

Mucosal immunization application to allergic disease: Sublingual immunotherapy

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ABSTRACT

Sublingual immunotherapy (SLIT) is an effective and safe treatment for respiratory allergy, and its mechanism of action currently is investigated with increasing attention. Studies of pharmacokinetics showed that allergen extracts administered via the sublingual route are not directly absorbed by the oral mucosa but are long retained at mucosal level, where the allergen molecules are captured by dendritic cells and, following their migration in the draining lymph nodes, presented to T cells. This seems to be the pivotal factor underlying the mechanisms of action of SLIT, at least for the long-term effects, and for the short-term efficacy, observed with ultrarush or coseasonal treatment, a down-regulation of mast cells resulting in hyporeactivity at the peak of the pollen season may be suggested. Regarding the clinically established long-lasting effects, the core mechanism is likely to consist of T regulatory (Treg) cell activation. In particular, Treg cells differentiate from naive T cells after application of soluble antigens to the mucosae, a crucial factor being the tolerogenic function of dendritic cells, and exert a suppressive effect on both Th1 and Th2 responses. Moreover, at least for the type 1 cells (Treg1), a production of IL-10 with consequent down-modulation of the immune response has been reported. Another characteristic of sublingual immunization is the absence of effectors cells, viz., mast cells, basophils, and eosinophils, in the oral mucosa of allergic subjects, which account for the excellent tolerability of SLIT.

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Key words: Allergic disease, mucosal immunization, oral mucosa, sublingual immunotherapy, tolerance

For nearly 100 years, allergen-specific immunotherapy has been an important tool in the treatment of respiratory allergy and has been fully validated in consensus documents by the World Health Organization.¹ Various efforts have been made, mainly for safety reasons, to obtain alternative administration to the classic form of subcutaneous immunotherapy; sublingual immunotherapy (SLIT), introduced in 1986² and now diffusely used in Europe, especially in Latin countries, and in Germany, fulfilled such requirement.³ SLIT may be performed by two techniques, the sublingual swallow, in which the allergen extract is kept under the tongue for a few minutes and then

swallowed, and the less-used sublingual spit, in which the allergen extract is spat out.

The efficacy of SLIT was shown, in a series of double-blind, placebo-controlled trials, and recently confirmed by a meta-analysis considering 22 controlled studies.⁴ In addition, its safety is excellent in children and in using cumulative doses of up to 500 times higher than those administered with subcutaneous immunotherapy.^{5,6}

In the first attempts of sublingual desensitization⁷ it was thought that the allergen extract could passively cross the oral mucosa and thus elicit systemic reactions, but now studies of pharmacokinetics have indicated that there is no allergen absorption at the oral level. This is confirmed by the fact that the typical side effects of SLIT are local, and, viz., in the mouth and in the gastrointestinal tract. Although the allergens administered by SLIT do not cross the oral mucosa, they induce in both systemic and respiratory compartments, which are involved in the aeroallergen hypersensitivity disease, immunologic changes such as the blunting of seasonal rise of specific IgE in the target organ, as shown in the nasal mucosa of grass allergic subjects treated with high-dose SLIT.⁸

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Epithelium Lamina propria Submucosa	CELL	DISTRIBUTION IN ORAL MUCOSA
	LANGERHANS' CELL	• LC are found in the oral mucosa • Oral LC expressed > MCH I - II , CD 40, CD 80/B7.1, CD86/B7.2 , than LC in the skin • Oral LC displayed FcγRIII/CD16 and FcγRI/CD64 • Oral LC constitutively expressed FcεRI in atopic and non atopic donors, in atopic was correlated with the serum IgE levels • Oral LC showed a higher allogenic stimulatory activity than skin LC
	LYMPHOCYTE	• TC are more present in the epithelium of oral mucosa than of the skin (37/1) • CD4/CD8 ratio is ½ in the epithelium of oral mucosa and ¼ in the skin • B cells are not found in the epithelium and papillary layer of the oral mucosa (2)
	EOSINOPHIL MAST CELL and BASOPHIL	• Not found in allergic patients also during the maintenance with High Dose SLIT • ECP not found in mucosal surface during SLIT with low and high dosage • Few cells ; no increase during the maintenance High dose SLIT • Triptase not found in mucosal surface during SLIT with low and high dose

Figure 1. Type of cells in oral mucosa.

It is important to note that a role for intestinal absorption in inducing tolerance is unlikely, because oral immunotherapy, in which the allergen extracts in gastro-resistant preparations are swallowed without any contact with the oral mucosa, failed to demonstrate a global clinical efficacy,³ despite some positive results with particular preparations.⁹ Thus, local mucosal mechanisms in the oral cavity must be invoked to explain the evident reduction of sensitivity that takes place in subjects treated with SLIT. In particular, Langerhans-like dendritic cells abundantly present in the oral mucosa¹⁰ and T regulatory (Treg) lymphocytes of the mucosa-draining lymph nodes seem to play a pivotal role in the complex mechanisms of tolerance, and their study offers new perspectives in the investigation of the immunotolerance mechanisms.

THE CELLS IN THE ORAL MUCOSA AND THE IMMUNE RESPONSE

The oral mucosa has been long considered a anatomic site devoid of immunologic activity with respect to tolerance to antigens, but, recently, thanks to studies

conducted on SLIT, new aspects on its peculiarities were highlighted. Under the anatomic aspect the oral mucosa is composed by three layers, epithelium, lamina propria, and submucosa, with an important role for the basal membrane, in which its particular thickness accounts for the permeability of this anatomic area. The cells populating the oral mucosa, directly related with its immunologic properties, are dendritic cells, T lymphocytes, and B lymphocytes (Fig. 1).

Increasing evidence attributes to dendritic cells a central role in the response to allergen molecules reaching the mouth. These cells are sparsely distributed in the mucosa, including the external epithelial layer, and show the anatomic features of the Langerhans cells present in various areas of the body with the function of antigen-presenting cells. They share with the Langerhans cells the content in Birbeck intracytoplasmic granules but are characterized by the expression of the high-affinity IgE receptor FcεRI related to subjects who are atopic to serum IgE levels.⁹ The link of dendritic cell's IgE receptors, including the low-affinity receptor CD23, with the allergen molecules elicits the produc-

tion of cytokines such as IL-10 and TGF- β and up-regulates the synthesis of indoleamine 2,3-dioxygenase, an enzyme that metabolizes tryptophan, which is essential for the biology of T cells, and thus reduces their proliferation.¹¹ Other receptors with higher expression on dendritic cells compared with skin Langerhans cells are MCH I-II, CD40, CD80/B7.1, and CD86/B7.2.

Regarding lymphocytes, the T cells are located mainly in perivascular spaces in the epithelial layer and they are represented ~37 times more in the oral mucosa than in the skin, whereas the B cells are not present in the epithelium and in the papillar layer of the mucosa.¹⁰ The role of T cells in orchestrating the immune response is well known. In particular, in tolerance to allergens they may exert suppressive but also regulatory activity. The latter currently is regarded as very important in the response to allergens.¹²⁻¹⁴ The cells with such activity are named Treg cells and include naturally occurring CD4⁺ and CD25⁺ T cells and cells induced by antigen exposure (e.g., Tr1 cells, Th3 cells, and CD8⁺ Treg cells). The presentation of allergens by dendritic cells is critical in inducing the differentiation of Treg cells,¹⁵ which in turn can down-regulate Th1 and Th2 immune responses,^{13,14} directly by cell contact or by production of immunosuppressive cytokines such as TGF- β (typical of Th3 cells)¹⁶ or IL-10 (typical of Tr1 cells).¹⁴

An association between atopy and a deficit in Treg functions is well documented. In one study, children with a deficit in CD4⁺ and CD25⁺ Treg cells developed eczema, elevated IgE levels, eosinophilia, and hypersensitivity to foods,¹⁷ and in an *in vitro* model the suppressive activity of CD4⁺ and CD25⁺ Treg cells was significantly lower in atopic individuals than in nonatopic individuals.¹⁸ Indeed, the prevalence in healthy individuals of allergen-specific Tr1 cells over IL-4-secreting Th2 cells is another demonstration of the tolerogenic role of Treg cells during natural exposure to allergens.¹⁹ In addition, in an ongoing allergic response Treg cells have a beneficial effect by the decrease of the IgE synthesis induced by IL-10 and the increase of IgG4 and IgA production induced by TGF- β .²⁰

A last remark about the immunity in the oral mucosa regards the effector cells of allergic inflammation, *i.e.*, eosinophils, mast cells, and basophils, these cells are abundantly found in sites targeted by allergy such as the nose, the eyes, and the lungs, but it has been shown that in the oral mucosa of allergic subjects they normally are absent or in negligible number.²¹ Of course, this does not exclude their involvement in other pathologies of the mouth; e.g., mast cells were found in the oral mucosa of subjects with local inflammation caused by oral lichen planus.²²

PHARMACOKINETICS OF THE ALLERGENS ADMINISTERED WITH SLIT

The fate of the allergen after its application on the sublingual mucosa is of obvious importance in suggesting how SLIT works. The group of Canonica investigated the pharmacokinetics of the allergens by using materials radiolabeled with ¹²³I, which included native and modified allergens, and comparing the administration *via* the oral or nasal mucosa.²³⁻²⁵

The results of such studies showed that the sublingually administered allergen (*viz.*, the major allergen from *Parietaria judaica* Par j 1) was not absorbed through the oral mucosa but was retained at mucosal level for up to 20 hours, and after swallowing, a gastrointestinal absorption occurred but the plasma radioactivity (peaking at 1.5-3 hours) was caused by free radioiodine and small peptides not by native allergen. Using a monomeric allergoid, the kinetics was comparable as to permanence in the oral mucosa and to absorption after swallowing, with plasma radioactivity peaking after 2 hours, but involving also the undegraded allergoid. Interestingly, the nasal administration of Par j 1 in *Parietaria* allergic patients is associated to a kinetics with rapid clearance from the nasal mucosa, little local persistence in the pharynx, and an increase in plasma radioactivity after swallowing.²³⁻²⁵

PUTATIVE TOLERANCE MECHANISMS TO ALLERGENS IN THE ORAL MUCOSA

The contact of the oral mucosa with the allergen administered with SLIT leads to activation of dendritic cells, which when reaching the most superficial layers phagocytate the allergen by macropinocytosis and transport it to the lymph nodes draining the anatomic area. Once migrated in the lymph nodes, dendritic cells express costimulatory molecules and interact with the naive CD8 and CD4 T cells,²⁶ a number of factors being critical in determining a T response or tolerance. Regarding allergens, in an *in vitro* model, the internalization of allergen molecules and control proteins was dose and time dependent and related to the immature state of the cells, with a higher efficiency for allergens, *viz.*, Bet v1 and Phl p1,²⁷ which is likely to depend on the linking by the Fc ϵ RI receptor. The immunologic effects on T cells might be related to two distinct mechanisms able to account for the clinical effectiveness of SLIT.

A first mechanism may be suggested for the short-term efficacy of SLIT observed with ultrarush or coseasonal treatment in patients with pollen allergy:^{28,29} as hypothesized by Bousquet, down-regulation of mast cells is more efficient with the high allergen doses administered in a short time and results in hyporeactivity at the peak of the pollen season.²⁸

A second mechanism might depend on Treg activation, which is likely to underlie the long-lasting effects characteristics of prolonged immunotherapy observed also with SLIT.³⁰ In particular, the differentiation in Treg of naive T cells is stimulated by the application of soluble antigens to the mucosae, a crucial factor being the tolerogenic function of dendritic cells.^{31,32} Antigen-specific Treg cells have a suppressive effect on both Th1 and Th2 response and, at least for the type 1 cells (Treg1), down-modulate the immune response producing IL-10.³³ A recent report on this issue showed that patients treated with prolonged SLIT with house-dust mite extract had a significant production of IL-10, which was absent in untreated patients with mite-induced rhinitis.³⁴

Another important aspect, as emphasized previously, is the negligible number in the oral mucosa of mast cells, eosinophils, and basophils, which if present in high number should prevent the administration of high allergen doses and thus the development of tolerance.^{8,21} The hyporeactivity of the oral mucosa was confirmed recently by a study that used recombinant allergens; this study showed that subjects with oral allergy syndrome tolerated doses of Bet v 1 and Phl p 1 applied to the mouth better than those needed to elicit allergic responses in the skin and in the nose.³⁵ Despite this, some subjects actually developed oral reactions to allergen extracts administered with SLIT; in a recent compliance study, ~6% of SLIT-treated subjects withdrew the therapy because of local reactions.³⁶ Future investigations are needed to determine which mechanisms trigger the development of such reactions.

CONCLUSIONS

Among the different routes of allergen immunotherapy, the sublingual route, which joins clinical efficacy to a high safety profile, currently is used increasingly as a valid option for treating respiratory allergy and offers promising perspectives of development. In fact, the effectiveness of SLIT could be further improved by a better knowledge of the mechanisms of mucosal tolerance leading to the introduction of materials and doses optimally fitting with both the pharmacokinetics and the immunizing capacity of sublingually administered allergens.

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