal rhinoconjunctivitis (13 with mild intermittent asthma) caused by *Parietaria* pollen and had experienced symptoms in at least the 2 previous years. Other sensitizations were excluded by skin testing and RAST (panel including mites, pet dander, grass, olive, birch, *Alternaria* species, and *Aspergillus* species). Exclusion criteria were systemic immunologic or metabolic disease, malignancies, major anatomic alterations of the upper airways, severe atopic dermatitis, chronic corticosteroid- $\beta$ -blocker treatment, and previous courses of immunotherapy. No pregnant or lactating women were admitted. All patients were informed in detail about the experimental procedure and provided written informed consent. Demographic data of the patients are summarized in Table I.

### Immunotherapy and concomitant treatments

The SLIT (ALK-Abello) was administered preseasonally (from October 15 to March 15), according to a rush schedule.<sup>5</sup> The vaccine was standardized by a RAST-inhibition procedure in comparison with an in-house reference, and potency was expressed in biologic units (BUs).<sup>18</sup> The content of the major allergen Par j 1 for the batch used was expressed in micrograms.<sup>19</sup> The extract in glyceri-

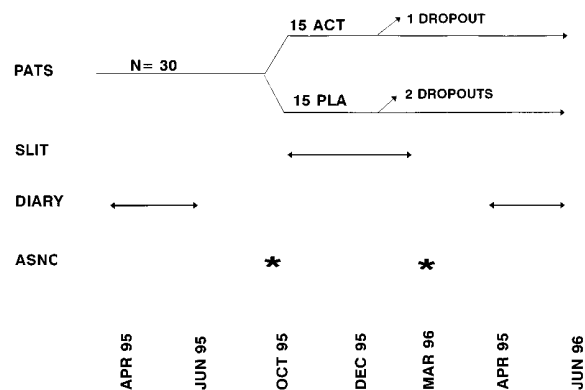


**FIG 1.** Study design.

nated-phenolated aqueous solution was prepared in 5 different vials at increasing concentrations (0.016, 0.08, 0.4, 2, and 10 BU/mL). During the build-up, the drops had to be taken twice daily, starting with one drop from the first vial and increasing up to 5 drops and then repeating the procedure with each vial until the maximum dose was reached (5 drops from the 10-BU/mL vial). This maintenance dose (corresponding to 0.12  $\mu$ g of Par j 1) was then administered daily until the beginning of the pollen season. The cumulative dose was 256 BU (about 16  $\mu$ g of Par j 1), corresponding to 7-fold the dose of a 5-month course of subcutaneous immunotherapy. During the build-up phase, the drops were taken in the morning and in the evening (at maintenance in the morning only) at least 15 minutes before eating. They had to be kept sublingually for 2 minutes and then swallowed. The placebo was undistinguishable from the active treatment. During the trial, the patients were allowed to take the following drugs if needed: cetirizine or loratadine (10-mg tablet once daily), beclomethasone nasal spray (200  $\mu$ g/d), inhaled albuterol on demand (100  $\mu$ g/puff, 1–3 puffs), azelastine eye drops, and short courses of oral prednisone (1 mg  $\cdot$  kg<sup>-1</sup>  $\cdot$  d<sup>-1</sup> for 3 days) in case of severe rhinitis.

### Diary card

The patients completed a daily record (April–June) of the presence and severity of the following symptoms: nasal obstruction, rhinorrhea, nasal itching, sneezing, conjunctival itching and hyperemia, palpebral edema, asthma attacks, and cough. The symptoms were graded as follows: 0, absent; 1, mild; 2, moderate; and 3, severe. The drug intake was scored daily as follows: 3 for each dose of corticosteroid, 2 for each dose of oral antihistamine, and 1 for each dose of the other drugs. The patients were also required to record any troublesome symptom possibly related to the treatment or to contact our service in case of any discomfort.

### Allergen-specific nasal challenge

The procedure was performed out of the pollen season, when patients were asymptomatic (before starting and after the end of treatment). The subjects had to discontinue any drug at least 15 days before the ASNC. The challenge was performed according to the procedure described by our group.<sup>17</sup> Increasing concentrations of the purified and standardized allergen (ALK-Abello) were sprayed into a nostril until a clinical reaction was observed. The reaction was assessed according to an arbitrary scale (0, absent; 1, mild; 2, moderate; and 3, severe) scoring each of the following symptoms: itching, sneezing, rhinorrhea, and obstruction. The dose eliciting a total score of 7 or more was considered as the threshold dose and was used in the subsequent ASNC. Nasal brushings were collected by a cotton tip 30 minutes after challenge from the medium third of

**TABLE II.** Rhinoconjunctivitis plus asthma score means

|                       | Before SLIT | After SLIT | P value |
|-----------------------|-------------|------------|---------|
| Active, April         | 95.3        | 53.9       | .003    |
| Placebo, April        | 75.4        | 47.4       | NS      |
| Active, May           | 121.8       | 79.1       | .05     |
| Placebo, May          | 110         | 80.4       | NS      |
| Active, June          | 84.6        | 56.2       | .05     |
| Placebo, June         | 80.1        | 63.7       | NS      |
| Active, whole season  | 301         | 189.2      | .016    |
| Placebo, whole season | 267         | 191        | NS      |

NS, Not significant.

**TABLE III.** Drug intake score means

|                       | Before SLIT | After SLIT | P value |
|-----------------------|-------------|------------|---------|
| Active, April         | 26.6        | 7.4        | .016    |
| Placebo, April        | 24.8        | 18.7       | NS      |
| Active, May           | 53.9        | 19.6       | .006    |
| Placebo, May          | 59.1        | 33         | NS      |
| Active, June          | 35          | 15.4       | .046    |
| Placebo, June         | 53.5        | 31.2       | NS      |
| Active, whole season  | 115.5       | 42.3       | .008    |
| Placebo, whole season | 137.4       | 83         | NS      |

NS, Not significant.

the inferior turbinate, transferred to glass slides, air-dried, and stained with May-Grunwald-Giemsa dye. The number of neutrophils and eosinophils was expressed as a mean of 10 fields in light microscopy (Leitz Laborlux microscope, 250 $\times$  focus). The ICAM-1 expression on nasal epithelium was determined by the alkaline phosphatase-antialkaline phosphatase (APAAP) method.<sup>20</sup> Briefly, specimens were incubated with an anti-ICAM-1 mAb (1  $\mu$ g/mL, 84H10, IgG1; Immunothec, Marseille, France) or anticytokeratine mAb (DAKO, Milan, Italy). After rinsing, specimens were incubated with rabbit anti-mouse IgG followed by APAAP complexes. Finally, the substrate, containing basic new fuchsin, naphthol AS-Bi phosphate, and levamisole (inhibitor of endogenous phosphatase), was added. Specimens were counterstained with Carazzi's hematoxylin, and the ICAM-1 positivity was graded as follows: 0, no positive cells; 1, mild positivity on 25% of cells; 2, mild positivity on 75% of cells; 3, intense positivity on 75% of cells; and 4, intense positivity on all cells.<sup>18</sup>

### Other paraclinical parameters

Soluble eosinophil cationic protein (sECP) levels were assessed in the nasal lavage fluid obtained after the ASNC. sECP was measured by means of a double-antibody RIA by using an iodine 125-labeled antibody (ECP-RIA; Pharmacia, Uppsala, Sweden). The values were expressed as micrograms per liter on the basis of a proper 6-point calibration curve. The EG1/EG2 ratio was also assessed in nasal specimens to verify the activation state of eosinophils. Evaluation was performed by using the APAAP method described above, with proper antibodies.

### Statistical analysis

The investigated parameters might be nonnormally distributed, and therefore the Mann-Whitney *U* test for intergroup comparison and the Wilcoxon test for intragroup comparisons were used when appropriate. Demographic data were analyzed by means of the Student *t* test.

**TABLE IV.** Symptoms plus drug intake score means

|                       | Before SLIT | After SLIT | P value |
|-----------------------|-------------|------------|---------|
| Active, April         | 121.9       | 62.26      | .003    |
| Placebo, April        | 100.2       | 66         | NS      |
| Active, May           | 175.6       | 98.6       | .011    |
| Placebo, May          | 169.1       | 113        | NS      |
| Active, June          | 119.6       | 71.6       | .035    |
| Placebo, June         | 134.2       | 94.8       | NS      |
| Active, whole season  | 417.1       | 231.5      | .006    |
| Placebo, whole season | 403.5       | 274.3      | .046    |

NS, Not significant.

## RESULTS

Of the 30 patients enrolled, 27 completed the study. One patient from the active group and 2 from the placebo group were excluded because of poor compliance with treatment schedule and visits. The remaining patients completed the study; no actual or suspected side effect were reported, and no dosage adjustment was required. The pollen counts for *Parietaria* extract (provided by Associazione Italiana di Aereobiologia) were superimposable in 1995 and 1996, and the area under the curve showed a slightly higher (7.8%) value in the second season.

After the SLIT course, the active group achieved a measurable clinical improvement versus the pretreatment conditions (Tables II-IV). This improvement was clearly significant when symptom (asthma plus rhinoconjunctivitis) scores were considered (before vs after SLIT, *P* = .015; placebo, *P* = not significant), as summarized in Table II. A superimposable result was obtained for drug intake scores (before vs after: active, *P* = .008; placebo, *P* = not significant), as shown in Table III. Considering the sum of symptoms plus drug intake, a significance was found also in the placebo group only for the global score (*P* = .046), but the active groups showed a higher level of significance (*P* = .006), as shown in Table IV.

The results from the ASNC are plotted in Fig 2. A significant reduction of the symptom score at the ASNC was observed in the actively treated group versus the placebo group after treatment (*P* = .0001); the intragroup comparison showed a significant improvement in both groups (active group, *P* = .001; placebo group, *P* = .01; Fig 2). A significant decrease in the number of inflammatory cells after treatment was observed only in the active patients (neutrophils, *P* = .001; eosinophils, *P* = .01), as shown in Fig 2. This reduction was paralleled by a decrease of ICAM-1 expression on epithelial cells (*P* = .04) in the active group. The intragroup comparison was significant for neutrophils (*P* = .05) and ICAM-1 (*P* = .05). No change was detectable in the sECP concentration in nasal lavage fluid. The sECP concentrations before and after SLIT were  $1.9 \pm 2.3$   $\mu$ g/L versus  $1.3 \pm 1.2$   $\mu$ g/L in the active group and  $2.6 \pm 2.5$   $\mu$ g/L versus  $4.3 \pm 3.5$   $\mu$ g/L in the placebo group. Finally, no significant change in the EG1/EG2 ratio was detectable as well (data not shown).

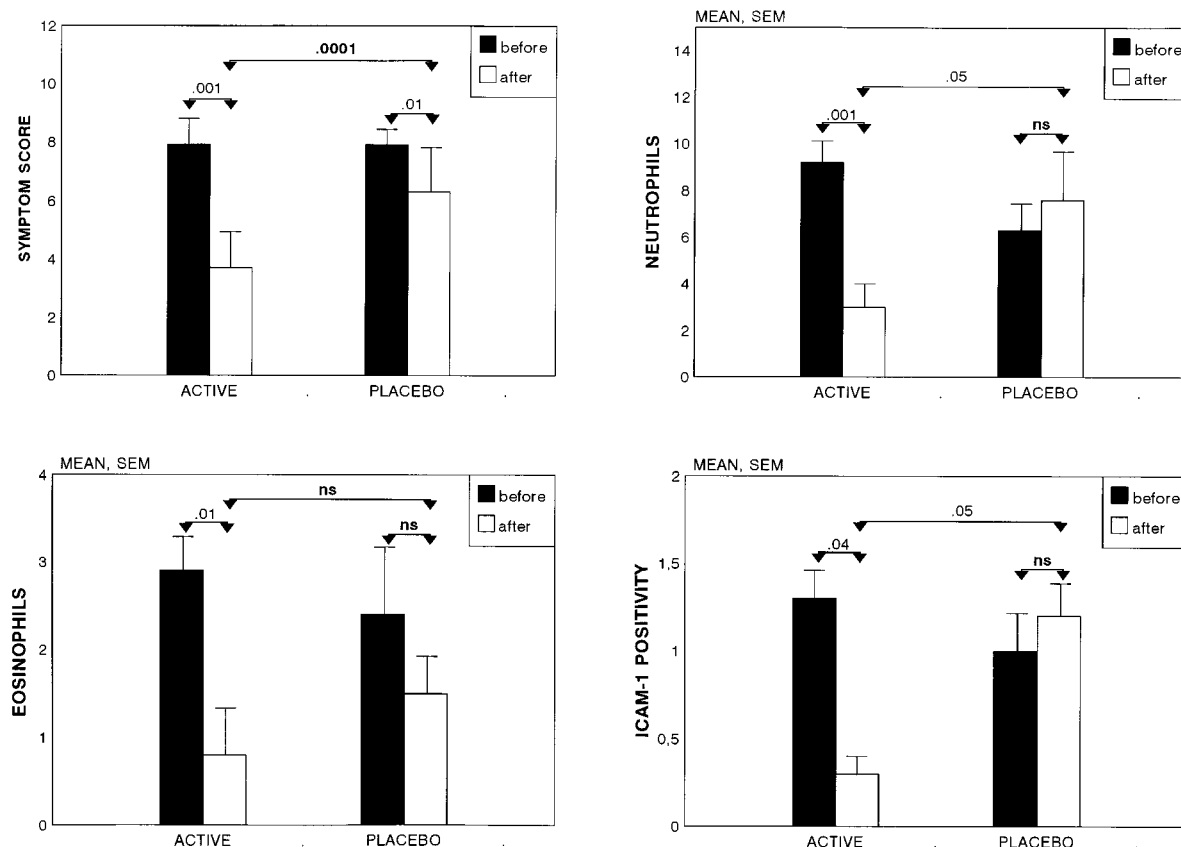


**FIG 2.** ASNC early-phase reaction. Symptoms (*upper left*), neutrophil count (*upper right*), eosinophil count (*lower left*), and ICAM-1 expression on nasal epithelium (*lower right*) before and after SLIT in the 2 groups (mean  $\pm$  SEM) are shown. The intragroup and intergroup comparisons are reported in the graph.

## DISCUSSION

The sublingual immunotherapy administration has been recently accepted by the World Health Organization for clinical use in adult patients.<sup>2,3</sup> On the basis of a preliminary kinetic study *in vivo*, the so-called sublingual-swallow route seems to be the more advantageous.<sup>14</sup> At present there is an increasing amount of data supporting the immunologic effects of subcutaneous immunotherapy, including the reduction of inflammatory cells and mediators in target organs,<sup>21-23</sup> whereas only sparse data are available on the mechanisms of action of noninjection routes. The nasal and the conjunctival allergen-specific challenges represent safe and reliable models for studying the allergen-driven inflammatory events<sup>24</sup> and therefore for assessing the effects of immunotherapy. We previously demonstrated that local immunotherapy reduces both inflammatory infiltrate and ICAM-1 expression on epithelia; these effects correlate well with clinical efficacy.<sup>9,15</sup> Although the clinical effects (ie, reduction of symptoms and drug intake) still represent the only objective parameters for evaluating the efficacy, the data about allergic inflammation may be of relevance for understanding the mechanisms of action of immunotherapy.

The investigated SLIT was able to significantly reduce

the clinical scores under natural pollen exposure, as evidenced by the analysis of diary cards; this fact is consistent with the results reported elsewhere in the literature.<sup>4-9</sup> Moreover, we observed a significant reduction of the severity of allergic inflammation during the early-phase reaction induced by ASNC. This effect was especially apparent on the ICAM-1 expression on epithelial cells.

The immunotherapy was well tolerated and accepted by patients. The rush schedule was easy to manage because the build-up phase took only 15 days, and the rapid dosage increase did not cause particular problems. The rush schedule, especially when preseasonal courses are required, allows simplification of the administration and therefore possibly enlarges the indications of SLIT. The standardization of the vaccine and the possibility of expressing its major allergen content allow objective comparison of the effectiveness of immunotherapy courses and achievement in the future of the optimal dose. The discontinuation of SLIT during the pollen season represents a precautionary approach; nevertheless, in a recent study performed in children,<sup>25</sup> it was seen that a coseasonal administration does not involve an increased risk of side effects. Finally, SLIT shows a relevant socioeconomic saving<sup>1</sup>; this aspect is not negligible for a prophylactic treatment, as is the case with immunotherapy.

In conclusion, we have provided experimental evidence that SLIT is able to reduce both the allergen-driven inflammation in the target organ after allergen nasal challenge and the symptom-medication score under natural pollen exposure. Further studies are needed to fully establish the efficacy and safety of rush schedules for SLIT and to verify its applicability to other allergens.

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