

Short communication

Sublingual tryptase and ECP in children treated with grass pollen sublingual immunotherapy (SLIT): safety and immunologic implications

Background: The clinical safety of sublingual immunotherapy (SLIT) has been repeatedly confirmed; nevertheless, the possible onset of local oral symptoms is still a concern, and nothing is known about the pathogenesis of this effect. We aimed to determine whether the administration of SLIT in allergic children can evoke an IgE-mediated reaction, by measuring the levels of sublingual tryptase and ECP.

Methods: Thirty children (7–12 years old) with allergic rhinitis/asthma due to grass pollen were prescribed SLIT. In these children, an allergen-specific nasal challenge was performed, and nasal tryptase and ECP were measured before and after. Sublingual ECP and tryptase were also assessed before the SLIT, after 1 month, and after 6 months of treatment. Ten matched allergic children and 10 healthy ones served as controls for the baseline levels of sublingual ECP and tryptase.

Results: The levels of nasal tryptase and ECP significantly increased after nasal challenge ($P < 0.001$), whereas no change during the SLIT course (at the beginning, after 1 month, and after 6 months) could be detected in sublingual tryptase either before or after SLIT administration. The sublingual ECP significantly decreased after 6 months of SLIT. The baseline levels of nasal tryptase and ECP were significantly higher in allergic subjects than in healthy controls, as was the level of sublingual ECP.

Conclusions: In the presence of an IgE-mediated reaction (ASNC), a significant increase of tryptase and ECP can be seen. When SLIT is administered, such a phenomenon does not occur; therefore, SLIT does not elicit any IgE reaction in the mouth. It is noteworthy that allergic subjects display higher levels of nasal ECP and tryptase than healthy subjects, even when symptom-free, and these observations may indicate the presence of subclinical inflammation.

**F. Marcucci¹, L. Sensi¹, F. Frati¹,
G. E. Senna², G. W. Canonica³,
S. Parmiani⁴, G. Passalacqua³**

¹Clinica Pediatrica, University of Perugia, Perugia;

²Allergy Service, Verona General Hospital, Verona;

³Allergy and Respiratory Diseases, DIMI, University of Genoa, Genoa; ⁴ALK-Abello, Ospiate di Bollate, Milan, Italy

Key words: ECP; nasal; nasal challenge; sublingual; sublingual immunotherapy; tryptase.

Giorgio Walter Canonica, MD
Allergy and Respiratory Diseases – DIMI
Pad. Maragliano, L.go R. Benzi 10
16132 Genoa
Italy

Accepted for publication 7 June 2001

After reports of severe side-effects from subcutaneous immunotherapy, the interest in noninjection routes for administration increased rapidly. In particular, during the last 10 years, a substantial number of randomized controlled clinical trials with sublingual immunotherapy (SLIT) were done (1), some of them also showing its effectiveness in asthma (2, 3). Due to the demonstrations of efficacy, this route has been accepted by the World Health Organization (WHO) and the European Academy of Allergology and Clinical Immunology (EAACI) for routine clinical use (1, 4, 5), although more data on pediatric indications are generally required.

The safety of SLIT in both adults and children was confirmed in large-population trials (6, 7) and no severe systemic side-effect was ever reported, while urticaria,

bronchospasm, and rhinitis were reported only occasionally. The most frequently reported adverse events were the oral itching/irritation and the gastrointestinal complaints. Because of its safety and tolerability, SLIT seems to be particularly suitable for pediatric patients (8, 9), although the local (oral) side-effects may still represent a major concern (10). These effects (oral itching, swelling, and irritation) usually appear within minutes; therefore, they are generally thought to be sustained by a local IgE-mediated reaction and a local allergic inflammation. Although the clinical safety of SLIT in patients with oral allergy syndrome has been reported (11), we still lack an objective assessment of its possible mucosal immunologic effect.

The mucosal content of tryptase and eosinophil cationic protein (ECP) is expected to reflect mast-cell

degranulation and, respectively, the activation of eosinophils, which are directly involved in the allergic inflammation. Their measurement *in situ* by appropriate methods would help elucidate the pathogenic mechanisms and the possible involvement of local IgE reactions.

We designed the present study to verify whether the administration of SLIT in children can elicit a local allergic reaction or an allergic inflammatory response, by measuring tryptase and ECP in parallel with the clinical assessment. The response to allergen-specific nasal challenge (ASNC) was used in the same patients as inner positive control to confirm that tryptase and ECP do increase during the allergen-induced mucosal reaction. A control group of healthy children were also evaluated at baseline.

Material and methods

Study design

A group of 40 children with seasonal allergy due to grass pollen was studied. In all these children, nasal and sublingual tryptase and ECP were evaluated at baseline, as well as in a control group of 10 healthy children. Thirty out of these children were prescribed SLIT. They underwent allergen-specific nasal challenge (ASNC) with measurement of nasal tryptase and ECP (30 min and 24 h, respectively) after challenge. Moreover, sublingual tryptase and ECP were measured before and after (30 min and 24 h, respectively) the SLIT administration. These latter measurements were repeated before beginning the treatment, at the end of the induction phase (1 month), and after 6 months of treatment.

Patients

Forty children suffering from seasonal allergic rhinitis, or rhinitis and asthma, solely due to grass pollen were studied, and 30 of them were designated to receive SLIT, whereas the remaining 10 patients served as controls for the baseline value of tryptase and ECP, together with a group of 10 nonallergic children. The demography of the subjects is summarized in Table 1. All patients were screened by a standard skin test panel (ALK Abellò, Milan, Italy), including mites, grasses, *Parietaria*, olive, cypress, *Alternaria*, *Cladosporium*, and cat and dog dander. The RAST (UniCAP IgE, FEIA, Pharmacia, Uppsala, Sweden) was also performed in allergic patients. A skin positivity of class ++ or higher (12) and a clinical history of at least 2 years were the inclusion criteria. All patients' parents gave their informed consent to the study, which was approved by the hospital ethics committee.

Allergen-specific nasal challenge (ASNC)

The ASNC was performed at the time of the first diagnosis in the 30 allergic children assigned to SLIT, outside the pollen season (October 1999). At that time, all patients were therefore symptom-free and did not take any drug. The ASNC was performed by spraying the vehicle into a nostril and then increasing concentrations of grass-pollen extract: 2, 4, and 8 BU/ml (ALK-Abellò, Milan, Italy). These concentrations correspond to about 0.016, 0.032, and 0.064 µg of the group 5 major allergens per puff. An arbitrary score was attributed to itching, sneezing, rhinorrhea, blockage, and lacrimation, as follows: 0=absent, 1=mild, 2=moderate, 3=severe. A total score of 5 or greater was used to define the threshold dose for the positive challenge.

Measurement of nasal and sublingual tryptase and ECP

Tryptase and ECP levels were determined by the ELISA (UniCAP Tryptase System FEIA and UniCAP ECP System FEIA, Pharmacia, Uppsala, Sweden), adapted for mucosal sampling (13). In the case of ASNC and SLIT administration, the measurement of tryptase was made 30 min after and that of ECP at 24 h after. Briefly, the sponges of the CAP System, coated with antitryptase or anti-ECP antibodies, were washed with physiologic solution, and then mounted on an appropriate plastic stick and fixed by a permeable envelope. The plastic stick carrying the sponges were then inserted into a nostril or the sublingual region and maintained in site for 5 min with a tape fixed on the skin of the nose or lips. The sponges were then removed and frozen in buffered (NaCl plus NaN₃ 0.1%) vials. At the end of the trial, all the assays were performed at the same time. The concentration of tryptase and ECP was expressed in µg/l, according to a calibration curve.

SLIT and concomitant treatments

The SLIT was administered as drops in glycerinated solution, standardized in STU (ALK-Abellò, Milan, Italy), where 1000 STU/ml = 2.5 µg/ml of group 5 major allergens. SLIT started at the end of October 1999: it had to be administered in the morning while the patients were fasting. The buildup (about 1 month) involved the administration of increasing amounts of allergen from five different vials (1.6, 8, 40, 200, and 1000 STU/ml), starting with one drop from the first vial, increasing by one drop a day until five drops, and then restarting the same schedule with the next vial (9). The maintenance dose (five drops from the 1000 STU/ml vial, corresponding to about 0.5 µg of allergen) was assumed every other day for about 6 months, until the middle of April 2000. All allergic patients, irrespective of SLIT, received appropriate drug therapy, such as oral antihistamines, nasal corticosteroids, inhaled corticosteroids, cromolyn, and salbutamol on demand. The parents of patients receiving SLIT were required to report on a diary card the possible occurrence of side-effects.

Statistical analysis

Since the values of tryptase and ECP might be nonnormally distributed, the Mann-Whitney test for the intergroup comparison and the Wilcoxon test for intragroup comparison were used. *P* values of 0.05 or less were considered significant.

Results

Two patients dropped out before the end of the study: one moved to another town, and one reported persistent oral itching after taking the fourth dose of the buildup (about 0.01 µg of allergen), although no objective sign could be detected. The parents of this patient decided to stop the SLIT administration. In the other patients, no other relevant side-effect possibly attributable to SLIT was reported in the diaries during the 6-month course of treatment, and no evidence of alteration of the oral mucosa, tongue, or lips could be detected at the clinical visits in all patients receiving SLIT.

Nasal and sublingual tryptase in the healthy controls was virtually absent. At baseline, the 40 allergic children had significantly higher levels of nasal tryptase (14.4 ± 2.5 µg/l vs <1 , $P=0.02$) and nasal ECP (46.8 ± 4.1 vs 9.3 ± 2 µg/l, $P=0.001$) than healthy controls. The baseline levels of sublingual tryptase were low and compar-

Table 1. Demographic data of patient population

	Allergic SLIT	Allergic controls	Healthy controls	Total
No. of patients	30	10	10	50
Mean age (years)	9.6	9.75	10.3	9.8
Age range (years)	7–12	7–12	8–12	7–12
Sex (M/F)	13/17	4/6	6/4	24/28
Rhinitis alone	3	1	–	4
Rhinitis + asthma	27	9	–	36

able in allergic and nonallergic subjects, whereas the sublingual ECP was higher in the allergic patients than in controls (44.3 ± 3.6 vs 5.1 ± 1.9 $\mu\text{g/l}$; $P=0.001$), as summarized in Table 2. After the ASNC performed in the group of 30 children assigned to SLIT, a significant increase ($P<0.001$) of both nasal tryptase and ECP was seen (Figs. 1 and 2). This increase paralleled the onset of clinical symptoms.

No significant change in the levels of sublingual tryptase was found during the SLIT course (beginning, after buildup, and after 6 months) either before or after SLIT administration: all the mean values remained below 1.5 $\mu\text{g/l}$. However, sublingual ECP was significantly decreased after 6 months of SLIT, compared to baseline and to the end of the buildup phase ($P<0.01$ for all comparisons), as shown in Fig. 3. Interestingly, in the only subject who reported a persistent oral itching since the beginning of SLIT, sublingual tryptase remained negative and ECP did not change after two repeated administrations of the dose (about 0.01 μg of allergen) which provoked symptoms.

Table 2. Levels of ECP and tryptase (mean $\pm$ SEM) ($\mu\text{g/l}$) in allergic and nonallergic subjects at baseline

	Allergic ($n=40$)	Healthy ($n=10$)	P (Mann-Whitney)
Nasal tryptase	14.4 ± 2.5	<1	0.02
Nasal ECP	46.8 ± 4.1	9.3 ± 2	0.001
Sublingual tryptase	1.1	1.1	NS
Sublingual ECP	44.3 ± 3.6	5.1 ± 1.9	0.001

Discussion

The safety of SLIT in adults and children has been well documented clinically. Nevertheless, the immunologic effects of the local administration of an allergen have never been investigated before, although the local side-effects of SLIT are well known from the literature and clinical experience. Oral itching, swelling, edema, and irritation in general are attributed to an IgE-mediated reaction, because they occur within minutes after the administration of the allergen. They also occur after an allergen-specific challenge in the nose or the conjunctiva (14).

When an IgE reaction takes place, the degranulation of mast cells is the first event, followed by the release of mediators, cellular infiltration, and inflammation in which eosinophils are mostly present. The mast-cell degranulation and the activation of eosinophils can be easily demonstrated by the increase of specific mediators, such as tryptase and ECP (15–18). In fact, a significant increase of nasal tryptase and ECP after ASNC (with an amount of 0.016–0.064 μg of allergen) occurred in the group of allergic patients. However, no increase of tryptase could be demonstrated after the administration of SLIT, even at the dose of 0.5 μg of allergen, thus giving serious reasons

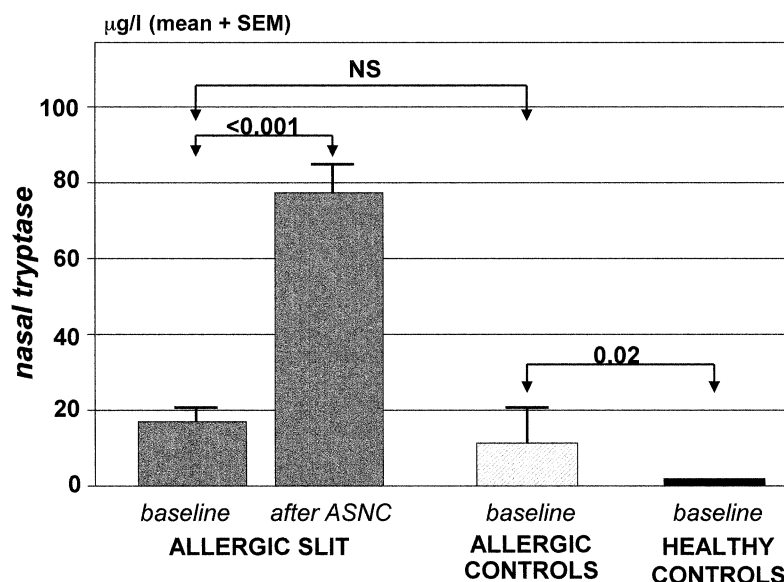


Figure 1. Nasal tryptase (mean $\pm$ SEM, $\mu\text{g/l}$) before and after ASNC in 30 children assigned to SLIT (Allergic SLIT) and baseline values in 10 allergic and 10 healthy controls.

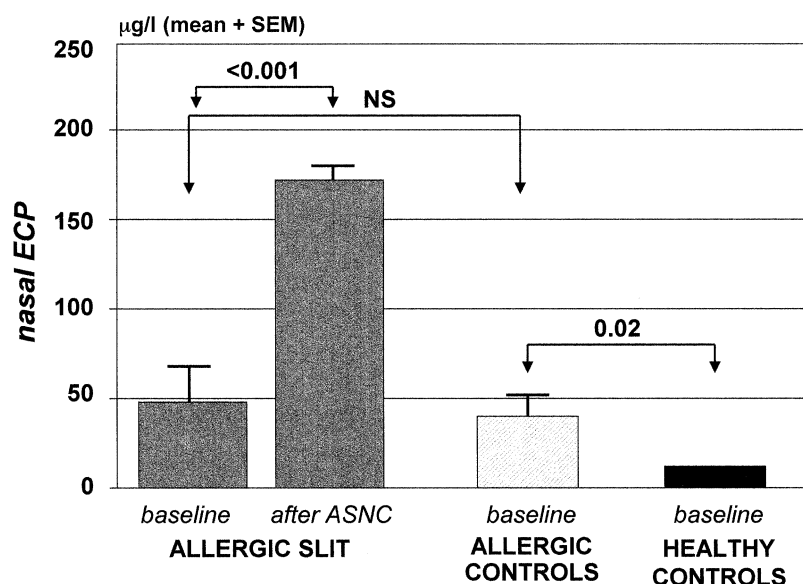


Figure 2. Nasal ECP (mean $\pm$ SEM, $\mu\text{g/l}$) before and after ASNC in 30 children assigned to SLIT (Allergic SLIT) and baseline values in 10 allergic and 10 healthy controls.

to doubt that an allergic reaction can occur in the mouth. Interestingly, after 6 months of SLIT therapy, the sublingual ECP was even significantly reduced in comparison to baseline and 1-month values. The only patient reporting oral itching troublesome enough to cause withdrawal from SLIT did not show any change in tryptase and ECP in his oral mucosa even under controlled conditions. These concerns about the pathogenic mechanisms of the local side-effects of SLIT are further corroborated by the complete absence of mast cells in sublingual biopsies from allergic patients (data not published).

Nevertheless, the oral mucosa is expected to play a relevant role in the mode of action of local immunotherapy. This is suggested by pharmacokinetics studies, finding long-lasting persistence of the allergen in the mouth (19), and by the demonstration of the activity of oral antigen-presenting cells (20).

In contrast, it is interesting that sublingual and nasal ECP was higher in allergic subjects than in normal controls, and that sublingual ECP was reduced after 6 months of SLIT. It is not possible to determine the origin of the ECP detected in the mouth, but it can be hypothesized that the presence of detectable levels of

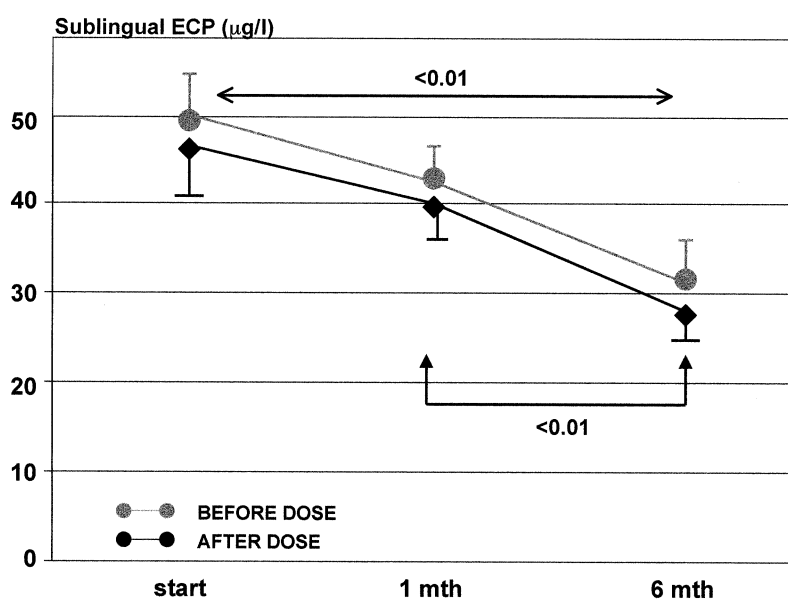


Figure 3. Sublingual ECP (mean $\pm$ SEM, $\mu\text{g/l}$) at different time points of SLIT course (beginning, end of buildup phase, and 6 months after), measured before dose delivery and 24 h later.

ECP may be due to the mucociliary clearance, which transports bronchial secretion from the bronchi to the

mouth (21). The increased levels of nasal ECP would suggest the presence of a minimal ongoing activation of eosinophils, even in the absence of allergenic stimuli.

Finally, the procedure of measurement of mucosal tryptase and

ECP was confirmed to be a valuable and simple tool to study the allergic reaction.

References

1. MALLING H-J, ABREU-NOGUEIRA L, ALVAREZ-CUESTA E, et al. EAACI/ESPACI position paper on local immunotherapy. *Allergy* 1998;**53**:833–844.
2. BOUSQUET J, SCHEINMANN P, GUINNEPAIN MT, et al. Sublingual swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. *Allergy* 1999;**55**:249–260.
3. PURELLO D'AMBROSIO F, GANGEMI S, ISOLA S, et al. Sublingual immunotherapy: a double-blind, placebo-controlled trial with *Parietaria judaica* extract standardized in mass units in patients with rhinoconjunctivitis, asthma or both. *Allergy* 1999;**54**:968–973.
4. BOUSQUET J, LOCKEY RF, MALLING H-J, editors. WHO position paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* 1998;**53** Suppl 44:1–42.
5. Allergic rhinitis and its impact on asthma. WHO position paper. BOUSQUET J, KALTHAEV N, VAN CAUWENBERGE P, editors. 2001 (in press).
6. DI RIENZO V, PAGANI A, PARMIANI S, PASSALACQUA G, CANONICA GW. Post-marketing surveillance study on the safety of sublingual immunotherapy in pediatric patients. *Allergy* 1999;**55**:1110–1113.
7. ANDRÉ C, VATRINET C, GALVAIN S, CARAT F, SICARD H. Safety of sublingual-swallow immunotherapy in children and adults. *Int Arch Allergy Immunol* 2000;**121**:229–234.
8. LA ROSA M, RANNO C, ANDRÉ C, CARAT F, TOSCA MA, CANONICA GW. Double blind placebo controlled evaluation of sublingual swallow immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 1999;**104**:425–432.
9. PAJNO GB, MORABITO L, BARBERIO G, PARMIANI S. Clinical and immunologic effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, placebo-controlled study. *Allergy* 2000;**55**:842–849.
10. FREW AJ, WHITE PJ, SMITH HE. Sublingual immunotherapy. *J Allergy Clin Immunol* 1999;**104**:267–270.
11. LOMBARDI C, CANONICA GW, PASSALACQUA G. Sublingual immunotherapy in OAS. *Allergy* 2000;**55**:817–818.
12. Società Italiana di Allergologia e Immunologia Clinica. Memorandum sulla diagnostica allergologica. *G Ital Allergol Immunol Clin* 1992;**2**:35–38.
13. SENSI LG, SERI A, SIRACUSA A, PERTICI A, MARCUCCI F. Allergic rhinitis in children: effects of flunisolide and disodium cromoglycate on nasal eosinophil cationic protein. *Clin Exp Allergy* 1997;**27**:270–276.
14. PASSALACQUA G, ALBANO M, FREGONESE L, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet* 1998;**331**:629–632.
15. DI LORENZO G, MANSUETO P, MELLUSO M, et al. Allergic rhinitis to grass pollen: measurement of inflammatory mediators of mast cells and eosinophils in native fluid lavage and in serum out of and during the pollen season. *J Allergy Clin Immunol* 1997;**100**:832–837.
16. BETTIOL J, RADMAKER M, SELE J, HENQUET M, CATALDO D, LOUIS R. Airway mast cell activation in asthmatics is associated with selective sputum eosinophilia. *Allergy* 1999;**55**:1188–1193.
17. WILSON SJ, LAU L, HOWARTH PH. Inflammatory mediators in naturally occurring rhinitis. *Clin Exp Allergy* 1998;**28**:220–227.
18. KLEINJAN A, MCEUEN AR, DIJKSTRA MD, et al. Basophil and eosinophil accumulation and mast cell degranulation in the nasal mucosa of patients with hay fever after local allergen provocation. *J Allergy Clin Immunol* 2000;**106**:677–686.
19. BAGNASCO M, MARIANI G, PASSALACQUA G, et al. Absorption and distribution kinetics of the major *Parietaria* allergen administered by noninjectable routes to healthy human beings. *J Allergy Clin Immunol* 1997;**100**:13–18.
20. VAN WILSEM EJ, VAN HOOGRATEN IM, BREVE J, et al. Dendritic cells of the oral mucosa and the induction of oral tolerance. A local affair. *Immunology* 1994;**93**:128–132.
21. FAHY JV, LIU J, WONG H, BOUSHEY HA. Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. *Am Rev Respir Dis* 1993;**147**:1127–1131.