Oral Cavity-Associated Immune System: What is New?

Mirta Ana Valentich[1], Thamara Analía Cafaro[2] and Horacio Marcelo Serra[*,2]

[1]*Institute of Cell Biology and Department of Cell Biology, Histology and Embryology, Faculty of Medical Sciences, National University of Cordoba, Córdoba, Argentina*

[2]*Immunology, Department of Clinical Biochemistry-CIBICI (CONICET), Faculty of Chemical Sciences, National University of Cordoba, Córdoba, Argentina*

Abstract: Although several excellent articles have reviewed different aspects of mucosal associated lymphoid tissues (MALT), there is not enough information about the oral cavity immune response. In this manuscript we highlight advances in the recent knowledge about this topic focusing on the anatomy/histology of the oral cavity, the associated immunological structures and the role of dendritic cells and Toll signalling, trying to envisage what happen when microorganisms or soluble antigens enter in the mouth. Oral mucosa has received attention in the last decade because it offers excellent accessibility and avoids degradation of proteins and peptides. Although the oral mucosa is continuous with the remaining gastrointestinal tract, structurally the oral mucosa has more in common with skin than with the gastrointestinal tract.

Keywords: Oral cavity, dendritic cells, toll like receptors.

1. THE ANATOMY AND HISTOLOGY OF THE ORAL CAVITY

The mouth or oral cavity is the opening through which the food is ingested. It is an anatomic space defined by hard and soft tissue structures that change with normal physiologic function such as speech, swallow, and respiration. The oral cavity is delimited anteriorly by the lips, laterally by the cheeks, superiorly by the hard and soft palate, and inferiorly by the tongue and the sheet of muscles attaching to the inner side of the mandible. The posterior wall is a hole called isthmus of the fauces, which connects the oral cavity with the pharyngeal cavity.

The mouth is divided into two sections: the vestibule (area between the cheeks and the teeth in the dental arches) and the oral cavity proper (Fig. **1**). The oral cavity and vestibule communicate with each other through the spaces between teeth and the retro molar space. The mouth includes the upper and lower dentition, the tongue, salivary glands (mayor salivary glands as well as numerous small minor salivary glands), and the mucosal tissue covering the hard palate, which bear the rugae.

The oral cavity and vestibule are entirely lined by mucous membranes that continue along the remaining gastrointestinal tract. The oral mucosa consists of connective tissue (the lamina propia) covered by a stratified epithelium either keratinized or no-keratinized. Anatomically, the oral mucosa is located between the mucosa lining of the gastrointestinal tract and the skin of the face, displaying properties of both tissues. In comparison to the mucosa of the gastrointestinal tract, the oral mucosa has no muscularis mucosae (typical thin layer of smooth muscle located outside the lamina propria mucosae and separating it from the submucosa found in most parts of the gastrointestinal trac) [1]. It has been shown that the oral mucosa is architecturally similar to the stratified squamous epithelium of the penile and glandular urethra, making the most versatile tissue for urethral replacement [2-4]. Alveolar mucosa is the mucosa membrane covering the alveolar process that extends from the mucogingival junction to the vestibular epithelium and from the mandible to the sublingual sulcus

The highly resilient buccal mucosa is frequently exposed to different forces such as compression and stretching. Different regions of the buccal mucosa have different functions, and support various degrees and types of stress during speech, mastication and facial expression. Thus, the structure of the oral mucosa varies in the thickness and type of epithelium, the degree of keratinization, the complexity of the epithelial-connective tissue interface, the composition of the lamina propria, and the presence or absence of the submucosal layer.

The lamina propia-oral epithelium interface shows extensive projections of connective tissue into the epithelial layer, which increase the surface interface's area and the oral mucosa's ability to resist overlying forces [5]. The oral mucosa thickness is directly associated with gender and indirectly with age [6]. Also, the thickness of the epithelium does not always depend on the degree of keratinization and thus, nonkeratinized mucosa is significantly thicker than keratinized mucosa [7, 8]. In the mouth we can distinguish three types of mucosa: the Simple Coating or linning mucosa, the masticatory mucosa, and the specialized mucosa:

- *Simple Coating or linning mucosa (60%)*: The lining mucosa covers the lips, cheeks, alveolar mucosa, floor of mouth, ventral surface of the tongue and soft palate. It is more mobile and compliant than the masticatory mucosa, and its connective tissue is more elastic and flexible because it is not subject to high

*Address correspondence to this author at the Immunology, Department of Clinical Biochemistry, CIBICI (CONICET), Faculty of Chemical Sciences, National University of Cordoba, Haya de la Torre y Medina Allende, 5000 Cordoba, Argentina; Tel: 351 4344973; Fax 351 4333048;
E-mail: hserra@fcq.unc.edu.ar

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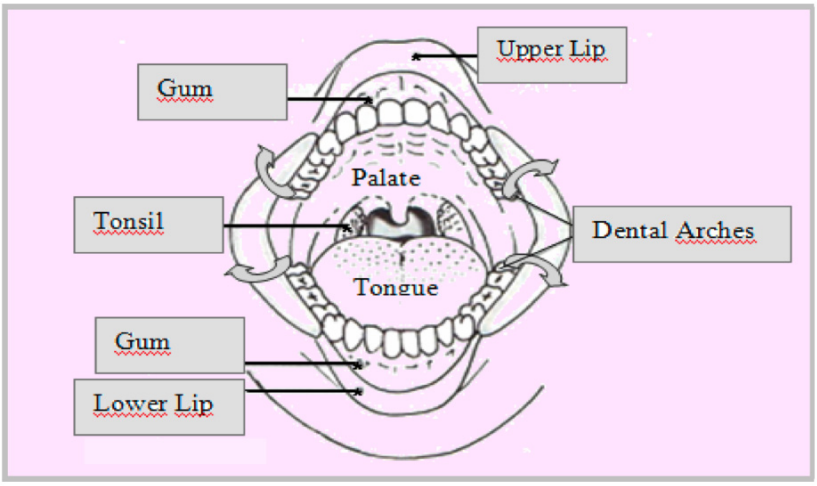

Fig. (1). Schematic representation of oral cavity.

levels of friction. The epithelium is stratified no-keratinized and has a loose lamina propria with collagen fibers in the network. In general, the lining mucosa has a submucosa [9].

- *Masticatory (25%):* The Masticatory mucosa covers the hard palate and the gum or gingiva which undergoes high compression and friction. It is covered by a keratinized epithelium tightly attached to the underlying bone tissue by a collagenous connective tissue or lamina propia.
- *Specialized (15%):* Specialized mucosa is found in two areas: the taste of the back mucosa of the tongue and the red area corresponding to the lips that forms the transition between the skin and oral mucosa [1].

Table **1** summarizes the general histological characteristics of the different areas of the oral cavity.

The oral epithelium lining the mouth is formed by different layers of cells (keratinocytes) conforming a keratinized or non keratinized or para-keratinized epithelium. A keratinized epithelium consists of the following layers: stratum basale or germinative, Stratum spinosum, stratum granulosum and stratum keratinized. Unlike the epithelium covering the skin (epidermis), the oral mucosa has not stratum lucidum. The various layers or strata of the oral epithelium represent a progressive maturation process. Thus,

Table 1. Regional Variations of Oral Mucosa

Mucosa Type	Region	Epithelium Thickness	Epithelium Keratinization	Lamina Propria Papillae	Lamina Propria Fibber Types	Sub Mucosa Density	Sub Mucosa Fixing Type
Linning or Simple Coating	Floor of mouth	Thin	No keratinized	Short, wide	Collagen, Elastic	Loose	Loosely attached to muscle
	Alveolar mucosa		Keratinized	Short or absent	Elastic	Loose	Loosely attached to periostio
	Ventral surface of the tongue		No keratinized	Short, numerous	Collagen, Elastic	Non-defined layer, coupled with the underlying muscle	
	Labial or buccal mucosa	Thick	Keratinized	Short, irregular	Collagen, Elastic	Dense	Strongly attached to underling muscle
	Soft palate		No keratinized	Short	Elastic	with loose binding to underlying tissues	
Masticatory	Hard palate	Thick	Keratinized	Long	On the lateral side of the hard palate the lamina propria contains dense collagen and on the middle side binds to the periosteum because it has not submucosa		
	Gingiva		Keratinized and parakeratinized	Long, narrow	Dense collagen attached to the periosteum	Without definite sub mucosa	
Specialized	Posterior third of the dorsal tongue surface	Variable	Generally no keratinized	Short or absent	Collagen, Elastic	Layer not well defined, attached to underling muscle	
	Free edge of the lips	Thin	No keratinized	Long, narrow	Collagen, Elastic	Dense	Strongly attached to underling muscle

more superficial cells are shedding continuously and they are replaced by cells from the lower layers. The cell turnover rate varies five times faster in the grooves along the surface of the teeth that in the cheeks. Basal layer cells, which divide by mitosis, originate daughter cells that migrate to the surface maturing and adopting the characteristic appearance of each layer that they pass. Basal layer cells contain keratin 19, but when migrate they synthesize a new type of keratin. In keratinized regions the mechanically tough stratum corneum serves to dissipate shearing forces, and in keratinized, para-keratinized and non-keratinized regions lipid-based permeability barriers protect the underlying tissues against the fluid loss and the entry of potentially harmful environmental agents. A thin (100μm) non-keratinized stratified squamous epithelium covers the ventral tongue and the floor of mouth and a thick (250μm) keratinized stratified squamous epithelium covers gingiva surrounding the teeth and palate, whereas a very thick (500μm) non-keratinized stratified squamous epithelium is found on labial and buccal mucosa

Regional differences into the epithelium 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 cornified 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. Thus, the membrane coating granules are believed to be responsible for the formation of the superficial permeability barrier in stratified squamous epithelium.

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 [10]. The Fig. (**2A**) shows a non-keratinized oral epithelium with nucleated cells in the superficial cell layer).

The oral epithelium also contains melanocytes, Langerhans and Merkel cells (Fig. **2B**). Melanocytes are regularly found in normal papillary epithelium, even in the absence of clinical signs of pigmentation. The basal epithelial melanocyte contains individual pigment organelles at different stages of maturation, and rudimentary half-desmosomes are found between the melanocyte and the basal membrane. The suprabasal Langerhans cells can be identified by their specific organelles and by the lack of tonofilaments and desmosomes. The Merkel cells are characterized by a horizontal orientation in the basal epithelial layer, the development of desmosomal attachments to keratinocytes, the lack of tonofilaments, the typical Merkel cell granules and an associated axon terminal [11].

Also, a number of inflammatory cells can often be seen in the nucleated cell layers. These cells are transient, although the presence of polimorfonuclear leukocytes and mast cells is not uncommon. The association between nonkeratinocytes and keratinocytes in oral mucosa, as in the skin, could represent a subtle and finely balanced relationship in which cytokines are the controlling factors [12].

The study of both, the structure and the relative area of the different types of mucosa, is important to understand the penetration of substances across the oral lining.

2. ASSOCIATED IMMUNOLOGICAL STRUCTURES

The different mucosal surfaces in the body are protected by a network of highly specialized and organized structures known as the mucosal associated lymphoepithelial tissue (MALT). These structures include isolated lymphoid follicles, the Waldeyer's ring, salivary glands, and Peyers patches. Some MALT, such as the gut, the bronchus, and the nasopharingeal-associated lymphoepithelial tissue have been fully described but the genital, conjunctiva and oral MALT remain partly understood.

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, lymphocytes zones with antigen presenting cells (APC) but also more diffuse areas where home effectors lymphocytes. 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. The route by which antigens cross the epithelial-cell barrier is likely to dictate the type of immune response that is induced, and the generation of effectors or suppressor cells may be related to the pathway used by the antigen to gain access to the host [13-15].

The Waldeyer´s ring is comprised of the nasopharyngeal tonsil, the paired tubal tonsils, the paired palatine tonsils, and the lingual tonsil arranged in a circular orientation around the wall of the throat. The tonsils can be considered an ''evolutionary novelty'', which did not appear before mammals and not all of them have a direct equivalent to the human tonsils. As reviewed by Serra *et al.* [16] 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. The later ones possess several unique characteristics: they are not fully encapsulated; they do not possess afferent lymphatics; they are lymphoepithelial organs; and the tonsillar epithelium not only provides a protective surface cover but also invaginates and lines the tonsillar crypts. The location of the palatine tonsils enables these structures to come in direct contact with potentially harmful inhaled and ingested material that exist in their native form since digestive enzymes are not present in the oral cavity. The tonsillar epithelium has an important protective role but it also plays a critical role in antigen-sampling. For antigens reaching the oral cavity one could speculate that tonsils are important *inductive sites* and recent studies have provided evidences that innate immune

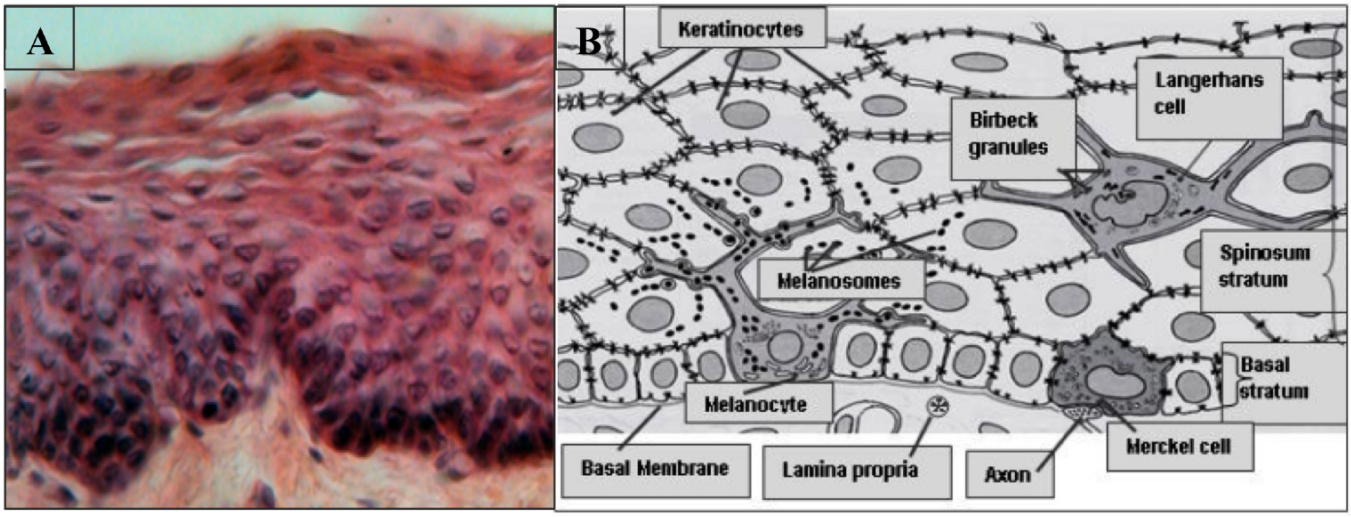

Fig. (2). A. Oral epithelium (HE, 400X); **B**. Basal and spinosum stratum of oral epithelium with typical keratinocytes and other cells as Melanocyte, Merckel and Langerhans cells (Photo (**A**) and diagram (**B**) modified of the correspondents given by Dra ME Ferraris, FO, UNC, Argentina).

mechanisms enable the tonsils to react to aggressions and also instruct the adaptive cellular immune components to elicit an appropriate response [17].

The entire oral cavity is moistening by the salivary glands secretion. There are three pairs of mayor salivary glands (parotid, submandibular and sublingual) and numerous small minor salivary glands (labial, genians, palatal and lingual) located in the submucosa. The minor salivary glands are primarily mucous secreting and are more prevalently found in the labial mucosa.

3. DENDRITIC CELLS, CENTRAL INTEGRATORS OF THE IMMUNE RESPONSE ON THE ORAL CAVITY

Dendritic cells (DC), term coined in 1973 by Ralph Steinman and Zanvil Cohn' [18], are highly efficient antigen-presenting cells that are central to the induction and regulation of most adaptive immune responses. Their specialized capacities for acquiring, processing, retaining, and finally presenting peptides on major histocompatibility complex (MHC) molecules are critical properties which account in part for their superior role in antigen presentation [19-22]. The role of these cells has been repeatedly highlighted in infectious diseases and cancer [23, 24]. Recently, there have been great insights into the origins of DC subsets [22] and their modulation by distinct cytokines from neighbouring cells [25]. In mice, as well as in human beings, they constitute a very heterogeneus cell population, in terms of phenotype and function. In human peripheral blood, there are at least two major groups ocurring: myeloid and plasmacytoid dendritic cells ($CD11c^+$ $CD123^-$ and $CD11c^-$ $CD123^+$, respectively) [26]. DC populations under steady conditions but especially under inflammatory ones, show a far bigger complexity in lymphoid and non-lymphoid organs than in blood [26-28]. DC differentiate into at least four pathways: Langerhans' cells (LC), myeloid DC, lymphoid (L) DC and plasmacytoid cells (pDC) [29]. Progenitors of DC in bone marrow migrate *via* the bloodstream and then home towards the peripheral tissues to confront invading pathogens [20-22]. In such environments, such as the skin and other mucosa, and in almost all tissues and organs including the lymphatic organs, DC constantly sense the environment for the arrival of pathogenic microorganisms, detecting either PAMPs by *pattern recognition receptors (PRRs*), including TLRs and others or self-danger-associated molecular patterns (DAMPs) by Nod-like receptors (NLRs), retinoid acid–inducible gene–1 (RIG-1)-like receptors (RLRs) and others [30]. They ingest antigens *via* several mechanisms that include phagocytosis [31] and receptor-mediated endocytosis [32]. DC internalize, process and present immunogenic peptides to various T-cell types [33, 34]. The ability of DC to initiate and orchestrate immune responses is a consequence of their localization within tissues and their specialized capacity for mobilization [35]. They have an immature phenotype in peripheral tissues, which is specialized for Ag uptake. However, upon recognition of PAMPs and/or DAMPs, DC migrate to T cell zones in secondary lymphoid organs and arrive as mature cells, where they provide the essential costimulatory molecules for activation of naive CD4+ and CD8+ T cells [21]. The response of DC to exposure to foreign Ag is part of the innate immune response, and by providing a link between Ag recognition and Ag processing for presentation to naive T cells, as well as a cross talk with other cells from the immune system, these cells form a bridge between the innate and adaptive immunity [21, 27, 30, 36-39].

Oral mucosa, exposed to large amounts of environmental antigens, food particles, commensal flora, and pathogens (bacteria, viruses, fungi and their by-products), is covered by stratified squamous epithelium that provide physical protective barriers as a first line of defense being the first dynamic interaction between microbes and the human host [40].

Buccal mucosa has two distinct types of DC: LC which reside within the epithelial layers and are properly oriented to sample the oral fluids and bacteria, with their dendrites toward the surface [41] and interstitial and submucosal DC

which are the counterpart of dermal DC in the skin and reside beneath the basement membrane of the mucosal epithelium. After capturing foreign antigens, DC migrate to immune inductive sites and initiate the adaptive immune response, but can also re-stimulate a local response in peripheral tissues, i.e., effector sites. In the case of the oral mucosa, significantly more number of LC are found in non-keratinized mucosa of the lip, vestibule, soft palate and ventral tongue than in keratinized mucosa of the hard palate and gingiva with the typical "tennis-racket" cytoplasmic organelles, named Birbeck granules, with a central linear density and a striated appearance [42]; very little is known about these inductive and effector sites. The typical oral inductive sites, contained within Waldeyer's ring, consist of oropharyngeal tissues and nasopharyngeal lymphoid tissue [43]. LC, a sentinel immunity cell lineage and a specialized subset of the bone marrow-derived DC, are located in the oral stratified squamous epithelial layer as with the skin. LC, are well situated and equipped to internalizing and processing foreign antigens that breach the oral mucosa protective layer [21]. The highest number of LC are found in non-keratinized mucosa of the soft palate, ventral tongue, lip, and vestibular mucosa, while the lowest counts are found in keratinized mucosa of the hard palate and gingival [42]. The lipid antigen-presenting molecule CD1a has been increasingly used to identify LC and immature DC in human tissues [41, 44-56].

The participation of LC/immature DC in oral mucosa diseases and more recently, dermal or intersticial and plasmacytoid DC are surmised, from different studies, in Table **2**.

Experimental murine studies suggest that buccal mucosa DC capture antigen and migrate to cervico-mandibular lymph nodes, where the antigen is presented [76]. However, the identity of the DC subsets that participate in oral mucosa immune responses or migrate to cervico-mandibular lymph nodes is not clear. CD1a+ LC infiltrate the oral/gingival epithelium, while a high number of dendritic-cell-specific ICAM-3-grabbing non integrin-positive interstitial DC infiltrate the gingival lamina propria, particularly in chronic periodontitis. Both LC and dermal or interstitial DC appear to contribute to the pool of maturing CD83+ DC in the lamina propria [75]. More recently, [77] have investigated the phenotypic and migrational properties of oral mucosal DC (OMDC) painting the mouse buccal mucosa with fluorescein isothiocyanate (FITC) and studing the expression patterns of functional molecules in FITC-bearing migrating DC within the regional lymph nodes (RLNs). They found three distinct subpopulations of OMDCs within the RLNs with different migrational kinetics: CD11c ^{hi} CD207- (F1), CD11 c $^{int/lo}$ CD207- (F2) and CD11 c $^{int/lo}$ CD207+ (F3). The F1 DC, expressed high levels of CD11b, reached the RLNs earlier (after 24 hr) but diminished immediately. The F2 DC migrated continuously to the RLNs and maintained the highest ratio of all three fractions. The F3 DC showed the highest CD205 expression levels of all three subsets and migrated slowly to the RLNs and demonstrated a late peak at 96 hr. All fractions of migrating OMDC lacked CD8a expression and expressed high levels of CD80, CD86 and major histocompatibility complex class II at high levels, suggesting that all OMDC are in a mature stage and have the potential for antigen presentation. The lack of CD207 identifies submucosal DC (F1 and F2); F1 DC are resident and F2 DC are newly recruited following FITC application. The F3 DC, which express CD207, are mucosal LC that migrate later. More studies are necessary to understand the role of DC sub-sets in the local immunity at the oral mucosa.

4. TOLL-LIKE RECEPTORS IN THE ORAL CAVITY

In this decade different studies have demonstrated that microbes can be recognized by the innate immune system

Table 2. Langerhans Cells/Dendritic Cells in Oral Mucosa Diseases

Oral Mucosal Langerhans Cells are Responsive to:	
• the accumulation of bacterial plaque (migrating into the gingival epithelium during early gingivitis)	[57]
• oral lichen planus	[58-62]
• lichenoid drug eruptions	[63]
• rhomboid median glossitis	[64]
• Verruciform xanthoma	[65]
• HIV infection	[66]
• oral squamous cell carcinoma	[67]
• oral skin grafts	[68]
• hairy leukoplakia of the tongue	[69]
Dendritic Cells Infiltrate the Lamina Propria of Oral Mucosa During:	
• oral bacterial infections, such as chronic adult periodontitis	[70]
• oral lichen planus	[71, 72]
• recurrent aphthous ulcers	[73]
• oral fibrovascular lesions	[74]
• chronic adult periodontitis (Dermal dendritic cells contribute to the CD83+ mature dendritic cell pool in the lamina propria)	[70, 75]
• oral lymphoid foci (Mature CD83+ dendritic cells form immune conjugates with CD4+ T-cells)	[75]

using different types of receptors. Toll-like receptors (TLRs) are germ line encoded, evolutionarily conserved pattern recognition receptors (PRRs) which expression has been extensively studied on the cell surfaces of macrophages and DC. They lack the sophisticated specificity and diversity of the lymphocyte receptors but are fully capable of recognizing pathogen associated molecular patterns (PAMPs). Upon ligation of TLRs, a signalling cascade is activated, which results in the initiation of innate, inflammatory, and immune defense mechanisms [78]. There are several types of TLR: TLR-1 recognizes triacyl lipopeptides, peptidoglycans and lipopeptides are mainly recognized by TLR2 and TLR-6, LPS is recognized by TLR4 and flagelin by TLR-5. Viral and/or bacterial nucleic acids are recognized by TLR-3, TLR-7, TLR-8, and TLR-9 located within the endosomal compartment [79, 80]. Other microbes' components such as desmuramylpeptides containing diaminopimelic acid and muramyldipeptide are recognized by intracellular receptors NOD1 and NOD2, respectively (Fig. **3**) [81, 82].

Since the oral epithelium constitutes the first barrier against aggression it should be endowed with innate immune receptors for microbes components. Different studies have been conducted in the last years in order to investigate if TLRs are expressed in the oral cavity. Uehara *et al.* found constitutive expression of TLR4, TLR2, NOD1 and NOD2 in normal oral epithelium. In inflamed conditions, cell surface localizations of TLR2 and TLR4 were more clearly observed than in healthy tissues. Upon stimulation with ligands for these receptors there was an enhance production of beta-defensin 2 and peptidoglycan recognition proteins (PGRPs). More over, they found that NOD1 and NOD2 agonists, in combination with TLR agonists, synergistically induced production of the antibacterial responses in the oral epithelium *via* NF-kappaB [83-85]. The TLR2-mediated immune responses in the oral cavity can also be modulated by the presence of soluble forms of TLR2 and CD14 in saliva as have been shown by Kuroishi T *et al.* [86].

The cellular expression and distribution of TLR-1 to TLR-10 have also been studied in gingival tissue samples from healthy controls and patients with periodontitis [87]. All TLRs except TLR-10 were found in the normal gingival epithelia with variable expression in the different epithelial layers for each TLR. Except for TLR-7 and TLR-8, all the

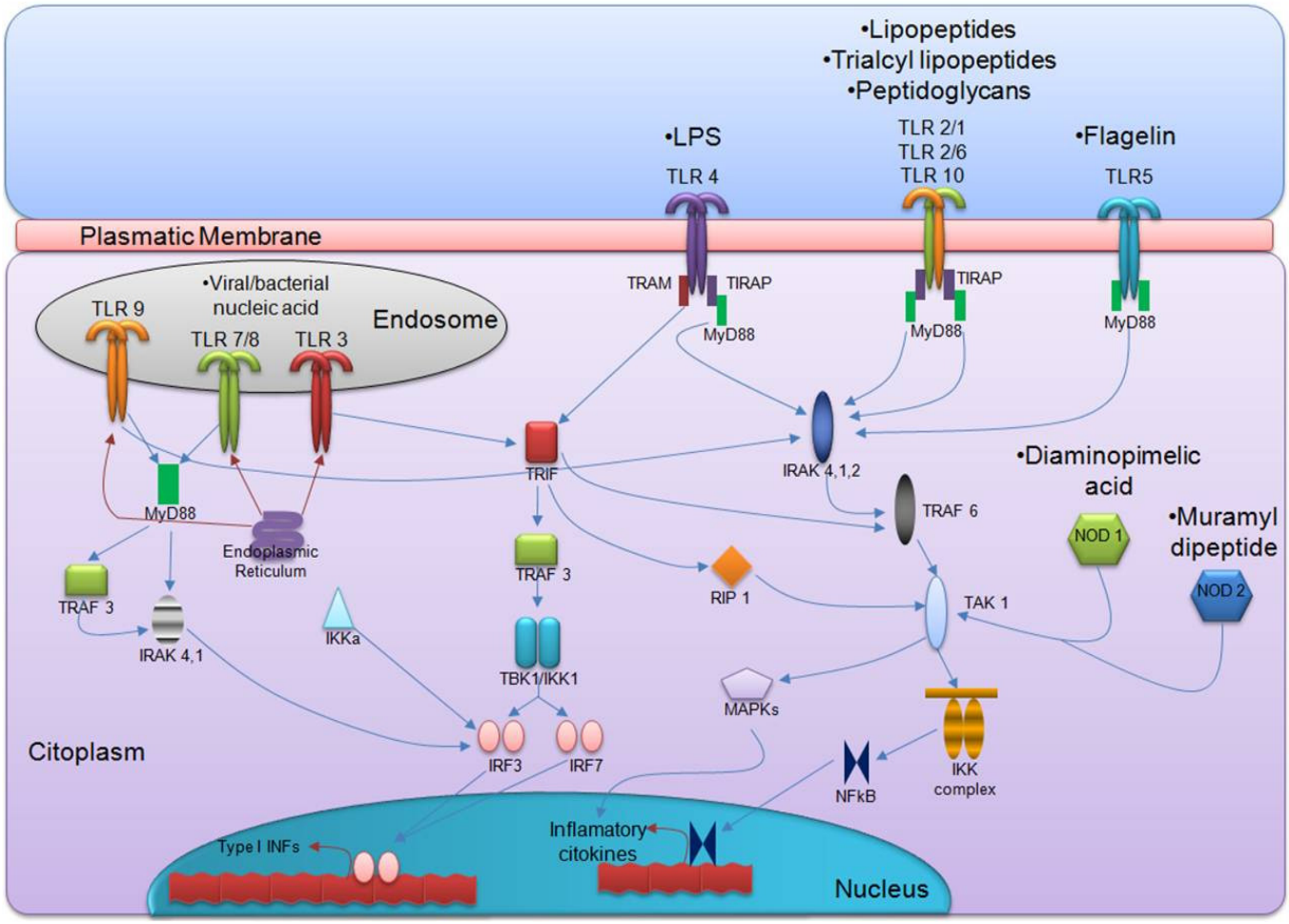

Fig. (3). Simplify drawing of Toll-like receptor (TLR) and Nod receptor (NOD) signaling pathways. Extracellular pathogen-associated molecular patterns are recognized by TLRs at the plasma membrane and endosomes, which signal through the adaptors MyD88 and Toll/interleukin-1 receptor–domain-containing adapter-inducing interferon beta (TRIF), as well as through interleukin-1 receptor–associated kinase (IRAK) proteins and tumor necrosis factor receptor–associated factor 6 (TRAF6). NOD1 and NOD2 sense Diaminopimelic acid and muramyl dipeptide, respectively, signaling pathways recruit TAK1, which mediates the activation of nuclear factor–kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), resulting in the transcriptional upregulation of proinflammatory genes.

other TLRs showed statistically significant differences between patients with periodontitis and healthy controls. In the disease samples the TLR expressing cells were most frequently found in the basal cell layer, and the frequency tapered off towards the more superficial epithelial layers. In a more recent manuscript this group of researchers studied and found that the interaction of pathogen-associated molecular patterns with TLR2 and TLR5 in gingival epithelial cells kept in cultures produced both interleukin (IL)-1 beta and TNF-alpha. The addition of IL-17 greatly enhanced the TLR ligand-induced pro-inflammatory cytokines release [88]. Similar results were obtained by Kocgozlu L *et al*. using RT-PCR analysis of human oral epithelial cells stimulated with Porphyromonas gingivalis LPS and showing that the major etiological agent of chronic periodontal diseases used Toll-like receptor 2 (TLR2) to activate the production of pro-inflammatory cytokines [89]. In a recent study Eskan MA *et al*. showed that pro-inflammatory cytokine production, including IL-6 and IL-8, was increased with LPS and concomitant S1P stimulation of human gingival epithelial cells. S1P1 and S1P3 expression was induced by adding LPS to these cells, and this elevated expression enhanced the influence of S1P in its cooperation with TLR4 to increase cytokine production [90].

Human oral epithelial cells are not only exposed to microorganisms but also to other harmful agents. Their responses to these different stimuli are critical in maintaining periodontal homeostasis. Interestingly, cigarette smoke extract has modulating effects on the innate immune responses of human gingival epithelial cells since it is capable of suppressing beta-defensin 2 and enhancing IL-8 production [30].

Gingival fibroblasts also constitutively expressed TLRs and NOD molecules, and upon stimulation with their ligands the production of pro-inflammatory cytokines, such as IL-6, IL-8, and monocyte chemoattractant protein-1, are markedly up-regulated [91]. The innate immune response observed in inflamed gingival can be amplified by histamine since human gingival fibroblasts mainly express histamine receptor H1R, and responded to histamine to produce IL-8. Stimulation of gingival fibroblasts with tumor necrosis factor-alpha, IL-1alpha, and lipopolysaccharide markedly induced IL-8 production, and the IL-8 production was synergistically augmented in the presence of histamine [92]. Treatment of human gingival fibroblasts with a low dose of interferon (IFN)-gamma in combination with TNF-alpha and IL-4 or IL-13 had synergistic effects on the production of CCL17 which is a potent chemokine for Th2 lymphocytes. More over, both CCL17 and its receptor (CCR4), were expressed in diseased periodontal tissues [93]. All these results have provided strong evidences that pattern recognition molecules present in the oral mucosa are functional and participate in bacterial clearance but might also be responsible for periodontal pathogenesis.

The role of TLRs in dental pulp, which is bounded by hard tissues, was largely unknown for many years until Mutoh N. *et al*. investigated the expression of TLR-2 and TLR-4 in experimentally inflamed pulp by quantitative real-time polymerase chain reaction and immunohistochemistry, and demonstrated that TLR-2 was mainly regulated during the early stage inflammation [94]. Hirao K. *et al.* have also shown that that TLR2, TLR4, NOD2, and NOD1 are functional receptors in human dental pulp fibroblasts since various adhesion molecules, chemokines, cytokines, prostaglandin E(2), COX-2, were up-regulated by stimulation with agonists of these receptors [95]. These and other data recently reviewed by Farges JC *et al.* [96] suggest that odontoblasts initiate immune / inflammatory events within the dental pulp in response to cariogenic bacteria. Since all TLR genes, many chemokines and chemokine receptors genes are expressed in the healthy human dental pulp there is not doubt that it is well prepared to combat pathogens entering this tissue

The present of many TLRs, type I and II IFN receptors, and their downstream signalling components has also been shown in lingual epithelium and more abundantly in the taste buds of mice. Since administration of TLR ligands activates the IFN signalling pathways it has been suggested that inflammation may disrupt normal taste transduction or cell renewal in taste buds [97].

Although several studies have demonstrated the ability of the palatine tonsils to produce pro inflammatory molecules [98, 99], it was not until recently that the expression and function of TLRs in tonsillar epithelial cell lines and primary tonsillar epithelial cells were investigated [100].

In this study, the authors presented evidences that tonsillar epithelial cells express TLR2 and TLR3 mRNA and proteins and that these TLRs were functional because upon stimulation with Pam3Cys and Poly I:C different cytokines were released.

The concerted action of all these immune and non immune agents in the oral cavity provides a multifunctional protective network that plays an important role in the homeostasis of the oral microbiota.

5. OTHER DEFENSE FACTORS THAT PROTECT ORAL TISSUES

Since the oral cavity mucosal surfaces are exposed to many species of microbes, they are equipped with different defense mechanisms. The oral cavity is flooded with saliva which is produced by the salivary glands. The secretory units of salivary glands are represented by acini or adenomeres, (which may be serous, mucous, or mixed) which discharge their secretion into the mouth through an excretory duct system. Both structures, acini and ducts, constitute the parenchyma or functional portion of the glands. The parenchyma is supported by connective tissue forming the stroma where are distributed tissue blood vessels and lymphatics. Only the major glands have a connective tissue capsule, which divide the parenchyma into lobes and lobules.

The human saliva contains several nonspecific and specific agents that apart of facilitate chewing and swallowing food, play other functions such as: lubrication, remineralisation, buffering, antimicrobial protection, cleansing and maintenance of mucosal integrity. The nonspecific defense factors include flushing, mucins, salivary glycoproteins, and peptides. The continuous flow of saliva increased by the muscular activity of the lips and tongue removes a large number of bacteria and products from teeth and mucosal surfaces into the gut and provide

continuous presence of other factors in the mouth. Among the growth factors provides by saliva is the epidermal grow factor (EGF) that plays a fundamental role in systemic and oral wound healing [101].

The major specific factors found in saliva are the dimeric secretory IgA (sIgA) and IgG antibodies. sIgA is synthesized by plasma cells (PCs) in salivary glands and is exported by the polymeric Ig receptor (pIgR). Most IgG in saliva is derived from serum although some is locally produced as well as little amount of IgM. The sIgA cooperates with lysozyme, lactoferrin, alpha-amylase, and host defense peptides (HDP) in order to provide an excellent defense against pathogens and antigens presented at mucosal surfaces. Whereas sIgA is transported into saliva across salivary glandular epithelial cells, alpha-amylase and lactoferrin are synthesized and secreted by acinar cells in the secretory end piece of the glands. The main sources of lysozyme and HDP are macrophages in the oral mucosa with low production by the basal cells of striated ducts in the parotid glands, and oral epithelia cells, respectively [102].

The sIgA response in submandibular/sublingual glands is better related to B cell induction in the intestinal environment whereas parotid sIgA is more linked to immune induction in adenoids and palatine tonsils [103].

The volume of the saliva and it constituent proteins are regulated by the parasympathetic and sympathetic nervous systems [104]. Since secretion and synthesis of sIgA depend on antigenic stimulation and also on neuroendocrine control, alterations in neuroendocrine functioning (drugs, hormonal changes, stress, and exercise) have been shown to affect sIgA levels [105-107].

Although it was originally thought that the oral epithelium provided defenses primarily as a mechanical barrier against microbial invasion it is now recognized that these cells play an important active role in the recognition of microbes, eliciting a defensive response similar to that found in the phagocytes cells of the innate immune response. Ligation of microbes' components to different receptors and co receptors results in the activation of a signal transduction pathway, transcription factors, and the expression of innate immune genes such as IL-1β, IL-8, and HDP. Among these peptides defensins are found in mucosal surfaces of the oral cavity and apart from their broad-spectrum antimicrobial activities new studies have shown they play an important role in the innate defense against pathogenic microbial colonization [108].

In one of the articles of this Hot Topic Li J., *et al*. have reviewed the recent progress in understanding the roles of defensins in combating ocular infection and in the modulation of inflammation as well as the biological activities and regulated expression of defensins in corneal and conjunctival epithelial cells.

In humans, α-defensins are found predominantly in the azurophilic granule of neutrophils and they are known as human neutrophil peptides (HNP 1-4). Since variable levels of HNP have been found in saliva and gingival crevicular fluid in healthy persons and in different oral diseases it has been suggested that they may be regulated by pathogens that affect neutrophil migration and function [109, 110].

β-defensin peptides, which are expressed in epithelial cells as well as certain cell types of the myeloid lineage, are found in human saliva where they play the very important role of maintenance of steady-state levels of flora in the oral cavity [111, 112]. In human gingival tissue, hBD-1 and hBD-2 are both expressed in normal uninflamed tissue at the highest levels at the gingival margin whereas hBD3 expression was observed primarily in the basal layer as well as in Merkel and Langerhans cells. Moreover, the expression of peptides and mRNA levels increase in biopsies from periodontitis tissue [113-115].

In vitro studies using cultured human gingival epithelial cells have demonstrated that different microbes induce the expression of different β-defensin peptides in a LPS independent way [116, 117]. There have also been a wide range of responses in cultured epithelial cells when some species associated with periodontal disease were compared with bacteria not associated with periodontal disease [118]. Using an animal model Kurland *et al.* [119] have demonstrated that introduction of both pathogenic bacteria and commensal bacteria can transiently induce β-defensin gene expression in rat gingival tissue.

Very recently Diamond G., *et al*. reviewed the differences and similarities between host defense peptides in the airway and the oral cavity and showed that β-defensin genes are induced in the airway by all bacteria and Toll-like receptor (TLR] agonists primarily through an NF-κB-mediated pathway, whereas the same genes are induced in the oral epithelium by only certain bacteria and TLR ligands, *via* different pathways [120].

Histatins, which is a class of related α- helical HDP with potent activity primarily against fungi, has also been shown to play an important function in the defense of the oral cavity again *C. albicans* [121].

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