

WHAT DO WE NEED TO LEARN TO OPTIMIZE THE SLIT ALTERNATIVE?

H.-M. Serra (1), D.-O. Alignani (1), G. Passalacqua (2), G.-W. Canonica (2), S.-G. Correa (1)

SUMMARY: Allergen-specific immunotherapy (IT) is the only treatment of allergy in adults and children capable of modifying the immune response at early steps. As a consequence, IT improves symptoms, prevents the onset of new sensitizations, reduces the risk of developing asthma and its clinical efficacy lasts many years. The main rationale for administering sublingually IT (SLIT) is to reduce the occurrence of side effects, still yet preserving the immunological effects. SLIT with the most common allergens have been used in many studies with significant clinical effectiveness in both asthma and rhinitis. The pharmacokinetic of allergens administered through non injection routes is complex. Peptide absorption across oral mucosa occurs mainly by passive diffusion but delivery of proteins has some limitations. Moreover, the molecular mechanisms responsible for the efficacy of SLIT are poorly defined. In this review we focus on the anatomy/histology of the oral cavity as well as on the associated immunological structures to envisage what may happen when an allergen is kept in the mouth. Moreover, the induction of immune responses in this particular immunological environment is also discussed.

ARE THERE IMMUNOTHERAPY ALTERNATIVES FOR ALLERGIC INDIVIDUALS?

Allergen-specific immunotherapy (IT), allergen avoidance and drug therapy are the cornerstones of the treatment of allergy in adults and children. Only IT is capable of modifying the immune response at early steps, and it is now clear that it acts as a biological response modifier. IT not only reduces symptoms and the need for medications, but can also prevent the onset of new sensitisations [1] and asthma [2], and its clinical efficacy lasts many years after discontinuation [3]. IT was administered for decades only by subcutaneous route, but the need for improved safety and the experimental evidence obtained with models of local induction of tolerance [4, 5] supported the local administration of allergen extracts to various mucous membranes. Over the last 20 years new methods of local application were sought and different non injection routes have been used in order to achieve desensitization. Assessing IT efficacy has been somewhere difficult, because of the multiplicity of the criteria used in different studies to decide what constitutes an improvement, and till now the clinical criterion represents the gold standard.

Oral IT (allergen immediately swallowed) was found to be poorly effective in clinical trials [6] and its use is no more recommended. The nasal mucosa is an important arm of the mucosal immune system since it is often the first point of contact for inhaled antigens and as a consequence, proper intranasal immunisation emerged as a possible route for vaccination [7]. Local nasal IT (LNIT) proved effective and safe in many controlled trials as far as rhinitis is concerned [8]. Nevertheless, it requires a particular administration technique, does not maintain its clinical efficacy once discontinued and is not effective on asthma, thus its use is slowly declining.

The original rationale for administering IT sublingually (SLIT) was to achieve a prompt and rapid absorption of the vaccine and to avoid possible gastrointestinal degradation. Different forms of SLIT using the most common allergens have been used in many double-blind controlled studies with significant clinical effectiveness for both asthma and rhinitis [9]. More recently, SLIT was shown to achieve a significant clinical benefit in birch pollinosis associated to reduction of the eosinophil infiltration in nasal mucosa, and to improved pulmonary function during the pollen seasons [10]. The efficacy, safety and long-lasting effects in paediatric patients have also been demonstrated [11, 12], and it has also recently been shown that SLIT can reduce the risk of onset of asthma [13]. At present, in Italy, France and Spain, SLIT is common in office practice. The ARIA guidelines offi-

(1) CIBICI, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre esquina Medina Allende, 5000, Córdoba (Argentina).

(2) Allergy and Respiratory Diseases, University of Genoa (Italy).

cially accepted SLIT as a routine clinical option in both adults and children, although whether this route is better than the subcutaneous route is still a subject of debate [14]. Moreover, as reviewed by Zwacka et al., an exact diagnosis of allergy, guidance and explanation by the specialist, reliable compliance of the patient, and strict monitoring of symptoms are necessary to get high efficacy of SLIT [15].

The molecular mechanisms responsible for the efficacy of subcutaneous IT have been extensively studied and elucidated. Among them, it can be mentioned the downregulation of cytokine and mediator release, the inhibition of activation and recruitment of effector cells, the modulation of Th1/Th2 balance and expansion of CD4+IL-10+ T regulatory cells expressing a peripheral tissue trafficking phenotype [16-23]. On the contrary, non injection IT has been studied mainly from a clinical point of view and the mechanisms of action remain poorly understood. It has been speculated that the persistence of the allergen at the mucosal level could play some role in the modulation of the immune response. Oral IT appears to produce systemic immunologic changes [24, 25], but nasal IT mainly exerts its effects locally [26-28].

Some studies in animal models have shown that dendritic cells of oral mucosa act as efficient antigen-presenting cells and produce IL-12, which directs the immune response towards a Th1 profile away from IgE-Th2 profile [29]. No significant difference in specific IgE and specific IgG4 was detected between the active and placebo groups in several studies [30, 31]. By contrast other studies demonstrated immunoglobulin changes resembling those of injection IT [32, 33]. Similarly, Canonica and co-workers reported a significant reduction of neutrophils, eosinophils and intercellular adhesion molecule 1 expression after specific nasal and conjunctival challenge [34]. More recently Ippoliti et al. found that SLIT in children with asthma due to allergy to *Dermatophagoides pteronyssinus* produced a reduction in the synthesis of Th2 cytokines as well as activation of T lymphocytes [35], and a more recent study evidenced that SLIT increases IL-10 production [36].

Looking at the literature, few studies have been done to comprehend the molecular basis of non injection IT. Moreover, it has been difficult to reach a conclusion because some studies have used different types of allergens and different administration routes with subsequent differences in processing and degradation pathways.

BUT WHAT DO WE KNOW ABOUT MUCOSAL SUBSTANCE DELIVERY?

Many of the drugs commercially available are administered via enteral route. The absorption of allergens through the intact intestinal mucosa has been explored and results have shown that corpuscular elements, macromolecules and proteins are absorbed in biologically and immunologically active forms [37-39]. The degree of absorption is influenced by both exogenous (medications) and endogenous factors (atopic disposition) [40]. However, is it possible to assume that these characteristics of the intestinal mucosa apply also by extension to other mucous membranes?

Because the oral mucosa is highly vascularised, drugs that are absorbed through the oral mucosa directly enter the systemic circulation, bypassing the gastrointestinal tract and first-pass metabolism in the liver. Some cardiovascular drugs, analgesics, sedatives and oestrogens have been developed for oral transmucosal delivery, although some limitations exist due to the characteristics of the oral mucosa and to the physicochemical and biological properties of the substance [41].

Oral mucosa has also received attention in the last decade because it offers excellent accessibility and avoids degradation of proteins and peptides. Peptide absorption occurs across oral mucosa by passive diffusion via the intercellular route, but delivery of proteins has some limitations and the addition of absorption promoters/permeabilizers is frequently needed [42].

The pharmacokinetics of allergens after local administration have been very controversial [43] and until 1997, there was a paucity of information about the *in vivo* kinetics of allergens administered through local routes [44-46]. Starting from 1997, Bagnasco and co-workers designed and performed several studies the absorption and fate of *Parietaria judaica* allergen (Par j 1) labelled with radioiodine in human beings. They showed that a fraction of the radiolabelled allergen persists at the mucosa level for a long time and gastrointestinal absorption of the degraded allergen appears to be the most relevant event [47]. When the pharmacokinetics of the same allergen and a monomeric allergoid for oromucosal IT in allergic volunteers was investigated, they found a similar behaviour of the allergoid and the allergen in terms of local kinetics, whereas plasma peak was higher with the allergoid than with the allergen. They concluded that the chemical modification of the allergen might affect its biodistribution, by making it less susceptible to enzymatic degradation [48]. The same group also

suggested that the more advantageous way to administer the allergen is the sublingual/swallow procedure, since the sublingual/spit procedure leads to a certain loss of the allergen administered [49].

If we really want to know what happens when an allergen is kept in the mouth in relation to the induction of any type of immune response we should focus the anatomy/histology as well as the immunological structures of this part of the body.

The MALT in higher mammals consists of secondary lymphoid tissues located beneath different epithelia and classified as *inductive sites*, where the priming of the immune system takes place, and *effector sites* in which antigen-specific T and B lymphocytes reside and perform their functions. Two major features distinguish MALT from other lymphoid tissues: (i) specializations of the overlying epithelium that facilitate antigen uptake, processing, and presentation for induction of mucosal immune responses; (ii) presence of organized regions that include subepithelial areas, B-cell zones with germinal centers, T-cell regions with antigen presenting cells (APC) but also more diffuse areas where home effector lymphocytes [50]. The MALT includes the gut-associated lymphoepithelial tissue (GALT), the bronchus-associated lymphoepithelial tissue (BALT), the nasopharyngeal-associated lymphoepithelial tissue (NALT), the genital tract and conjunctiva whose integration is only partly understood.

The epithelium of the MALT plays an active role in both innate and adaptive types of mucosal immunity. Due to the physical proximity of epithelial cells to the external milieu and to the site of initial antigen exposure they play a central role in defining the antigens with which the MALT is confronted and regulating the ultimate immune response [51]. The route by which antigens cross the epithelial-cell barrier is likely to dictate the type of immune response that is induced [52], and the generation of effector or suppressor cells may be related to the pathway used by the antigen to gain access to the host [51, 52].

WHAT DO WE KNOW ABOUT THE ORAL CAVITY?

The moist lining tissue of the oral cavity, the oral mucosa, is continuous with the remaining gastrointestinal tract. However, structurally the oral mucosa has more in common with skin than with the gastrointestinal mucosa. Oral mucosa in regions of the mouth subject to mechanical forces associated with mastication, such as gingival and hard palate, is covered

by a keratinized epithelium tightly attached to the underlying tissues by a collagenous connective tissue or lamina propria. The mucosa lining the floor of the mouth, which must be flexible to accommodate chewing or speech, is termed lining mucosa, and is covered with a nonkeratinized epithelium. The connective tissue of lining mucosa is more elastic and flexible than the connective tissue in the masticatory mucosa [42, 53].

A number of inflammatory cells can often be seen in the nucleated cell layers. These cells are transient, and mainly Langerhans' cells, although the presence of polymorphonuclear leukocytes and mast cells is not uncommon. The association between nonkeratinocytes and keratinocytes in skin and oral mucosa could represent a subtle and finely balanced relationship in which cytokines are the controlling factors [54].

The primary function of oral epithelium is the protection of the underlying tissue. In the keratinized regions, the mechanically tough *stratum corneum* serves to dissipate shearing forces, and in both keratinized and nonkeratinized regions lipid-based permeability barriers protect the underlying tissues against the fluid loss and the entry of potentially harmful environmental agents.

Regional differences arise during the process of differentiation as epithelial cells move from the basal region to the surface. As cells leave the basal layer, they become larger and begin to flatten and to accumulate lipids and cytoplasmic protein filaments, the cytokeratins. At the boundary between the granular layer and the surface layer, small organelles known as membrane-coating granules or lamellar granules, migrate to the apical region of keratinocytes and the bounding membrane of the organelle fuses with the cell plasma membrane, so that the lipid in lamellae are extruded into the intercellular spaces (figure 1). Thus the membrane coating granules are believed to be responsible for the formation of the superficial permeability barrier in stratified squamous epithelium.

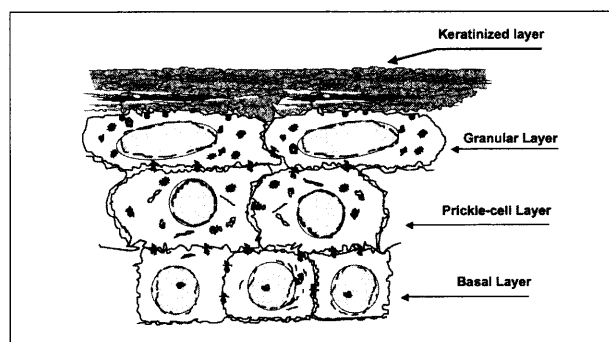


Figure 1: Scheme of the differentiation process in keratinized oral epithelium.

lium. In nonkeratinized epithelia, the accumulation of lipids and cytokeratin in the keratinocytes is less evident and the change in morphology is less far marked than in keratinized epithelia. The mature cells are large and flat, retain nuclei and other organelles and the cytokeratine do not aggregate to form bundles of filaments as seen in keratinized epithelia. As cells reach the upper third or quarter of the epithelium, membrane coating-granules appear to fuse with the plasma membrane to extrude their contents into the intercellular space. In contrast to the intercellular space of stratum corneum, that of the superficial layer of nonkeratinized epithelia contains electron lucent material which may represent non lamellar phase lipid, with only occasional short stacks of lipid lamellae [42]. Both the structure and the relative area of the different types of mucosa will influence the penetration of substances across the oral lining.

WHERE TAKES PLACE THE INITIAL CONTACT OF ANTIGENS FROM THE ORAL CAVITY WITH THE IMMUNE SYSTEM?

Waldeyer recognized the presence of lymphoid tissue in the human pharynx in 1884, and described its specific arrangement as a 'ring' of lymphoid tissue. The ring comprises the nasopharyngeal tonsil, attached to the roof of the pharynx; the paired tubal tonsils, located at the pharyngeal openings of the Eustachian tubes; the paired palatine tonsils positioned in the oropharynx; the lingual tonsil on the posterior third of the tongue and aggregations of lymphoid cells located in the lamina propria of the pharyngeal wall that complete a circular structure. This structure is situated at the entrance of the alimentary and respiratory tracts and has been considered as a first-line defense against the organisms entering these passages (figure 2).

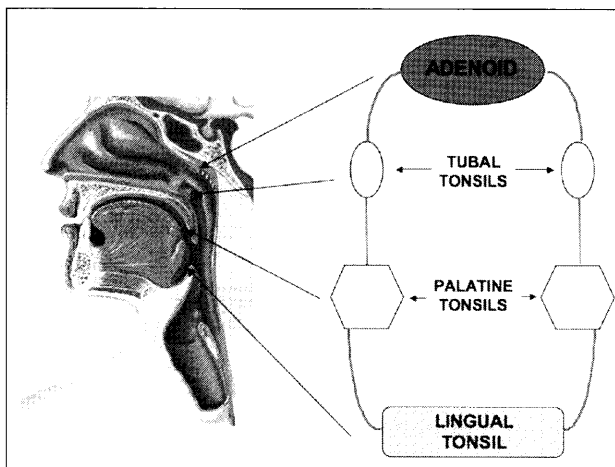


Figure 2: Pharyngeal lymphoid tissue of Waldeyer's ring comprises the adenoid, the tubal tonsils (TT), the palatine tonsils (PT) and the lingual tonsil (LT).

This defense structure emerged in connection with the developing rostral part of the digestive tube, exposed to the environmental antigens. At the evolutionary stage of mammals, it also became a mother region of the tonsils [55]. In that context, the tonsils can be considered an "evolutionary novelty", which did not appear before mammals. Situated in the same region where in lowest vertebrates the thymus develops, the tonsils are a modern phyletic successor of the thymus, as there is a gap of at least 300 million years between the appearance of these organs in evolution [56, 57]. Such time gap also exists in ontogeny because in humans all other branchiogenic organs develop within the third week of gestation, whereas the tonsillar crypts start to develop in the form of epithelial buds and later cords in the third month and continues their formation during the whole pregnancy [58, 59]. Waldeyer's ring continues developing during childhood but starts to disappear during adolescence [55].

Not all animals have a direct equivalent to the human tonsils and comparisons need to be conducted carefully. Since Waldeyer's ring is also present in pigs those animals have been used for experimental studies [7]. Studying the influence of antigen stimulation in the development of the palatine tonsils in germ-free (GF) and conventional piglets it has been shown that in newborn piglets and all GF postnatal stages studied the palatine tonsils were formed by a narrow strip of lymphoid tissue extended into the lateral part of the soft palate. The lymphoid infiltration of the connective tissue stroma occurred mostly around the tips of these crypts. However, the first primary follicles formed never developed into secondary follicles, even if mitogens were applied experimentally in GF piglets. The study demonstrated that the tonsils in GF piglets retain their fetal character during postnatal development, and that their full development depends on the antigenic stimulation [60].

One structural characteristic of tonsils is the existence of crypts which are narrow epithelial diverticula, varying in arrangement from the monocryptic units of the lingual tonsil to the polycryptic, sometimes branching crypts of the nasopharyngeal tonsils and palatine tonsils. In healthy tonsils the openings of the crypts are fissure-like, and the walls of the lumen are in apposition. In the centre of the palatine tonsils are tightly packed ramified crypts that join with each other, while on the periphery there is a rather simple and sparse arrangement [61]. The pharyngeal surfaces of the nasopharyngeal tonsil and the tubal tonsils are covered mainly with a ciliated respiratory epithelium, whereas those of the palatine tonsils and lingual tonsil are protected by stratified squamous non-

keratinized epithelium, respectively. A band of thick connective tissue containing many vessels, nerves and lymphatics underlines the surface epithelia. By contrast, the epithelium lining the crypts is not uniform and is underlined with disrupted basement membrane but no thick connective tissue band. Epithelium covering the cavities is usually not penetrated by blood vessels. Importantly, in the tonsillar lymphoepithelium, there is a network of intraepithelial blood vessels arranged in loops oriented perpendicularly to the crypt surface, and high endothelial venules are located in the lower border regions of the reticulated patches of epithelium (figure 3).

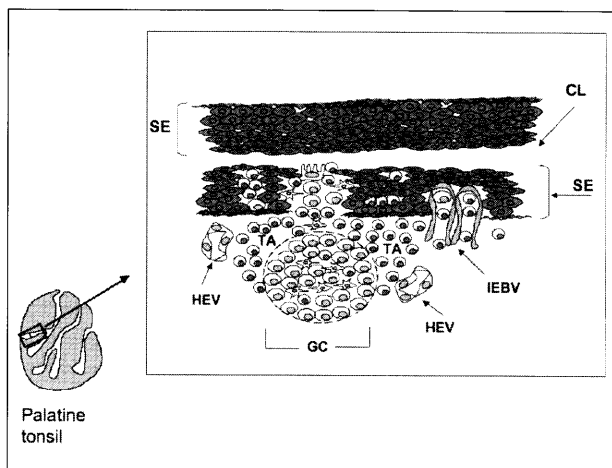


Figure 3: Lymphoepithelium of the palatine tonsils PT (A) and the adenoids (B). CL: Crypt Lumen, SE: Squamous Epithelium, HEV: High Endothelial Venules, IEBV: Intraepithelial Blood Vessels, GC: Germinal Centres, TA: T Cells Areas, Respiratory Epithelium: RE .

ARE THE TONSILLAR CRYPTS A BARRIER OR AN ANTIGENIC SAMPLER?

Due to its complex structure the epithelium covering the crypts has been termed in different ways: lymphoepithelium, reticulation of crypt epithelium, or follicle-associated epithelium (FAE), referring to an epithelium that can sample antigens and translocate them to the underlying lymphoid tissue [62].

The mesh of the epithelial cells has been graphically compared with a sponge with interstices holding different immune cells such as T cells, IgG-, IgA- and IgM-producing B cells, macrophages, interdigitating dendritic cells (DC), Langerhans cells and M-cells [62]. There is a crucial numerical balance between epithelial and nonepithelial cells, having the first ones the capacity to synthesize secretory component or the polymeric Ig receptor (pIgR/SC) to stabilize and trans-

port secretory IgA to the mucosal surface. Patches of the crypt epithelium at the nasopharyngeal tonsils express pIgR/SC but not in palatine tonsils [63]. The infiltrating nonepithelial cells are constantly exposed to alimentary and airborne antigens after birth. The ultrastructure and function of M-cells in the human nasopharyngeal tonsils and their capacity for antigen uptake have also been studied [64].

The cellular architecture of tonsils resembles that of lymph nodes, although the lack of afferent lymphatics predisposes them to direct interactions with environmental antigens. Tonsils contain, apart from the reticular crypt epithelium, other specialized lymphoid compartments such as the extrafollicular area (T cell areas), the mantle zones and the follicular germinal centers. Although the tonsils contain the complete set of cellular components necessary for primary and secondary immune responses, the precise contribution of these lymphoid structures to the induction and regulation of mucosal immune responses is not completely understood.

For antigens reaching the oral cavity one could speculate that tonsils are important inductive sites. The reticular crypt epithelium contains DC that can transport exogenous antigens to the extrafollicular T-cell areas and to the B-cell follicles. Extrafollicular T cells are mainly CD4+ of both naïve (CD45RA+) and memory (CD45RO+) subsets, and some express the IL-2 receptor (CD25) as a marker of recent activation. Naïve B cells are stimulated in the extrafollicular zones through cognate help from activated CD4+ T cells to which DC have presented antigens. Cytokines and microenvironmental factors determining isotype differentiation and coexpression of J chains in B cells remain obscure.

Classical memory B cells (sIgD-sIgM+) with strong CD80/CD86 expression are also found at extrafollicular sites where they might likewise exert an antigen-presenting function. Stimulated B cells colonize the primary lymphoid follicles and form the germinal center (GC) where antibody secreting cell (ASC) producing IgG (55-72%), or IgA (30-18%) predominate. These cells and ASC producing IgM and IgD often show concurrent expression of J chain (13-80%). The J-chain expressing capacity of IgA-ASC in tonsils is better than in peripheral lymph nodes (LN) and lower than in mesenteric LN [65].

After the tonsillar B-cell differentiation process only few of the leaving IgA-ASC terminate their differentiation as extrafollicular cells or join the systemic immune system. Most of them home to regional secretory *effector sites* for terminal differentiation there.

The finding of a similar distribution of IgA1 and IgD ASC among nasal mucosae, salivary and lacrimal glands, and tonsils, supports the notion that these regional secretory effector sites are seeded mainly by B-cell blasts generated in GC of Waldeyer's ring [50, 66-68].

HOW IS THE CIRCULATION FROM TONSILS TO EFFECTOR SITES ESTABLISHED?

Attempts have been made to track directly the dissemination of B cells from human tonsils by means of molecular markers. Because the human herpes virus Epstein-Barr virus (EBV) preferentially establishes persistent infection within memory/effector B cells of Waldeyer's ring, their migration to other compartments may be mapped by DNA analysis for latent EBV infection. On the basis of this approach, B-cell trafficking has been suggested to take place from Waldeyer's ring to peripheral blood and to a lesser extent into systemic lymphoid organs such as mesenteric lymph nodes and the spleen [50]. Using another DNA marker, namely Ig heavy chain C μ gene deletion [68, 69] Brandtzaeg et. al. showed that memory/effector B cells undergoing non-classical CH switching to IgD immunocyte differentiation, preferentially home from Waldeyer's ring through cervical lymph nodes to the upper airway mucosa and associated glands [65].

IgA-ASC precursors present in the oral cavity, and upper digestive tract preferentially populate the salivary glands with low levels of trafficking to the intestine. ASC in mucosal lymph tissues can upregulate the expression of two chemokine receptors, CCR9 and CCR10, which are associated with the trafficking patterns of these cells. However, whereas responsiveness to the CCR9 ligand (CCL25) is not characteristic in the upper digestive tract the CCR10 ligand, CCL28 is profusely produced in epithelial cells in various mucosal tissues. Almost all IgA ASC express CCR10 and respond to CCL28, and the reduced number of IgG or IgM ASC expressing CCR 10 supports a selective association of this receptor with IgA ASC. Differences in dissemination of antigen-specific IgA after nasal or oral immunization is likely due to differential migration properties [70].

Possibly, cell maturation in the course of several proliferative cycles results in reduced expression of J chain promoting the monomeric production of IgA. In fact, repeated tonsillar inflammation is associated with decreased J-chain expression by B cells, compromising their putative contribution to the regional secretory IgA system. Tonsillitis appears to speed the

age-related involution of the tonsils as immunological organs possibly related to increased shedding of antigen transporting M cells in the crypt epithelium, also influencing the balance between expansion of J positive (early) and J negative (mature) effector B cells.

IS THE IMMUNE RESPONSE TO ORALLY ADMINISTERED ANTIGENS LIMITED TO THE WALDEYER'S LYMPHOID STRUCTURES?

Orally applied proteins underlie the activity of salivary enzymes, proteases and the low pH, which can inactivate most of the relevant epitopes of antigens before they reach the APC of the gut. For fed antigens the Peyer's patches (PP) seem to be the main inductive sites. They are organized lymphoid structures, which are distributed mainly in the distal ileum. This mucosal microenvironment appears to be particularly geared toward the induction of T helper cells producing type 2 and type3 cytokines. This ability is consistent with the three prominent features on immune responses to oral antigens: the propensity of resident DC for secretion of anti-inflammatory cytokines unless they are activated by maturation signals, the production of secretory IgA, and the induction of regulatory T cells.

In the normal intestine most antigens after traversing the large surface area of the epithelium are processed and presented on MHC molecules by intestinal epithelial cells, absorbed into capillaries that culminate in the portal vein and the liver or captured by resident APC and carried to draining lymph nodes. Dendritic cells can also extend processes into the lumen to capture antigens directly and then migrate to the mesenteric lymph nodes (MLN). Another important portal of entry for antigens is the specialized M cells of the epithelium. Antigens either passing through the epithelium into blood capillaries or carried through lymphatic vessels to the mesenteric lymph nodes ultimately converge into the spleen [51].

The particular reasons why the mucosal environment may be especially conducive to the differentiation of T cell into a Th2/Th3 pathway are unclear. One possibility is the presence of particular APC subsets capable of the Th2 induction in the mucosa. In the GALT, DC are found in the PP, lamina propria, and draining MLN. In humans, rat and mouse one DC subset is located underneath the epithelium to take up luminal antigens transported by M cells. It is also scattered through the B cell follicle but excluded from the germinal centers [71]. Other DC subset is present in the interfollicular regions in close association with

more mature or differentiated T cells. The behavior of DC in organized PP is unique because the site of initial antigen entry and capture is in close proximity to organized T and B cell zones [72]. These DC are responsible for the priming of T cells since they come in close contact with naive T cells. Results suggest that there is a primary difference between DC from PP and those from spleen related to their ability to induce distinct T cell responses. IL-10 and possibly TGF β are produced by PP DC when interaction with naive T cells occurs and these cytokines directly or indirectly drive T cells to differentiate into T cells producing Th2 cytokines and TGF- β [73].

UNMET NEEDS

Based upon what we have discussed in this review it is very clear that there is a need to perform different studies in order to know the following issues:

- The precise molecular mechanisms of allergen degradation and absorption.
- Which are the molecular mechanisms involved in induction of regulatory immune cells in the oral cavity.
- Is SLIT effective in treatment and/or prevention of atopic disorders?
- How early could SLIT be applied?

Acknowledgements: This work was supported by grants of Agencia Córdoba Ciencia, Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) and Secretaría de Ciencia y Tecnología (SECYT-UNC). Authors would like to thank Dr. C. Baena-Cagnani for helpful suggestions.

Bibliographie

1. Purello-D'Ambrosio F., Gangemi S., Merendino R.-A., Isola S., Puccinelli P., Parmiani S., Ricciardi L. - Prevention of new sensitizations in monosensitized subjects submitted to specific immunotherapy or not. A retrospective study. *Clin Exp Allergy* 2001;31:1295-302.
2. Moller C., Dreborg S., Ferdousi H.-A., Halken S., Host A., Jacobsen L. et al. - Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 2002;109:251-6.
3. Eng P.-A., Reinhold M., Gnehm H.-P. - Long-term efficacy of preseasonal grass pollen immunotherapy in children. *Allergy* 2002;57:306-12.
4. Holt P.-G., Batty J.-E., Turner K.-J. - Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. *Immunology* 1981;42:409-17.
5. Holt P.-G., Vines J., Britten D. - Sublingual allergen administration. I. Selective suppression of IgE production in rats by high allergen doses. *Clin Allergy* 1988;18:229-34.
6. Bousquet J., Lockey R., Malling H.-J. - Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998;102:558-62.
7. Davis S.-S. - Nasal vaccines. *Adv Drug Deliv Rev* 2001;51:21-42.
8. Kagi M.-K., Wuthrich B. - Different methods of local allergen-specific immunotherapy. *Allergy* 2002;57:379-88.
9. Canonica G.-W., Passalacqua G. - Noninjection routes for immunotherapy. *J Allergy Clin Immunol* 2003;111:437-48.
10. Marogna M., Spadolini I., Massolo A., Canonica G.-W., Passalacqua G. - Clinical, functional, and immunologic effects of sublingual immunotherapy in birch pollinosis: a 3-year randomized controlled study. *J Allergy Clin Immunol* 2005;115:1184-8.
11. Passalacqua G., Baena-Cagnani C.-E., Berardi M., Canonica G.-W. - Oral and sublingual immunotherapy in paediatric patients. *Curr Opin Allergy Clin Immunol* 2003;3:139-45.
12. Di Rienzo V., Marcucci F., Puccinelli P., Parmiani S., Frati F., Sensi L. et al. - Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: a 10-year prospective study. *Clin Exp Allergy* 2003;33:206-10.
13. Novembre E., Galli E., Landi F., Caffarelli C., Pifferi M., De Marco E. et al. - Coseasonal sublingual immunotherapy reduces the development of asthma in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2004;114:851-7.
14. Guez S. - Efficacy of desensitization via the sublingual route in mite allergy. *Chem Immunol Allergy* 2003;82:62-76.
15. Zwacka G., Markert U.-R. - Therapeutic procedures of sublingual immunotherapy in clinical practice. *Chem Immunol Allergy* 2003;82:44-52.
16. Akdis C.-A., Blaser K. - Mechanisms of allergen-specific immunotherapy. *Allergy* 2000;55:522-30.
17. Durham S.-R., Till S.-J. - Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998;102:157-64.
18. Secrist H., DeKruyff R.-H., Umetsu D.-T. - Interleukin 4 production by CD4+ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *J Exp Med* 1995;181:1081-9.
19. Varney V.-A., Hamid Q.-A., Gaga M., Ying S., Jacobson M., Frew A.-J. et al. - Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993;92:644-51.
20. Gabrielsson S., Soderlund A., Paulie S., van der Pouw Kraan T.-C., Troye-Blomberg M., Rak S. - Specific immunotherapy prevents increased levels of allergen-specific IL-4- and IL-13-producing cells during pollen season. *Allergy* 2001;56:293-300.
21. Benjaponpitak S., Oro A., Maguire P., Marinkovich V., DeKruyff R.-H., Umetsu D.-T. - The kinetics of change in cytokine production by CD4 T cells during conventional allergen immunotherapy. *J Allergy Clin Immunol* 1999;103:468-75.
22. Gardner L.-M., Thien F.-C., Douglass J.-A., Rolland J.-M., O'Hehir R.-E. - Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4+ CD25+ interleukin-10+ T cells expressing peripheral tissue trafficking markers. *Clin Exp Allergy* 2004;34:1209-19.
23. Rak S., Hakanson L., Venge P. - Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season. *J Allergy Clin Immunol* 1990;86:706-13.
24. Moller C., Dreborg S., Lanner A., Bjorksten B. - Oral immunotherapy of children with rhinoconjunctivitis due to birch pollen allergy. A double blind study. *Allergy* 1986;41:271-9.
25. Leng X., Fu Y.-X., Ye S.-T., Duan S.-Q. - A double-blind trial of oral immunotherapy for Artemisia pollen asthma with evaluation of bronchial response to the pollen allergen and serum-specific IgE antibody. *Ann Allergy* 1990;64:27-31.
26. Georgitis J.-W., Nickelsen J.-A., Wypych J.-I., Barde S.-H., Clayton W.-F., Reisman R.-E. - Local intranasal immunotherapy with high-dose polymerized ragweed extract. *Int Arch Allergy Appl Immunol* 1986;81:170-3.
27. Andri L., Senna G., Betteli C., Giovanni S., Andri G., Falagiani P. - Local nasal immunotherapy for Dermatophagoides-induced rhinitis: efficacy of a powder extract. *J Allergy Clin Immunol* 1993;91:987-96.
28. Passalacqua G., Albano M., Ruffoni S., Pronzato C., Riccio A.-M., Di Berardino L. et al. - Nasal immunotherapy to Parietaria: evidence of reduction of local allergic inflammation. *Am J Respir Crit Care Med* 1995;152:461-6.

29. Pajno G.-B., Peroni D.-G., Barberio G., Boner A.-L. - Efficacy of sublingual immunotherapy in asthma and eczema. *Chem Immunol Allergy* 2003;82:77-88.
30. Purello-D'Ambrosio F., Gangemi S., Isola S., La Motta N., Puccinelli P., Parmiani 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;54:968-73.
31. Rolinck-Werninghaus C., Kopp M., Liebke C., Lange J., Wahn U., Niggemann B. - Lack of detectable alterations in immune responses during sublingual immunotherapy in children with seasonal allergic rhinoconjunctivitis to grass pollen. *Int Arch Allergy Immunol* 2005;136:134-41.
32. La Rosa M., Ranno C., Andre C., Carat F., Tosca M.-A., Canonica G.-W. - Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 1999;104:425-32.
33. Bahceciler N.-N., Arikan C., Taylor A., Akdis M., Blaser K., Barlan I.-B., Akdis C.-A. - Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. *Int Arch Allergy Immunol* 2005;136:287-94.
34. Passalacqua G., Albano M., Riccio A., Fregonese L., Puccinelli P., Parmiani S., Canonica G.-W. - Clinical and immunologic effects of a rush sublingual immunotherapy to *Parietaria* species: A double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 1999;104:964-8.
35. Ippoliti F., De Santis W., Volterrani A., Lenti L., Canitano N., Lucarelli S., Frediani T. - Immunomodulation during sublingual therapy in allergic children. *Pediatr Allergy Immunol* 2003;14:216-21.
36. Ciprandi G., Fenoglio D., Cirillo I., Vizzaccaro A., Ferrera A., Tosca M.-A., Puppo F. - Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol* 2005;95:38-44.
37. Hemmings W.-A. - Antigen absorption by the gut. 1978; Lancaster: MTP Press.
38. Hemmings WA, Williams EW. Transport of large breakdown products of dietary protein through the gut wall. *Gut* 1978;19:715-23.
39. Warshaw A.-L., Bellini C.-A., Walker W.-A. - The intestinal mucosal barrier to intact antigenic protein. Difference between colon and small intestine. *Am J Surg* 1977;133:55-8.
40. Bjarnason I., Williams P., So A., Zanelli G.-D., Levi A.-J., Gumpel J.-M. et al. - Intestinal permeability and inflammation in rheumatoid arthritis: effects of non-steroidal anti-inflammatory drugs. *Lancet* 1984;2:1171-4.
41. Zhang H., Zhang J., Streisand J.-B. - Oral mucosal drug delivery: clinical pharmacokinetics and therapeutic applications. *Clin Pharmacokinet* 2002;41:661-80.
42. Senel S., Kremer M., Nagy K., Squier C. - Delivery of bioactive peptides and proteins across oral (buccal) mucosa. *Curr Pharm Biotechnol* 2001;2:175-86.
43. Falagiani P., Mistrello G. - Pharmacokinetics of allergens after local administration. *Allergy* 1997;52:17-21.
44. Einarsson R., Renck B., Taudorf E. - In vitro studies of degradation of birch and timothy pollen allergen preparations by human duodenal juice. *Allergy* 1988;43:469-72.
45. Mistrello G., Roncarolo D., Gentili M., Zanoni D., Falagiani P. - Modified par j I allergen from *P. judaica* pollen and its rate of absorption in rats. *Immunol Lett* 1994;40:31-6.
46. Swarbrick E.-T., Stokes C.-R., Soothill J.-F. - Absorption of antigens after oral immunisation and the simultaneous induction of specific systemic tolerance. *Gut* 1979;20:121-5.
47. Bagnasco M., Mariani G., Passalacqua G., Motta C., Bartolomei M., Falagiani P. 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;100:122-9.
48. Bagnasco M., Passalacqua G., Villa G., Augeri C., Flamigni G., Borini E. et al. - Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers. *Clin Exp Allergy* 2001;31:54-60.
49. Passalacqua G., Villa G., Altrineti V., Falagiani P., Canonica G.-W., Mariani G., Bagnasco M. - Sublingual swallow or spit? *Allergy* 2001;56:578.
50. Brandtzaeg P., Farstad I.-N., Haraldsen G. - Regional specialization in the mucosal immune system: primed cells do not always home along the same track. *Immunol Today* 1999;20:267-77.
51. Mayer L. - Mucosal immunity. *Pediatrics* 2003;111:1595-600.
52. Mayer L., Shao L. - Therapeutic potential of oral tolerance. *Nat Rev Immunol* 2004;4:407-19.
53. Squier CKM. - Biology of the oral mucosa and esophagus. *Journal of the National Cancer Institute Monographs* 2001;7:15.
54. Feliciani C., Gupta A.-K., Sauder D.-N. - Keratinocytes and cytokine/growth factors. *Crit Rev Oral Biol Med* 1996;7:300-18.
55. Slipka J. - Palatine tonsils—their evolution and ontogeny. *Acta Otolaryngol Suppl* 1988;454:18-22.
56. Slipka J., Pospisilova V., Slipka J., Jr. - Evolution, development and involution of the thymus. *Folia Microbiol (Praha)* 1998;43:527-30.
57. Slipka J., Slipka J., Jr. - The palatine tonsil as an evolutionary novelty. *Acta Otolaryngol Suppl* 1996;523:8-11.
58. Slipka J. - The development and involution of tonsils. *Adv Otorhinolaryngol* 1992;47:1-4.
59. von Gaudecker B., Muller-Hermelink H.-K. - The development of the human tonsilla palatina. *Cell Tissue Res* 1982;224:579-600.
60. Slipka S., Jaroslav, Slipka J., Jaroslav. - Palatine tonsils are they branchiogenic organs? *International Congress Series* 2003;1257:71-74.
61. Abbey K., Kawabata I. - Computerized three-dimensional reconstruction of the crypt system of the palatine tonsil. *Acta Otolaryngol Suppl* 1988;454:39-42.
62. Perry M., Whyte A. - Immunology of the tonsils. *Immunol Today* 1998;19:414-21.
63. Brandtzaeg P., Korsrud F.-R. - Significance of different J chain profiles in human tissues: generation of IgA and IgM with binding site for secretory component is related to the J chain expressing capacity of the total local immunocyte population, including IgG and IgD producing cells, and depends on the clinical state of the tissue. *Clin Exp Immunol* 1984;58:709-18.
64. Fujimura Y. - Evidence of M cells as portals of entry for antigens in the nasopharyngeal lymphoid tissue of humans. *Virchows Arch* 2000;436:560-6.
65. Brandtzaeg P. - Immunology of tonsils and adenoids: everything the ENT surgeon needs to know. *Int J Pediatr Otorhinolaryngol* 2003;67 Suppl 1:S69-76.
66. Brandtzaeg P. - Immunological functions of adenoids: Kugler Publications, The Hauge, 1998:233-46pp.
67. Brandtzaeg P., Farstad I.-N., Johansen F.-E., Morton H.-C., Norderhaug I.-N., Yamanaka T. - The B-cell system of human mucosae and exocrine glands. *Immunol Rev* 1999;171:45-87.
68. Brandtzaeg P., Baekkevold E.-S., Farstad I.-N., Jahnsen F.-L., Johansen F.-E., Nilsen E.-M., Yamanaka T. - Regional specialization in the mucosal immune system: what happens in the microcompartments? *Immunol Today* 1999;20:141-51.
69. Arpin C., de Bouteiller O., Razanajaona D., Fugier-Vivier J., Briere F., Banchereau J. et al. - The normal counterpart of IgD myeloma cells in germinal center displays extensively mutated IgVH gene, Cmu-Cdelta switch, and lambda light chain expression. *J Exp Med* 1998;187:1169-78.
70. Kunkel E.-J., Butcher E.-C. - Plasma-cell homing. *Nat Rev Immunol* 2003;3:822-9.
71. Novak N., Allam J.-P., Betten H., Haberstock J., Bieber T. - The role of antigen presenting cells at distinct anatomic sites: they accelerate and they slow down allergies. *Allergy* 2004;59:5-14.
72. Neutra M.-R., Mantis N.-J., Kraehenbuhl J.-P. - Collaboration of epithelial cells with organized mucosal lymphoid tissues. *Nat Immunol* 2001;2:1004-9.
73. Iwasaki A., Kelsall B.-L. - Mucosal immunity and inflammation. I. Mucosal dendritic cells: their specialized role in initiating T cell responses. *Am J Physiol* 1999;276:G1074-8.