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Danger Signals in Oral Cavity-Related Diseases

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Abstract

The oral cavity is a unique environment containing teeth juxtaposed with soft tissues, all of which are constantly bathed in microbial products and host-derived factors. While microbial dysbiosis in the oral cavity clearly leads to oral inflammatory disease, recent advances find that endogenous danger-associated molecular patterns (DAMPs) released from oral and salivary tissue also contribute to the progression of inflammatory and autoimmune disease, respectively. In contrast, DAMPs produced during oral fungal infection actually promote the resolution of infection. Here we present a review of the literature suggesting a role for signaling by DAMPs, which may intersect with pathogen-associated molecular pattern (PAMP) signaling, in diseases that manifest in the oral cavity, specifically periodontal disease, oropharyngeal candidiasis and Sjögren's Syndrome.

Summary sentence:

Review of how activation of the immune system through signaling of danger signals contributes to the progression or resolution of oral diseases.

GRAPHICAL ABSTRACT

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Conflict of Interest Disclosure

The authors declare no conflict of interest.

Keywords

Periodontal disease; Sjögren's syndrome; candidiasis; DAMP; PAMP

Introduction

The innate immune system is fundamental to host survival and overall health, being the first line of defense against pathogens as well as fundamental in maintaining tissue homeostasis. This is especially true in the complex environment of the oral cavity, which is a unique anatomical structure characterized by the juxtaposition of soft and hard tissues constantly bathed in microbial products and host-derived factors. Many ecological niches are present in the oral cavity reflecting the variety of tissues present including teeth and multiple keratinized and non-keratinized epithelial surfaces. Each niche has varying microenvironments due to differing immune capacity and physiological conditions: one distinct feature of the oral cavity is the presence of hard non-shedding tooth enamel surfaces which are ideal to promote the growth and maintenance of complex microbial biofilms. The oral mucosa, comprised of epithelium and underlying connective tissue, generally functions as an effective mechanical barrier despite being constantly exposed to, and in direct interaction with, environmental stimuli. However, despite continuous turnover of superficial oral epithelial layers, these mucosal surfaces are colonized abundantly with organisms [1].

The mucosa immediately adjacent to the teeth, termed the gingival sulcus, normally forms a tight seal against the teeth that prevents microbial invasion. This periodontal tissue is highly vascular and thus the gingival sulcus is bathed in the serum-like exudate termed gingival crevicular fluid (GCF) which carries components such as complement, antibodies and immune cells that function to maintain homeostasis at the biofilm-gingival sulcus interface [1].

The mucosal tissues and tooth surfaces of the oral cavity are also constantly bathed in saliva, a watery substance produced by the salivary glands. Saliva functions to provide lubrication but also serves as source of nutrients and proteins for initial formation and succession of the oral biofilm community. Saliva is a key component of the oral defense system as it contains a plethora of innate components that aim to maintain bacterial symbiosis. The importance of saliva is illustrated in patients with salivary hypofunction, including the autoimmune disease Sjögren's syndrome (SS). In this disease, the salivary glands display immune-mediated dysfunction and patients often lose salivary flow, leading to rampant dental decay [2]. Thus, saliva plays a key role in oral homeostasis and alterations in the amount produced or the composition has profound effects on the integrity of the oral tissues.

The oral cavity is under constant microbial pressure, with a diverse microbiome composed of bacteria, viruses, fungi and protozoa. The bacterial community in the mouth is highly complex, with over 1000 potential species present; it is second only to the gut in microbial diversity in the human body [3]. Likewise, a vast diversity in the fungal microbiome is observed [4]. The continual presence of microbes in the oral cavity, even in health, necessitates constant immune surveillance to maintain a characteristic homeostatic steady-state. A rich immunological cellular and cytokine network is present at the gingival tissue

interface and supporting oral mucosa [5, 6] and a large number of neutrophils are recruited to the gingival crevice in health, which are significantly increased during inflammation. These neutrophils play a role in initial microbial surveillance as well as orchestrating the overall immune response to maintain oral health. Mononuclear populations in the gingiva are comprised of resident macrophages and dendritic cells with increased recruitment and activation of these cells along with monocytes in response to microbial dysbiosis during oral disease progression [7]. These cells are involved in directly defending the tissue barrier against bacterial insults as well as promoting immune regulation through cross-talk with adaptive immune cells and their antigen presentation functions. In health, gingival lymphocyte populations consist of minimal B-cells and a prominent resident T-cell infiltrate with increased proportions of various T- and B-cell subsets in disease, where some subsets, such as T_H17 cells, may drive pathogenesis while others such as T_{reg} , may mediate a protective response [6].

Like the gingival crevice, other oral mucosal tissues including the tongue, palate and buccal surface have similar immune constituents, however slight differences in cellular proportions and subsets may occur in different mucosal areas reflecting regional differences. Generally, the salivary glands are a sterile location with tightly regulated immune responses. In addition to IgA-producing plasma cells, macrophages, dendritic cells, innate lymphoid cells, and T-cells are present in salivary gland tissue to contribute to protection of oral tissues. In pathoses such as SS, increased infiltration and hyperactivity of these immune cell populations contribute to disease [8].

It is well known that the immune response involves complex signaling systems that 1) recognize pathogens and tolerate commensals while initiating defense responses to control infection and 2) properly recognize self versus non-self under homeostatic conditions. The first line of immune defense involves detection of conserved molecular motifs expressed by members of the microbiome referred to as pathogen-associated molecular patterns (PAMPs) or more generally microbial-associated molecular patterns (MAMPs). Conversely, danger-associated molecular patterns (DAMPs) are endogenous factors released upon cellular damage or tissue disruption [9]. These PAMPs and DAMPs are recognized by a variety of germ-line encoded membrane-bound and cytoplasmic sensors termed pattern-recognition receptors (PRRs). PRRs are expressed on tissue resident cells and infiltrating immune cells and include Toll-like receptors (TLRs), C-type-lectin receptors, nucleotide-binding and oligomerization domain (NOD1 and NOD2), NOD-like receptors (NLR), receptor for advanced glycation end-products (RAGE), retinoic acid-inducible gene-I (RIG-I) receptors and the inflammasome, among others [10, 11]. Signaling through PRRs induces downstream activation of signaling pathways to modulate generally pro-inflammatory responses, though polymorphisms can alter the signaling responses from PRRs. Indeed, recent studies have begun to associate links between polymorphisms in PRRs and the development of periodontal disease and SS [12–14].

It is well established that microbial products contribute to oral disease. However, we have also recently come to appreciate that DAMPs activate PRRs expressed by host cells, thereby also serving as potent inflammatory stimuli that can help promote a robust immune response in order to clear the pathogen, or promote excessive inflammation and exacerbate disease.

Here we present a review of the literature suggesting a role for DAMPs in diseases that manifest in the oral cavity, specifically periodontal disease, oropharyngeal candidiasis and SS (Figure 1 and 2). Modulation of DAMPs themselves or pathways activated by DAMPs may serve as novel therapeutic strategies for these debilitating diseases.

Periodontal Disease

Periodontal disease is the most prevalent chronic inflammatory diseases, with nearly 50% of those over 30 years of age in the US being affected [15]. While periodontal disease is a multifactorial disease with development dependent on oral care, environmental triggers and host genetic makeup, it is well understood that overgrowth and transformation of the subgingival oral microbiome into a dysbiotic state is ultimately required to initiate and sustain chronic inflammation; this chronic inflammation in turn is the principal driver of tissue destruction and disease [16, 17]. The involvement of the oral microbial community leads to a largely PAMP-driven inflammatory response and disease development [18]. While largely extracellular [10], there is evidence of intracellular PAMP responses during the development of disease.

Levels of IL-1 β , the prototypical cytokine released upon intracellular PAMP and DAMP recognition and inflammasome activation [19], are linked with periodontal disease progression [20, 21], as is the expression of the NLRP3 inflammasome [22, 23]. A number of periodontal pathogens, including *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, *Treponema denticola* and *Tannerella forsythia* are capable of eliciting an IL-1 β response from immune cells [24–26] as well as from non-immune cells including gingival epithelial cells and fibroblasts [27, 28]. In addition to whole bacterial interactions, recent studies have found outer membrane vesicles (OMVs) from *P. gingivalis*, *T. denticola* and *T. forsythia* are capable of inducing the inflammasome and IL-1 β release [29, 30]. Although the majority of studies indicate signaling through the intracellular NLRP3 inflammasome is required [31], the exact bacterial components required to fully activate complement and IL-1 β release, or if DAMPs such as ATP are additionally required [27, 32], remains uncertain.

P. gingivalis, the most extensively studied oral pathogenic bacterium, has a large number of pathogenic factors contributing to its ability to disrupt both the oral microbiota and the immune system, to initiate dysbiosis and promote an environment within which it can flourish [16]. There have been conflicting reports suggesting *P. gingivalis* may have a unique ability to inhibit the activation of the inflammasome and release of IL-1 β , even when in the presence of other inflammasome activating oral pathogens [33, 34]. Indeed, there is precedent for this pathogen interfering with IL-8 production by releasing into the cytoplasm of invaded epithelial cells a phosphatase to reduce NF κ B RelA phosphorylation [35]. However, recent studies suggest that *P. gingivalis* activates the inflammasome, but that the presence of bacterial-produced proteases (gingipains) are responsible for destroying much of the cytokine produced [36, 37].

A number of oral pathogens, including *P. gingivalis*, are able to survive within both immune and non-immune cells [28, 38, 39]. While the exact mechanisms of survival are still unclear,

an ability to survive within mammalian cells provides a potential means for transport of bacterial components into the cytoplasm leading to internal PAMP and DAMP signaling [40]. Further, though much focus has been on the role of oral microbes with a traditional pathogenic phenotype, recent evidence highlights a potential role for traditionally commensal oral microbes, including *Streptococcus sp.* and *Enterococcus sp.* in the development of disease [41]. While there is an essential role for the adherence of the traditional pathogens such as *P. gingivalis* to the commensal flora [42], there is emerging evidence for commensal-host immune cell interactions changing during inflammation, with increased survival within phagocytes potentially leading to increased PAMP signaling within the phagocyte cytoplasm [43].

Extensive tissue destruction is a hallmark of periodontal disease and therefore tissue-released DAMPs may contribute to disease (Figure 1). *T. denticola*, *P. gingivalis*, *T. forsythia* and *F. nucleatum* have been implicated in inducing the release of DAMPs, including ATP, HSP60, fibronectin and HMGB1 from mammalian cells [28, 44]. ATP can function as a secondary internal signal through the P₂X₇ receptor (P₂X₇R) to promote the activation of the inflammasome of cells when in the presence of oral pathogens [27]. Whether such signaling is required *in vivo* for periodontal disease development is currently unclear, however *P. gingivalis* can secrete a nucleoside diphosphate kinase-like enzyme that can remove ATP and inhibit such signaling [45]. In addition, recent studies have begun to indicate a role for the DAMPs serum amyloid A and HMGB1 in periodontal disease, as these are increased in tissue and GCF [46–48] and can activate TLR signaling in mouse models of periodontal disease [48, 49]. Systemic administration of anti-HMGB1 inhibited periodontal inflammation and bone loss in a bacterial induced murine model, highlighting a potential role in periodontal progression [50].

Finally, neutrophil extracellular traps (NETs) can be induced in the oral cavity and may be involved in maintaining oral health, as their impaired formation due to genetic mutations or bacterial DNase production may exacerbate periodontal disease. Similarly, exaggerated NET production occurs in the periodontal pocket, obstructing removal of cellular debris and potentially PAMPs and DAMPs by preventing efficient flushing of the pocket by GCF flow leading to bystander tissue damage (reviewed in [51]). While DAMPs such as HMGB1 and histones released by injured liver hepatocytes can induce NET formation through TLR activation [52], and NETs with associated IL-1B are linked with active ulcerative colitis [53], to our knowledge the involvement of DAMPs in NET formation within the oral cavity is currently unknown.

Sjögren's Syndrome (SS)

SS is an autoimmune disease that primarily affects women [2]. The disease is characterized by loss of saliva and tear production, although patients may also exhibit many serious extra-glandular disease manifestations such as B-cell lymphoma [2]. Like many other autoimmune diseases, the cause of SS is likely multifactorial. While PRRs and PRR-mediated signaling pathways are clearly dysregulated in SS, adult SS patients do not appear to be particularly susceptible to microbial infections [54] and so it is unlikely that the activating ligands in SS

are derived from exogenous sources. Thus, it is probable that PRR activation is mediated by endogenous triggers, as is the case for other autoimmune diseases [55].

Evidence in both SS models and patients shows that DAMPs may be released through the pathologic degradation of exocrine tissue [56–58] (Figure 2). Indeed, extracts from SS salivary biopsy tissue showed elevated proteolysis of extra-cellular matrix molecules (ECM) proteins, such as fibronectin and laminin [57]. Moreover, fibronectin is dysregulated in salivary tissue from SS mice and is also elevated in saliva from SS patients [59, 60]. Studies show the ECM proteins decorin and biglycan are degraded by saliva derived from an SS mouse model [58]. Importantly, each of these ECM molecules can activate TLRs [61–63]. In addition, the ECM molecule versican is increased in salivary gland epithelial cell lines derived from SS patients as compared to those from healthy controls [64]. Versican activates TLR2/6 heterodimers [55] and is regulated by type I interferon (IFN) in lung tissue [65]. This finding may have particular relevance to SS pathogenesis, since a subset of SS patients display an IFN gene signature [14]. Significantly, 2 separate GWAS studies revealed a suggestive association between variants in the TNFAIP3 gene locus with SS [14]. While the functional consequence of this is unclear at present, TNFAIP3 encodes a negative regulator of TLR signaling and dysregulation of TLR-mediated networks could contribute to DAMP-driven inflammation in disease.

In addition to ECM molecules, elevated levels of the DAMP S100A8/A9 (calprotectin) are seen in the sera, salivary tissue, and saliva of SS patients [66–69]. Treatment of PBMCs from SS patients with S100A8/A9 causes significant TNF α secretion [68]. Studies in an infection model demonstrate S100A9 activates TLR4 [70]. Aberrant mucin localization may also activate TLR4 in SS. Of note, acinar cells in SS display loss of polarity and this has profound effects on cell function [71]. As a consequence of this loss of polarity, salivary mucins are secreted into the ECM at the basolateral aspect of the cell rather than from the apical pole [71]. Studies in HSG cells revealed that the mucins MUC5B and MUC7 activate TLR4 signaling [71], suggesting that ligation of epithelial cell-derived TLR4 by mucins may provide a continuous endogenous source of unremitting salivary inflammation in SS.

Finally, recent work to identify factors that upregulate IFN in autoimmunity identified a family of endogenous virus-like genomic retroelements, termed long interspersed nuclear element 1 (LINE-1 or L1) that are increased in minor salivary gland (MSG) tissue from SS patients [72]. L1 contains 2 open reading frames, and inappropriate expression of this retroelement activates innate immunity [72]. Significantly, L1 transcripts correlate with type I IFN in MSG tissue of SS patients [72]. Expression of L1 in pDCs or monocytes results in upregulation of *IFN α* and inhibition of TLR-7/TLR-8 reduced this expression in pDCs [72]. Significantly, L1 may also contribute to the female disease predilection observed in SS, as these sequences are located on the X chromosome and are known to escape X-chromosome inactivation [73]. Thus, endogenous genetic elements may serve as DAMPs in SS through activation of endosomal TLRs. Altogether, these studies provide evidence that host-derived mediators of inflammation may stimulate TLRs in SS patients, thereby precipitating and/or exacerbating disease.

In addition to TLR agonism, there is evidence that the P₂X₇R is activated by ATP in SS. ATP may be released from host cells as a result of necrosis or apoptosis. This results in inflammasome activation and subsequent secretion of pro-inflammatory cytokines including IL-1 β and IL-18 [74]. Indeed, P₂X₇R activation causes salivary gland inflammation and studies in a murine model of SS show that blockade of this receptor reduces IL-1 β expression [75]. Corroborative work using human samples found that P₂X₇R expression was higher in salivary tissue and PBMCs from SS patients compared with controls [76, 77]. In addition, they found that inflammasome components and IL-18 were elevated in the salivary tissue and saliva of SS patients, respectively [76]. Moreover, studies using CD14⁺ PBMCs from SS patients found that ATP treatment resulted in upregulation of P₂X₇R expression [78]. Taken together, these data suggest that activation of the P₂X₇R may contribute to both local and systemic inflammation in SS.

Oropharyngeal Candidiasis

Candida albicans is normally commensal in many healthy individuals, however, *C. albicans* is a significant cause of morbidity in individuals immunocompromised due to AIDS, neutropenia, diabetes mellitus or immunosuppressive drug treatment [79]. In addition, many primary immune deficiencies result in increased susceptibility to fungal infection, including neutropenia and patients with deficiencies in NADPH oxidase complex reactive oxygen production, leukocyte adhesion, and IL-17-related immunity [80]. Production of IL-1 cytokines is also critical to anti-fungal defenses [81]. Recent evidence also indicates there may also be a role for IL-1 cytokines, and their production by DAMPs, in distinguishing commensal yeast colonization from pathogenic invasion by immune cells [82].

The ability of *C. albicans* to escape from the phagosome of phagocytes is clear [83, 84]. This is achieved through filamentation and elongation of the yeast, an essential component of *C. albicans* pathogenicity [85]. Cell wall changes that accompany this filamentation are then responsible for the activation of the NLRC4 and NLRP3 inflammasomes that are also required for effective immunity [86, 87]. Importantly however, before significant interactions with phagocytes occur, as it is normally present in the oral cavity as a commensal, the yeast to hyphal transition allows *C. albicans* to invade the epithelium and grow within the host [88]. This invasion of the epithelial cells can cause the release of a number of endogenous DAMPs, including IL-1 α , S100A8 and S100A9 [89, 90] (Figure 1). In the case of mucosal candidiasis, including OPC, release of DAMPs and internal signaling from DAMPs and PAMPs are important in mounting an effective immune response and in contrast to the above examples of periodontal disease and SS, we are unaware of any evidence suggesting cellular DAMP signaling induces increased *Candida* virulence.

Concluding Remarks

The oral cavity is a complex tissue environment with a variety of bacterial-mediated and autoimmune conditions that can occur. While immune responses to maintain tissue homeostasis and respond to dysbiotic bacterial communities at other body sites are documented, knowledge of immune responses in the unique oral environment are not as well understood. Emerging evidence suggests endogenous signals play an important role in

disease progression and effective immune responses in the oral cavity. However, further studies are needed to understand the way in which these DAMPs are generated, the subsequent intracellular signals that result, and whether pharmacologic modulation of DAMP-dependent pathways has therapeutic efficacy.

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Abbreviations

DAMP	Damage-associated molecular pattern
ECM	Extracellular matrix
GCF	Gingival crevicular fluid
HMGB1	High mobility group box 1
LINE-1 or L1	Long interspersed nuclear element 1
MAMP	Microbial-associated molecular patterns
MSG	Minor salivary gland
NET	Neutrophil extracellular trap
NLR	NOD-like receptors
NOD	Nucleotide-binding and oligomerization domain
PAMP	Pathogen-associated molecular pattern
RAGE	Receptor for advanced glycation end-products
RIG-I	Retinoic acid-inducible gene-I
SS	Sjögren's syndrome
SSA	Serum amyloid A
TLR	Toll-like receptor

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Figure 1:

Immunity at oral mucosal surfaces. Mucosal surfaces such as the tongue and periodontium are sites of rich immune networks due to the constant presence of a diverse microbiome. Tongue epithelium is colonized with normally commensal organisms including the dimorphic fungi *Candida albicans*. If overgrowth persists in a susceptible host, hyphae can invade the epithelium and the host normally mounts an effective cellular immune response through release of DAMPs. In health, the periodontium tissue surrounding the tooth and associated gingival crevice is in homeostatic balance while dysbiosis of the bacterial community drives destructive cellular inflammation with activation of DAMP signaling. DAMP production through matrix degradation and cellular damage can help to enhance inflammation and promote characteristic tissue destruction of periodontal disease.

Figure 2:
DAMPs associated with SS-related inflammation in salivary tissue. DAMPs implicated in SS and the innate and adaptive immune cells that may be activated as a result of DAMP interactions are shown. SGECs = Salivary Gland Epithelial Cells.