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Review Article

Epithelial Antimicrobial Peptides: Guardian of the Oral Cavity

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Gingival epithelium provides first line of defence from the microorganisms present in dental plaque. It not only provides a mechanical barrier but also has an active immune function too. Gingival epithelial cells participate in innate immunity by producing a range of antimicrobial peptides to protect the host against oral pathogens. These epithelial antimicrobial peptides (EAPs) include the β-defensin family, cathelicidin (LL-37), calprotectin, and adrenomedullin. While some are constitutively expressed in gingival epithelial cells, others are induced upon exposure to microbial insults. It is likely that these EAPs have a role in determining the initiation and progression of oral diseases. EAPs are broad spectrum antimicrobials with a different but overlapping range of activity. Apart from antimicrobial activity, they participate in several other crucial roles in host tissues. Some of these, for instance, β-defensins, are chemotactic to immune cells. Others, such as calprotectin are important for wound healing and cell proliferation. Adrenomedullin, a multifunctional peptide, has its biological action in a wide range of tissues. Not only is it a potent vasodilator but also it has several endocrine effects. Knowing in detail the various bioactions of these EAPs may provide us with useful information regarding their utility as therapeutic agents.

1. Introduction

Oral cavity is a diverse ecosystem where several microbes, commensal and pathogenic, interact and proliferate in the moist and warm environment. It is perhaps the only ecosystem of the human body which is dynamic, open, and close at the same time. The uniqueness of the oral cavity also lies in the presence of specialized interface which in the hard tissue (tooth) extrudes through the underlying soft tissue (gingival epithelium) [1]. The gingival epithelium constitutes oral epithelium, sulcular epithelium, and junctional epithelium. Since the tooth surface is invariably covered by a layer of microbial biofilm termed "dental plaque," the surrounding epithelium is continuously exposed to microorganisms and their products [2]. The role of gingival epithelium has been under the scanner because of this close interaction. Previously considered to be an innocent bystander, its active role in host microbial interaction has been elucidated with recent research [3].

Gingival epithelium works in many ways for the protection of underlying connective tissue. First, it provides a physical barrier which does not allow microbes to invade while permitting selective transaction with oral environment. Second, upon interaction with microbes it secretes cytokines as well as chemokines to activate the influx of neutrophils and other immune cells into the sulcular area [4]. Third, the gingival epithelium has been found to be a source of antimicrobial products termed epithelial antimicrobial peptides (EAP). These EAPs are considered to be important for the innate immunity of the host. These peptides are known to possess a wide spectrum of antimicrobial activity acting against Gram-positive and Gram-negative bacterial species as well as yeast and some viruses [5]. Thereby, EAPs have the potential to prevent various oral diseases of microbial origin such as periodontitis, dental caries, candidiasis, and herpetic gingivostomatitis.

Since gingival epithelial cells are in close proximity with microbes and their virulence factors, the response of epithelium to these insults determines the health of the gingival sulcus. Several families of antimicrobial peptides have been identified in oral cavity which includes α-defensins, β-defensins, calprotectin, adrenomedullin, histatins, and

cathelicidin. Of these α-defensins are of nonepithelial origin, with their major source being the neutrophils migrating into the gingival sulcus as primary host response, whereas histatins are secreted in saliva, produced by parotid and submandibular salivary gland duct cells [6, 7]. The EAPs work in tandem with other salivary antimicrobial agents such as histatins, immunoglobulins, and lysozyme to strengthen first line of host defence. These antimicrobial peptides are expressed in oral epithelium constitutively and are inducible; thus, they have both functions, of surveillance and of host defence [8].

2. Epithelial Antimicrobial Peptides in Oral Cavity

Several antimicrobial peptides produced by epithelial cells are widespread in animal kingdom ranging from Cecropins in insects to Magainins in frogs and Bactenecins in cattle [9–11]. All these antimicrobial peptides although different in structure and amino acid composition share a common trait of being broad spectrum antibiotics and essentially the first line of defence for host. First mammalian antimicrobial peptide identified in oral epithelium was the lingual antimicrobial peptide (LAP) by the works of Schonwetter et al. on bovine tongue [12]. LAP was found to have antibiotic activity against Gram-positive and Gram-negative bacteria as well as antifungal properties [12]. After LAP, several more EAPs have been identified in mammals including humans. The EAPs identified in humans are β-defensin family, cathelicidin (LL-37), calprotectin, and adrenomedullin. The major characteristics of these EAPs are compared in Table 1.

3. The β-Defensin Family

Beta-defensins are universally expressed in all human epithelial cells [13]. In the oral cavity, they have been found in oral mucosa, gingiva, and tongue epithelium along with salivary glands [14]. They were first described in bovine tracheal pseudostratified epithelium [15]. Beta-defensins share certain structural similarities with α-defensins although they are a bit larger than α-defensins. However, both α- and β-defensins are cationic in nature because of presence of arginine and lysine residue in their structure [16]. Out of several β-defensins (hBDs) known, four have been characterized elaborately and termed as hBD-1, hBD-2, hBD-3, and hBD-4. Out of this only hBD-1, hBD-2, and hBD-3 are expressed in the oral cavity [17]. hBD-1 and hBD-2, found in the suprabasal layer of epithelium, can be localized to differentiated epithelial cells, whereas hBD-3 is expressed in undifferentiated epithelial cells found in the basal layer of epithelium [18, 19]. Apart from hBD-1, which is constitutively expressed, the rest of the β-defensins are inducible upon stimulation with microbes or proinflammatory cytokines [20]. Recently another β-defensin hBD-9 has been identified and localized in gingival epithelium [21].

3.1. Genes Encoding β-Defensin. The first human β-defensin was isolated from hemofiltrate passing through kidney [22]. The gene encoding for hBD-1 is present on chromosome 8 and is termed DEFB1. It lies in close proximity to the neutrophil α-defensin gene (DEFA 1), arranged approximately 100–150 kilobases apart. DEFB1 has two exons and one intron which is translated into a hBD-1 propeptide [23]. This propeptide undergoes posttranslational modifications and forms several hBD-1 mature peptides upon cleavage which are about 36–47 amino acids long [24].

The second human β-defensin was first isolated in the psoriatic skin keratinocytes [25]. hBD-2 is encoded via gene EFB4 which is also located on chromosome 8 in close proximity to DEFB1. Similar to DEFB1, it has two exons and one intron which encodes a signal peptide which is 23 amino acids long and a mature peptide which is 41 amino acids long [26].

DEFB103, which is the gene for hBD-3, has been localized on chromosome 8 above the hBD-2 gene. The translational product of this gene consists of a propeptide with a signal peptide domain and a mature peptide of 22 and 45 amino acids in length, respectively. The amino acid sequence of hBD-3 shares some amount of similarity with hBD-2 [27].

3.2. Induction of Genetic Expression. Human β-defensin-1 is expressed at a low level within gingival and buccal epithelium, dental pulp, and salivary gland tissue [5]. It has been observed that hBD-1 is not regulated in response to infection or other stimuli, which means that it is not inducible. Otherwise expressed at a very low level, hBD-2 and hBD-3 are, on the other hand, inducible in response to microbes and their products [28]. Their amplified expression in response to infection has already been confirmed in gingivitis and periodontitis [29, 30]. IL-1β, tumor necrosis factor (TNF)-α, and IL-17 have been implicated in the induction of hBD-2 and hBD-3 expression in epithelial cells [31]. Their induction via cytokines confirms their role in innate immunity. IL-17 also induces β-defensin expression against the colonization of *Candida* in oral cavity [32]. Thus, β-defensins expressed within the oral cavity not only check an overgrowth of commensal microorganisms but also prevent colonization of pathogens.

3.3. Structure. The structure of β-defensins is characterized by an antiparallel β-pleated sheet stabilization via three intramolecular disulphide bonds formed between six cysteine amino acids. Also, hBD-2 and hBD-3 contain an α-helical domain at their N terminus. Since β-defensin peptide contains both hydrophilic and hydrophobic domains in their structure, they are amphiphatic in nature [33]. The amino acid sequences of these defensins and other oral EAPs are provided in Table 2.

3.4. Antimicrobial Activity. The salivary concentration of hBD-1 and hBD-2 varies in a range of nondetectable to 39 ng/mL and 33 ng/mL, respectively [34]. On the other hand, hBD-3 exists at a concentration range of 0.31 μg/mL in saliva [35]. Several in vitro assays have revealed β-defensins to be active against broad range of microbes including Gram-positive and Gram-negative bacteria, enveloped viruses, and fungi [36–39]. They are also efficacious against periodontal pathogens such as *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* [37]. A recent study demonstrated extermination

Table 1: Comparison of chief characteristics of oral epithelial antimicrobial peptides.

	Human β-defensins			Cathelicidin (LL-37)	Calprotectin	Adrenomedullin
	hBD-1	hBD-2	hBD-3			
Genes	DEFB1 on chromosome 8	EFB4 on chromosome 8	DEFB103 on chromosome 8	CAMP gene on chromosome 3	Dimer of two peptides MRP8 and MRP14 encoded by genes S100A8 and S100A9, respectively, on chromosome 1	Adrenomedullin (ADM) gene located on chromosome 11
Number of amino acids	36–47	41	45	37	MRP8: 93 MRP14: 114	52
Secondary structure	Antiparallel β-pleated sheet stabilization via three intramolecular disulphide bonds formed between six cysteine amino acids			Random coil in aqueous solutions with intramolecular hydrogen bonding forms a α-helix secondary structure	Noncovalently complexed heterodimer of MRP8 and MRP14. Each monomer has helix-loop-helix calcium ion binding domain, EF hands, and a hinge region	Amino acid chain with a single disulphide bridge between residues 16 and 21
Expression in oral cavity	Oral mucosa, gingiva, and tongue epithelium along with salivary glands			Orogranulocytes, inflamed gingival tissues, buccal mucosa, and tongue epithelium	Oral epithelium, sulcular epithelium, and immune cells	Oral epithelium along with salivary gland epithelium and immune cells
Mechanism of antimicrobial action	Upon interaction with anion lipids of bacterial cell membrane leads to formation of multimeric pores and permeation of the membrane			Overlaps bacterial cell membrane in a carpet-like manner and dissolves it similar to detergent	Binds with trace metal ions essential for microbial functioning, thus inhibiting microbial growth	Promotes intramembranous pore formation in bacterial cell membrane
Antimicrobial spectra	Gram-negative and Gram-positive bacteria, enveloped viruses and fungi			Gram-negative and Gram-positive bacteria including many periopathogens	Gram-negative and Gram-positive bacteria including periopathogen *P. gingivalis*. Antifungal and antiviral activity	Acts against both Gram-positive and Gram-negative bacteria of oral cavity, lacking antifungal activity, antiviral activity unknown

Table 2: Amino acid sequences of oral epithelial antimicrobial peptides.

Human β-defensins	
hBD-1	DHYNCVSSGG QCLYSACPIF TKIQGTCYRG KAKCCK
hBD-2	GIGDPVTCLK SGAICHPVFC PRRYKQIGTC GLPGTKCCKK P
hBD-3	GIINTLQKYY CRVRGGRCAV LSCLPKEEQI GKCSTRGRKC CRRKK
Human cathelicidin (LL-37)	LLGDFFRKSK EKIGKEFKRI VQRIKDFLRN LVPRTES
Calprotectin	
MRP8	MLTELEKALN SIIDVYHKYS LIKGNFHAVY RDDLKKLLET ECPQYIRKKG ADVWFKELDI NTDGAVNFQE FLILVIKMGV AAHKKSHEES HKE
MRP14	MTCKMSQLER NIETIINTFH QYSVKLGHPD TLNQGEFKEL VRKDLQNFLK KENKNEKVIE HIMEDLDTNA DKQLSFFEFI MLMARLTWAS HEKMHEGDEG PGHHHKPGLG EGTP
Adrenomedullin	YRQSMNNFQG LRSFGCRFGT CTVQKLAHQI YQFTDKDKDN VAPRSKISPQ GY

of *F. nucleatum* with in gingival epithelial cells via hBD-2 and hBD-3 [40]. Their spectrum of activity also includes *Candida albicans* along with other *Candida* spp. in oral cavity [38]. In an experiment carried out to assess the antimicrobial activity of hBD-1, hBD-2, and hBD-3 against periodontopathic and cariogenic bacteria, it was suggested that Gram-negative bacteria except *F. nucleatum* were less susceptible to antimicrobial peptides than Gram-positive organisms. Except for hBD-1, all peptides demonstrated 100% bactericidal activity with concentration of >10 mg/L of peptides. hBD-1 and hBD-2 were significantly less effective than hBD-3 in their antimicrobial activity. The minimum inhibitory concentration (MIC) for hBD-3 was around 100–200 mg/L for *A. actinomycetemcomitans*, *P. gingivalis*, and *Prevotella intermedia*, whereas it was 12.5 mg/L for *F. nucleatum* [41]. In another study, the MIC of hBD-2 and hBD-3 was found to be in a range of 3.9 to >250 μg/mL and 1.4 to >250 μg/mL, respectively, against *A. actinomycetemcomitans*, *F. nucleatum*, *P. gingivalis*, *Peptostreptococcus micros*, *Streptococcus mutans*, *S. sanguis*, and *Candida* spp. [36].

It has also been observed that certain periodontal pathogens have developed resistance against β-defensins, thereby enhancing their pathogenicity. These pathogens include *P. gingivalis* and *Treponema denticola* [42, 43]. It is assumed that *T. denticola* interacts with the signal transduction pathway to suppress the expression of β-defensins [43]. Also, recent data demonstrates that hBD-2 and hBD-3 expression in adult gingival epithelial cells inactivates human immunodeficiency virus (HIV) suggesting an important role of β-defensins in antiviral defence [39].

3.5. Mechanism of Antimicrobial Action. β-defensins belong to a group of cationic antimicrobial peptides (CAPs), known to target the bacterial cell membrane. Since β-defensins are positively charged, they bind to the negatively charged sites on the bacterial cell membrane. In Gram-negative bacteria, the target is lipopolysaccharide (LPS), whereas in Gram-positive bacteria it is teichoic acid. Apart from these, membrane rich phospholipids (phosphatidyl glycerol) which are common to both Gram-positive and Gram-negative bacteria are also targeted by β-defensins. As the eukaryotic cell membrane is rich in phosphatidylcholine rich phospholipids which are zwitterionic in nature, they are exempted from the action of β-defensins [8]. It has been hypothesized that defensins upon interaction with anion lipids of bacterial cell membrane leads to formation of multimeric pores and permeation of the membrane (Figure 1). This permeation leads to the loss of vital contents of the bacterial cell leading to cell death [44].

3.6. Other Roles. Various roles of β-defensins are summarized in Figure 2. Since β-defensins exhibit similarities to cytokines structurally, they show potent chemotactic activity to a range of immune cells [45, 46]. This activity takes place at a much lower level of β-defensin concentration than required for its antimicrobial activity. hBD-1 activates immature dendritic cells and memory T cells, hBD-2 is able to recruit mast cells and neutrophils, and hBD-3 is chemotactic for neutrophils, dendritic cells, mast cells, and monocytes. hBD-2 and hBD-3 also induce degranulation of mast cells [47–49]. Recently, it has been demonstrated that β-defensins are able to bind to CXCR 4 receptors on T cells, leading to internalization of receptor. This receptor is crucial for HIV-1 infection of T cell, thereby its internalization provides a defence against HIV-1 infection [39].

Beta-defensins have a role in inducing the resident as well as nonresident cells to release chemokines. They induce the release of several chemokines from keratinocytes such as monocyte chemotactic protein-1, interferon-γ inducible protein-10, and macrophage inflammatory protein 3α [50]. Beta-defensins are known to inhibit production of chemokines and cytokines such as TNF-α and IL-6, thereby exhibiting a role in anti-inflammatory activities and suppression of immune response [51, 52]. They also have a role in promotion of wound healing, where the growth factors secreted by various immune cells in the area of injury can stimulate keratinocytes to release hBD-3. These growth factors include insulin-like growth factor- (IGF-) 1, transforming growth factor- (TGF-) α, and epidermal growth factor (EGF) [53]. Also, β-defensins act as a connecting link between innate and adaptive immunity. Epithelial cells release β-defensins along with chemokines and cytokines, to signal Langerhans

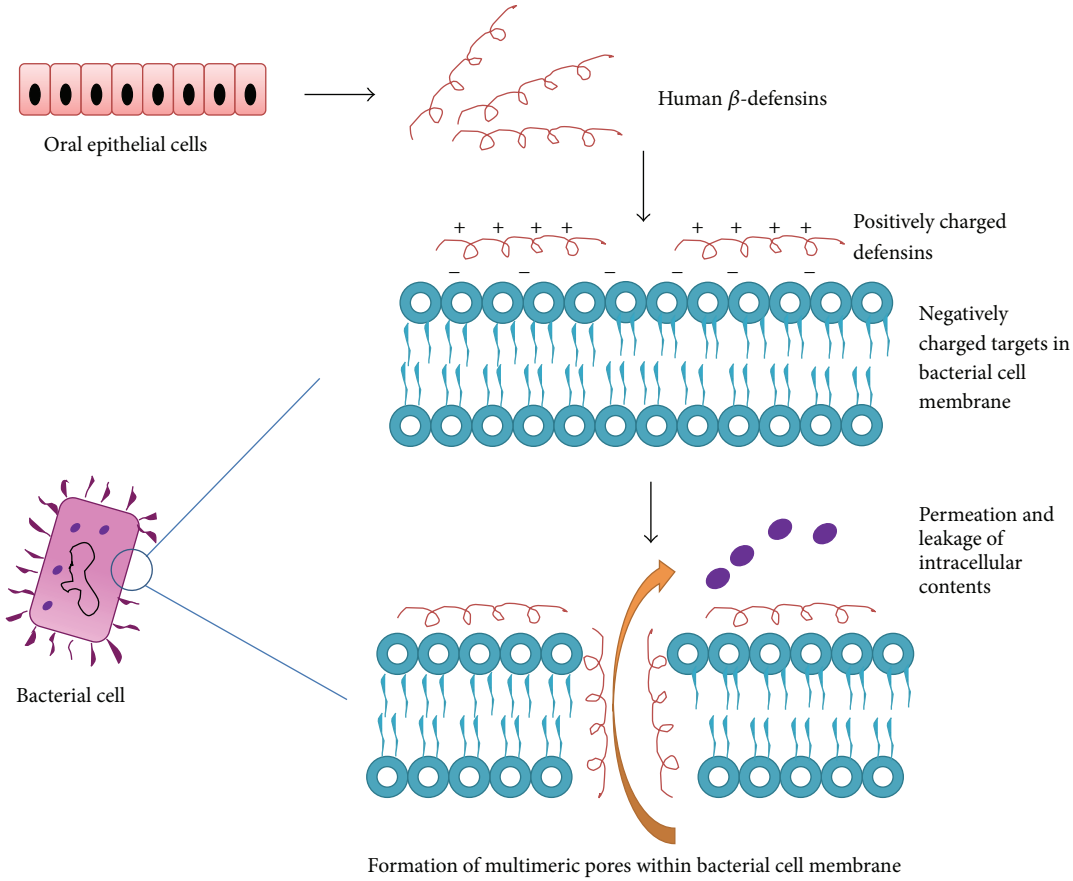

Figure 1: Mechanism of antimicrobial action of human beta-defensins.

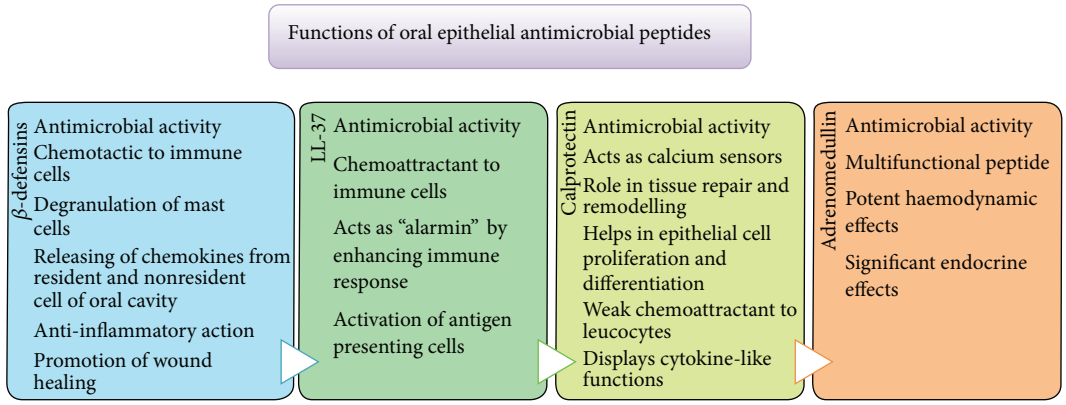

Figure 2: Functions of oral epithelial antimicrobial peptides.

cells which are antigen presenting cells within the epithelium. Also, hBD-1 and hBD-2 act as a chemoattractant for T cells and dendritic cells [54].

4. Human Cathelicidin (LL-37)

Cathelicidin family of antimicrobial peptides consists of a "cathelin" domain at their N terminus and a mature peptide at their C terminus [55]. The amino acid sequence of the cathelin domain is highly conserved and thereby similarity is observed despite species or cells it is obtained from [56]. The mature peptide however demonstrates considerable variation in its size, amino acid sequence, and three-dimensional structures [57]. The cathelin domain derives its name from a porcine neutrophil protein of the same name, since both share sequence homology [57]. The only antimicrobial peptide of Cathelicidin family expressed in humans is LL-37. It was cloned from human bone marrow cDNA and derives its name from its length of 37 amino acids with two leucine residues in the beginning (Table 2) [58]. It is a cationic antimicrobial

peptide of 18 Kda size, therefore also termed hCAP 18 [59]. It is expressed in epithelial cells lining the respiratory, gastrointestinal, and urogenital tract as well as oral cavity, although its main source in oral cavity is from neutrophilic granules and to a lesser extent from epithelial cells [18]. In oral cavity, LL-37 is expressed in inflamed gingival tissues, buccal mucosa, and tongue epithelium [60]. It has also been identified in saliva and GCF [61, 62]. The concentration of LL-37 is found to increase with increasing depth of gingival sulcus [63]. It has also been proposed that LL-37 detected in gingival epithelium may be the product of neutrophil migration through gingival epithelium rather than epithelial cells themselves [18].

4.1. Gene Encoding LL-37. The gene encoding LL-37 is located on chromosome 3 at location 3p21.3. It has been named cathelicidin antimicrobial peptide (CAMP) gene. LL-37 gene has four exons and three introns. First three exons encode for the signal sequence and cathelin region of the peptide while the fourth exon translates into mature peptide [54]. In intron and promoter region of LL-37, there exist binding sites for acute phase response factors, which establish the upregulation of LL-37 in inflammation [64].

4.2. Induction of Gene Expression. LL-37 expression in various cell types has been found to be upregulated on exposure to growth factors, differentiating agents, and microorganisms. Insulin-like growth factor-1 which is known to promote wound healing upregulates LL-37 expression [65]. Also, vitamin D which is a differentiating agent has been found to amplify LL-37 activity [66]. Increased level of LL-37 in gingival tissues in response to inflammation correlates positively with depth of gingival crevice [63]. In a comparative study, levels of LL-37 in GCF were found to be significantly elevated in chronic periodontitis patients than in gingivitis patients and healthy volunteers [67].

4.3. Structure. The cathelicidin gene in humans is translated into an inactive precursor protein termed as hCAP-18. Upon posttranslational processing an active C terminus peptide with 37 amino acids is released from precursor protein. This cleavage is carried out via proteolytic enzyme elastase or proteinase-3 [68, 69]. This peptide has a net positive charge at physiologic pH and more than 50% of its residues are hydrophilic in nature. Structurally, it exists as a random coil in aqueous solutions. Many of its amino acids form intramolecular hydrogen bonds, acquiring an α-helix secondary structure. It is supposed that antibacterial activity of LL-37 is correlated with α-helicity [70].

4.4. Antimicrobial Activity. Found in saliva in a concentration of 0.14–3 μg/mL, LL-37 is active against both Gram-negative and Gram-positive bacteria including established periopathogens *P. gingivalis* and *A. actinomycetemcomitans* [71]. Its MIC was 30–60 μg/mL and 125 μg/mL against *A. actinomycetemcomitans* and *P. gingivalis*, respectively [37]. LL-37 maintains its antimicrobial activity even in presence of gingipains with some salivary components providing it with protection [72]. It is ascertained that LL-37 inhibits the inflammatory response in gingival fibroblasts to *P. gingivalis* and its products [73]. LL-37 binds directly to bacterial lipopolysaccharide [59]. Morbus Kostmann syndrome is a genetic form of periodontal disease which was shown to have a near absence of LL-37 [74]. This suggests the importance of LL-37 as an antimicrobial peptide in oral cavity.

4.5. Mechanism of Antimicrobial Action. LL-37 belongs to the category of amphiphatic α-helical antimicrobial peptides. It has been proposed that these peptides do not function via formation of transmembrane pores within the lipid biolayers of microbial cell membrane as is the case with defensins. Although the exact mechanism of action of Cathelicidins is not clear, some members of this family overlap the bacterial cell membrane in a carpet-like manner and dissolve it similar to a detergent by micelles formation [75] (Figure 3).

4.6. Other Roles. LL-37 acts as a chemoattractant and causes influx of neutrophils, monocytes, and T cells to the site of inflammation [76]. Some researchers believe that it acts as an "alarmin" rather than an antimicrobial by enhancing the immune response leading to activation of antigen presenting cells [77]. The major roles of LL-37 are described in Figure 2.

5. Calprotectin

Calprotectin, also known as calgranulin, is a heterodimer of two anionic peptides MRP8 and MRP14 [78]. Several nomenclatures have been proposed for these individual peptides as well as calprotectin dimer but controversy regarding nomenclature still exists. These belong to S100 family of calcium binding proteins [79]. Calprotectin has been found to be constitutively expressed in cells of immune function such as neutrophils, monocytes, macrophages, and epithelial cells [80–82]. Its level increases in plasma, saliva, and synovial fluid during infectious and inflammatory diseases [83–85]. Also, it is highly responsive to any kind of stress in epidermal cells [86].

5.1. Gene Encoding for Calprotectin. The HGNC approved names of genes encoding for MRP8 and MRP14 are S100A8 and S100A9, respectively. The genes are from a closely located cluster of thirteen genes on chromosome 1 at location 1q21 [87]. The organization of these genes is evolutionarily conserved. Both of these peptides have three exons separated by two introns. Exon 1 is untranslated, whereas exons 2 and 3 encode into an N-terminal and a C-terminal EF hand motif, respectively [88].

5.2. Induction of Genetic Expression. Calprotectin expression is upregulated in epithelial cells upon induction of stress and exposure to ultraviolet radiation and in wound healing [86]. Also, its level heightens via exposure to complement factor C5a and proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 [89]. Calprotectin levels have been found to positively correlate with severity of periodontitis in gingival crevicular fluid (GCF) [90]. The origin of calprotectin in GCF is from sulcular epithelium as well as immune cells. Calprotectin expression amplifies with increased pocket depth

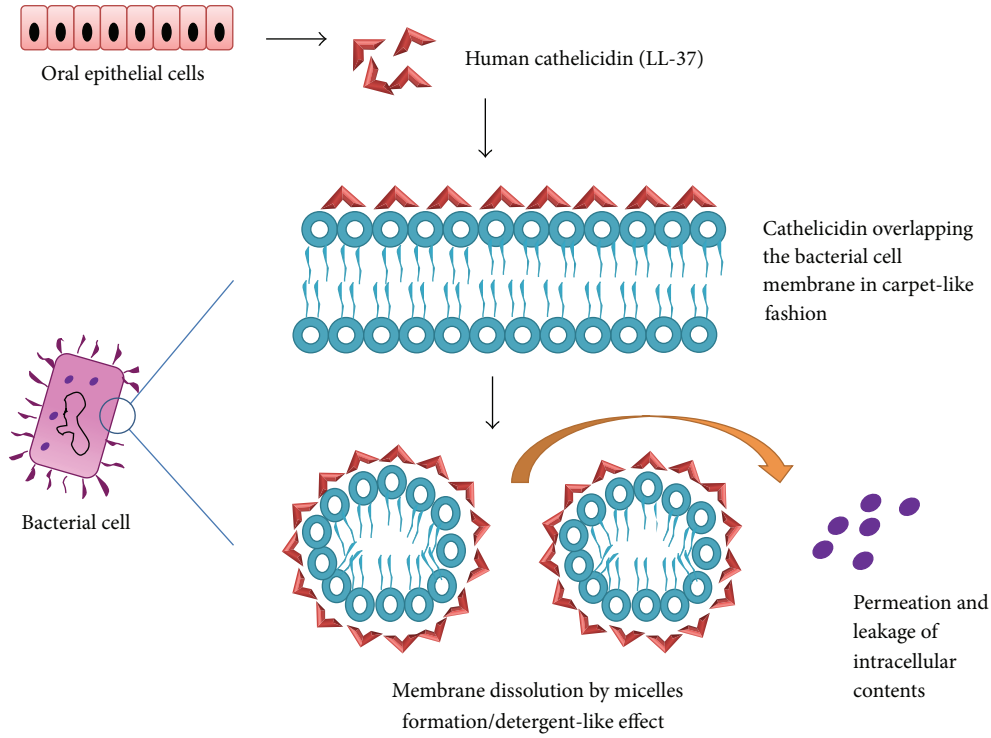

Figure 3: Mechanism of antimicrobial action of human cathelicidin (LL-37).

as well as with increasing levels of aspartate aminotransferase, IL-1β, prostaglandin E2, and collagenase in GCF [91]. Immunohistochemical analysis reveals its increased expression in pocket epithelium of periodontitis patients [92].

5.3. Structure. Calprotectin exists as a noncovalently complexed heterodimer of two calcium and zinc binding proteins MRP8 and MRP14. Calprotectin is a 36.5 KDa molecule with two subunits: 8.3 KDa light chain MRP8 and 13.3 KDa heavy chain MRP14. MRP8 is a 93-amino-acid-long chain whereas MRP14 has 114 amino acids, respectively (Table 2). Each monomer has two helix-loop-helix calcium ion (Ca^{2+}) binding domains, EF hands and a hinge region separating two hydrophobic regions [93]. The C-terminal region contains a zinc ion (Zn^{2+}) binding site as well as a phosphorylation site [94].

5.4. Antimicrobial Activity. Calprotectin is present in a concentration of 22 mg/L in stimulated whole saliva whereas its concentration in parotid saliva is around 3.2 mg/L [95]. The biostatic activity of calprotectin has been demonstrated in few bacteria such as *Staphylococcus aureus*, *S. epidermis*, and *Escherichia coli*. Against these organisms, calprotectin has a MIC of 64–256 mg/L. For *Cryptococcus neoformans*, the MIC was found to be 4–128 mg/L and 2–4 times this concentration was fungicidal [96]. It was observed in an *in vitro* experiment that calprotectin when expressed in oral epithelial cells confers protection against invasion by *P. gingivalis*, an established periodontal pathogen [97]. Its antifungal activity against *C. albicans* is also beneficial in maintaining oral health [98]. Similarly, increased calprotectin expression has been detected in oral keratinocytes infected with Epstein-Barr virus and herpes simplex virus [99].

5.5. Mechanism of Antimicrobial Action. The antimicrobial activity of calprotectin can be attributed to its trace metal binding activity. As discussed before, calprotectin has sites for binding of calcium and zinc ions as well as other dipositively charged trace metal ions such as copper and manganese [100]. Whenever epithelial cells are encountered with microbial insults, calprotectin is released into the interstitial milieu. There it may act via chelation of zinc or other divalent ions. These ions are essential for usual microbial functioning; thus, calprotectin provides a growth inhibitory type of host defence as an antimicrobial agent [101].

5.6. Other Roles. Calprotectin is termed as calcium sensor together with other S100 proteins as they change their conformation in response to calcium influx [102]. It appears to have a role in tissue repair and remodeling. It has been hypothesized that calprotectin participates in epithelial cell proliferation as well as differentiation [103]. It acts as a chemoattractant for leucocytes although being weak [104]. They display cytokine-like functions and act as ligand for toll-like receptor- (TLR-) 4 and receptor for advanced glycated end products (RAGE) [105]. Figure 2 summarizes various roles of calprotectin.

6. Adrenomedullin

Adrenomedullin belongs to the category of regulatory peptides with a wide array of biological action. Adrenomedullin was first purified and sequenced in 1993 from many peptides

extracted from pheochromocytoma of a Japanese patient. It was termed adrenomedullin since it was derived from adrenal medulla [106]. But now it is known that it is a ubiquitous peptide with many cell types producing the peptide. Since the time of its discovery, adrenomedullin has been measured in various diseases such as cardiovascular, liver and renal diseases, and preeclampsia [107, 108]. It is believed that rise in levels of adrenomedullin is actually a consequence rather than cause of pathology.

6.1. Gene Encoding Adrenomedullin. Adrenomedullin is expressed in many cells including adrenal medulla, kidney, and lung as well as epithelial lining of skin, gut, and oral cavity [109, 110]. It is produced as a precursor molecule preproadrenomedullin. The gene encoding for preproadrenomedullin is termed adrenomedullin (ADM) gene which is located on chromosome 11. This gene is composed of four exons and three introns. There are binding sites for NF-$\kappa\beta$ on promoter region of this gene [111]. Preproadrenomedullin is 185 amino acids long, with a 21-amino-acid-long N terminal signal peptide and a 20-amino-acid-long amidated peptide [106]. It has also been hypothesized that another biologically active peptide adrenotensin may also be product of adrenomedullin gene. Adrenotensin is proteolytic product of the adrenomedullin precursor from amino acids 153–185. It is believed to have opposite actions to that of adrenomedullin [112].

6.2. Induction of Genetic Expression. Adrenomedullin is constitutively expressed and secreted by the epithelial cells of the oral cavity. The expression is further induced when epithelial cells come in contact with microbes. Proinflammatory cytokines such as IL-1 and TNF-α also tend to upregulate the expression of adrenomedullin gene. Lipopolysaccharide also provides a potent stimulus to its secretion. Thus, its induction via lipopolysaccharide and cytokines proves its significant role in infection and immunity [113, 114].

6.3. Structure. Human adrenomedullin peptide is 52 amino acids long (Table 2) after posttransnational changes in preproadrenomedullin. It has a single disulphide bridge in between the residues at 16 and 21. Also, it has an amidated tyrosine at the carboxy terminus. Since structurally adrenomedullin peptide shares homology with calcitonin gene related peptide, it has been included into calcitonin peptide family [106].

6.4. Antimicrobial Action. They share some functional parallels with the β-defensins despite the fact that they are encoded via different genes which translate into structurally different proteins. Its salivary concentration is around 55–65 pg/mL [115]. It acts against both Gram-positive and Gram-negative bacteria of the oral cavity. The MIC of adrenomedullin is around 500 pmol/L against *P. gingivalis* while it is 12.5 μg/mL against *E. coli* [115, 116]. Little is known about its antiviral activity but adrenomedullin seems to lack antifungal action [110].

6.5. Mechanism of Antimicrobial Action. Adrenomedullin shares its mechanism of antimicrobial activity with other cationic antimicrobial peptides. Although exact mechanism is still not elucidated, it is thought to promote intramembranous pore formation in bacterial cell membrane. With the disruption of bacterial cell membrane, there is stoppage of critical intracellular processes and finally cell death [116].

6.6. Other Roles. Adrenomedullin is a multifunctional peptide with a wide array of roles (Figure 2). It has potent haemodynamic effects resulting in a sustained hypotension from markedly reduced peripheral resistance [117]. It is also believed to be potent vasodilator in uterine circulation [118]. It has significant endocrine effects where it has a role in inhibiting ACTH release from pituitary, effecting secretory activity of adrenal cortex along with consequence on insulin secretion from the pancreas [119–121].

7. Probiotics and EAPs

Millions of microbes reside within the oral cavity and gastrointestinal track of humans. These microbes coexist with the host and are harmful to the host only when the immunity of the host is altered or there is loss of sensing and defense mechanisms of epithelial lining. These commensal bacteria are known to possess immunomodulatory capacities [122, 123]. Also, they prevent the colonization of host by pathogenic microorganisms [124]. Identification of these endogenous bacteria and their benefits has led to the development of "probiotics." Probiotics are viable bacteria that are administered to host to competitively populate the host sites and confer health benefits. This modality is being applied for the treatment and prevention of many infectious and inflammatory diseases [125]. Such organisms include *Streptococcus salivarius*, *Lactobacillus* spp., and *Bifidobacterium* spp.; it has always been an area of speculation as to how epithelial tissues interact with commensal bacteria and/or probiotics and how they differentiate between pathogenic and nonpathogenic bacteria. Several studies have been conducted to elaborate on the interaction of probiotic organisms with epithelium. It has been suggested that there are different signaling pathways that are initiated by probiotic and pathogenic bacteria [126, 127]. The probiotic bacteria carry out immunomodulation via three ways: via an alteration in toll-like receptor (TLR) signaling, through inhibition of NF-$\kappa\beta$ pathway, and via release of interleukin-10 cytokine [128–131]. With these mechanisms, probiotics block the proinflammatory pathways and also the production of EAPs and are protected against host defence. Thus, probiotics not only are well tolerated by host but also promote oral epithelial health, integrity, and homeostasis [132].

8. Future Perspective

Since the oral epithelial antimicrobial peptides are produced locally against the oral microbes and are potent in countering such insults, they are under investigations for control of oral infections. These peptides operate not only by keeping the commensal organisms in check but also by acting against pathogenic microbiota too. Thus, a systematic insight into their function, mechanism of action, and potential side

effects is essential to develop them into therapeutic agents to counter oral infections such as periodontitis. The levels of these antimicrobial peptides are found to increase locally in periodontitis, and their external application may provide protection from progression of disease. Oral EAPs can be developed in a variety of ways for their therapeutic benefits. These can be produced in form of gels, mouthwashes, and gum paints for local application over periodontal tissues to not only prevent the development of periodontal disease but also reverse the existing disease. EAPs can also be developed as local drug delivery agents within the periodontal pockets to curtail the effects of periopathogenic bacteria. Because of their antiviral and antifungal effects, EAPs can be used in immunocompromised individuals against opportunistic infections such as candidiasis and herpetic gingivostomatitis. The activity of β-defensins against HIV holds potential for drugs that are more efficacious with lesser side effects than existing drugs for AIDS. Iseganan hydrochloride, a synthetic cathelicidin, is under investigation for prevention of ulcerative oral mucositis and has shown promising results [133]. Iseganan hydrochloride is the salt of 17-amino-acid-long synthetic protegrin-1, an 18-amino-acid antimicrobial peptide isolated from porcine leucocyte. Iseganan HCl is available as oral solution by the name of IB-367 rinse and is a broad spectrum antimicrobial and acts rapidly by disrupting cell membranes of microorganisms including bacteria, fungi, and viruses [134].

Epithelial cells are believed to behave differently when exposed to commensal and pathogenic bacteria. This could mean that antimicrobial peptide secreted from epithelial cells may have differential action against commensals and pathogens. In view of the fact that these antimicrobial peptides are broad spectrum and rapidly acting, they provide little chance of development of resistance in microbes against them. This property could be fruitful in developing novel therapeutic agents that lack resistance compared to conventional antibiotics. Finally, these antimicrobial peptides could be developed as biomarkers for oral disease diagnosis and prognosis. Therefore, there remains a whole unexplored world of therapeutic benefits of epithelial antimicrobial peptides which needs to be ventured into.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] M. T. Pöllänen, J. I. Salonen, and V.-J. Uitto, "Structure and function of the tooth-epithelial interface in health and disease," *Periodontology 2000*, vol. 31, no. 1, pp. 12–31, 2003.

[2] H. E. Schroeder and M. A. Listgarten, "The gingival tissues: The architecture of periodontal protection," *Periodontology 2000*, vol. 14, no. 1, pp. 91–120, 1997.

[3] M. S. Tonetti, "Molecular factors associated with compartmentalization of gingival immune responses and transepithelial neutrophil migration," *Journal of Periodontal Research*, vol. 32, no. 1, pp. 104–109, 1997.

[4] B. A. Dale, "Periodontal epithelium: A newly recognized role in health and disease," *Periodontology 2000*, vol. 30, no. 1, pp. 70–78, 2002.

[5] G. Diamond, N. Beckloff, and L. K. Ryan, "Host defense peptides in the oral cavity and the lung: similarities and differences," *Journal of Dental Research*, vol. 87, no. 10, pp. 915–927, 2008.

[6] T. Ganz, M. E. Selsted, D. Szklarek et al., "Defensins. Natural peptide antibiotics of human neutrophils," *The Journal of Clinical Investigation*, vol. 76, no. 4, pp. 1427–1435, 1985.

[7] F. G. Oppenheim, T. Xu, F. M. McMillian et al., "Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on Candida albicans," *Journal of Biological Chemistry*, vol. 263, no. 16, pp. 7472–7477, 1988.

[8] A. Weinberg, S. Krisanaprakornkit, and B. A. Dale, "Epithelial antimicrobial peptides: review and significance for oral applications," *Critical Reviews in Oral Biology and Medicine*, vol. 9, no. 4, pp. 399–414, 1998.

[9] H. G. Boman, "Cecropins : antibacterial peptides from insects and pigs," in *Phylogenetic Perspectives in Immunity: The Insect-Host Defense*, J. Hoffmann, S. Natori, and C. Janeway, Eds., pp. 24–37, Landes Biomed, Austin, Tex, USA, 1994.

[10] M. Zasloff, "Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 15, pp. 5449–5453, 1987.

[11] D. Romeo, B. Skerlavaj, M. Bolognesi, and R. Gennaro, "Structure and bactericidal activity of an antibiotic dodecapeptide purified from bovine neutrophils," *Journal of Biological Chemistry*, vol. 263, no. 20, pp. 9573–9575, 1988.

[12] B. S. Schonwetter, E. D. Stolzenberg, and M. A. Zasloff, "Epithelial antibiotics induced at sites of inflammation," *Science*, vol. 267, no. 5204, pp. 1645–1648, 1995.

[13] B. A. Dale and S. Krisanaprakornkit, "Defensin antimicrobial peptides in the oral cavity," *Journal of Oral Pathology and Medicine*, vol. 30, no. 6, pp. 321–327, 2001.

[14] M. Mathews, H. P. Jia, J. M. Guthmiller et al., "Production of β-defensin antimicrobial peptides by the oral mucosa and salivary glands," *Infection and Immunity*, vol. 67, no. 6, pp. 2740–2745, 1999.

[15] G. Diamond, M. Zasloff, H. Eck, M. Brasseur, W. Lee Maloy, and C. L. Bevins, "Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: peptide isolation and cloning of a cDNA," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 9, pp. 3952–3956, 1991.

[16] B. L. Kagan, T. Ganz, and R. I. Lehrer, "Defensins: a family of antimicrobial and cytotoxic peptides," *Toxicology*, vol. 87, no. 1–3, pp. 131–149, 1994.

[17] Y. Abiko, M. Saitoh, M. Nishimura, M. Yamazaki, D. Sawamura, and T. Kaku, "Role of β-defensins in oral epithelial health and disease," *Medical Molecular Morphology*, vol. 40, no. 4, pp. 179–184, 2007.

[18] B. A. Dale, J. R. Kimball, S. Krisanaprakornkit et al., "Localized antimicrobial peptide expression in human gingiva," *Journal of Periodontal Research*, vol. 36, no. 5, pp. 285–294, 2001.

[19] Q. Lu, L. P. Samaranayake, R. P. Darveau, and L. Jin, "Expression of human β-defensin-3 in gingival epithelia," *Journal of Periodontal Research*, vol. 40, no. 6, pp. 474–481, 2005.

[20] S. Krisanaprakornkit, A. Weinberg, C. N. Perez, and B. A. Dale, "Expression of the peptide antibiotic human β-defensin 1 in cultured gingival epithelial cells and gingival tissue," *Infection and Immunity*, vol. 66, no. 9, pp. 4222–4228, 1998.

[21] P. Premratanachai, S. Joly, G. K. Johnson, P. B. McCray Jr., H. P. Jia, and J. M. Guthmiller, "Expression and regulation of novel human β-defensins in gingival keratinocytes," *Oral Microbiology and Immunology*, vol. 19, no. 2, pp. 111–117, 2004.

[22] K. W. Bensch, M. Raida, H. J. Mägert, P. Schulz-Knappe, and W. G. Forssmann, "hBD-1: a novel β-defensin from human plasma," *FEBS Letters*, vol. 368, no. 2, pp. 331–335, 1995.

[23] L. Liu, C. Zhao, H. H. Q. Heng, and T. Ganz, "The human β-defensin-1 and α-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry," *Genomics*, vol. 43, no. 3, pp. 316–320, 1997.

[24] T. Ganz, "Defensins: antimicrobial peptides of innate immunity," *Nature Reviews Immunology*, vol. 3, no. 9, pp. 710–720, 2003.

[25] J. Harder, J. Bartels, E. Christophers, and J. M. Schröder, "A peptide antibiotic from human skin," *Nature*, vol. 387, no. 6636, p. 861, 1997.

[26] J. Harder, R. Siebert, Y. Zhang et al., "Mapping of the gene encoding human β-defensin-2 (DEFB2) to chromosome region 8p22-p23.1," *Genomics*, vol. 46, no. 3, pp. 472–475, 1997.

[27] H. P. Jia, B. C. Schutte, A. Schudy et al., "Discovery of new human β-defensins using a genomics-based approach," *Gene*, vol. 263, no. 1-2, pp. 211–218, 2001.

[28] J. Harder, J. Bartels, E. Christophers, and J.-M. Schröder, "Isolation and characterization of human betadefensin-3, a novel human inducible peptide antibiotic," *The Journal of Biological Chemistry*, vol. 276, no. 8, pp. 5707–5713, 2001.

[29] S. Offenbacher, S. P. Barros, D. W. Paquette et al., "Gingival transcriptome patterns during induction and resolution of experimental gingivitis in humans," *Journal of Periodontology*, vol. 80, no. 12, pp. 1963–1982, 2009.

[30] Q. Lu, L. Jin, R. P. Darveau, and L. P. Samaranayake, "Expression of human β-defensins-1 and -2 peptides in unresolved chronic periodontitis," *Journal of Periodontal Research*, vol. 39, no. 4, pp. 221–227, 2004.

[31] G. Diamond, N. Beckloff, A. Weinberg, and K. O. Kisich, "The roles of antimicrobial peptides in innate host defense," *Current Pharmaceutical Design*, vol. 15, no. 21, pp. 2377–2392, 2009.

[32] H. R. Conti, F. Shen, N. Nayyar et al., "Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis," *Journal of Experimental Medicine*, vol. 206, no. 2, pp. 299–311, 2009.

[33] M. E. Selsted, Y.-Q. Tang, W. L. Morris et al., "Purification, primary structures, and antibacterial activities of β-defensins, a new family of antimicrobial peptides from bovine neutrophils," *The Journal of Biological Chemistry*, vol. 268, no. 9, pp. 6641–6648, 1993.

[34] M. S. Gardner, M. D. Rowland, A. Y. Siu, J. L. Bundy, D. K. Wagener, and J. L. Stephenson Jr., "Comprehensive defensin assay for Saliva," *Analytical Chemistry*, vol. 81, no. 2, pp. 557–566, 2009.

[35] R. Tao, R. J. Jurevic, K. K. Coulton et al., "Salivary antimicrobial peptide expression and dental caries experience in children," *Antimicrobial Agents and Chemotherapy*, vol. 49, no. 9, pp. 3883–3888, 2005.

[36] S. Joly, C. Maze, P. B. McCray Jr., and J. M. Guthmiller, "Human β- defensins 2 and demonstrate strain selective activity against oral microorganisms," *Journal of Clinical Microbiology*, vol. 42, no. 3, pp. 1024–1029, 2004.

[37] S. Ji, J. Hyun, E. Park, B.-L. Lee, K.-K. Kim, and Y. Choi, "Susceptibility of various oral bacteria to antimicrobial peptides and to phagocytosis by neutrophils," *Journal of Periodontal Research*, vol. 42, no. 5, pp. 410–419, 2007.

[38] Z. Feng, B. Jiang, J. Chandra, M. Ghannoum, S. Nelson, and A. Weinberg, "Human beta-defensins: differential activity against candidal species and regulation by *Candida albicans*," *Journal of Dental Research*, vol. 84, no. 5, pp. 445–450, 2005.

[39] Z. Feng, G. R. Dubyak, M. M. Lederman, and A. Weinberg, "Cutting edge: human β defensin 3—a novel antagonist of the HIV-1 coreceptor CXCR4," *The Journal of Immunology*, vol. 177, no. 2, pp. 782–786, 2006.

[40] S. Ji, J. E. Shin, Y. C. Kim, and Y. Choi, "Intracellular degradation of *Fusobacterium nucleatum* in human gingival epithelial cells," *Molecules and Cells*, vol. 30, no. 6, pp. 519–526, 2010.

[41] K. Ouhara, H. Komatsuzawa, S. Yamada et al., "Susceptibilities of periodontopathogenic and cariogenic bacteria to antibacterial peptides, β-defensins and LL37, produced by human epithelial cells," *Journal of Antimicrobial Chemotherapy*, vol. 55, no. 6, pp. 888–896, 2005.

[42] C. E. Shelburne, W. A. Coulter, D. Olguin, M. S. Lantz, and D. E. Lopatin, "Induction of β-defensin resistance in the oral anaerobe *Porphyromonas gingivalis*," *Antimicrobial Agents and Chemotherapy*, vol. 49, no. 1, pp. 183–187, 2005.

[43] J. E. Shin and Y. Choi, "*Treponema denticola* suppresses expression of human β-defensin-2 in gingival epithelial cells through inhibition of TNFα production and TLR2 activation," *Molecules and cells*, vol. 29, no. 4, pp. 407–412, 2010.

[44] Y. Agawa, S. Lee, S. Ono et al., "Interaction with phospholipid bilayers, ion channel formation, and antimicrobial activity of basic amphipathic α-helical model peptides of various chain lengths," *Journal of Biological Chemistry*, vol. 266, no. 30, pp. 20218–20222, 1991.

[45] A. M. Cole, T. Ganz, A. M. Liese, M. D. Burdick, L. Liu, and R. M. Strieter, "Cutting edge: IFN-inducible ELR- CXC chemokines display defensin-like antimicrobial activity," *Journal of Immunology*, vol. 167, no. 2, pp. 623–627, 2001.

[46] D. Yang, A. Biragyn, L. W. Kwak, and J. J. Oppenheim, "Mammalian defensins in immunity: more than just microbicidal," *Trends in Immunology*, vol. 23, no. 6, pp. 291–296, 2002.

[47] D. Yang, O. Chertov, S. N. Bykovskaia et al., "β-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6," *Science*, vol. 286, no. 5439, pp. 525–528, 1999.

[48] F. Niyonsaba, H. Ogawa, and I. Nagaoka, "Human β-defensin-2 functions as a chemotactic agent for tumour necrosis factor-α-treated human neutrophils," *Immunology*, vol. 111, no. 3, pp. 273–281, 2004.

[49] F. Niyonsaba, A. Someya, M. Hirata, H. Ogawa, and I. Nagaoka, "Evaluation of the effects of peptide antibiotics human β-defensins-1/-2 and LL-37 on histamine release and prostaglandin D_2 production from mast cells," *European Journal of Immunology*, vol. 31, no. 4, pp. 1066–1075, 2001.

[50] F. Niyonsaba, H. Ushio, N. Nakano et al., "Antimicrobial peptides human β-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines," *Journal of Investigative Dermatology*, vol. 127, no. 3, pp. 594–604, 2007.

[51] K. G. Kohlgraf, L. C. Pingel, D. E. Dietrich, and K. A. Brogden, "Defensins as anti-inflammatory compounds and mucosal adjuvants," *Future Microbiology*, vol. 5, no. 1, pp. 99–113, 2010.

[52] F. Semple, S. Webb, H.-N. Li et al., "Human β-defensin 3 has immunosuppressive activity in vitro and in vivo," *European Journal of Immunology*, vol. 40, no. 4, pp. 1073–1078, 2010.

[53] O. E. Sorensen, J. B. Cowland, K. Theilgaard-Mönch, L. Liu, T. Ganz, and N. Borregaard, "Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors," *The Journal of Immunology*, vol. 170, no. 11, pp. 5583–5589, 2003.

[54] D. Yang, A. Biragyn, D. M. Hoover, J. Lubkowski, and J. J. Oppenheim, "Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense," *Annual Review of Immunology*, vol. 22, pp. 181–215, 2004.

[55] B. Ramanathan, E. G. Davis, C. R. Ross, and F. Blecha, "Cathelicidins: microbicidal activity, mechanisms of action, and roles in innate immunity," *Microbes and Infection*, vol. 4, no. 3, pp. 361–372, 2002.

[56] M. Zaiou and R. L. Gallo, "Cathelicidins, essential gene-encoded mammalian antibiotics," *Journal of Molecular Medicine*, vol. 80, no. 9, pp. 549–561, 2002.

[57] R. Gennaro and M. Zanetti, "Structural features and biological activities of the cathelicidin-derived antimicrobial peptides," *Biopolymers*, vol. 55, pp. 31–49, 2000.

[58] G. H. Gudmundsson, B. Agerberth, J. Odeberg, T. Bergman, B. Olsson, and R. Salcedo, "The human gene *FALL39* and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes," *European Journal of Biochemistry*, vol. 238, no. 2, pp. 325–332, 1996.

[59] J. W. Larrick, M. Hirata, R. F. Balint, J. Lee, J. Zhong, and S. C. Wright, "Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein," *Infection and Immunity*, vol. 63, no. 4, pp. 1291–1297, 1995.

[60] M. F. Nilsson, B. Sandstedt, O. Sørensen, G. Weber, N. Borregaard, and M. Stahle-Backdahl, "The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6," *Infection and Immunity*, vol. 67, no. 5, pp. 2561–2566, 1999.

[61] M. Murakami, T. Ohtake, R. A. Dorschner, and R. L. Gallo, "Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva," *Journal of Dental Research*, vol. 81, no. 12, pp. 845–850, 2002.

[62] O. Turkoglu, G. Emingil, N. Kutukçuler, and G. Atilla, "Gingival crevicular fluid levels of cathelicidin ll-37 and interleukin-18 in patients with chronic periodontitis," *Journal of Periodontology*, vol. 80, no. 6, pp. 969–976, 2009.

[63] I. Hosokawa, Y. Hosokawa, H. Komatsuzawa et al., "Innate immune peptide LL-37 displays distinct expression pattern from beta-defensins in inflamed gingival tissue," *Clinical and Experimental Immunology*, vol. 146, no. 2, pp. 218–225, 2006.

[64] M. Zanetti, R. Gennaro, M. Scocchi, and B. Skerlavaj, "Structure and biology of cathelicidins," *Advances in Experimental Medicine and Biology*, vol. 479, pp. 203–218, 2000.

[65] O. E. Sorensen, J. B. Cowland, K. Theilgaard-Mönch, L. Liu, T. Ganz, and N. Borregaard, "Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors," *Journal of Immunology*, vol. 170, no. 11, pp. 5583–5589, 2003.

[66] G. Weber, J. D. Heilborn, C. I. C. Jimenez, A. Hammarsjö, H. Törmä, and M. Ståhle, "Vitamin D induces the antimicrobial protein hCAP18 in human skin," *Journal of Investigative Dermatology*, vol. 124, no. 5, pp. 1080–1082, 2005.

[67] O. Türkoğlu, G. Emingil, N. Kütükcxüler, and G. Atilla, "Gingival crevicular fluid levels of cathelicidin ll-37 and interleukin-18 in patients with chronic periodontitis," *Journal of Periodontology*, vol. 80, no. 6, pp. 969–976, 2009.

[68] A. Panyutich, J. Shi, P. L. Boutz, C. Zhao, and T. Ganz, "Porcine polymorphonuclear leukocytes generate extracellular microbicidal activity by elastase-mediated activation of secreted proprotegrins," *Infection and Immunity*, vol. 65, no. 3, pp. 978–985, 1997.

[69] O. E. Sorensen, P. Follin, A. H. Johnsen et al., "Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3," *Blood*, vol. 97, no. 12, pp. 3951–3959, 2001.

[70] V. Nizet and R. L. Gallo, "Cathelicidins and innate defense against invasive bacterial infection," *Scandinavian Journal of Infectious Diseases*, vol. 35, no. 9, pp. 670–676, 2003.

[71] G. Bachrach, G. Chaushu, M. Zigmond et al., "Salivary LL-37 secretion in individuals with down syndrome is normal," *Journal of Dental Research*, vol. 85, no. 10, pp. 933–936, 2006.

[72] M. Gutner, S. Chaushu, D. Balter, and G. Bachrach, "Saliva enables the antimicrobial activity of LL-37 in the presence of proteases of *Porphyromonas gingivalis*," *Infection and Immunity*, vol. 77, no. 12, pp. 5558–5563, 2009.

[73] M. Inomata, T. Into, and Y. Murakami, "Suppressive effect of the antimicrobial peptide LL-37 on expression of IL-6, IL-8 and CXCL10 induced by Porphyromonas gingivalis cells and extracts in human gingival fibroblasts," *European Journal of Oral Sciences*, vol. 118, no. 6, pp. 574–581, 2010.

[74] K. Pütsep, G. Carlsson, H. G. Boman, and M. Andersson, "Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study," *The Lancet*, vol. 360, no. 9340, pp. 1144–1149, 2002.

[75] Z. Oren and Y. Shai, "Selective lysis of bacteria but not mammalian cells by diastereomers of melittin: structure-function study," *Biochemistry*, vol. 36, no. 7, pp. 1826–1835, 1997.

[76] O. Chertov, D. F. Michiel, L. Xu et al., "Identification of defensin-1, defensin-2, and CAP37/azurocidin as T-cell chemoattractant proteins released from interleukin-8-stimulated neutrophils," *Journal of Biological Chemistry*, vol. 271, no. 6, pp. 2935–2940, 1996.

[77] D. Yang and J. J. Oppenheim, "Alarmins and antimicrobial immunity," *Medical Mycology*, vol. 47, pp. S146–S153, 2009.

[78] C. Pröpper, X. Huang, J. Roth, C. Sorg, and W. Nacken, "Analysis of the MRP8-MRP14 protein-protein interaction by the two- hybrid system suggests a prominent role of the C-terminal domain of S100 proteins in dimer formation," *Journal of Biological Chemistry*, vol. 274, no. 1, pp. 183–188, 1999.

[79] J. R. Dorin, M. Novak, R. E. Hill, D. J. Brock, D. S. Secher, and V. van Heyningen, "A clue to the basic defect in cystic fibrosis from cloning the CF antigen gene," *Nature*, vol. 326, no. 6113, pp. 614–617, 1987.

[80] I. Dale, P. Brandtzaeg, M. K. Fagerhol, and H. Scott, "Distribution of a new myelomonocytic antigen (L1) in human peripheral blood leukocytes. Immunofluorescence and immunoperoxidase staining features in comparison with lysozyme and lactoferrin," *The American Journal of Clinical Pathology*, vol. 84, no. 1, pp. 24–34, 1985.

[81] K. Odink, N. Cerletti, J. Bruggen et al., "Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis," *Nature*, vol. 330, no. 6143, pp. 80–82, 1987.

[82] P. Brandtzaeg, I. Dale, and M. K. Fagerhol, "Distribution of a formalin-resistant myelomonocytic antigen (L1) in human tissues. II. Normal and aberrant occurrence in various epithelia," *The American Journal of Clinical Pathology*, vol. 87, no. 6, pp. 700–707, 1987.

[83] J. Sander, M. K. Fagerhol, J. S. Bakken, and I. Dale, "Plasma levels of the leucocyte L1 protein in febrile conditions: relation to aetiology, number of leucocytes in blood, blood sedimentation reaction and C-reactive protein," *Scandinavian Journal of Clinical & Laboratory Investigation*, vol. 44, no. 4, pp. 357–362, 1984.

[84] M. Cuida, A.-K. Halse, A. C. Johannessen, T. Tynning, and R. Jonsson, "Indicators of salivary gland inflammation in primary Sjögren's syndrome," *European Journal of Oral Sciences*, vol. 105, no. 3, pp. 228–233, 1997.

[85] H. B. Hammer, T. K. Kvien, A. Glennas, and K. Melby, "A longitudinal study of calprotectin as an inflammatory marker in patients with reactive arthritis," *Clinical and Experimental Rheumatology*, vol. 13, no. 1, pp. 59–64, 1995.

[86] C. Marionnet, F. Bernerd, A. Dumas et al., "Modulation of gene expression induced in human epidermis by environmental stress in vivo," *Journal of Investigative Dermatology*, vol. 121, no. 6, pp. 1447–1458, 2003.

[87] S. G. Gregory, K. F. Barlow, K. E. McLay et al., "The DNA sequence and biological annotation of human chromosome 1," *Nature*, vol. 441, pp. 315–321, 2006.

[88] C. Kerkhoff, M. Klempt, and C. Sorg, "Novel insights into structure and function of MRP8 (S100A8) and MRP14 (S100A9)," *Biochimica et Biophysica Acta—Molecular Cell Research*, vol. 1448, no. 2, pp. 200–211, 1998.

[89] D. Benet Bosco Dhas, B. Vishnu Bhat, and D. Bahubali Gane, "Role of calprotectin in infection and inflammation," *Current Pediatric Research*, vol. 16, no. 2, pp. 83–94, 2012.

[90] J.-I. Kido, T. Nakamura, R. Kido et al., "Calprotectin in gingival crevicular fluid correlates with clinical and biochemical markers of periodontal disease," *Journal of Clinical Periodontology*, vol. 26, no. 10, pp. 653–657, 1999.

[91] T. Nakamura, J.-I. Kido, R. Kido et al., "The association of calprotectin level in gingival crevicular fluid with gingival index and the activities of collagenase and aspartate aminotransferase in adult periodontitis patients," *Journal of Periodontology*, vol. 71, no. 3, pp. 361–367, 2000.

[92] R. S. Gómez, P. Langer, M. Pelka, P. von den Driesch, A. C. Johannessen, and M. Simon Jr., "Variational expression of functionally different macrophage markers (27E10, 25F9, RM3/1) in normal gingiva and inflammatory periodontal disease," *Journal of Clinical Periodontology*, vol. 22, no. 5, pp. 341–346, 1995.

[93] P. A. Hessian, J. Edgeworth, and N. Hogg, "MRP-8 and MRP-14, two abundant Ca(2+)-binding proteins of neutrophils and monocytes," *Journal of Leukocyte Biology*, vol. 53, no. 2, pp. 197–204, 1993.

[94] C. W. Heizmann, G. Fritz, and B. W. Schäfer, "S100 proteins: structure, functions and pathology," *Frontiers in Bioscience*, vol. 7, pp. d1356–d1368, 2002.

[95] M. Cuida, J. G. Brun, T. Tynning, and R. Jonsson, "Calprotectin levels in oral fluids: the importance of collection site," *European Journal of Oral Sciences*, vol. 103, no. 1, pp. 8–10, 1995.

[96] M. Steinbakk, C.-F. Naess-Andresen, E. Lingaas, I. Dale, P. Brandtzaeg, and M. K. Fagerhol, "Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin," *The Lancet*, vol. 336, no. 8718, pp. 763–765, 1990.

[97] K. Nisapakultorn, K. F. Ross, and M. C. Herzberg, "Calprotectin expression in vitro by oral epithelial cells confers resistance to infection by Porphyromonas gingivalis," *Infection and Immunity*, vol. 69, no. 7, pp. 4242–4247, 2001.

[98] A. R. K. Murthy, R. I. Lehrer, S. S. L. Harwig, and K. T. Miyasaki, "In vitro candidastatic properties of the human neutrophil calprotectin complex," *Journal of Immunology*, vol. 151, no. 11, pp. 6291–6301, 1993.

[99] L. R. Eversole, K. T. Miyasaki, and R. E. Christensen, "Keratinocyte expression of calprotectin in oral inflammatory mucosal diseases," *Journal of Oral Pathology and Medicine*, vol. 22, no. 7, pp. 303–307, 1993.

[100] S. M. Damo, T. E. Kehl-Fie, N. Sugitani et al., "Molecular basis for manganese sequestration by calprotectin and roles in the innate immune response to invading bacterial pathogens," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 10, pp. 3841–3846, 2013.

[101] P. Brandtzaeg, T.-O. Gabrielsen, I. Dale, F. Muller, M. Steinbakk, and M. K. Fagerhol, "The leucocyte protein L1 (calprotectin): a putative nonspecific defence factor at epithelial surfaces," *Advances in Experimental Medicine and Biology*, vol. 371, pp. 201–206, 1995.

[102] D. B. Zimmer, J. O. Eubanks, D. Ramakrishnan, and M. F. Criscitiello, "Evolution of the S100 family of calcium sensor proteins," *Cell Calcium*, vol. 53, no. 3, pp. 170–179, 2013.

[103] C. Kerkhoff, A. Voss, T. E. Scholzen, M. M. Averill, K. S. Zänker, and K. E. Bornfeldt, "Novel insights into the role of S100A8/A9 in skin biology," *Experimental Dermatology*, vol. 21, no. 11, pp. 822–826, 2012.

[104] W. Nacken, J. Roth, C. Sorg, and C. Kerkhoff, "S100A9/S100A8: myeloid representatives of the S100 protein family as prominent players in innate immunity," *Microscopy Research and Technique*, vol. 60, no. 6, pp. 569–580, 2003.

[105] A. González-López, A. Aguirre, I. López-Alonso et al., "MMP-8 deficiency increases TLR/RAGE ligands S100A8 and S100A9 and exacerbates lung inflammation during endotoxemia," *PLoS ONE*, vol. 7, no. 6, Article ID e39940, 2012.

[106] K. Kitamura, K. Kangawa, M. Kawamoto et al., "Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma," *Biochemical and Biophysical Research Communications*, vol. 192, no. 2, pp. 553–560, 1993.

[107] B. Cheung and R. Leung, "Elevated plasma levels of human adrenomedullin in cardiovascular, respiratory, hepatic and renal disorders," *Clinical Science*, vol. 92, no. 1, pp. 59–62, 1997.

[108] T. Hata, K. Miyazaki, and K. Matsui, "Decreased circulating adrenomedullin in preeclampsia," *The Lancet*, vol. 350, no. 9091, p. 1600, 1997.

[109] K. Kitamura, J. Sakata, K. Kangawa, M. Kojima, H. Matsuo, and T. Eto, "Cloning and characterization of cDNA encoding a precursor for human adrenomedullin," *Biochemical and Biophysical Research Communications*, vol. 194, no. 2, pp. 720–725, 1993.

[110] R. P. Allaker, C. Zihni, and S. Kapas, "An investigation into the antimicrobial effects of adrenomedullin on members of the skin, oral, respiratory tract and gut microflora," *FEMS Immunology and Medical Microbiology*, vol. 23, no. 4, pp. 289–293, 1999.

[111] T. Ishimitsu, M. Kojima, K. Kangawa et al., "Genomic structure of human adrenomedullin gene," *Biochemical and Biophysical Research Communications*, vol. 203, no. 1, pp. 631–639, 1994.

[112] B. Gumusel, J.-K. Chang, A. Hyman, and H. Lippton, "Adrenotensin: an ADM gene product with the opposite effects of ADM," *Life Sciences*, vol. 57, no. 8, pp. PL87–PL90, 1995.

[113] S. Kapas, A. Bansal, V. Bhargava et al., "Adrenomedullin expression in pathogen-challenged oral epithelial cells," *Peptides*, vol. 22, no. 9, pp. 1485–1489, 2001.

[114] S. Kapas, M. Luisa Tenchini, and P. M. Farthing, "Regulation of adrenomedullin secretion in cultured human skin and oral keratinocytes," *Journal of Investigative Dermatology*, vol. 117, no. 2, pp. 353–359, 2001.

[115] S. Kapas, K. Pahal, A. T. Cruchley, E. Hagi-Pavli, and J. P. Hinson, "Expression of adrenomedullin and its receptors in human salivary tissue," *Journal of Dental Research*, vol. 83, no. 4, pp. 333–337, 2004.

[116] R. P. Allaker, P. W. Grosvenor, D. C. McAnerney et al., "Mechanisms of adrenomedullin antimicrobial action," *Peptides*, vol. 27, no. 4, pp. 661–666, 2006.

[117] H. He, H. Bessho, Y. Fujisawa et al., "Effects of a synthetic rat adrenomedullin on regional hemodynamics in rats," *European Journal of Pharmacology*, vol. 273, no. 3, pp. 209–214, 1995.

[118] A. Friedman, L. Todd, R. S. Baker, and K. E. Clark, "Uterine vascular effects of adrenomedullin," *Journal of the Society for Gynecologic Investigation*, vol. 5, p. F449, 1998.

[119] W. K. Samson, T. Murphy, and D. A. Schell, "A novel vasoactive peptide, adrenomedullin, inhibits pituitary adrenocorticotropin release," *Endocrinology*, vol. 136, no. 5, pp. 2349–2352, 1995.

[120] G. G. Nussdorfer, "Paracrine control of adrenal cortical function by medullary chromaffin cells," *Pharmacological Reviews*, vol. 48, no. 4, pp. 495–530, 1996.

[121] A. Martínez, C. Weaver, J. López et al., "Regulation of insulin secretion and blood glucose metabolism by adrenomedullin," *Endocrinology*, vol. 137, no. 6, pp. 2626–2632, 1996.

[122] J. J. Cebra, "Influences of microbiota on intestinal immune system development," *The American Journal of Clinical Nutrition*, vol. 69, no. 5, pp. 1046S–1051S, 1999.

[123] L. V. Hooper, M. H. Wong, A. Thelin, L. Hansson, P. G. Falk, and J. I. Gordon, "Molecular analysis of commensal host-microbial relationships in the intestine," *Science*, vol. 291, no. 5505, pp. 881–884, 2001.

[124] S. K. Mazmanian, H. L. Cui, A. O. Tzianabos, and D. L. Kasper, "An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system," *Cell*, vol. 122, no. 1, pp. 107–118, 2005.

[125] G. Reid, J. Jass, M. T. Sebulsky, and J. K. McCormick, "Potential uses of probiotics in clinical practice," *Clinical Microbiology Reviews*, vol. 16, no. 4, pp. 658–672, 2003.

[126] W. O. Chung and B. A. Dale, "Innate immune response of oral and foreskin keratinocytes: utilization of different signaling pathways by various bacterial species," *Infection and Immunity*, vol. 72, no. 1, pp. 352–358, 2004.

[127] Y. Hasegawa, J. J. Mans, S. Mao et al., "Gingival epithelial cell transcriptional responses to commensal and opportunistic oral microbial species," *Infection and Immunity*, vol. 75, no. 5, pp. 2540–2547, 2007.

[128] A. S. Neish, A. T. Gewirtz, H. Zeng et al., "Prokaryotic regulation of epithelial responses by inhibition of IκB-α ubiquitination," *Science*, vol. 289, no. 5484, pp. 1560–1563, 2000.

[129] S. Rakoff-Nahoum, J. Paglino, F. Eslami-Varzaneh, S. Edberg, and R. Medzhitov, "Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis," *Cell*, vol. 118, no. 2, pp. 229–241, 2004.

[130] M.-T. Tien, S. E. Girardin, B. Regnault et al., "Antiinflammatory effect of *Lactobacillus casei* on Shigella -infected human intestinal epithelial cells," *Journal of Immunology*, vol. 176, no. 6, pp. 1228–1237, 2006.

[131] C. Grangette, S. Nutten, E. Palumbo et al., "Enhanced antiinflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified teichoic acids," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 29, pp. 10321–10326, 2005.

[132] C. Cosseau, D. A. Devine, E. Dullaghan et al., "The commensal *Streptococcus salivarius* K12 downregulates the innate immune responses of human epithelial cells and promotes host-microbe homeostasis," *Infection and Immunity*, vol. 76, no. 9, pp. 4163–4175, 2008.

[133] F. J. Giles, C. B. Miller, D. D. Hurd et al., "A phase III, randomized, double-blind, placebo-controlled, multinational trial of Iseganan for the prevention of oral mucositis in patients receiving stomatotoxic chemotherapy (PROMPT-CT Trial)," *Leukemia & Lymphoma*, vol. 44, no. 7, pp. 1165–1172, 2003.

[134] S. Elad, J. B. Epstein, J. Raber-Durlacher, P. Donnelly, and J. Strahilevitz, "The antimicrobial effect of Iseganan HCl oral solution in patients receiving stomatotoxic chemotherapy: analysis from a multicenter, double-blind, placebo-controlled, randomized, phase III clinical trial," *Journal of Oral Pathology & Medicine*, vol. 41, no. 3, pp. 229–234, 2012.