

Original article

Effects on inflammation parameters of a double-blind, placebo controlled one-year course of SLIT in children monosensitized to mites

Background: Clinical documentation about effects on local markers of inflammation of sublingual immunotherapy (SLIT) in children is still poor.

Methods: Twenty-four children (age range 4–16 years, average 8.5 years) monosensitized to house dust mites (HDMs) were randomized to receive active or placebo SLIT for this allergen according to a double-blind, placebo-controlled design. Before treatment and 10–12 months later the following parameters were checked: ECP and tryptase in sputum and nasal secretion, serum and nasal mite-specific IgE (sIgE), allergen-specific nasal challenge test (sNCT), nasal symptoms and tryptase after sNCT.

Results: Nasal tryptase and nasal IgE in basal conditions were unchanged in treated children but significantly increased in untreated children ($P = 0.0156$ and $P = 0.0313$, respectively). The threshold for sNCT was unchanged in both groups of children, but the symptom score after sNCT was unchanged in the placebo group and significantly decreased in the active group ($P = 0.0084$). The nasal tryptase after sNCT was unchanged in the active group and significantly increased in the placebo group ($P = 0.0218$). Intergroup comparison showed a significant difference in oral tryptase and nasal tryptase after sNCT in favour of the active group.

Conclusions: These interim results after only 1 year of treatment show that SLIT in children monosensitized to HDMs is able to avoid the spontaneous increase in both nasal sIgE antibodies and in local allergic inflammation in basal conditions. These outcomes are confirmed and supported by the decrease of symptoms in the active group combined with the increase of nasal tryptase only in the control group in both cases after sNCT.

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Key words: children; double-blind placebo-controlled study; eosinophil cationic protein; nasal challenge test; nasal IgE; nasal symptoms; sublingual immunotherapy; tryptase.

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Accepted for publication 28 February 2003

Much effort has been devoted in the last decade to study and document sublingual immunotherapy (SLIT) and the publication of several positive double-blind placebo-controlled (DBPC) studies run in adults have led to the official recognition by the WHO of the efficacy of this form of therapy (1). Relatively fewer studies have been run in children, but nonetheless the last WHO ARIA document (2) states as 'evidence based' the efficacy of SLIT for seasonal allergens in children. There is still, on the contrary, no official position about the efficacy of SLIT for perennial allergens in children because of the lack of adequate clinical evidence.

Most published studies have considered clinical scores as main parameters, whereas only in a few cases immunological and/or inflammation parameters have been studied.

The local production of IgE and the amount of inflammation mediators detectable in basal conditions or released upon allergen-specific provocation are, on the other hand,

valuable tools to check by means of objective parameters the changes elicited by specific immunotherapy.

Our group has previously shown that in children submitted to SLIT with grass allergens there is no increase of sublingual eosinophil cationic protein (ECP) and tryptase, demonstrating the safety of SLIT (3). Its capability to decrease allergen-driven inflammation at mucosal level was also evidenced.

We designed this study to check at the nasal level the change, if any, in immunological/inflammation parameters in children monosensitized to house dust mites (HDMs), submitted or not to SLIT. We sampled also the sputum of treated children to evaluate possible changes in lower airways. Sputum was preferred to saliva because it allows for an indirect estimation of the inflammation mediators released not only in the mouth but also in the respiratory mucosa. Here we report the results of the first year of the study, run according to a DBPC design; the experimental protocol includes also a

second year of treatment with all patients under active treatment, according to an open design. The results at the end of the second year of treatment will be reported in a separate paper.

Materials and methods

Study design

Twenty-four children with respiratory symptoms due to monosensitization to HDMs were enrolled in the study.

They were randomized by means of a computer-generated code to receive SLIT (13 children) or placebo (11 children), according to a DBPC design for 1 year.

Before starting the treatment and 10–12 months after, the following determinations were performed: ECP and tryptase in sputum, nasal and serum mite-specific IgE, and nasal ECP. An allergen-specific nasal challenge was also performed before starting the treatment and 10–12 months later, with the assessment of the following parameters: allergen threshold dose, nasal symptoms after nasal challenge and nasal tryptase before and 30 min after nasal challenge.

Patients

Children were considered eligible for the study if monosensitized to HDMs, with a clinical history of at least 2 years of rhinitis and/or asthma related to perennial allergens and never treated with specific immunotherapy before.

Demographic data are shown in Table 1. The children were submitted to the standard skin prick test screening panel, including biologically standardized allergenic extracts of mites, grasses, *Parietaria*, olive, cypress, *Alternaria*, *Cladosporium*, cat and dog dander (ALK-Abellò, Milan, Italy). Wheals at least half the weal elicited by histamine hydrochloride 10 mg/ml were considered as positive (4) and the skin prick test results were confirmed by *in vitro* IgE determination (UniCAP IgE, FEIA, Pharmacia, Uppsala, Sweden).

The experimental protocol was approved by the hospital Ethics Committee, and all patients' parents were asked to sign an informed consent to the study before enrolment.

Allergen-specific nasal challenge test (sNCT)

The sNCT was performed just before the beginning of the treatment, the patient being free of allergic symptoms and not taking any drug possibly able to interfere with the test.

The test was begun by spraying the solvent of the allergenic solution (10% w/v glycerol in saline) into the nostril, followed by increasing concentrations of mite allergens: 2, 4 and 8 BU/ml (ALK-Abellò, Milan, Italy). These concentrations correspond to

about 0.064, 0.128 and 0.256 µg of the major mite allergen Group 1 and the half of the major mite allergen Group 2 per actuation.

An arbitrary score-system was used for itching, sneezing, rhinorrhea, nasal blockage and tearing: 0 = no symptom, 1 = mild, 2 = moderate, 3 = severe. If a total score of at least 5 was not reached with the lowest concentration, the following ones were sequentially used. Symptoms were registered for 20 min after the administration of each test solution.

Assessment of tryptase and ECP in nasal mucosa and in sputum

Tryptase and ECP in sputum and nasal secretion was determined using ELISA (UniCAP Tryptase System FEIA and UniCAP ECP System FEIA, Pharmacia, Uppsala, Sweden), adapted for mucosal sampling, as previously reported (5). ECP and tryptase were first determined in basal conditions both in sputum and in nasal secretion, whereas tryptase was again determined in nasal secretion 30 min after the sNCT.

Briefly, the sponges of the CAP system, coated with anti-tryptase or anti-ECP antibodies, after washing with saline solution, were mounted and fixed by a permeable envelope on an appropriate plastic stick. The plastic stick carrying the sponge was then inserted into a nostril or the oral cavity and maintained in site for 5 min by means of a tape fixed on the skin of the nose or lips. Just before positioning the stick in the mouth, children were asked to cough 10 times in order to enhance the presence of bronchial secretions at the oral level. The sponge was 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 to avoid interanalysis variations. The concentration of tryptase and ECP was expressed in micrograms per litre, according to a calibration curve. For values below the sensitivity limit of the method or outside the calibration curve, i.e. <2 and >200 for ECP and <1 and >200 for tryptase, statistics was performed taking into account the corresponding limit value (2–200 for ECP and 1–200 for tryptase, respectively).

Assessment of serum and nasal mite-specific IgE

Mite-specific IgE in sera were determined by means of the UniCAP IgE FEIA method (Pharmacia, Uppsala, Sweden), according to the instructions of the supplier.

Nasal mite-specific IgE were determined according to a method previously described by our group (6, 7). Briefly, the sponges coupled with mite allergens used for the mite-specific IgE determination in sera were mounted on a plastic applicator, covered with a permeable membrane and allowed to incubate for 5 min in contact with the nasal mucosa at the level of the anterior part of the lower turbinate. After washing with a 1% v/v Tween 20 solution in saline (0.9% w/v NaCl in distilled water), the sponges were put in test tubes containing saline solution preserved with sodium azide (0.02% w/v) and stored at –20°C. Nasal IgE determinations were thereafter performed using the same technique used for serum IgE.

In statistical evaluations, for values below the detection limits, i.e. <0.35 and >200 kU/l, the limit value was considered (0.35–200).

SLIT and concomitant treatments

The SLIT was prepared from standardized allergens (1 ml of the top-dose vial = 1000 STU/ml, corresponding to 4 µg of the major mite allergen Group 1 and 2 µg of the major mite allergen Group 2) and administered in the mornings before breakfast as drops in

Table 1. Demographic data

Group	Number	Age range, years (average)	Male/female	Pathology
Active	13	4–11 (7.7)	6 M/7 F	2 RC, 2 A, 9 RCA
Placebo	11	3–15 (7.3)	10 M/1 F	2 RC, 9 RCA
Total	24	3–15 (7.5)	16 M/8 F	4 RC, 2 A, 18 RCA

RC, rhinoconjunctivitis; A, asthma; RCA, rhinoconjunctivitis + asthma.

aqueous solution containing 50% w/v glycerol, 0.9% w/v NaCl and 0.3% w/v phenol as preservative (ALK-Abellò, Milan, Italy). The placebo treatment had the same composition and presentation of the active treatment, but obviously contained no allergen. Patients and their relatives were instructed to keep the allergen drops in the mouth for at least 2 min and then to swallow (sublingual-swallow technique). The build-up phase was completed in 30 days with the administration of increasing amounts of allergenic solution from five different vials (1.6, 8, 40, 200, 1000 STU/ml), starting from one drop of the first vial up to five drops of the last vial. The maintenance dose of five drops from vial 5, corresponding to around 0.8 µg of mite allergen Group 1 and 0.4 µg of mite allergen Group 2, was thereafter administered every other day for at least 12 months. On a yearly base, the cumulative dose of allergen was around 110 µg of mite allergen Group 1 and around 55 µg of mite allergen Group 2.

All children, whatever treatment group they belonged to, received an appropriate pharmacological therapy (oral antihistamines, nasal corticosteroids, inhaled corticosteroids, cromolyn and salbutamol) to be used on need to control their allergic symptoms.

Statistical analysis

Statistical analysis was performed by means of nonparametric tests (Wilcoxon test for intragroup comparison and Mann–Whitney *U*-test for intergroup comparison), because there was no chance to check all parameters under consideration for normal distribution. Because of the high interindividual variability of the baseline values, intergroup analysis were run on the basis of the variation T1 vs T0 in each patient of the parameter under observation.

Statistical analysis was performed with a standard statistical software (BMDP Inc., Los Angeles, USA). *P* values of 0.05 or less were considered as statistically significant.

Results

Taking into account the average values in each group, the nasal ECP decreased from 59.4 to 42.5 µg/l in the active group, whereas the same parameter increased from 52.6 to 69.7 µg/l in the placebo group. The same comparison for ECP in sputum showed a slight increase from 16.1 to 19.7 µg/l in the active group and a sharp increase from 11.1 to 53.8 µg/l in the placebo group (Table 2).

Tryptase in sputum showed, on the contrary, a statistically significant decrease from baseline after 12 months of active treatment from an average value of 2.5–1.2 µg/l (*P* = 0.0078), whereas an almost stable value was detected for the placebo group (2.3 µg/l at the baseline vs 2.2 µg/l after 12 months; Fig. 1). The intergroup analysis showed a statistically significant difference in favour of the active group (*P* = 0.013).

Nasal tryptase in basal conditions showed a statistically nonsignificant increase in the active group (average baseline value 7.5 µg/l, average value after 12 months 12.9 µg/l) and a statistically significant increase in the placebo group during the same period (from 2.7 to 41.3 µg/l on average, *P* = 0.0156; Fig. 2). No difference was found by means of the intergroup analysis (*P* = 0.246).

Table 2. Values (mean and range) for the investigated parameters

Parameter	Active group	Placebo group
Nasal ECP	T0: 59.4 (12.0–122) T1: 42.5 (2.0–138) <i>P</i> = n.s. (<i>n</i> = 13)	T0: 52.6 (2.0–125) T1: 69.7 (2.0–200) <i>P</i> = n.s. (<i>n</i> = 11)
ECP in sputum	T0: 16.1 (2.0–41.8) T1: 19.7 (2.0–54.9) <i>P</i> = n.s. (<i>n</i> = 11)	T0: 11.1 (2.0–21.2) T1: 53.8 (2.0–200) <i>P</i> = n.s. (<i>n</i> = 10)
Tryptase in sputum	T0: 2.5 (1.0–3.6) T1: 1.2 (1.0–2.06) <i>P</i> = 0.0078 (<i>n</i> = 9)	T0: 2.3 (1.0–4.9) T1: 2.2 (1.0–5.68) <i>P</i> = n.s. (<i>n</i> = 9)
Nasal tryptase (before sNCT)	T0: 7.5 (1.0–36.1) T1: 12.9 (1.0–45.6) <i>P</i> = n.s. (<i>n</i> = 12)	T0: 2.7 (1.0–9.12) T1: 41.3 (1.0–200) <i>P</i> = 0.0156 (<i>n</i> = 10)
Nasal tryptase (30 min after sNCT)	T0: 61.9 (12.9–200) T1: 44.2 (1.0–200) <i>P</i> = n.s. (<i>n</i> = 11)	T0: 24.0 (1.0–150) T1: 52.0 (2.4–200) <i>P</i> = 0.0218 (<i>n</i> = 10)
Nasal IgE	T0: 2.1 (0.35–10.5) T1: 1.35 (0.35–10.6) <i>P</i> = n.s. (<i>n</i> = 13)	T0: 0.5 (0.35–0.96) T1: 1.12 (0.35–6.51) <i>P</i> = 0.0313 (<i>n</i> = 11)
Nasal symptom scores after sNCT	T0: 8.8 (5–12) T1: 5.6 (1–10) <i>P</i> = 0.0084 (<i>n</i> = 13)	T0: 7.0 (4–11) T1: 4.4 (0–12) <i>P</i> = n.s. (<i>n</i> = 11)
Serum IgE	T0: 37.1 (0.36–100) T1: 38.0 (0.48–100) <i>P</i> = n.s. (<i>n</i> = 13)	T0: 19.4 (0.36–100) T1: 19.5 (0.37–100) <i>P</i> = n.s. (<i>n</i> = 11)

T0, basal condition (before starting of the DBPC treatment); T1, after 11 ± 1 months; *n*, data analysed.

The same trend could be shown for nasal tryptase 30 min after the sNCT during the same period. This parameter had a nonsignificant decrease in the active group (from 61.9 to 44.2 µg/l on average), whereas a statistically significant increase was detected in the placebo group after 12 months (from 24.0 to 52.0 µg/L on average, *P* = 0.0218; Fig. 3). The intergroup analysis showed a statistically significant difference in favour of the active group (*P* = 0.003).

Serum mite-specific IgE remained almost stable in both groups. Nasal-specific IgE showed, on the contrary, an opposite trend in the two groups. The basal value of 2.1 kU/l decreased to 1.35 kU/L after 12 months without reaching a statistical significance, whereas the increase from 0.5 to 1.12 kU/l in the placebo group was statistically significant (*P* = 0.0313).

No statistically significant change between basal values and values at the end of the 12-month treatment period could be shown in either group for both nasal and sputum ECP.

No change could be detected in either group for the sNCT threshold (data not shown). The symptom scores after sNCT decreased nonetheless significantly from 8.8 to 5.6 (*P* = 0.0084) in the active group, whereas the decrease in the placebo group from 7.0 to 4.4 did not reach statistical significance.

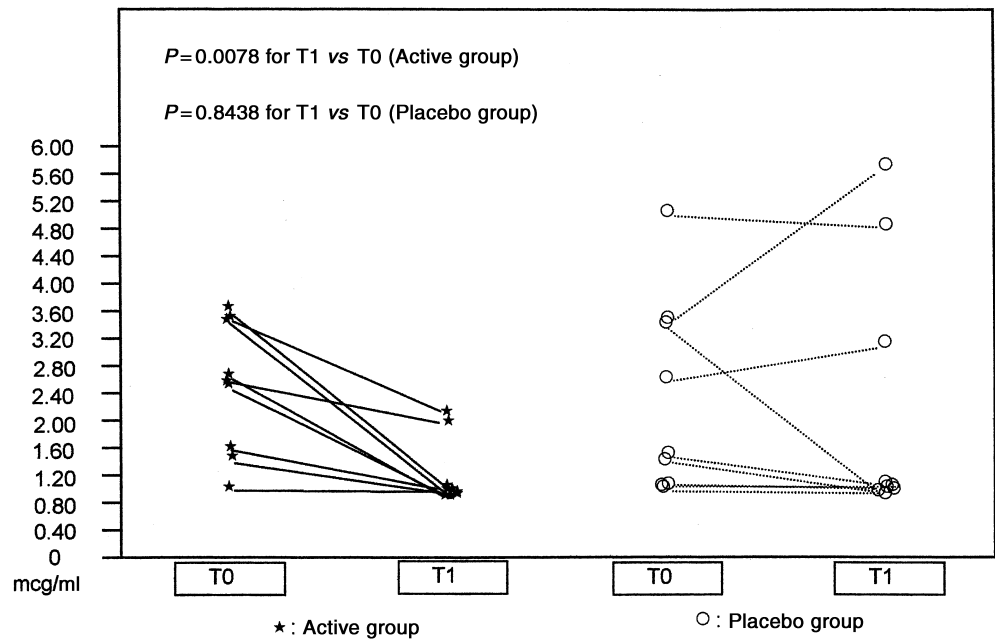


Figure 1. Evolution of tryptase in sputum from T0 to T1.

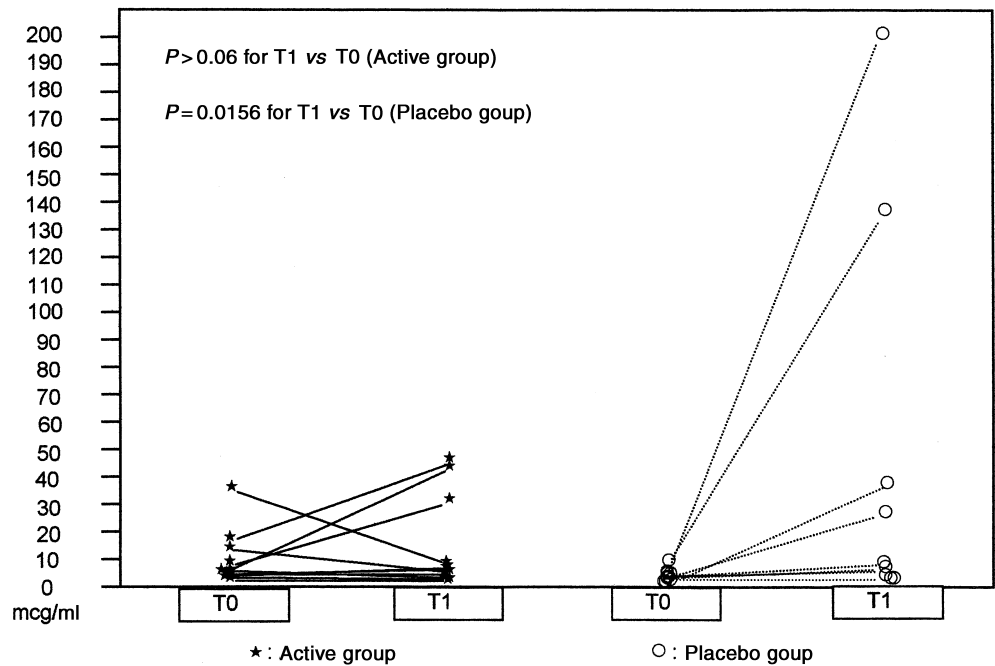


Figure 2. Evolution of nasal tryptase from T0 to T1 in basal conditions (before SNPT).

Discussion

This study was run according to a DBPC design to investigate the effects of a 12-month course of SLIT on local parameters of allergic inflammation in a group of children sensitized to HDMs. Clinical data and their relationships with the inflammatory parameters will be

reported in a separate paper at the end of the second year of treatment.

We have chosen to follow the variation of local rather than systemic parameters of allergic inflammation because in our previous experience we have shown that, if investigated with an appropriate method, both specific IgE and ECP in the nasal mucosa showed a better

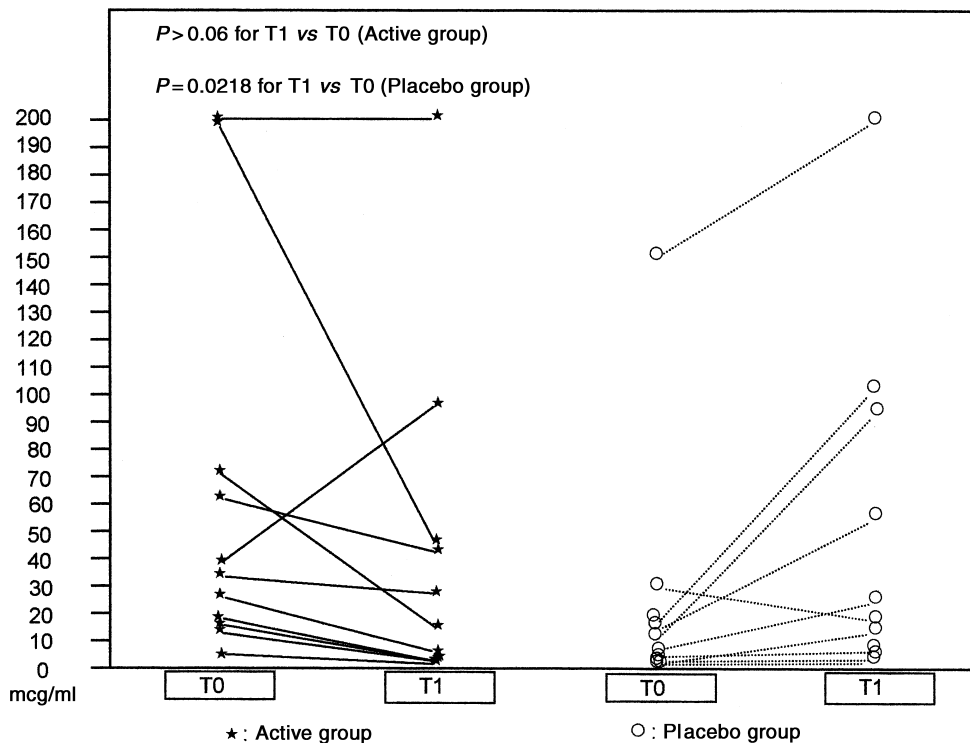


Figure 3. Evolution of nasal tryptase from T0 to T1 30 minutes after SNPT.

correlation with allergen exposure than serum evaluations (7). The nasal mucosa is currently recognized as a site of ongoing IgE synthesis and a persistent IgE synthesis has been demonstrated in explanted cultured cells obtained from grass pollen-sensitive patients. Moreover, a higher proportion of IgE produced by this method in comparison to serum IgE is allergen specific (8).

The randomization key followed did not allow for a good balancing for gender between groups but we believe that this had little or no effect on the final outcomes.

Our data show that SLIT in comparison to placebo is able to significantly decrease one inflammation parameter or at least to contrast the increase of other inflammation parameters. After a 12-month course of treatment children under placebo showed a significant increase in nasal tryptase both in basal conditions as well as 30 min after sNCT, and in nasal allergen-specific IgE, whereas a decrease or a statistically nonsignificant increase for the same parameters was seen in children under active therapy. ECP in sputum and nasal secretion showed no statistically significant changes in both group, but values were decreasing or stable in the active group as compared to a small or a fivefold increase in the placebo group.

Tryptase in sputum significantly decreased in actively treated children, whereas the same parameter showed an almost stable value in the placebo group.

No change was detected in the threshold value for the sNCT but, in spite of this, children under active therapy

showed a statistically significant decrease in symptom scores after the challenge, whereas the decrease in placebo-treated children was not statistically significant.

These clinical observations in our opinion fit well and are consistent with the results obtained for inflammation parameters in the two groups of children.

In a previous study in children under active SLIT therapy for grass pollen, we reported a decrease of ECP and no variation of tryptase in saliva (3), whereas in this trial we have detected a significant decrease of tryptase and no change in ECP in sputum in the active group. The higher ECP levels can be explained with the different samples used for detection, which also included bronchial-derived mediators.

Moreover, the difference in allergens under investigation (grass as opposed to mites), the different size of the trials, the different length of the treatments may have played in our opinion a relevant role. Anyway, the difference between active and placebo groups seem to confirm our previous data about the safety of SLIT. Both perennial and seasonal allergens have been associated to minimal persistent inflammation even if objective clinical signs are not detected, but for pollens it is obviously limited to the pollen season, whereas for perennial allergens the continuous exposure to the natural allergen gives chronic inflammation (9, 10). This may account for the need of a longer treatment with allergen-specific immunotherapy in patients sensitized to mites as compared to patients sensitized to pollens.

Negative or poor results from the clinical point of view have been indeed obtained with specific immunotherapy for perennial allergens in short-term studies (11, 12), whereas long-term studies (i.e. lasting more than 18 months) with these allergens have shown good or excellent results (13–16).

Changes in an inflammation parameter such as ICAM-1 expression before and after conjunctival challenge have been shown after 12 and 24 months of sublingual therapy in mite-sensitized subjects, and these changes were associated to changes in eosinophils and neutrophils after

conjunctival challenge at 12 and 24 months and before challenge at 24 months (14).

The size of the population of children investigated by us and the short duration of the observation period of our study do not allow to draw definitive conclusions, but our data are consistent with other published trials and confirms that SLIT is able to decrease or to contrast the increase of some inflammation parameters.

These laboratory results should be ideally matched with clinical outcomes in future allergen-specific immunotherapy studies to be correctly interpreted and used.

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