

Allergic Inflammation and the Oral Mucosa

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Abstract: Allergic inflammation is initiated by the contact between allergen(s) and specific IgE antibodies, driven by regulatory cells such as antigen presenting cells and T lymphocytes, which orientate and orchestrate the response, and sustained by effector cells such as mast cells, basophils, and eosinophils. Among tissues and organs targeted by allergy, the nose, the lungs and the skin have the property to spread in distant sites the initially local reaction, thus resulting in systemic disease. By contrast, the oral mucosa seems to be a tolerogenic site regarding the immunologic response to allergens. This mucosa is characterized by abundance of dendritic cells, which are antigen presenting cells specialized in uptaking, processing and presenting the antigens to T cells, and particularly to T regulatory cells which in turn can downregulate Th1 and Th2 immune responses by direct cell contact or by production of immunosuppressive cytokines.

The other important aspect of the oral mucosa is the negligible presence of effector inflammatory cells, namely mast cells and eosinophils, which accounts for the reportedly good safety of sublingual administration of allergen immunotherapy. These peculiar aspects and patents have important implications in treatment and prevention of allergic diseases.

Keywords: Allergic inflammation, oral mucosa, dendritic cells, sublingual immunotherapy.

INTRODUCTION

Allergic inflammation is caused by an immune response to environmental allergens driven by regulatory cells such as antigen presenting cells and T lymphocytes, which orientate and orchestrate the response, and sustained by effector cells such as mast cells, basophils, and eosinophils. Various tissues are involved in such inflammation according to the site of contact with the specific allergen and to the target organs, which are triggered by natural exposure but may also be stimulated and studied by specific allergen challenges. The kind of cellular response to allergens was investigated in the nose [1], in the lungs [2], and in the skin as well [3], demonstrating evident increase of inflammatory cells in reaction to the introduction of the allergen - generally done by specific challenges - while recent data suggest that the oral mucosa, which is a set site of contact to a large spectrum of allergens including food and inhalant antigens, has a completely different type of response.

THE IMMUNOLOGIC RESPONSE IN THE ORAL MUCOSA

Anatomically, the oral mucosa is composed by three layers, epithelium, lamina propria, and submucosa, with a particular importance of the basal membrane, whose thickness accounts for the reduced permeability of this anatomical area.

The cells present in the oral mucosa, which are directly related with its immunologic properties, are dendritic cells, T lymphocytes, and B lymphocytes.

A bulk of evidence attributes to dendritic cells the role of chief character in the response to allergen molecules

reaching the mouth. These cells originate from bone marrow-derived leucocytes which migrate into the tissues and specialize in uptaking, processing and presenting the antigens to T cells [4-8]. Dendritic cells are scattered in the oral mucosa including the external epithelial layer and show the features of the Langerhans cells observed in various areas of the body with the function of antigen presenting cells. Dendritic cells share with the Langerhans cells the content in Birbeck intracytoplasmatic granules but are characterized by the expression of the high affinity IgE receptor FcεRI, very important in allergic inflammation [9, 10]. In fact, the link of dendritic cell's IgE receptors, including the low affinity receptor CD23, with the allergen molecules elicits the production of a cytokine pattern including IL-10 and TGFβ, and up-regulates the synthesis of indoleamine 2,3-dioxygenase, an enzyme which metabolizes tryptophan, which is essential for the biology of T cells [11]. Other receptors with higher expression on dendritic cells compared to skin Langerhans cells are MHC I-II, CD 40, CD 80/B7.1, and CD 86/B7.2. Dendritic cells are able to internalize the antigen molecules by macropinocytosis and to concentrate them in the major histocompatibility complex class II compartment for presentation to T cells [12,13]. Regarding allergens, it has been reported that the internalization is more efficient in presence of an high allergen load [14]. The subsequent migration of the dendritic cell to draining lymph nodes is essential for the interaction with naive CD4 and CD8 T cells [8], the kind of presentation being critical to result in either to priming of effector and memory T cells or to functional inactivation and T cell tolerance [15].

Regarding lymphocytes in the oral mucosa, the T cells are mainly located in perivascular spaces in the epithelial layer, and about 40 times more represented in the oral mucosa than in the skin, while the B cells are virtually absent in the epithelium and in the papillar layer of the mucosa [9].

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The role of T cells in orchestrating the immune response in allergic inflammation is well described [16]. Recent studies showed that tolerance to allergens may take place by a suppressive but also by a regulatory activity, the latter being currently considered as very important in the response to allergens [17,18]. The cells with such activity are known as regulatory T cells (TREG) and include naturally occurring CD⁴⁺CD²⁵⁺ T cells but also cells induced by antigen exposure (such as Tr1 cells, Th3 cells, and CD8+ regulatory T cells). The allergen presentation by dendritic cells is critical in inducing the differentiation of regulatory T cells [19], which in turn can downregulate Th1 and Th2 immune responses [17,18], by direct cell contact or by production of immunosuppressive cytokines such as TGF β - characteristic of Th3 cells [20], or IL-10 - characteristic of Tr1 cells [18].

A relationship between allergic inflammation and deficit in TREG functions is well documented. In one study, children with a deficit in CD⁴⁺ CD²⁵⁺ regulatory T cells developed eczema, elevated IgE levels, eosinophilia and hypersensitivity to foods [21], and in an *in vitro* model the suppressive activity of CD⁴⁺ CD²⁵⁺ regulatory T cells was significantly lower in atopic than in non atopic individuals [22]. Moreover, the prevalence in healthy individuals of allergen-specific Tr1 cells over IL4-secreting Th2 cells is a further demonstration of the tolerogenic role of Treg cells during natural exposure to allergens [23]. Treg have a beneficial effect also in an ongoing allergic inflammation by decreasing the IgE synthesis induced by IL-10 and the increase of IgG4 and IgA production induced by TGF β [24].

A point of great interest about the immunity in the oral mucosa regards the effector cells of allergic inflammation, i.e. eosinophils, mast cells, and basophils, which are abundantly found in organs targeted by allergy such as the nose [25] and the lung [26]. With the model of allergen immunotherapy - which is the practice of administering increasing doses of allergen extracts to reduce the clinical reactivity to the specific allergens [27] - it has been shown by skin biopsies that the cellular response to the subcutaneous injection of the allergen extract was followed by infiltration of inflammatory cells, especially lymphocytes and activated eosinophils in the site of injection [28]. By contrast, in the oral mucosa of allergic subjects treated with sublingual immunotherapy, by which the allergen extract is held under the tongue before being swallowed [29] could not be detected tryptase and ECP, which are the markers of mast cell and eosinophils activation [30]. Also the direct evaluation by biopsy of the oral mucosa in a subject with local reactions to sublingual immunotherapy confirmed the negligible presence of inflammatory cells [31]. It is important to underline that such cells are not whatever excluded from inflammatory response in the mouth: for example, mast cells were found in the oral mucosa of subjects with local inflammation caused by oral lichen planus [32].

THE CONCEPT OF SUBLINGUAL TOLERANCE

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 [33]. Therefore, the lack of allergic inflammation in the oral mucosa upon contact with the specific allergen suggests that

the mouth is likely to be a tolerogenic site, and this 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.

It is important to distinguish the concepts of sublingual and oral tolerance: the latter originated from the studies of Besredka almost one century ago [34] and was recently revised according to latest findings [35]. Oral tolerance is aimed at inducing a local and systemic hyporesponsiveness to ingested protein antigens, which ensures the prevention of food allergies. Also for the intestinal mucosa there is ample evidence that dendritic cells are fundamental in taking up dietary proteins and migrating to the draining mesenteric lymph node, where they induce regulatory CD4 T-cell differentiation. Tolerized T cells are likely to maintain the homeostasis of the gut microenvironment by conditioning dendritic cells to stay quiescent, with a possible contribute by commensal bacteria in inhibitory signalling.

Thus, the pivotal role of dendritic cells seems to outline an apparent similarity between sublingual and oral - including gastrointestinal - immunologic response to antigens. Again, the model of allergen immunotherapy may be helpful in understanding the differences between oral and sublingual tolerance. In fact, oral immunotherapy, i.e. the administration of the allergen extract in tablets or capsules to be swallowed bypassing the sublingual contact, proved to be ineffective despite the use of doses thousands of times higher than those received with conventional subcutaneous immunotherapy [36] and is no more considered a feasible option of immunotherapy [37]. Different compositions has been reported for the prevention and treatment of oral mucosal infections [38-41]. Concerning hypersensitivity, it is the sublingual contact between allergens and dendritic cells that elicits, at least for inhalant allergens, the immune mechanisms explained before, which are summarized in Fig. 1. In addition, the high doses administered by oral immunotherapy caused important local reactions including gastrointestinal bleeding, possibly able to interfere with the antigen absorption and thus with immunization, while it is evident that sublingual mucosa is able to tolerate allergen amounts very much higher than the nose or the skin [42].

Studies on the kinetics of sublingually administered allergens further highlight the issue: the group of Canonica investigated such kinetics by using materials radiolabeled with I¹²⁵, which included native and modified allergens, and comparing the administration via the oral or nasal mucosa [43,44]. It was possible to observe that the sublingually administered allergen (namely 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 that after swallowing a gastrointestinal absorption occurred but that the plasma radioactivity (peaking at 1.5-3 hours) was due to free radioiodine and small peptides but not to native allergen. Using a monomeric allergoid, the kinetics was comparable regarding the permanence in the oral mucosa and the absorption after swallowing, with plasma radioactivity peaking after two hours, but involving also the undegraded allergoid.

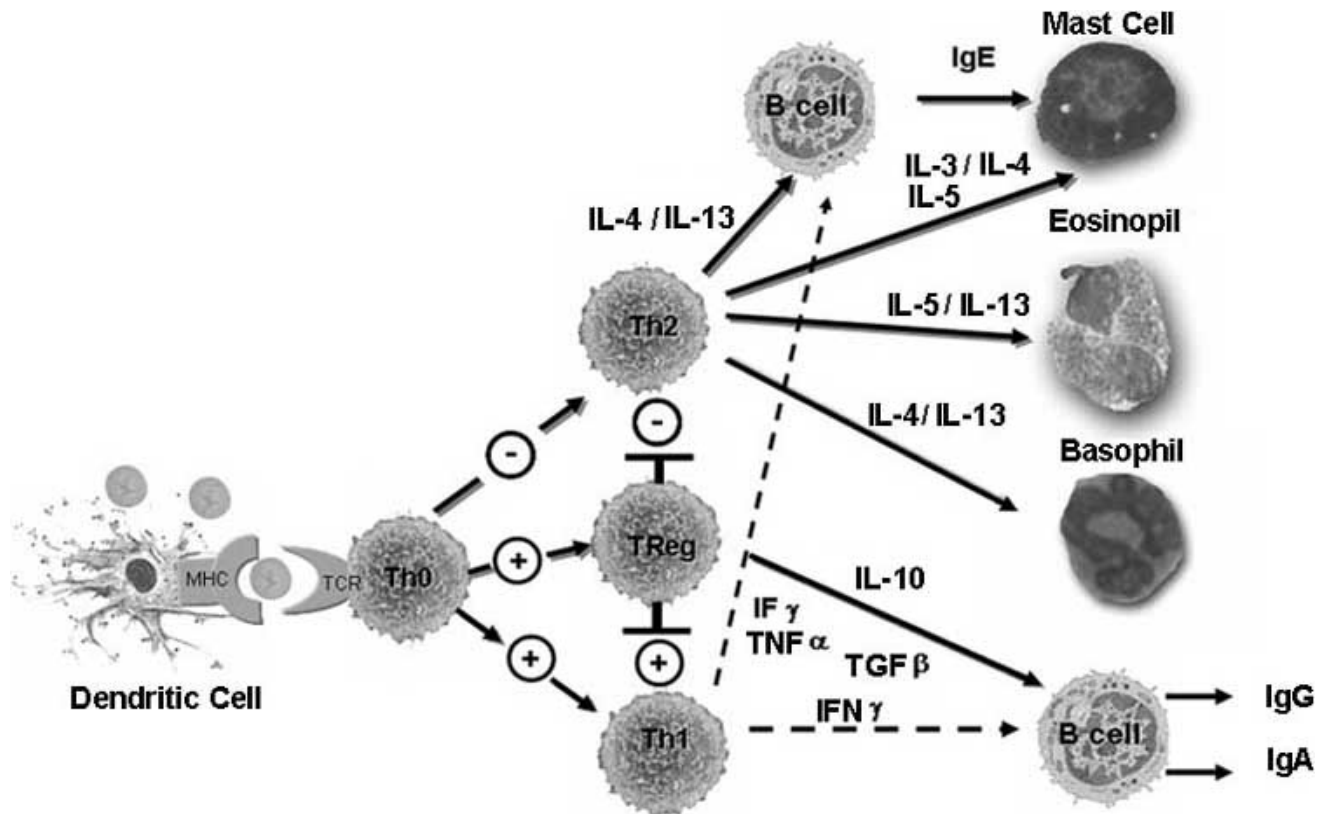


Fig. (1). From dendritic cells in the oral mucosa to systemic tolerance.

CURRENT & FUTURE DEVELOPMENTS

Allergic inflammation is activated, though not exclusively, by the interaction between allergens coming into contact with the body systems - and particularly respiratory organs, gastrointestinal tract, and skin - and specific IgE antibodies. The factors determining such activation, the kind of response and the target organs involved in individual subjects are still largely unknown. However, the concept of allergy as a systemic disease [33] may improve the understanding of some pathophysiological aspects, such as the rapid development of bronchial hyperresponsiveness, which is associated to inflammation, following nasal challenge with the specific allergen with no evidence of allergen deposition into the lungs [45], or the worsening of atopic dermatitis following inhalation of dust mite allergens [46] or ingestion of specific foods [47].

The most convincing explanation of these phenomena is the release by the first cells contrasting allergen penetration, especially the mast cells, of soluble mediators, such as histamine and leukotrienes, and inflammatory cytokines, such as IL-4, IL-5 and TNF- α , which are able to elicit the allergic cascade by entering blood circulation or by activating leucocytes when they pass through the site of the original reaction [33].

Under this vision, respiratory and skin tissues are the most efficient in triggering inflammation in distant organs, while the oral mucosa has a poor ability to achieve this outcome. The immunologic event related to such response

seems to be the interplay between dendritic cells and regulatory T cells [48] once the allergen is captured by the former in the oral mucosa. This peculiar aspect has important implication in the treatment, particularly in allergen sublingual immunotherapy [49], and prevention of allergic diseases, which is currently under evaluation even regarding primary prevention through sublingual immunization of newborn at risk of allergy [50].

ABBREVIATIONS

Th	=	T helper
CD	=	Cluster of differentiation
IL	=	Interleukin
TGF β	=	Transforming growth factor beta
MHC	=	Major histocompatibility complex
Tr	=	T regulatory
ECP	=	Eosinophil cationic protein
TNF	=	Tumor necrosis factor

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