

The immune privilege of the oral mucosa

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Despite high bacterial colonization and frequent allergen contact, acute inflammatory and allergic reactions are rarely seen in the oral mucosa. Therefore we assert that immune tolerance predominates at this site and antigen presenting cells, such as dendritic cells and different T cell subtypes, serve as key players in oral mucosal tolerance induction. In this article we describe the mechanisms that lead to tolerance induced in the oral mucosa and how they differ from tolerance induced in the lower gastrointestinal tract. Furthermore we discuss ways in which novel nonparenteral approaches for immune intervention, such as allergen-specific immunotherapy applied by way of the sublingual route, might be improved to target the tolerogenic properties of the sophisticated oral mucosal immune network.

Introduction

The oral mucosal epithelium comprises masticatory and lining mucosa and represents the entry port to the gastrointestinal tract (GIT), where immune tolerance induction towards commensal microbes as well as to different foreign antigens from food proteins predominates to maintain immune homeostasis. In view of the high level of exposure of the oral mucosa to foreign antigens, we believe it is more than likely that corresponding protolerogenic mechanisms take place in this tissue. Furthermore, antigen presenting cells as well as different T cell subtypes play central roles in oral mucosal tolerance (see Glossary) induction. Therefore, in recent years, interest in understanding the immunology of the oral mucosal tissue has increased to target the protolerogenic properties of this easily accessible organ for therapeutic purpose. Herein, we will review the mechanisms that lead to tolerance induced in the oral mucosa and how they differ from tolerance induced in the lower GIT. In addition, we will discuss one of the most recent approaches of tolerance induction by way of the oral mucosal route, represented by sublingual allergen application, referred to as sublingual immunotherapy (SLIT), which is aimed at the induction of allergen specific tolerance in sensitized individuals. We will describe how existing therapies such as SLIT might be improved and which new therapeutic approaches including novel routes for antiviral vaccines might be developed in the near future.

Components of the oral mucosa

The oral mucosa consists of a physical barrier with integrated immunological elements that prevent the invasion

of pathogenic organisms (Figure 1a) [1]. A stratified squamous epithelium lines the oral cavity, which is subdivided into masticatory mucosa and lining mucosa. Masticatory mucosa is orthokeratinized without a prominent granular layer and covers regions exposed to strong shear forces such as tongue, attached gingiva and hard palate. Lining mucosa features a nonkeratinized epithelium that lines the remaining part of the oral cavity. In contrast to skin epithelium, all oral mucosal regions are characterized by high vascularization and permeability and indistinguishable papillary and reticular dermis. The high permeability of oral mucosa results in frequent contact of both potentially harmful and harmless antigens with local immune cells. Local immune cells must ensure continuous uptake of antigens, while preventing entry of harmful pathogens. Simultaneously, they need to avoid immune reactions towards harmless foreign substances or particles such as nutrients. Even though great scientific progress has been made in elucidating tolerogenic mechanisms in the intestinal mucosa of the gut within recent years, only little is known about the immune system of the oral mucosa, which represents much more than a simple entry port to the GIT. In light of the more than 500 different bacterial species comprising commensal and pathogenic

Glossary

Adjuvants: substances added to allergens to improve the desired immune response because of their capability of redirecting Th2 prone immune responses into modified, more Th1 dominated immune responses.

Follicular dendritic cells: part of lymphoid follicles in secondary lymphoid tissues. They are not of hematopoietic origin but resemble dendritic cells and play a role in B cell maturation.

M cells: epithelial cells that sample antigens from the gastrointestinal lumen to antigen presenting cells and lymphocytes at the basolateral site.

Mucosa associated lymphoid tissue (MALT): the MALT represents inductive sites for T and B cells in the gut mucosa comprising B cell follicles and M cell containing lymphoid epithelium, which pass the uptaken antigens to antigen presenting cells including dendritic cells, macrophages (MC), B cells and follicular dendritic cells (FDC). Memory B and T cells migrate by way of the blood to effector sites in the gut.

Oral mucosal tolerance: refers to tolerogenic mechanisms that are induced by contact of the antigens and adjuvants to the oral mucosa but not lower gastrointestinal tract.

Oral vaccines: boost immune responses after being swallowed and resorbed by the gut mucosa.

Sublingual immunotherapy (SLIT): refers to the application of allergen solutions or tablets to the sublingual mucosa. The sublingual region is easily accessible and allows maintenance of allergen solutions or tablets for a defined time without premature swallowing and degradation of proteins. Aim of sublingual immunotherapy is the induction of allergen specific tolerance.

T regs: regulatory T cells that express CD4⁺CD25⁺ and Foxp3 and produce TGF- β and IL-10.

Th3: suppress immune responses and produce mainly TGF- β .

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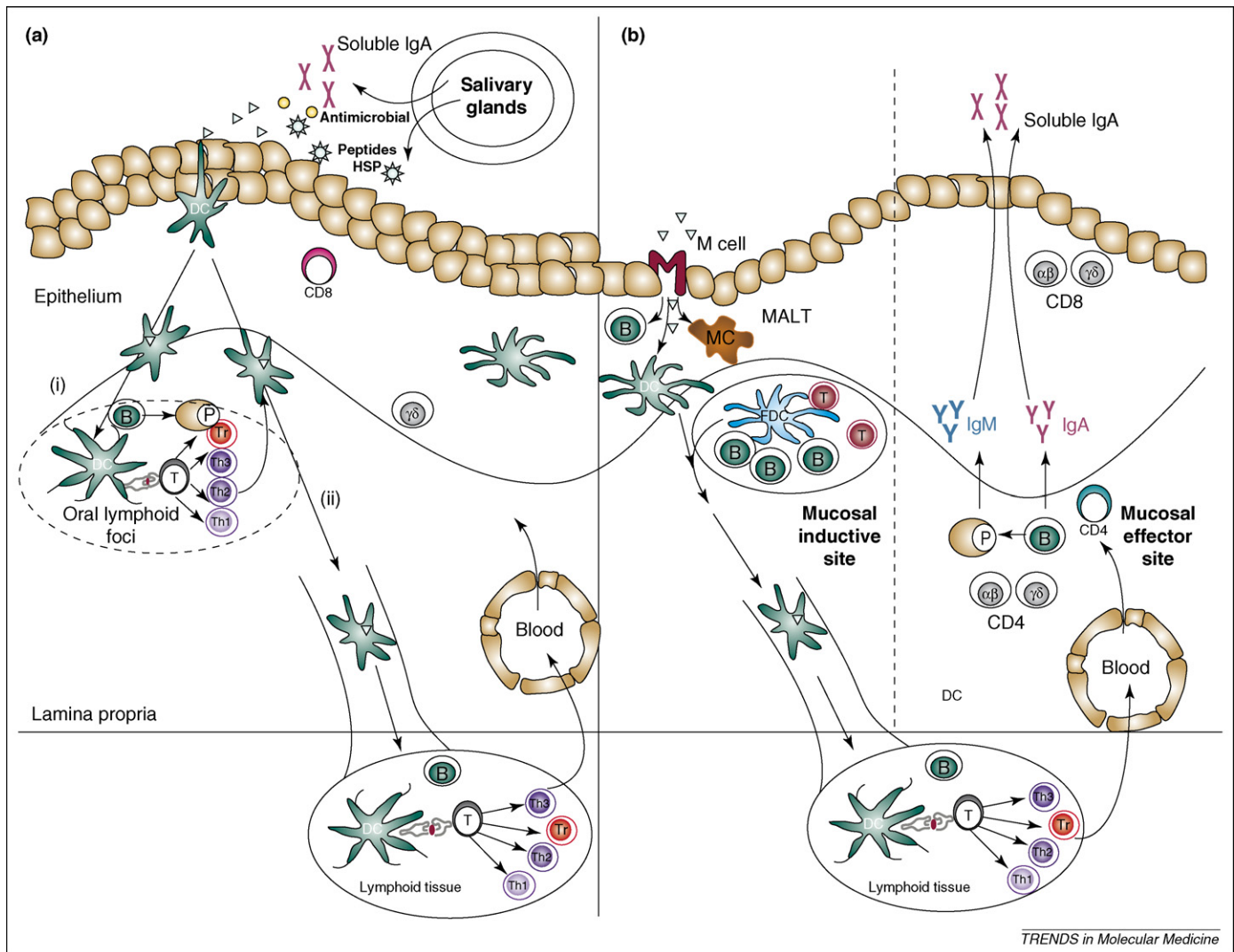


Figure 1. Inductive and effector sites in the oral mucosa versus the gut mucosa. (a) Because classical mucosa associated lymphoid tissue (MALT), defined as the inductive site of the immune response, is absent from the oral mucosa, it has been hypothesized that dendritic cells (DCs) in the epithelium take up antigens (represented by light blue triangles), mature partially and migrate to the basal lamina, where they present the antigens in the oral lymphoid foci to T cells to directly induce an effector response (i) or DCs migrate to regional lymphoid organs such as tonsils to prime naïve T cells and to induce effector immune responses (ii). (b) By contrast, gut mucosa inductive sites for T and B cells are comprised of MALT with B cell follicles and M cell containing lymphoid epithelium, which pass the uptaken antigens to antigen presenting cells including DCs, macrophages, B cells and follicular dendritic cells. As in the oral mucosa, DCs migrate to regional lymphoid tissue to prime naïve T cells and induce effector immune responses. Memory B and T cells migrate by way of the blood to effector sites in the gut. The distribution of $\alpha\beta$ - and $\gamma\delta$ -CD4 and CD8 positive T lymphocytes is depicted, as well as differentiation of B cells into IgA and IgM producing plasma cells. In addition, soluble IgA is generated. Abbreviations: B, B cell; DC, dendritic cell; FDC, follicular dendritic cell; HSP, heat shock protein; IgA, immunoglobulin A; MC, macrophage; P, plasma cell; T, T cell; Th1, T helper cell 1; Tr, T reg.

microbes [2] and the high frequency of contact with antigens from food proteins, we assert it is more than likely that local homeostasis in the oral mucosa is maintained by a sophisticated network of active, protolerogenic mechanisms, as described below.

Tissues of the oral versus the gut mucosa

Mucosal tissues in general harbour a specialized immune network composed of inductive and effector sites (Figure 1b). The latter include the lamina propria mucosae, the stroma of exocrine glands and surface epithelia, whereas inductive sites comprise mucosa-associated lymphoid tissue (MALT) as well as local and regional draining lymph nodes. The histological architecture of MALT is similar to the structure of lymph nodes, although MALT lacks afferent lymphatics. Antigens are captured and processed directly from the mucosal luminal side through a

specialized follicle-associated epithelium (FAE) containing so called microfold or membrane (M) cells. These cells deliver antigens to MALT antigen presenting cells (APCs), which are able to stimulate naïve B and T cells. Within effector sites of mucosal tissue, effector cells such as T cells contribute for instance to the formation of secretory immunoglobulin A (IgA) [3].

'Oral tolerance' is a term commonly used to describe tolerance induction by gut MALT [4]. By contrast, little is known about the oral mucosa, which corresponds to effector sites but lacks inductive sites represented by MALT [5]. Some researchers consider oral mucosa to be part of cranial-, oral- and nasal-associated tissue (CONALT) containing oropharyngeal and nasopharyngeal tissue as well as Waldeyer's ring and cervical lymph nodes [6,7]. Other researchers prefer the term cranial-, oral- and nasal-associated lymph nodes (CONALN) to emphasize the

absence of lymphoid structures or sampling of antigens directly from mucosal surfaces through M cells and to acknowledge the induction of immune responses within local and regional lymph nodes [3]. However, the term 'oral mucosal tolerance' is meant to denote tolerance induced within the oral cavity, and several key mechanisms have been discovered that both initiate and perpetuate tolerance. These take place concomitantly within the mucosal epithelium, the oral lamina propria mucosa and the salivary glands (Figure 1a). The role of organized lymphoid tissue and regional lymph nodes in the induction of oral mucosal tolerance is unclear. Although it is unlikely that an association between tonsillectomy and hypersensitivity to bacteria or food allergens exists, a clear connection between the absence or presence of draining lymphoid structures and tolerance induction by way of the nasal mucosa in murine models has been observed [8].

Comparing the nature of enzymes in the oral mucosa with the environment of the gut, antigens (e.g. those derived from food particles) must pass through gastric digestion, which causes cleavage by gastric acid or pepsin in the GIT, whereas food particles come into contact with α -amylase and lingual lipase in the oral cavity [9].

Cell type differences between oral and gut mucosa

Mucosal dendritic cells (DCs) are the most important arbiters of mucosal tolerance because they have to meet conflicting interests: on the one hand, they need to warrant an effective defense against the invasion of harmful pathogens; while on the other hand they must limit immune reactions in such a way that, even after antigen contact, health benefits conferred by commensal bacteria or by the uptake of food are not counteracted by an uncontrolled activation of the host's immune system. Resident DCs can be found throughout the entire oral mucosal epithelium and are composed of myeloid DCs from the Langerhans cell (LC) subtype expressing CD1a and the LC specific lectin Langerin (CD207). Only in respect to expression of costimulatory molecules, such as B7.1 (CD80) and B7.2 (CD86), and other myeloid markers, such as CD11b, do the oral mucosal DCs resemble DCs in gut MALT [10–12].

Murine DCs of the lamina propria, the Peyer's patches (PP) and gut mucosa are characterized by the expression of the CX₃CR1 (receptor for the chemokine fractalkine) and are derived from CX₃CR1^{high} Gr1[−] monocytes, which migrate spontaneously into noninflamed tissue [13]. Murine DCs of the subepithelial region of the PP are CD11c⁺, CD11b⁺, CD8a[−] and CCR6⁺, whereas DCs in the mesenteric lymph or T cell zones are CD11c⁺, CD8a⁺ and CCR7⁺. Double negative CD11b[−]/CD8a[−] murine DCs can be found at both of the latter sides and inside the epithelium.

Although the oral cavity comprises a relatively small surface area, numbers of resident CD1a⁺ DCs vary depending on the oral mucosal region. The largest numbers of CD1a⁺ DCs are located in the vestibulum, bucca, hard palatum and lingua, whereas fewer numbers of CD1a⁺ DCs reside in the gingiva and sublingual region [14]. Density of DCs and dendritic morphology of DCs is related to age and decreases in older age groups [15], whereas the number of DCs and their maturation stage increases at inflammatory sites [16]. Furthermore, the diversity of the

numbers of cells might result from a different thickness of mucosal epithelium as suggested by an older study [17], but also points to regions with higher antigen frequency and altered balance between newly recruited and emigrated DCs. The latter assumption is supported by the observation that DCs are rapidly recruited into buccal epithelial tissue after adjuvant application [18]. In contrast to nasal mucosal epithelium, plasmacytoid DCs (pDCs) have not been found within noninflamed oral mucosa, whereas under certain pathological conditions, such as oral lichen planus, the number of pDCs in the mucosa increases [19,20].

Induction of tolerance: definition of oral mucosal tolerance

In light of the large number of food antigens and antigens from commensal bacteria of the oral microflora, tolerance induction in the oral mucosa represents an active process that includes delayed type hypersensitivity and antibody formation. Oral mucosal tolerance emerges actively from complex regulatory mechanisms. These include: deletion of T cells, which results from the induction of T cell apoptosis; anergy as the outcome of functional inactivation of T cells; active inhibition by co-inhibitory receptors; as well as the development of antigen specific T cells that selectively suppress the stimulation and activation of the immune system by specific antigens.

A classical example of tolerance induced by way of the oral mucosa is the suppression of T cell mediated immune reactions against the contact allergen nickel, induced by frequent contact with nickel-releasing substances in the oral mucosa at an early age in individuals with oral braces [21]. In contrast to physiologic tolerance, tolerance induced by sublingual allergen specific immunotherapy elicits allergen specific tolerance in an individual who is already sensitized against this allergen, mirrored by clinical manifest allergic reactions after allergen contact, allergen specific IgE and other immunological markers.

Oral mucosal tolerance: the role of DCs

Not only the type of antigen, but also the dose and combination with other components, frequency of antigen contact as well as the immune status of the microenvironment determines the outcome of mucosal tolerance [22]. Oral mucosal DCs are equipped with a specific receptor repertoire and sense the environment for invading pathogens to induce an effective defense. Apparently, even allergens belong to the repertoire of antigens that can be taken up by oral mucosal DCs, because these cells bear high amounts of IgE bound to the high affinity receptor for IgE (Fc ϵ RI) on their cell surface [11,19]. Interestingly, Fc ϵ RI cross-linking on DCs results in induction of both proinflammatory and antiinflammatory mediators such as IL-10 [23] or indoleamine 2,3-dioxygenase (IDO) [24], which are crucially involved in tolerance induction and silencing of T cell responses.

Besides Fc ϵ RI, oral mucosal LCs express high and low affinity receptors for IgG [11]. It has been reported that signalling by way of IgG receptor CD32B, which contains an immunoreceptor tyrosine inhibitory motif, is necessary for the induction of antigen-specific regulatory T cells in

the nasal mucosa in a murine model [25]. Therefore, tolerance induction by way of FcεRI, CD32B or both on mucosal DCs might represent crucial mechanisms to sustain immunobalance within oral mucosal tissue *in vivo*. However, whether this is also of relevance in the human system awaits further evaluation because murine and human CD32B differ structurally and functionally [26].

Moreover, mucosal DCs express Toll-like receptor (TLR)2 and TLR4 [27]. As an example, TLR2 and TLR4 are downregulated from the surface of antigen presenting cells after repetitive stimulation with lipopolysaccharides (LPS) from *Porphyromonas gingivalis* *in vitro* [28]. Concomitantly, production of proinflammatory cytokines in response to these stimuli decreases [28]. *In vivo*, this mechanism might be partially responsible for 'endotoxin tolerance', which is suggested to develop as an unspecific regulatory mechanism to avoid uncontrolled cell activation and inflammation despite chronic bacterial colonization of the oral mucosa. In addition, other components of the bacterial cell wall, such as peptidoglycans, upregulate peptidoglycan recognition proteins on oral mucosal epithelial cells to initiate host defence against bacteria, which does not include an inflammatory reaction [29].

Expression of LPS receptor CD14, TLR2 and TLR4 by LCs within the noninflamed oral mucosa might be crucial for the initiation of counter-regulatory, tolerogenic mechanisms [27]. Regarding this point it has been shown that activation of TLR4 on *ex vivo* isolated oral mucosal DCs induced upregulation of immunosuppressive interleukin (IL)-10 production, which in turn is required for the induction of regulatory T lymphocytes [30]. Indeed, upon TLR4 ligation, oral LCs induce forkhead box protein (Foxp)3- and IL-10-expressing as well as transforming growth factor (TGF)-β-producing regulatory T lymphocytes, which are key players in oral mucosal tolerance (Figure 2) [27]. Antigen presenting cells that reside in the mucosa are kept in an immature state in the presence of commensals, whereas antigen presenting cells newly recruited to the mucosa in case of invasion of pathogenic organisms induce an active immune response [31]. Therefore another hypothesis regarding how immunotolerance might be maintained in the oral mucosa is similar to that in the gut, programming of DCs by their microenvironment determines whether tolerance or an active immune response is induced [31]. Based on this concept, 'missing recruitment of proinflammatory cells' is to some degree able to prohibit overactivation of the mucosal immune system.

It is assumed that antigen presentation by DCs takes part mainly in regional draining lymph nodes [22]. However, under certain pathologic conditions such as chronic periodontitis, DC-, B lymphocyte- and T lymphocyte-rich infiltrates called 'oral lymphoid foci' are detectable within the gingival lamina propria, which suggests that local antigen presentation, apart from antigen presentation in MALT and regional draining lymph nodes [7], might play an important role in local tolerance induction (Figure 1).

Impact of salivary glands

An important part of maintenance of a state of tolerance as well as induction of specific tolerance is the release of

secretory IgA produced by plasma cells within the salivary glands such as the parotid and submandibular glands, as well as the numerous minor salivary glands distributed throughout the entire oral mucosa that produce over 1000 ml saliva per day [32]. TGF-β, IL-4, IL-10 and some CD4⁺ T cells support the switch of the constant region of the IgA heavy chain from Cμ to Cα to promote B cell commitment to IgA [33], whereas IL-5 or IL-6 support the generation of IgA-producing plasma cells from B cells [33]. IgA is the most prominent antibody class that lines the oral mucosal surfaces and represents a kind of fishing rod that catches antigens to circumvent attachment of foreign pathogens to the mucosal surface at a very early stage. This defense strategy is called 'immune exclusion' [34] (Figure 2) and is primarily responsible for the prevention of colonization of the mucosa by microorganisms. Moreover, IgA is capable of removing immune complexes and neutralizing intracellular viruses and products released by bacteria, such as enzymes or toxins, by way of opsonization (Figure 2). Besides IgA, saliva contains other protective components that belong to the innate immune system, such as antimicrobial peptides [35], or cytoprotective extracellular chaperones, such as Hsp70 [36], that are involved in host defense (Figure 2).

The contribution of T cell responses to oral mucosal tolerance

T cell responses can be limited at different levels, such as directly after antigen contact by apoptosis or later during activation and proliferation. This can take place on the level of DCs by transfer of less prostimulatory signals, more co-inhibitory signals, or both, as well as release of cytokines, which leads to incomplete or dampened T cell activation. Constitutively higher expression of inhibitory B7-H molecules on the surface of oral mucosal LCs in comparison with LCs in the epidermis of the skin and further upregulation after microbial stimulation might contribute to the immunosilencing functions of oral LCs (Figure 2) [27]. However, it is unclear how long tolerance is maintained in the oral mucosa and whether some type of memory T regulatory cells exist at this site as well as systemically. On the level of T cells, CD4⁺ T cells seem to be important for the perpetuation of oral mucosal tolerance, whereas CD8⁺ T cells are less important [22]. It is believed that in particular the γδ T cell subtype plays an important role in mucosal tissue homeostasis in the murine model. These cells migrate from the thymus to epithelial tissue and are activated in response to stress or tissue damage [37].

Clinical applications: immunotherapeutic approaches that target the oral mucosa

Most of the studies that have highlighted the mechanisms of 'oral tolerance' are actually concerned with the oral application of a vaccine that then has to reach the lower gastrointestinal mucosa to induce immunoprotective mechanisms. By contrast, sublingual administration of vaccines to prevent chronic infectious diseases [38] and of allergens as in SLIT is independent from swallowing the allergen and from any contact of the allergen with the mucosa of the lower gastrointestinal tract [39].

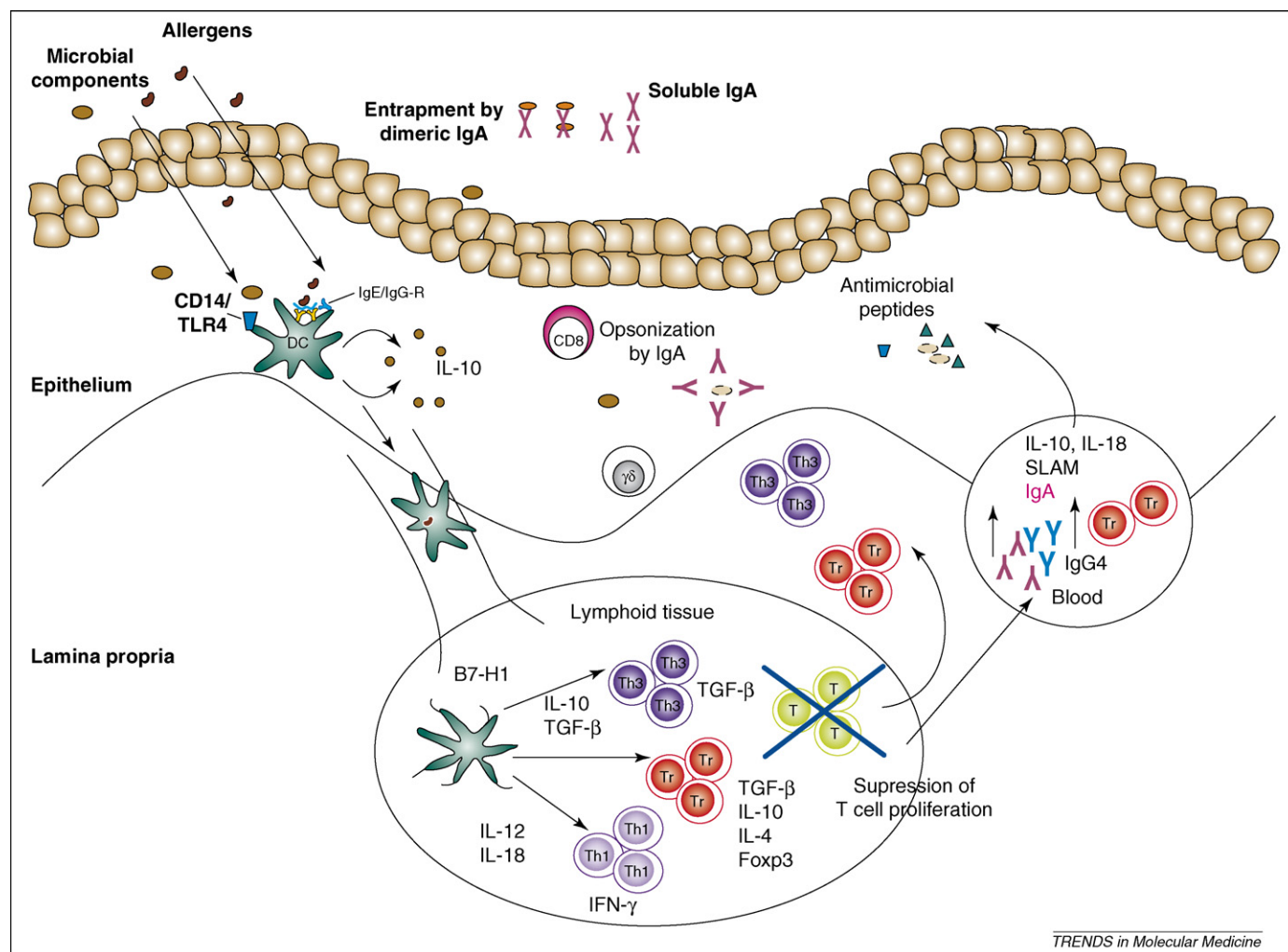


Figure 2. Inductive and regulatory protolerogenic mechanisms in the oral mucosa. The schematic depicts normal physiological tolerance induction after contact with microbial stimuli and allergens and tolerance induction during sublingual immunotherapy (SLIT). Tolerance toward microbial antigens is induced via entrapment or opsonization of microbial components by IgA, innate immune responses mediated by antimicrobial peptides released within the oral mucosa and stimulation of pattern recognition receptors on antigen presenting cells. Allergens applied during sublingual immunotherapy (SLIT) are most likely to be taken up by the IgE receptor bearing antigen-specific dendritic cells (DCs). These allergens might co-activate IgE and IgG receptors and thereby induce stimulatory and inhibitory signals simultaneously. In addition, co-stimulation of pattern recognition receptors, such as Toll-like receptor (TLR)4 and CD14, on oral mucosal DCs by microbial components or adjuvants used in the context of SLIT could increase the induction of tolerogenic mechanisms in the oral mucosa. DCs activated by allergens or microbial antigens release IL-10, migrate to the lymphoid tissue and upregulate coinhibitory molecules such as B7-H1 on their cell surface. Within the lymphoid tissue they might induce TGF- β producing T cells (Th3), interferon- γ producing T cells (Th1) and T cells with characteristics of regulatory T cells (Tr), causing these cells to produce IL-4, IL-10 and TGF- β and to express forkhead box protein (Foxp)3. Furthermore, these DCs are capable of suppressing the proliferation of T cells. Enhancement of Foxp3⁺CD4⁺CD25⁺ Tr cells and IL-10 in the peripheral blood, as well as enhancement of IL-18 and the signalling lymphocytic activation molecule (SLAM) in peripheral blood mononuclear cells (PBMCs), are changes observable in the peripheral blood during SLIT that might indicate the induction of tolerance. Abbreviations: CD14/TLR4, CD14 associated with TLR4; DC, dendritic cell; Foxp3, forkhead box protein 3; IgA, immunoglobulin A; IgG-R, immunoglobulin G receptor; SLAM, signalling lymphocytic activation molecule; TGF- β , transforming growth factor β ; Th1, T helper cell 1; Th3, T helper cell 3 (TGF- β producing regulatory T cell); TLR, Toll-like receptor; Tr, T reg (CD4⁺CD25⁺Foxp3⁺ regulatory T cell).

Targeting the tolerogenic properties of the oral mucosa in a noninvasive way represents a promising strategy to induce wanted immunomodulation and protective as well as tolerogenic immune responses to a certain antigen. Furthermore, the mucosal route is a physiologic way for antigen application because most infections and environmental allergens are taken up by way of this organ. Protection throughout the body, not limited to the application site of the antigens, is the major goal of oral mucosal immunotherapeutic strategies [34].

The main problems with antigens applied to the oral mucosa are that the antigens need to be protected from enzymatic digestion and from excessive dilution by saliva. In addition, there are problems with the low adhesion of antigens to the mucosa. This results in uncontrollable

application sites and application times. Therefore, relatively high amounts of antigens are required, which is very cost intensive. A yet unaddressed question is the optimal location for antigen application. Depending on the expression patterns of the structures targeted by the antigens, the optimal application site most likely profoundly varies. As an example, although allergen solutions or tablets are currently applied to the sublingual region, a recent study revealed the highest expression of the high affinity receptor for IgE on LCs within the vestibulum [14]. By contrast, a relatively high number of mast cells was detected in the sublingual region in particular within the sublingual glands, which might explain swelling of the sublingual caruncle observed as one of the most frequent unwanted side effects of SLIT. In view of these

data, the application sites for SLIT should be carefully reconsidered.

Mechanisms induced during SLIT

The sublingual region is easily accessible and allows maintenance of allergen solutions or tablets for a defined time without premature swallowing and degradation of proteins. Further on, the thinner epithelium allows better resorption and uptake of allergens by DCs.

For some years now, SLIT has represented an alternative to classical subcutaneous immunotherapy for patients with allergic rhinitis and mild asthma [40]. Mechanistic data about the exact mode of action of SLIT are limited, but the underlying mechanisms are most likely very similar to the mechanisms assumed to be responsible for the effect of its subcutaneous variant. We believe that it is more than likely that IgE receptor-bearing DCs in the oral mucosa take up allergens from allergen solutions and tablets and induce T cell responses. In contrast to subcutaneous allergen specific immunotherapy, effects observable locally at mucosal sites predominate during SLIT. For instance the amount of allergen-specific IgE in the nasal mucosa decreases during SLIT [41]. Mice sensitized against the timothy grass (*Phleum pratense*) allergen and treated with SLIT displayed reduced sneezing and reduced eosinophil recruitment to the nasal mucosa, as well as reduced allergen specific IgE in the nasopharyngeal lavage fluid when challenged intranasally afterwards [42]. An increase of serum IgG4 and IgA, a reduced number of inflammatory cells infiltrating target organs, as well as a reduction of eosinophilic cationic protein and a very heterogeneous influence on T cells in the peripheral blood in terms of T cell suppression occur with SLIT [39,40,43,44]. Other groups reported an enhancement of Foxp3⁺CD4⁺CD25⁺ T regulatory cells (Treg) and IL-10 in the peripheral blood, as well as enhancement of IL-18 and the signalling lymphocytic activation molecule (SLAM) in peripheral blood mononuclear cells (PBMCs) after SLIT, which suggests a downregulation of tolerance induction as well as Th2 immune responses [45,46]. In one study T cell proliferation decreased, whereas levels of IgA in serum and bronchoalveolar and nasal lavage fluid increased after SLIT [47]. Intraoral administration of a T cell epitope peptide in sensitized mice was more effective in tolerance induction than a T cell epitope administered to the gastrointestinal mucosa, which implies that tolerance induction by way of the oral mucosa is superior to tolerance induction by way of the gut mucosa [48]. Concerning clinical studies, a meta analysis of 22 randomized clinical studies that included a total of 979 adult patients [49] as well as a meta analysis that covered ten studies conducted in a total of 577 children [50] revealed that SLIT represents an effective treatment alternative to the subcutaneous route in the clinical practice. Aeroallergens such as birch or grass pollen have been used in most of the studies but there are also first approaches with other allergen types such as food allergens [51].

Future directions of immunotherapeutic approaches that target the oral mucosa

Various vector systems are used in immunotherapeutic approaches to reduce the amount of antigen and to

optimize the delivery of the antigen to target cells. In addition, substances capable of redirecting Th2 prone immune responses into modified, more Th1 dominated immune responses are used as adjuvants to provide co-signals to T cells. Presumably, even substances administered to the oral mucosa will be supported by these vector systems and adjuvants in the near future. Most of these tools target innate immunity receptors such as TLRs. In this context, mimicking the physiologic way of tolerance induction toward bacterial products in the oral mucosa by addition of TLR ligands, such as monophosphoryl lipid A (MPL) or CpG, to the vaccine represents a very successful strategy [14,27,52]. Data from human oral DCs stimulated with MPL *in vitro* hold promise that the combination of allergens with TLR ligands might increase the efficacy of SLIT [14,27].

Bacterial toxins are able to enter the cytosol and are therefore useful adjuvants to deliver antigens to major histocompatibility pathways [53]. In a mouse model, conjugation of an antigen to cholera toxin B as adjuvant showed higher efficacy than antigen alone administered to the sublingual mucosa, in terms of suppression of T cell proliferation as well as increased TGF- β serum levels and antigen specific Foxp3 expressing regulatory T cells [54]. However, the toxicity sometimes limits the application of bacterial toxins in the human system, so detoxified or nontoxic variants have been generated within the last years to circumvent this problem [53].

In the context of adjuvants applicable to mucosal surfaces, nonpathogenic food-grade bacteria such as lactic acid bacteria [53] might represent a more physiologic alternative. Co-application of allergens with lactic acid bacteria to the mucosa in a murine model induced increased levels of allergen-specific IgG₂ in the sera as well as higher Interferon- γ production by Th1 cells. Sublingual application of 1,25-dihydroxyvitamin D3, dexamethason or *Lactobacillus plantarum* induced IL-10 production by DCs and enhanced SLIT efficacy in mice [55].

Furthermore, approaches using virus like particles and plasmid DNA [53] represent other strategies that follow the physiologic ways of pathogen transmission. Plasmid-DNA lipid complexes applied intranasally have been shown to induce effective immunity against pathogens transferred by way of mucosal surfaces and hold promise of application to the oral mucosa in the future [56]. There are also some recent approaches aimed to optimize mucosal adhesion of antigens with the help of ovalbumin combined with maltodextrin to enhance mucosal adhesion. These studies revealed reduced airway hyperresponsiveness, IL-5 and IgE production in a mouse asthma model [57]. In addition, the usage of mucoadhesive additives might allow for the reduction of the allergen dosage, owing to longer contact times with the mucosa [57]. This is of practical importance, because only a very small fraction of the allergen remains detectable locally in the mucosa up to 20 h after sublingual administration [58].

Also, agents that increase the permeability of the oral mucosa, which differs depending on the region or grade of keratinization, have been developed to enhance antigen uptake [59].

Box 1. Existing knowledge and outstanding questions on oral mucosal tolerance

Existing knowledge

- Dendritic cells play a central role in mucosal tolerance induction and are capable of taking up antigens in the oral mucosa and of acquiring tolerogenic properties mirrored by production of IL-10 and upregulation of co-inhibitory molecules such as B7-H1.
- Immune exclusion takes place by way of IgA.
- Induction of TGF- β -producing Th3 cells occurs with oral mucosal tolerance.
- High amount of IL-10 and TGF- β -producing T regs occurs during oral mucosal tolerance induction.
- There is an increase of IgG4, IgA, IL-10, IL-18 and SLAM in the peripheral blood during oral mucosal tolerance induction.
- There is lower proliferation of T cells.

Outstanding questions

- Which location is optimal for antigen application within the oral mucosa?
- How long is tolerance maintained in the oral mucosa?
- Do local or systemic protolerogenic mechanisms predominate?
- Which roles do oral lymphoid tissue and oral lymphoid foci play in tolerance induction?
- Which adjuvants are most promising in targeting the protolerogenic potential of the oral mucosa?

Concluding remarks

Because oral mucosa tissue is easily accessible, it represents not only an attractive application site for immunotherapies, but also an interesting field for basic research. Most scientific effort so far has been directed towards investigating pathologic mechanisms in inflammatory diseases of the oral mucosa, but not much is known about physiological pathways of tolerance induction at this site. Therefore, upcoming investigations should focus on achieving a better understanding of the numerous protolerogenic mechanisms of this mucosal tissue to improve our knowledge about natural mechanisms of tolerance induction, which might be successfully targeted in immunotherapeutic approaches of the future (Box 1).

Disclosure statement

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