

Original article

Preseasonal local allergoid immunotherapy to grass pollen in children: a double-blind, placebo-controlled, randomized trial

Background: We assessed the efficacy of preseasonal local allergoid immunotherapy in a group of children with asthma and/or rhinitis and/or rhinoconjunctivitis due to grass pollen.

Methods: We randomly assigned 24 children allergic to grass pollen to receive local allergoid immunotherapy for 3 months before the pollen season and 24 such patients to receive identically appearing placebo. The immunotherapy consisted of tablets of monomeric allergoid grass pollen allergens held in the mouth until they dissolved and then swallowed. The study was double-blind. Symptoms and medications were scored on diary cards during the pollen season. Nasal eosinophil cationic protein levels were measured by the monoclonal antibodies EG1 and EG2 outside the pollen season and at low and at high pollen concentration during the pollen season.

Results: The active-treatment group had a statistically significant reduction of total symptoms ($P < 0.05$), especially bronchial symptoms ($P < 0.05$), in comparison with the placebo group. Immunotherapy was well tolerated and compliance was good. Nasal levels of EG2 and EG1 increased significantly during the pollen season, but there was no difference between groups. EG2/EG1 increased significantly only in the placebo group during natural allergen exposure ($P < 0.01$).

Conclusions: Our results suggest that this immunotherapy is effective for the treatment of asthma due to grass pollen in children.

C. Caffarelli

Pediatric Department, Parma

L. G. Sensi, F. Marcucci

Pediatric Department, Perugia

G. Cavagni

Pediatric Department Modena Sud, Sassuolo, Italy

Key words: asthma; children; grass pollen; local immunotherapy; rhinitis.

Dr Carlo Caffarelli
Clinica Pediatrica
Via Gramsci, 14
43100 Parma
Italy

Accepted for publication 29 June 2000

Subcutaneous specific immunotherapy is considered to be effective for allergic diseases (1). However, injection immunotherapy may be inconvenient and, in rare cases, causes severe systemic reactions (1). With a view to improving the safety and acceptability of treatment, renewed interest is being shown in noninjected allergenic extracts, such as the oral and sublingual routes. Both the WHO (2) and the EAACI-ESPACI Working Group on Local Immunotherapy (3) have stated that there is evidence of the clinical efficacy of sublingual-swallow immunotherapy (SLIT) (4–13), but not of oral or sublingual-split immunotherapy. SLIT and injective immunotherapy were equally effective in adults with grass pollen allergy (14). The EAACI-ESPACI position paper (3) did not recommend SLIT for normal clinical use in children, since only a few controlled clinical trials had evaluated the efficacy and the safety of SLIT in children. However, a post-marketing surveillance study has recently found that SLIT is safe in children (15).

Children can hardly keep the extracts under the tongue. There is a lack of data showing that the sublingual mucosa can absorb allergenic molecules better than other mucosal sites (dorsal surface of the tongue, labia, cheeks, and soft palate). In the present

study, the immunotherapy dosage consisted of tablets of monomeric allergoid grass pollen allergens to be held in the mouth until they dissolved, after which the residue was swallowed (oromucosal-swallow immunotherapy [OSI]) (16).

A reduction in both the cellular infiltration and the expression of intercellular adhesion molecule (ICAM-1) in the conjunctiva after allergen challenge was found after 1 year of SLIT (9). Furthermore, a reduction of neutrophils, eosinophils, and ICAM-1 expression after specific nasal challenge was detected after preseasonal SLIT with *Parietaria* (17).

Activation of eosinophils occurs during the pollen season. Nasal levels of eosinophil cationic protein (ECP) have been found to increase significantly during natural allergen exposure in patients allergic to pollen (18, 19). Moqbel et al. found that the anti-ECP monoclonal antibody EG2 recognized activated eosinophils and a minority of resting eosinophils while the anti-ECP monoclonal antibody EG1 recognized both activated and resting eosinophils (20). Therefore, the EG2/EG1 ratio is considered to be more reliable in identifying activated eosinophils.

We are not aware of studies on immunotherapy with

grass pollen administered orally in children. Therefore, we performed a randomized, placebo-controlled study in children allergic to grass pollen to evaluate the clinical efficacy and the safety of local allergoid immunotherapy. We also determined nasal levels of ECP by monoclonal antibodies (EG1 and EG2) outside the pollen season and during the season in order to assess allergic inflammation.

Material and methods

Patients

Forty-eight consecutive children (21 boys and 27 girls) aged 4–14 years (mean 8.4 years), outpatients at the pediatric departments of Parma, Perugia, and Brescia (Italy), were enrolled. The children had rhinitis and/or conjunctivitis and/or bronchial asthma in the grass pollen season, serum grass-specific IgE antibodies, and a positive skin prick test with grass pollens, including pollens contained in extracts for immunotherapy. Forty-three children had asthma, 34 rhinitis, and 27 conjunctivitis (Table 1). Sensitizations to allergens other than grass pollens (mites, pellitory, cat and dog dander, birch, mugwort, *Alternaria*, and *Aspergillus*) were excluded on the basis of clinical symptoms and negative skin prick test reactions.

Patients with perennial asthma and/or rhinitis who had received specific immunotherapy in the 3 years before the beginning of the present study and patients under treatment with systemic steroids were excluded from the study. Other exclusion criteria were the contraindications for immunotherapy of the European Academy of Allergy and Clinical Immunology (EAACI) (1).

Children were randomly assigned by a computer-generated list to receive either grass-pollen allergoid oral soluble tablets or placebo. Neither the investigators nor the patients were aware of the treatment assignments.

All parents gave written informed consent.

Skin prick tests and grass-specific IgE

Skin prick tests were performed with commercial extracts (Lofarma S.p.A, Milan, Italy). The extract for skin prick testing was not from the same allergen batch employed for immunotherapy. The diluent was used as negative control. Histamine 10 mg/ml was used as positive control. The reaction was considered positive if the wheal size was at least 3 mm after subtracting the diameter of the negative control wheal. Circulating grass-specific IgE was determined by the CAP System (Pharmacia, Uppsala, Sweden) and considered positive if at least class 2.

Table 1. Data of patients at entry

Characteristic	Group		P value
	Immunotherapy n = 24	Placebo n = 20	
Sex (M/F)	12/12	13/7	NS
Age (years)*	8.7 (3.3)	8.1 (2.7)	NS
Asthma	7	3	NS
Rhinitis	1	–	NS
Asthma and rhinitis	3	3	NS
Asthma, rhinitis, and conjunctivitis	13	14	NS

* Mean (SD).

NS: not significant.

Immunotherapy

Immunotherapy consisted of a mixture of monomeric allergoid grass-pollen allergens (33% *Holcus lanatus*, 33% *Phleum pratense*, and 33% *Poa pratensis*) incorporated in commercially available tablets (LAIS, Lofarma S.p.A, Milan, Italy). The procedure for chemical modification and the immunologic features of the final product have been described by Mistrello et al. (21). Briefly, the allergen is reacted with potassium cyanate (KCNO) at basic pH to obtain partial substitution of the amino groups and a substantial loss of its capacity to react with IgE antibodies, as measured by RAST inhibition (22). SDS-PAGE showed that the molecular size of carbamylated allergens corresponded to the native allergens. The LAIS preparation was titrated in allergenic units (AU) and standardized by RAST inhibition in comparison with an in-house reference preparation (IHR). The AU is a biologic unit originally established to titrate products for intranasal immunotherapy and is defined as 1/40 of the mean provocative dose by specific nasal challenge in a significant number of allergic volunteers (23). The tablets were prepared in doses of 25, 100, 300, and 1000 AU. KCNO-modified allergens have low allergenic potency but fully preserved immunogenic properties (21). They also maintain the native monomeric size, making them particularly suitable for use by the transmucosal route. The large molecular size of polymerized allergoids (e.g., by glutaraldehyde) presumably makes them unable to cross biologic membranes.

The placebo consisted of tablets that were indistinguishable from active treatment.

Immunotherapy was administered according to a preseasonal schedule from January until 15 April. The children took tablets containing either active treatment or placebo in the morning, on an empty stomach, and kept them in the mouth until they dissolved (1–2 min). In the buildup phase, children treated with active extracts had increasing doses of allergens: 25, 50, 100, 200, 300, 600, and 1000 AU. The tablets were given every other day (three times a week) until the maximum dosage was reached (1000 AU). This dosage was

repeated three times a week until the beginning of the pollen season, when immunotherapy was stopped. The cumulative dosage was 37 250 AU.

Symptom score and symptomatic treatment

Parents were instructed to record symptoms and any medications taken on a daily diary card during the pollen season, from 15 April to 15 June. Every month, a physical examination was done and the diary card was checked.

The weekly symptom score (*nasal symptoms*: itching, sneezing, nasal discharge, nasal obstruction; *eye symptoms*: itching, redness; *bronchial symptoms*: cough, difficulty in breathing, wheezing) was obtained by rating symptoms reported daily by parents according to the following scale: 0: no symptom; 1: mild; 2: moderate; 3: severe symptoms.

Patients were allowed to use the following drugs when necessary: local (both nasal sprays and eye-drops) or systemic antihistamines, inhaled β_2 -agonists, inhaled steroids, and theophylline.

The medication recorded by patients in their diary cards was rated on the following scale: one point for each application of local antihistamines; two points for each dose of systemic antihistamines, inhaled β_2 -agonists, inhaled steroids, or theophylline. Each patient's weekly drug consumption was recorded by counting the daily doses, scoring as above.

Safety and compliance

Each patient was evaluated for safety during the treatment period. Parents were asked to call the department if children experienced side-effects from treatment and to report them on the diary card. Safety was evaluated in terms of the number of side-effects experienced during treatment after administration of the allergen. For each patient the type of symptom, the interval from the dose, the duration of the reaction, the dosage that provoked the reaction, and the concomitant presence of any disorder were reported. Compliance with treatment was evaluated by counting leftover tablets at each visit.

ECP

Before the start of immunotherapy (January), at low pollen concentrations (April), and at moderate pollen concentrations (beginning of June), nasal levels of ECP were measured with monoclonal antibodies against EG1 and EG2.

Nasal sampling was performed by the method of *in situ* incubation (24). Briefly, a sponge covalently bound with the monoclonal antibodies EG1 and EG2 (Pharmacia, Uppsala, Sweden) was employed as solid phase. The sponge was cut in half and blotted on Whatman paper, and the halves were inserted in the nasal applicator, covered by a membrane. The appli-

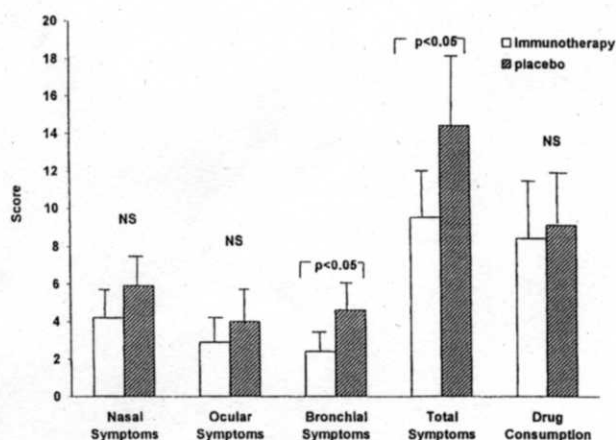


Figure 1. Weekly mean (95% CI) of symptom and medication scores in immunotherapy and placebo groups during pollen season. Columns represent mean values. Differences between two groups for weekly mean total symptom score and weekly mean bronchial symptom score were significant ($P < 0.05$).

cator was left at the level of the lower turbinate for 10 min. Then the sponge was put in a test-tube with 1 ml 0.9% (w/v) NaCl solution containing NaN_3 0.02% (w/v) as preservative. Samples were stored at -20°C until incubation with conjugate anti-ECP monoclonal antibodies (Pharmacia, Uppsala, Sweden).

Pollen counts

Grass pollen counts for the pollen season from 15 April to 15 June were obtained from Agenzia Regionale Prevenzione Ambientale.

Statistical analysis

Analysis of variance was used for continuous variables and chi-square or Fisher's exact test for categorical variables. Symptoms, drug intake, and EG1 and EG2 values were statistically analyzed by nonparametric tests; the Wilcoxon test was used for intragroup analysis and the Mann-Whitney U-test for intergroup analysis. The EG2/EG1 ratio was obtained for each child. The ratio was calculated as follows: $\text{EG2/EG1} \times 100$. Then, the median value of the ratios of each group was calculated.

Results

Forty-four out of 48 patients (91.6%), all 24 in the active treatment group and 20 of 24 given placebo, completed the study and reached the cumulative dosage of 37 250 AU. Four patients in the placebo group dropped out, three because they moved away, and one because of a mild side-effect (abdominal pain). Characteristics of the immunotherapy and placebo groups are listed in Table 1. There were no statistically significant differences between the two groups at entry into the study.

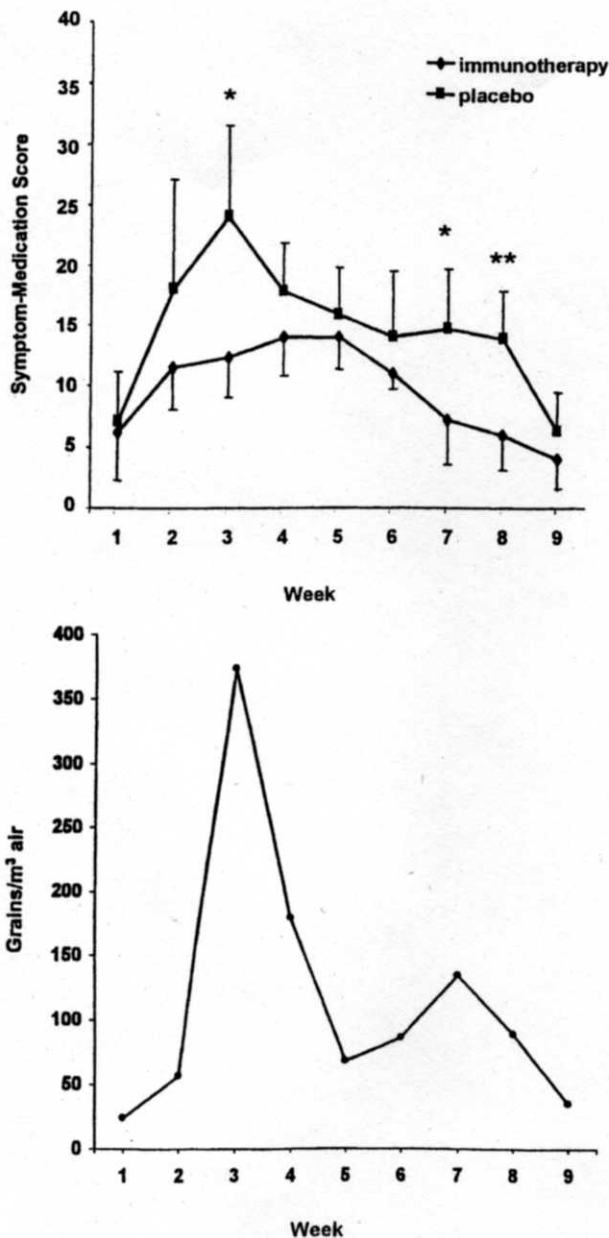


Figure 2. Mean (95% CI) symptom-medication scores and pollen counts in grass-pollen season. Significance of differences between immunotherapy and placebo groups: * $P < 0.05$; ** $P < 0.03$.

Two 4-year-old children were included in the study. One child took placebo, and the other received active treatment.

Neither systemic nor local adverse reactions were observed during treatment. Compliance with treatment was very good for all patients.

Total symptom scores during the pollen season (as weekly mean) were lower in the treated group than with placebo, the difference being statistically significant (mean [SD] 9.5 [7.2] vs 14.5 [8.3]; $P < 0.05$) (Fig. 1). The scores related to each nasal, ocular, and bronchial symptom were lower in the treated group, but the

difference was statistically significant only for bronchial symptoms (2.4 [2.7] vs 4.6 [3.5]; $P < 0.05$) (Fig. 1). Drug consumption scores (weekly mean) did not differ appreciably between the two groups (Fig. 1).

The symptom-medication score in the immunotherapy group was lower than in the placebo group during the pollen season in all weeks. This difference was statistically significant at week 3 ($P < 0.05$), week 7 ($P < 0.05$), and week 8 ($P < 0.03$) (Fig. 2). Mean weekly grass-pollen counts for the period of exposure to grass pollen from the centers participating in the study are shown in Fig. 2. Because of the weekly pollen concentration, five of the weeks were considered to have a very high pollen concentration (average weekly pollen count more than 80 pollen grains per cubic meter), and four to have a high pollen concentration. The symptom-medication score for weeks with elevated pollen concentration was significantly lower in the immunotherapy group than in the placebo group (10.1 [3.3] vs 16.84 [4.3]; $P < 0.03$). There was no significant difference between actively treated and placebo patients for the symptom-medication score under moderate pollen exposure (8.75 [4.6] vs 11.5 [6.2]; $P > 0.05$).

In the immunotherapy group, we found a significant increase during the pollen season for nasal levels of EG1 and EG2 (Table 2). We observed a similar increase in the placebo group. Comparison of EG1 and EG2 levels showed no difference between the placebo and immunotherapy groups (Table 2).

The EG2/EG1 ratio showed a significant increase at low pollen concentrations in comparison with baseline (T_0) in the placebo group (Table 2). In the immunotherapy group, the EG2/EG1 ratio did not change in the pollen season (Table 2); the between-groups comparison showed no difference.

Table 2. Median (range) and mean (standard deviation [SD]) values of nasal levels of EG1, EG2, and EG2/EG1 ratio in immunotherapy and placebo groups, at baseline, before starting immunotherapy treatment (January) (T_0), at low pollen concentrations (T_1) and at moderate pollen concentrations (T_2)

	Median (range) Mean (SD)		
	T_0	T_1	T_2
Immunotherapy group			
EG1 ($\mu\text{g/l}$)	6.6 (0.5-7.5) 10.3 (9.7)	19.2 (2.1-105)** 28.9 (29.6)	17.5 (0.1-200)* 53.6 (74.7)
EG2 ($\mu\text{g/l}$)	0.1 (0.1-12.8) 1.9 (3.4)	3.6 (0.1-41)* 8.7 (11.5)	2.1 (0.1-97.2)* 12 (24.5)
EG2/EG1 (%)	14.7 (0.4-100) 19.0 (25)	20.8 (1.4-85.4) 28.0 (27)	22.7 (0.2-100) 31.6 (35)
Placebo group			
EG1 ($\mu\text{g/l}$)	6.9 (0.1-31) 10.3 (9.8)	11.6 (0.1-135)* 30 (39)	8.3 (0.1-200) 36.6 (59.5)
EG2 ($\mu\text{g/l}$)	0.1 (0.1-3.8) 0.8 (1.3)	2.3 (0.1-38.3)* 5.7 (10.3)	0.1 (0.1-200)* 16.0 (48)
EG2/EG1 (%)	4.0 (0.5-100) 14.9 (29)	10.0 (1.2-100)** 18.0 (23)	13.5 (0.3-100) 38.0 (41)

* T_0 vs T_1 and T_0 vs T_2 : $P < 0.05$.

** T_0 vs T_1 : $P < 0.01$.

Discussion

Our results show that OSI for grass pollen is effective in reducing respiratory symptoms, particularly asthma, after 3½ months' preseasonal treatment in children. However, the improvement in nasal and ocular symptoms did not reach statistical significance. The symptom-medication score was reduced in the immunotherapy group compared with the placebo group during the pollen season in all weeks. However, a significant reduction was reached only when patients were exposed to high levels of pollen. The latter observations may limit the clinical relevance of a short preseasonal course of OSI.

The efficacy of oral immunotherapy in double-blind, placebo-controlled studies has been recently reviewed (2). In 2/6 studies, there was evidence of clinical efficacy. Controlled trials with SLIT have shown its effectiveness in adults allergic to grass pollen with rhinitis (5, 7, 8) and asthma (8, 13). SLIT was associated with a clinically significant improvement in children with asthma due to mites (9) or olive pollen (12). Our trial differs from previous investigations because: 1) we studied children with grass-pollen hypersensitivity; 2) the allergenic product was an original formulation; 3) the allergenic product was applied to the oral cavity and then swallowed.

The oral-soluble tablets have advantages over conventional drops of aqueous extracts in that it is easier to define the dosage and harder to make mistakes, as in counting the drops.

The mechanisms through which sublingual or oral immunotherapy acts are unclear (25). Kinetic studies of radiolabeled allergens showed that allergens that are kept under the tongue and then swallowed stay in the oral mucosa for a long time, up to 18–20 h after swallowing (26). The ingested portion of the allergen is quickly absorbed in the gastrointestinal tract. This suggests that both mucosa-associated lymphoid tissue and gut absorption capacity are involved (27).

Animal studies showed that high sublingual allergen doses suppressed IgE production (28). Fanta et al. (29) showed a significant decrease of the proliferative response of peripheral blood lymphocytes to grass pollen after sublingual-split immunotherapy. Moreover, a significant increase in the levels of serum specific IgG, IgG4, and IgE was observed (29). However, some studies found that SLIT did not affect serum specific IgE, IgG, and IgG4 or skin test reactions to grass pollen (13, 14). Oral immunotherapy has been found to reduce specific IgE levels and enhance levels of IgG4 and IgG1 in children (30).

We determined nasal levels of ECP outside the pollen season and during natural pollen exposure in order to evaluate whether immunotherapy could

modify eosinophil activity, which is a relevant part of allergic inflammation. Our results showed that nasal ECP significantly increased both in the placebo group and in actively treated patients during the pollen season. These findings agree with the results of an open study that showed a significant enhancement of ECP in nasal secretions during the pollen season in both drug-treated patients and in patients treated with injective immunotherapy (18).

The percentage of activated ECP is more reliably evaluated by the EG2/EG1 ratio, since the monoclonal antibodies EG1 and EG2 have been shown to detect, respectively, total and activated ECP (19). We found that the EG2/EG1 ratio significantly increased only in the placebo group during the pollen season. The augmentation of the EG2/EG1 ratio was greater in the placebo group than in the immunotherapy group under natural pollen exposure, even if this was not statistically significant. Taken together, these results may indicate a possible role of immunotherapy in decreasing eosinophil activation. This may be consistent with studies reporting a decrease of the cellular infiltrate of neutrophils and eosinophils and of ICAM-1 expression on epithelia after SLIT (10, 17). We found, however, that nasal levels of EG1 and EG2 were not associated with immunotherapy, suggesting that former observations may represent chance findings. Further studies comparing ECP with symptoms and pollen data throughout the grass-pollen season are warranted to clarify the interpretation of these parameters. These studies must also take into account the fact that different methods of sample preparation may influence the reactivity of EG1 and EG2 (31).

Systemic reactions to oral (2) and sublingual immunotherapy (5–9, 15), principally gastrointestinal symptoms, urticaria, rhinitis, and asthma, appear to be rare. Anaphylaxis never occurred. In this study, only one mild reaction was observed as a consequence of OSI.

The present study concludes that a preseasonal course of OSI with grass pollen allergens is more effective than placebo in the treatment of asthmatic symptoms in allergic children. However, our findings require further confirmation by investigating a greater number of children.

Acknowledgments

We thank Mr R. Brunori and M. Bernin for skillful technical assistance and Dr L. Cassoni, of Agenzia Regionale Prevenzione Ambientale, Parma, Italy, for providing the pollen count.

References

- MALLING H-J, WEEKE B. Immunotherapy position paper. *Allergy* 1993;48 Suppl 14:9-35.
- WHO position paper. Allergen immunotherapy: therapeutic vaccines for allergic disease. *Allergy* 1998;53 Suppl 44:1-42.
- MALLING H-J, ABREU-NOGUEIRA J, ALVAREZ-CUESTA E, et al. Local immunotherapy position paper. *Allergy* 1998;53:933-944.
- SCADDING K, BROSTOFF J. Low dose sublingual therapy in patients with allergic rhinitis due to dust mite. *Clin Allergy* 1986;16:483-491.
- VAN NIEKERK CH, DE WET JI. Efficacy of grass-maize pollen oral immunotherapy in patients with seasonal hay fever: a double blind study. *Clin Allergy* 1987;17:507-513.
- TARI MG, MANCINO M, MONTI G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double blind study. *Allergol Immunopathol (Madr)* 1990;18:277-284.
- SABBAH A, HASSOUN S, LE SELLIN J, ANDRÉ C, SICARD H. A double-blind, placebo-controlled trial by the sublingual route of immunotherapy with a standardized grass pollen extract. *Allergy* 1994;49:309-313.
- FELIZIANI V, LATTUADA G, PARMIANI S, DALL'AGLIO PP. Safety and efficacy of sublingual rush immunotherapy with grass allergen extracts. A double blind study. *Allergol Immunopathol (Madr)* 1995;23:224-230.
- NOVEMBRE E, MARANO E, BERNARDINI R, CARIA M, DINI L, VIERUCCI A. Studio controllato sull'immunoterapia per via sublinguale nel trattamento dell'asma allergico del bambino. *Riv Ital Pediatr* 1991;17:75-78.
- PASSALACQUA G, ALBANO M, FREGONESE L, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet* 1998;351:629-632.
- TROISE C, VOLTOLINI S, CANESSA A, PECORA S, NEGRINI AC. Sublingual immunotherapy in *Parietaria* pollen-induced rhinitis: a double-blind study. *J Invest Allergol Clin Immunol* 1995;5:25-30.
- VOURDAS D, SYRIGOU E, POTAMIANOU P, et al. Double-blind placebo controlled evaluation of sublingual immunotherapy with standardized olive pollen extract in pediatric patients with allergic rhinoconjunctivitis and mild asthma due to olive pollen sensitization. *Allergy* 1998;53:662-672.
- CLAVEL R, BOUSQUET J, ANDRÉ C. Clinical efficacy of sublingual-swallow immunotherapy: a double-blind, placebo-controlled trial of a standardized five-grass pollen extract in rhinitis. *Allergy* 1998;53:493-498.
- QUIRINO T, IEMOLI E, SICILIANI E, PARMIANI S, MILAZZO F. Sublingual versus injective immunotherapy in grass pollen allergic patients: a double-blind (double dummy) study. *Clin Exp Allergy* 1996;26:1253-1261.
- 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.
- European Economic Community (EEC). CPMP Working party on quality of medicinal products. Note for guidance. III/3593/91-EN, 1991.
- PASSALACQUA G, ALBANO M, RICCIO A, et al. Clinical and immunologic effects of a rush sublingual immunotherapy to *Parietaria* species: a double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 1999;104:964-968.
- SVENSSON C, ANDERSSON M, PERSSON CG, VENGE P, ALKNER U, PIKORIN U. Albumin, bradykinins, and eosinophil cationic protein on the nasal mucosal surface in patients with hay fever during natural allergen exposure. *J Allergy Clin Immunol* 1990;85:828-833.
- KLIMEK L, WOLF H, MEWES T, et al. The effect of short term immunotherapy with molecular standardized grass and rye allergens on eosinophil cationic protein and tryptase in nasal secretions. *J Allergy Clin Immunol* 1999;103:47-53.
- MOQBEL R, BARKANS J, BRADLEY BL, DURHAM SR, KAY AB. Application of monoclonal antibodies against major basic protein (BMK-13) and eosinophil cationic protein (EG1 and EG2) for quantifying eosinophils in bronchial biopsies from atopic asthma. *Clin Exp Allergy* 1992;22:265-273.
- MISTRELLO G, BRENNAN O, RONCAROLO D, ZANONI D, GENTILI M, FALAGIANI P. Monomeric chemically-modified allergens: immunologic and physico-chemical characterization. *Allergy* 1996;51:8-15.
- YMAN L, PONTERIUS G, BRANDT R. RAST based allergen assay methods. *Dev Biol Stand* 1975;1:51.
- GIANNARINI L, MAGGI E. Decrease of allergen-specific T-cell response induced by local nasal immunotherapy. *Clin Exp Allergy* 1998;28:404-412.
- SENSI LG, SERI A, SIRACUSA A, PERTICI L, MARCUCCI F. Allergic rhinitis in children: effects of flunisolide and disodium cromoglycate on nasal eosinophil cationic protein. *Clin Exp Allergy* 1997;27:270-276.
- HOLT PG. A potential vaccine strategy for asthma and allied atopic disease during early childhood. *Lancet* 1994;344:456.
- BAGNASCO M, MARIANI G, PASSALACQUA G, et al. Absorption and distribution kinetics of the major *Parietaria judaica* allergen (Par j 1) administered by noninjectable routes in healthy human beings. *J Allergy Clin Immunol* 1997;100:122-129.
- HOLT PG, SLY PD, SMITH W. Sublingual immunotherapy for allergic respiratory disease. *Lancet* 1998;351:613-614.
- HOLT PG, VINES J, BRITTEN D. Sublingual allergen administration. I. Selective suppression of IgE production in rats by high allergen doses. *Clin Allergy* 1988;18:229-234.
- FANTA C, BOHLE B, HIRT W, et al. Systemic immunological changes induced by administration of grass pollen allergens via the oral mucosa during sublingual immunotherapy. *Int Arch Allergy Immunol* 1999;120:218-224.
- GIOVANE AL, BARDARE M, PASSALACQUA G, CANONICA W. A three-year double-blind placebo-controlled study with specific oral immunotherapy to *Dermatophagoides*: evidence of safety and efficacy in paediatric patients. *Clin Exp Allergy* 1993;24:53-59.
- NAKAJIMA H, LOEGERING DA, KITA H, KEPHART GM, GLEICH GJ. Reactivity of monoclonal antibodies EG1 and EG2 with eosinophils and their granule proteins. *J Leukoc Biol* 1999;66:447-454.