

LACK OF INFLAMMATORY CELLS IN THE ORAL MUCOSA OF SUBJECTS UNDERGOING SUBLINGUAL IMMUNOTHERAPY

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The cells involved in allergic inflammation, such as mast cells, basophils, and eosinophils, have been thoroughly studied in the nose, the lungs and the skin, demonstrating an evident increase in response to the introduction of the specific allergen, while little is known in the mucosal system and particularly in the oral mucosa. We investigated such tissue by using the model of sublingual immunotherapy (SLIT), by which high doses of the specific allergen enter the mouth. Oral biopsies were carried out on seven subjects allergic to grass pollen and treated with SLIT by a grass extract. In biopsies carried out before SLIT there was a very low number of mast cells and eosinophils both in the epithelium and subepithelium layers, and insignificant changes were detected after SLIT. These findings show the lack of allergic inflammation in the oral mucosa upon contact with the specific allergen and confirm the role of the mouth as a tolerogenic site, which is conceivable considering the different attitude of the mouth, where the antigens transit to undergo digestion, in respect to the airways or the skin, where the antigen absorption is potentially dangerous.

Allergic inflammation is caused by an immune response to environmental allergens driven by regulatory cells, such as antigen presenting cells and T lymphocytes, and sustained by effector cells that include mast cells, basophils, and eosinophils. Various tissues are involved in inflammation according to the site of contact with the specific allergen and the target organ. The presence of inflammatory cells was investigated in the nose (1),

lungs (2), and skin (3), demonstrating an increase in response to the introduction of the allergen, generally made by specific challenge; however, little is known about the accumulation of such cells in the mucosal system and particularly in the oral mucosa. Oral challenge is clinically used to evaluate the response to foods to which the patients are suspected to be allergic, but is not feasible with inhalant allergens, which instead are the cause of allergic inflammation

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in respiratory tissues. We therefore used the model of sublingual immunotherapy (SLIT), which is suitable to evaluate possible immunologic changes in oral mucosa. This treatment permits high doses of the specific allergen to be introduced in the mouth; oral biopsies, involving epithelium and subepithelium, may thus directly investigate the cellular response to the allergen at its site of contact. To compare the oral response with the response of the respiratory mucosa to the same allergen, a nasal challenge was carried out on the same subjects, and the changes were assessed before and after treatment.

MATERIALS AND METHODS

Allergen immunotherapy

Seven adult patients (4 males, 3 females, mean age 38.7 years) suffering from allergic rhinoconjunctivitis caused by sensitization to grass pollen, as assessed by positive skin prick tests and CAP System (Phadia, Uppsala, Sweden), and not currently treated with drugs, were included in the study. SLIT was performed using high dose grass pollen extract (Staloral 300, Stallerg enes, Antony, France), standardized in IR (index of reactivity) (4). SLIT was started in January via a build-up schedule over 11 days as suggested by the manufacturer (starting at 1 IR and concluding at 300 IR), followed by a maintenance treatment with administration of four drops of the vial with a concentration of 300 IR, each day for six months until June. The patients were instructed to hold the allergen extract under the tongue for at least two minutes and then swallow it, in the morning before breakfast.

Nasal challenge

The nasal challenge was carried out by spraying into one nostril, first a negative control (a glycerol saline solution) and then increasing concentrations (0.2, 0.5, 0.8, 1.8 and 2.8 IR) of the same grass allergen extract. A score system from 0 to 3 for each nasal symptom (sneezing, runny nose, nasal blockage) was used, with a total score of at least 5 defining a positive test. Tryptase in nasal secretion following each allergen concentration was determined using the UniCAP Tryptase System FEIA (Phadia) adapted for mucosal sampling as previously described (5).

Biopsies

The biopsies were carried out at baseline, before starting the treatment, and 12 hours following the SLIT dose after three months of maintenance treatment, by cutting a small piece (about 3 mm of diameter) of the oral mucosa under the tongue with a lancet,

following infiltration by lidocain. All patients gave their informed consent and the University of Perugia Ethical Committee approved the study. The biopsies were fixed in paraformaldehyde (PFA) 4% diluted in PBS, heated at 56-60°C with PFA dissolved with a stirrer until the complete clearing of the solution. The PFA solution was frozen in small aliquots, and thawed just before use. Biopsies were allowed to remain 3 hours in this solution at room temperature and changed twice to a solution of PBS/sucrose (15%) for one hour; they were then stored by blocking them on cork, with the epithelium positioned perpendicularly to the cork's surface, frozen in isopentane cooled in liquid nitrogen, and kept at -80°C. Semi-serial 5 micron sections were stained with hematoxylin and eosin to detect eosinophils, and immunohistochemically by using CD117 (Dako, Milan, Italy) as the primary antibody to identify mast cells.

RESULTS

In the biopsies carried out before SLIT there was a very low number of mast cells and eosinophils both in the epithelium and subepithelium layers, and insignificant changes were detected after SLIT for mast cells (Fig. 1) both prior (A) and after (B) SLIT, and eosinophils (Fig. 2) both prior (A) and after (B) SLIT. All subjects had a positive response to nasal challenge, and a mean nasal secretion tryptase level of $59.46 \pm 40.12 \mu\text{g/l}$ before SLIT and $15.43 \pm 16.68 \mu\text{g/l}$ after the treatment was measured ($p = 0.02$).

DISCUSSION

The inflammation underlying allergic disease is triggered by the contact with the specific allergen to which allergic subjects are sensitized. The contact may occur by natural exposure, but may also be reproduced by specific allergen challenges as well as by administration of allergen extracts during the early phases of specific immunotherapy. During this procedure, increasing doses of the causative allergen are administered to sensitized individuals to induce clinical tolerance (6-7). Various options to classical subcutaneous administration were proposed, and sublingual immunotherapy (SLIT) was demonstrated to be effective and safe in both adults and children (8-10). In general, receiving the specific allergen causes inflammatory responses mainly elicited by the release of mediators by effector cells such as mast cells, basophils, and eosinophils, until

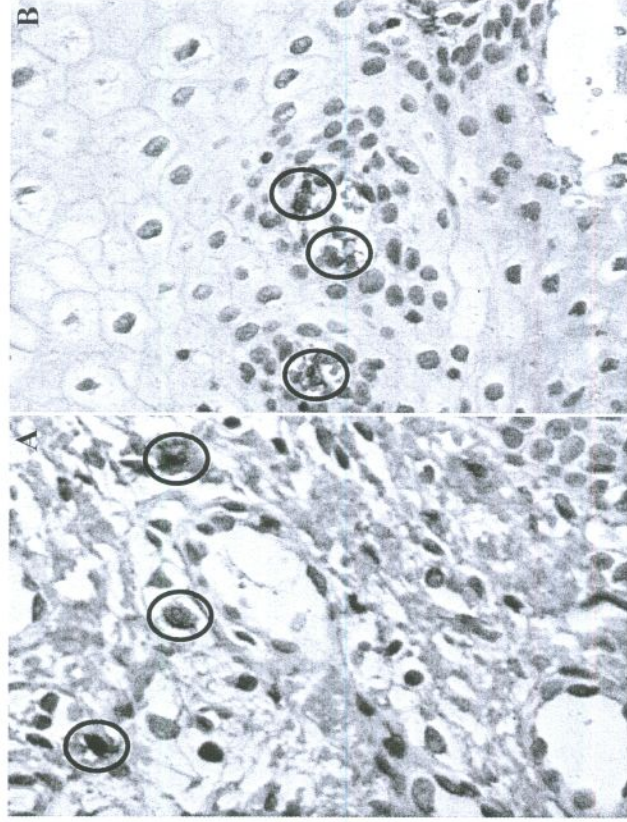


Fig. 1. Mast cells (indicated by circles) in one oral biopsy obtained prior (A) and after (B) sublingual immunotherapy.

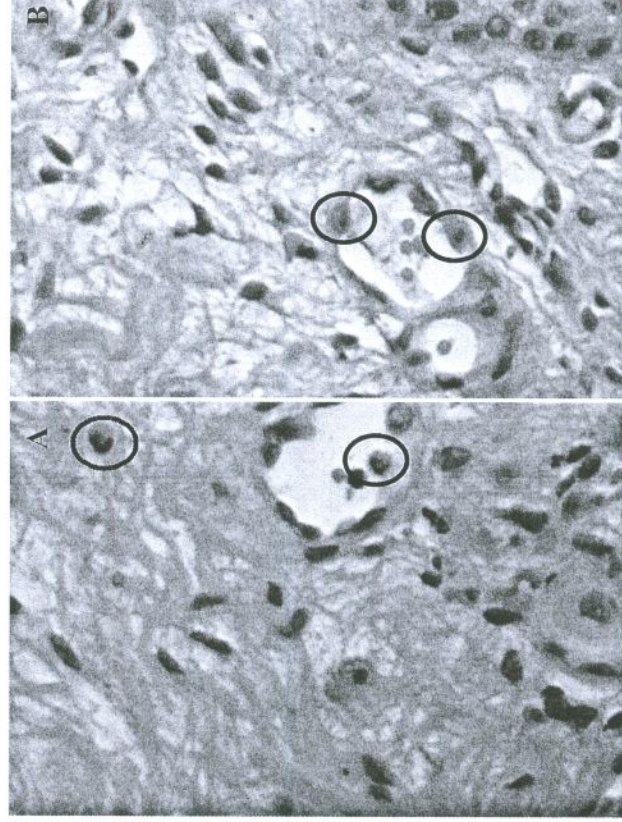


Fig. 2. Eosinophils (indicated by circles) in one oral biopsy obtained prior (A) and after (B) sublingual immunotherapy.

tolerance arises. As soon as the mechanism of action of immunotherapy takes place, currently suggested by a switch of the Th cells regulating the immune response from a Th2 dominance, typical of the atopic phenotype, to a Th1 dominance, and to generation of regulatory T cells (11), the number and activation of

effector cells in targeted tissues starts to decrease. The findings of our study indicate that SLIT, at least with the high doses we administered, induces significant decrease of tryptase – marker of mast cell activation – in the nose challenged with the specific allergen. The high tryptase level at baseline following the

challenge confirms the role of mast cells in allergic inflammation in the nasal mucosa (1). The striking observation of the study is that in the oral mucosa there was a negligible number of mast cells and eosinophils, not only before but also after the treatment. This confirms a previous study reporting that tryptase was detectable in very low amounts in oral fluid of subjects allergic to grass pollen either before or after SLIT (12). Different results were reported for subcutaneous route, where cellular response to the allergen extract was evaluated by skin biopsies and revealed that inflammatory cells, especially lymphocytes and activated eosinophils, significantly rose 24 hours after the allergen injection (13). Recently, evidence has been obtained that a local allergic reaction can lead to peripheral blood cell activation and thus to systemic effects of an apparently local allergic disease (14). Therefore, the lack of allergic inflammation in the oral mucosa upon contact with the specific allergen suggests that the mouth is a tolerogenic site, and this is conceivable considering the different role of the mouth, where the antigens transit to undergo digestion, in contrast to the airways or the skin, where the antigen absorption is potentially dangerous. Moreover, the fact that the effector cells typical of allergic inflammation are present in very low numbers in the oral mucosa may also account for a particular aspect of SLIT, its high safety, which emerged from the systematic review of more than 20 controlled studies (10). Local reactions in the mouth are quite common, and sometimes they may hamper the continuation of treatment. However, we have recently demonstrated that also in such reactions, negligible presence of mast cells and eosinophils can be detected (15).

REFERENCES

1. Igarashi Y, Goldrich MS, Kaliner MA, Irani AM, Schwartz LB, White MV. Quantitation of inflammatory cells in the nasal mucosa of patients with allergic rhinitis and normal subjects. *J Allergy Clin Immunol* 1995; 95:716-20.
2. Amin K, Ludviksdottir D, Janson C, Nettelbladt O, Björnsson E, Roomans GM, Boman G, Seveus L, Venge P. Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. *Am J Respir Crit Care Med* 2000; 162:2295-9.
3. Zweiman B, Haralabos IC, Pham NC, David M, von Allmen C. Sequential patterns of inflammatory events during developing and expressed skin late-phase reaction. *J Allergy Clin Immunol* 2000; 105: 776-82.
4. 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; 53:493-98.
5. Sensi L, 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; 27:270-5.
6. Bousquet J, Lockey R, Malling HJ (eds). WHO Position Paper: Allergen immunotherapy: therapeutical vaccines for allergic diseases. *Allergy* 1998; (S54):40-8.
7. Di Gioacchino M, Cavallucci E, Di Sciascio MB, Di Stefano F, Verna N, Raimondo S, Lobefalo L, Conti P, Cuccurullo F. Allergen immunotherapy: an effective immune-modifier. *Int J Immunopathol Pharmacol* 1999; 12:1-5.
8. Madonini E, Agostini F, Barra R, Berra A, Donadio D, Stefani E, Pappacoda A, Tierno E. Safety and efficacy evaluation of sublingual allergen-specific immunotherapy: a retrospective, multicenter study. *Int J Immunopathol Pharmacol* 2000; 13:77-81.
9. Della Volpe A, D'Agostino GW, Varicchio AM, Mansi N. Sublingual allergen-specific immunotherapy in allergic rhinitis and related pathologies: efficacy in a paediatric population. *Int J Immunopathol Pharmacol* 2002; 15:35-40.
10. Wilson DR, Torres-Lima M, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005; 60:4-9.
11. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2007; 119: 780-4.
12. Marcucci F, Sensi L, Frati F, Senna GE, Canonica GW, Permiani S, Passalacqua G. Sublingual tryptase and ECP in children treated with grass-pollen sublingual immunotherapy (SLIT): safety and immunological implications. *Allergy* 2001; 56:1091-6.
13. Eberlein-Koenig B, Jung C, Rakoski J, Ring J. Immunohistochemical investigation of the cellular

infiltrates at the sites of allergoid-induced late-phase cutaneous reactions associated with pollen allergen-specific immunotherapy. *Clin Exp Allergy* 1999; 29: 1641-6.

14. Togias A. Systemic effects of local allergic disease J

Allergy Clin Immunol 2004; 113:8-14.

15. Marcucci F, Sensi L, Incorvaia C, Di Cara G, Moingeon P, Frati F. Oral reactions to sublingual immunotherapy: a bioptic study. *Allergy* 2007; 62: 1475-7.