

Sublingual immunotherapy-induced IL-10 production is associated with changed response to the decongestion test: Preliminary results

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ABSTRACT

Sublingual immunotherapy (SLIT) has been shown to be capable of inducing a regulatory T-cell response as evidenced by IL-10 production. Decongestion testing consists of spraying an intranasal vasoconstrictor drug to evaluate the recovery of nasal airflow limitation. The aim of this study was to assess the association of SLIT-induced IL-10 production with nasal airflow recovery after decongestion testing. Nineteen patients with perennial allergic rhinitis (PAR) were studied: 9 patients successfully assumed SLIT for 3 years and 10 patients were considered as control. In vitro IL-10 production was evaluated after SLIT. Rhinomanometry and decongestion testing were performed in all subjects before and after 3 years. After 3 years, SLIT patients showed a significant decrease of nasal resistances (0.048) and reduced response to decongestion testing ($p = 0.044$ for absolute values and $p = 0.0051$ for Δ). The comparison with nontreated allergic patients shows significant differences concerning both pretest values ($p = 0.02$) and Δ percentages ($p = 0.00466$). In addition, the decrease of nasal airflow resistance values and the percentages of reversibility were significantly associated with IL-10 levels ($p = 0.0016$ and $p = 0.00294$, respectively). This preliminary study provides the first evidence of a changed response to decongestion testing after SLIT that is associated with IL-10 production in patients with PAR.

(Allergy Asthma Proc 28:574–577, 2007; doi: 10.2500/aap2007.28.3044)

Key words: Allergic rhinitis, decongestion test, IL-10, dust mite, nasal airflow resistance, rhinomanometry, SLIT, sublingual immunotherapy

Allergic rhinitis is characterized by eosinophilic Th2-dependent inflammation.¹ Recently, a variety of T-cell subpopulations that can modulate T-cell responses has been described: T regulatory (T reg) cells.² A defect of T reg cells has been reported in allergic subjects.³ Specific immunotherapy (SIT) is able to induce a T reg cell response as detected by IL-10 production.⁴ Recently, it has been reported also that sublingual immunotherapy (SLIT) induces IL-10 production and reduces T-cell proliferation associated with clinical improvement in patients with perennial allergic rhinitis (PAR).^{5,6} Nasal obstruction is a key symptom in patients with allergic rhinitis,⁷ is related to allergic inflammation,¹ and may be objectively measured by rhinomanometry.⁸

The nasal decongestion test consists of evaluating the percentage of reversibility of nasal airflow limitation after administering an intranasal vasoconstrictor (such

as α -adrenoreceptor stimulant). It has been shown to provide different responses, taking into consideration the type and number of sensitizations,⁹ the degree of allergic inflammation,¹⁰ and some bronchial parameters.¹¹ Thus, this preliminary study aimed at evaluating nasal airflow response to decongestion testing and comparing it with IL-10 production in successfully SLIT-treated PAR patients.

MATERIALS AND METHODS

The study was approved by the Review Board of the La Spezia Navy Hospital and was performed with the informed consent of all participants. The donor panel consisted of 9 healthy subjects (as the control group), 10 allergic subjects, and 9 patients receiving SLIT for house-dust mite allergen extract (Table 1). Skin-prick test was performed with standardized allergen extracts (Stallergenes, Milan, Italy), *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, grasses, *Parietaria*, dog, cat, *Alternaria*, *Ambrosia*, *Aspergillus fumigatus*, birch, olive tree, and hazel, according to validated criteria.¹²

Allergic donors were all symptomatic, with a clear history of PAR. Subjects undergoing SLIT had received house-dust mite allergen extract (Staloral 100 IR; Stallergenes) for at least 3 years and were well matched to

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Table 1 Demographic aspects

Subjects	Age (yr)	Sex	Skin-Prick Test	Nasal Obstruction
UA 1	31	Male	HDM	2
UA 2	22	Male	HDM	3
UA 3	33	Female	HDM, G	2
UA 4	19	Male	HDM, G, P	3
UA 5	25	Male	HDM, C	3
UA 6	20	Female	HDM	3
UA 7	36	Male	HDM, G, P	2
UA 8	40	Male	HDM, C, G, P	2
UA 9	21	Male	HDM	2
UA 10	18	Male	HDM, G, P	3
TA 1	35	Male	HDM	2
TA 2	28	Male	HDM, P	2
TA 3	19	Female	HDM	1
TA 4	37	Male	HDM, G	1
TA 5	24	Female	HDM, P	2
TA 6	27	Male	HDM, G, P	1
TA 7	19	Male	HDM	2
TA 8	22	Male	HDM, P	1
TA 9	32	Male	HDM	1

Nasal obstruction: 1 = mild, 2 = moderate, and 3 = severe. C = cat; G = grass; HDM = house-dust, mite; P = *Parietaria*; TA = treated allergic patients; UA = untreated allergic patients.

allergic donors for age, sex, and skin-prick test response (Table 1). Six subjects of the untreated group and five subjects of the treated group were polysensitized, but the sensitization for other allergens was not because of symptom occurrence, as provided both by their history and negative allergen-specific conjunctival challenge. The study was performed during the winter, which is outside the pollen season.

Patients were assessed for a change in symptoms. The following symptoms were assessed by the patient, answering the questions made by the investigator: nasal obstruction, sneezing, rhinorrhea, and itchy nose. Each symptom was evaluated on the following scale: 0 = absent, 1 = mild (symptom was present but was not annoying or troublesome), 2 = moderate (symptom was frequently troublesome but did not interfere with either normal daily activity or sleep), and 3 = severe (symptom was sufficiently troublesome to have interfered with normal daily activity or sleep).

Patients did not use intranasal or oral corticosteroids within the previous 4 weeks and antihistamines, anti-

leukotrienes, and decongestants during the week preceding the examination. The decongestion test was performed as previously reported.⁹⁻¹¹ Briefly, after baseline rhinomanometry (ZAN 100 Rhino Flow Handy II; ZAN Messgeraete GmbH, Oberthulba, Germany), two sprays of naphazoline (0.1%) were applied per nostril, a second rhinomanometry was performed 10 minutes later. No patients were receiving pharmacotherapy since 1 month before testing.

In vitro IL-10 production was stimulated by *D. farinae* or *Candida albicans* after 3-day incubation of peripheral mononucleated cells.^{6,7} The quantitative determination of IL-10 was performed by a commercial ELISA kit (Pantec s.r.l., Turin, Italy) following the manufacturer's instructions. The statistical analysis was performed using the χ^2 -square test, the Spearman test, and the ANOVA test.

RESULTS

Nasal symptoms significantly improved in comparison with baseline in SLIT-treated patients only ($p < 0.01$). *In vitro* IL-10 production was significantly higher in SLIT-treated patients in comparison with allergic nontreated patients: *Candida*-induced IL-10 ($p = 0.002$) and *D. farinae*-induced IL-10 ($p = 0.04$).

At baseline, the mean pretest nasal airflow resistances were 0.39 ± 0.17 Pa/mL per second in the SLIT group and 0.38 ± 0.22 Pa/mL per second in the control group ($p = 0.995$). After decongestion test, nasal resistances diminished both in the SLIT group (0.286 ± 0.08 ; Δ , $-21.7 \pm 0.156\%$) and in the control group (0.292 ± 0.18 ; Δ , $-22.9 \pm 0.147\%$), without any difference between the two groups ($p = 0.995$ for absolute values and $p = 0.986$ for Δ ; Fig. 1).

After 3 years, the pretest nasal airflow resistances significantly decreased in SLIT-treated patients (0.247 ± 0.04 ; $p = 0.048$); however, there was no significant change in control group (0.337 ± 0.09 ; $p = 0.788$). The intergroup analysis shows a statistical significant difference ($p = 0.02$). After decongestion test, nasal resistances slightly diminished in the SLIT group (0.227 ± 0.03 ; Δ , $-8.4 \pm 0.8\%$), with significant difference in comparison with baseline findings ($p = 0.044$ for absolute values and $p = 0.0051$ for Δ). On the other hand, nasal resistances were significantly affected by decongestion in the control group (0.252 ± 0.03 ; Δ , $-22.4 \pm 0.11\%$) without a difference in comparison with baseline data ($p = 0.229$ for absolute values and $p = 0.996$ for Δ).

The intergroup analysis shows that there is significant difference concerning Δ percentages ($p = 0.00466$). In addition, the decrease of nasal airflow resistance values and the Δ percentages of nasal airflow resistance values were significantly associated with IL-10 production ($p = 0.0016$ and $p = 0.00294$, respectively; Fig. 1).

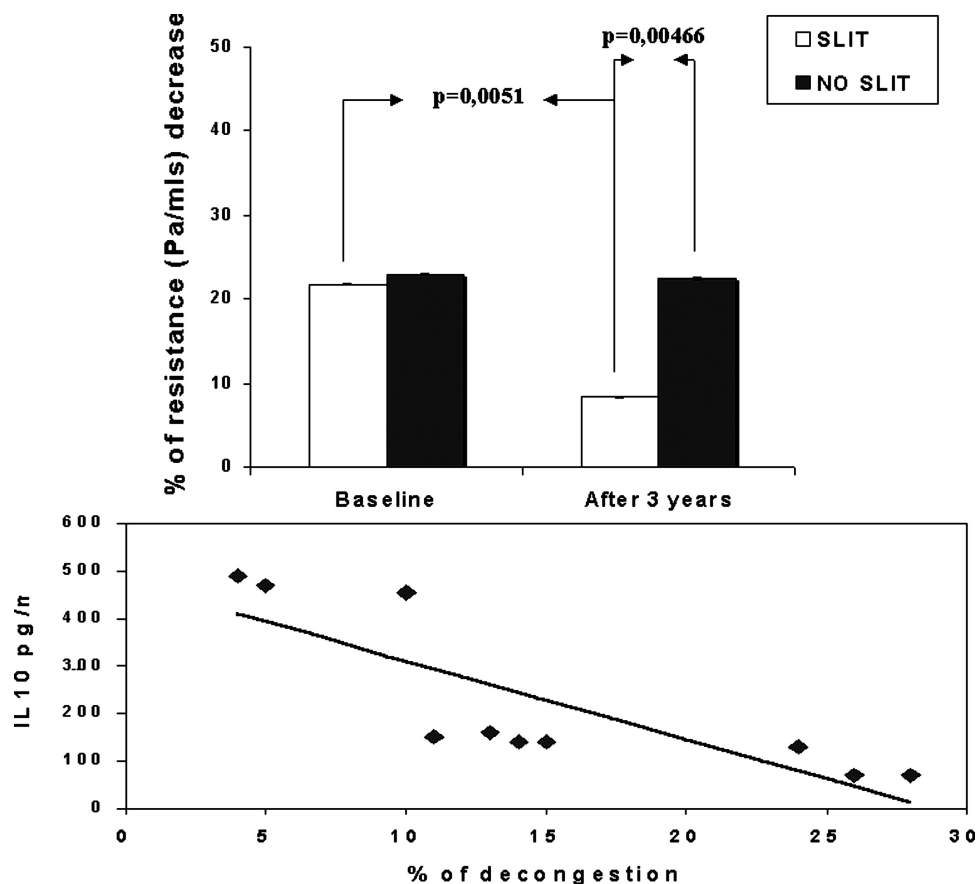


Figure 1. (Upper Panel) Nasal airflow resistances (expressed as percent of decrease) evaluated before and after 3 years in patients treated or not treated with SLIT. Data are expressed as means $\pm$ SD. (Lower Panel) IL-10 levels and resistance decrease (expressed as percent) in SLIT-treated patients after 3 years.

DISCUSSION

Nasal airflow resistances may be considered a reliable marker of nasal obstruction in patients with allergic rhinitis.¹ Moreover, SIT has been shown capable of inducing IL-10 production by T reg cells.⁵ IL-10 exerts a number of documented activities that are relevant for understanding the mechanisms of action of SIT: the switch of allergen-specific antibody production from IgE to IgG4, the inhibition of allergen-dependent mast cell activation, the suppression of activation and survival of eosinophils, the reduction of proallergic cytokines, and the hyporeactivity to allergens such as the induction of specific tolerance.⁵

SLIT is a safe and effective route of administration of allergen extracts in allergic patients. However, its mechanism of action is still poorly understood, because there are few data concerning the induced immunologic changes. We previously provided evidence that SLIT was able of inducing a T reg cell-dependent response, such as concerning IL-10 *in vitro* production, which was related to the observed clinical and immunologic changes.^{6,7}

This pilot study provides preliminary evidence that SLIT improves rhinomanometric impairment in pa-

tients with PAR as documented by decreased nasal airflow resistance values. Moreover, SLIT significantly affects the response to decongestion testing. This finding may mean that the nasal airflow after SLIT responds less to vasoconstrictor agents because nasal cavities show an improved patency, as shown by the reduced resistances. Thus, decongestant is less effective in this situation, because there is less relevant nasal obstruction.

The most important finding of this study is the association among IL-10 production, the nasal resistance, and the Δ reversibility to decongestion testing. These immunologic changes seem to be clinically relevant because the association with the functional effects underlines the value of these findings. Indeed, there is an inverse relationship between functional parameters and IL-10 production. It means that the improved nasal function may be dependent on immunologic changes because it has been previously recently reported that SLIT induced a reduction of nasal eosinophils.¹³ The reduced eosinophilic infiltration was associated and related to the increased IL-10 levels.¹³ Thus, the inverse relationship between nasal function and IL-10 may explain the clinical effect.

In conclusion, this preliminary study shows that SLIT is capable of both improving nasal obstruction and affecting the response to decongestion testing in patients with PAR and these functional effects are associated with immunologic changes, such as the induction of IL-10 production. Of course, additional controlled studies need to be performed to confirm these preliminary findings.

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