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Host Defense Peptides in the Oral Cavity and the Lung: Similarities and Differences

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Abstract

Peptides with broad-spectrum antimicrobial activity are found in the mucosal surfaces at many sites in the body, including the airway, the oral cavity, and the digestive tract. Based on their *in vitro* antimicrobial and other immunomodulatory activities, these host defense peptides have been proposed to play an important role in the innate defense against pathogenic microbial colonization. The genes that encode these peptides are up-regulated by pathogens, further supporting their role in innate immune defense. However, the differences in the local microbial environments between the generally sterile airway and the highly colonized oral cavity suggest a more complex role for these peptides in innate immunity. For example, β-defensin genes are induced in the airway by all bacteria and Toll-like receptor (TLR) agonists primarily through an NF-κB-mediated pathway. In contrast, the same genes are induced in the gingival epithelium by only a subset of bacteria and TLR ligands, *via* different pathways. Furthermore, the environments into which the peptides are secreted—specifically saliva, gingival crevicular fluid, and airway surface fluid—differ greatly and can effect their respective activities in host defense. In this review, we examine the differences and similarities between host defense peptides in the oral cavity and the airway, to gain a better understanding of their contributions to immunity.

Keywords

defensin; cathelicidin; innate immunity; antimicrobial peptide; host-pathogen interaction

Introduction

The human airway and the oral cavity both represent mucosal surfaces open to the environment. This allows for constant exposure to the micro-organisms that could colonize and lead to disease. Primary among the mechanisms to prevent such pathogenic colonization is the innate immune system, which provides a non-specific, rapid defense against invading pathogens. However, the airway is predominantly a sterile environment, while the oral cavity is host to hundreds of species of micro-organisms, many of which are commensal. An examination of the similarities and differences between these two epithelial surfaces will help us understand the roles played by innate immune mediators. These include several classes of molecules initially identified as antimicrobial peptides (AMP). With the increasing discovery of their multiple activities, they will here be referred to as host defense peptides (HDPs).

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The cells which comprise the innate immune response are primarily phagocytes, including neutrophils and macrophages, and the cells that line the epithelial mucosa. It had been originally thought that the epithelium provided defense primarily as a barrier against microbial invasion. However, it has been more recently recognized that these cells play an important active role in the recognition of microbes, eliciting a defensive response similar to that found in cells of the myeloid lineage. In general, the innate immune response is driven by the recognition of microbe-associated molecular patterns, such as lipopolysaccharide (LPS) or bacterial DNA, or by Toll-like receptors (TLRs), which are specific for these patterns. Other receptors, such as Dectin-1, a glucan receptor, or mannose-binding lectin (Takahashi *et al.*, 2006), also participate in this recognition. Recognition of the patterns by the receptors, often with co-receptors such as CD14 or MD2, usually results in the activation of a signal transduction pathway, which ultimately activates transcription factors, often including NF-κB, and in turn the induction of innate immune gene expression. These genes include pro-inflammatory cytokines such as IL-1β and IL-8, and host defense peptides such as defensins, cathelicidins, and histatins.

Host Defense Peptides

Defensins

Initially identified in phagocytic cells, defensins have been predicted to play a major role in innate antimicrobial host defense. These peptides are 3-5 kDa in size, highly cationic, and have a characteristic 6-cysteine motif, which results in a three-disulfide-bonded secondary structure. There are two main subgroups, the α- and β-defensins, which differ in their cysteine motifs, but share a similar secondary structure and are both rich in cationic residues (Fig. 1). Peptides in both groups are encoded by unique genes, which are primarily localized to a region of human chromosome 8p. The peptides are expressed as larger precursors, with a putative signal sequence at the N-terminus of the precursor. α-defensins have an acidic pro-region which undergoes a secondary cleavage to release the mature peptide, while mature β-defensins are predicted to mature by signal peptidase (Beckloff and Diamond, 2008).

The α-defensins are 29-35 residues in length and exhibit broad-spectrum activity against Gram-positive and Gram-negative bacteria (Ganz and Lehrer, 1995), fungi (Lehrer *et al.*, 1986), viruses (Klotman and Chang, 2006), and mycobacteria (Kisich *et al.*, 2001). In humans, they are found predominantly in the neutrophils, where the 4 α-defensins [known as human neutrophil peptides (HNP) 1, 2, 3, and 4] make up approximately 30% of the total protein in the azurophilic granule (Liu *et al.*, 1997). The presence of neutrophils during airway inflammation can result in the introduction of high concentrations of HNP into inflamed tissue. Increases in HNP levels in broncho-alveolar lavage fluid has been observed in lung inflammatory diseases (Ashitani *et al.*, 2007a,b; Mukae *et al.*, 2007). Similarly, an increase in HNP levels in the saliva has been observed in oral diseases (Mizukawa *et al.*, 1999, 2000). However, variability in levels of HNP in gingival crevicular fluid in both healthy persons and those with periodontitis (Lundy *et al.*, 2004) suggests that they may be regulated by pathogens that affect neutrophil migration and function, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans.*

β-defensins were initially discovered in a study to characterize the role of antimicrobial peptides in the innate immune response in the airway (Diamond *et al.*, 1991). Human β-defensins (hBD)1-4, the 4 human β-defensin peptides characterized to date, are all expressed in epithelial cells as well as certain cell types of the myeloid lineage (reviewed in Diamond *et al.*, 2004). These peptides are slightly larger than the α-defensins, at 36-42 amino acid residues in length. Both classes of peptide exhibit broad-spectrum activity against Gram-positive and Gram-negative bacteria, mycobacteria, and fungi (reviewed in Lehrer and Ganz, 2002). Since they are expressed by epithelial cells, the peptides have been found in the airway surface fluid

that lines the respiratory tract (Laube *et al.*, 2006), and in saliva (Mathews *et al.*, 1999; Sahasrabudhe *et al.*, 2000). Thus, they have been proposed to play a role in either the prevention of bacterial colonization in the airway, or in the maintenance of steady-state levels of flora in the oral cavity (Weinberg *et al.*, 1998).

Cathelicidins

In contrast to defensins, which are characterized by a specific sequence motif in the mature peptide, cathelicidins are defined by a conserved sequence of about 100 residues in the N-terminus of the precursor, named the cathelin domain (Zanetti *et al.*, 1995). After proteolytic cleavage, an active C-terminal domain is released. This active peptide is highly heterogeneous between species (reviewed in Zanetti, 2004). Each mammalian species examined has cathelicidins, but each contains a different set of related genes. The sole human cathelicidin, LL-37, is a 37-residue peptide found at the C-terminus of the human cationic antimicrobial protein 18 (hCAP-18) encoded by the cathelicidin-related antimicrobial peptide gene (Larrick *et al.*, 1995; Gudmundsson *et al.*, 1996). LL-37 peptide was initially found in the specific granules of neutrophils at approximately 1 μM (Sorensen *et al.*, 1997). The peptide was subsequently identified in monocytes, T-cells (Agerberth *et al.*, 2000), the surface epithelia of conducting airways (Bals *et al.*, 1998b), and in broncho-alveolar lavage fluid (Agerberth *et al.*, 1999).

LL-37 has broad-spectrum activity similar to that of defensins, against both Gram-positive and Gram-negative bacteria, as well as *Candida albicans* (Larrick *et al.*, 1995). Furthermore, the N-terminal cathelin domain was also found to have distinct antimicrobial activity after the proteolytic maturation step (Zaiou *et al.*, 2003). This cathelin segment is active against bacterial strains that are resistant to LL-37, including methicillin-resistant *Staphylococcus aureus*, suggesting a complementary host defense mechanism by a single gene.

Histatins

A component of saliva from humans and higher primates, histatins are a class of related α-helical HDPs with potent activity primarily against fungi, including both forms of *C. albicans* (reviewed in Edgerton and Koshlukova, 2000). These peptides bind to a surface protein on Candida, and translocate into the cytoplasam, where they interact with cellular processes, ultimately killing the cell (Edgerton *et al.*, 1998; Koshlukova *et al.*, 1999). Since they are not found in the airway, they represent a unique peptide-based antifungal defense of the oral cavity.

Defense Against Microbial Infections

Antibacterial Activity

Most HDPs generally carry an overall structural cationic charge that allows them to bind to negatively charged prokaryotic cellular membranes. Direct correlations between cationicity and antimicrobial activity have been demonstrated, suggesting a mechanism for the selectivity of microbes, as opposed to the host cell membranes, which are composed of more neutral phospholipids (Matsuzaki *et al.*, 1995). Once bound to the microbial surface, the peptides are predicted to lead to membrane disruption by insertion, but may also translocate into the microbe and kill by intracellular mechanisms (reviewed in Brogden, 2005).

In general, the peptides exhibit broad-spectrum antimicrobial activity. Each peptide, however, has differential activity against different micro-organisms, as measured by *in vitro* assays. Predominant bacterial pathogens in human lung infections include *Pseudomonas aeruginosa, Haemophilus influenzae, Klebsiella pneumoniae, Burkhoderia cepacia, Bordetella pertussis*, and *Mycobacterium tuberculosis*. Minimal inhibitory concentrations (MICs) for defensins and

LL-37 against these pathogens can range from less than 10 μg/mL to greater than 250 μg/mL (Miyakawa *et al.*, 1996; Saiman *et al.*, 2001; Starner *et al.*, 2005) (Table 1). However, the susceptibility of a given species is often strain-specific and dependent on conditions, including salt concentration and interaction with other antimicrobials (Bals *et al.*, 1998a; Garcia *et al.*, 2001b; Starner *et al.*, 2002; Fattorini *et al.*, 2004).

Similarly, in the oral cavity, activity of peptides against bacterial species is highly variable. The α-defensins are generally inactive against most oral bacteria tested (Miyasaki *et al.*, 1990). Human β-defensins, however, are active, but demonstrate strain-specific variability, with greater activity in general against aerobic species compared with anaerobic species (Joly *et al.*, 2004). Furthermore, a study has suggested that periodontopathogenic bacteria were more resistant to both LL-37 and hBD3 than were non-pathogenic bacteria (Ji *et al.*, 2007a). Results from the same strain, however, vary between laboratories (Table 1).

While HDPs were initially identified as peptides with potent antimicrobial activity *in vitro*, most of these antimicrobial assays were carried out in low-salt conditions, and in the absence of host fluids such as serum or saliva. Subsequent investigations have determined that the activity of many HDPs is inhibited by high levels of salt and serum proteins, which inhibit the potential for the initial electrostatic interactions necessary for activity (Dorschner *et al.*, 2006). For example, saliva reduces the activity of hBD1-3 and LL-37 by 20-50% (Mineshiba *et al.*, 2003; Ouhara *et al.*, 2005), compared with an 80% reduction by serum (Mineshiba *et al.*, 2003). Cationic antimicrobial peptides are apparently active in airway fluid (Cole *et al.*, 2002b), although the effect of airway fluid on activity is unknown for most peptides. It has been observed that LL-37 can bind to airway mucins, which can inhibit its activity (Felgentreff *et al.*, 2006). A full examination of the species-specificity and activity in respective fluids (*e.g.*, oral pathogens in saliva, or airway pathogens in airway fluid) remains to be carried out.

Defense against Viral Infections

While initially examined as antibacterial peptides, more recently defensin activity against viral infections has been studied. Interestingly, initial experiments demonstrating the role of defensins as direct antiviral agents suggested a selectivity against enveloped viruses, but analysis of recent data has shown that defensins and other antimicrobial peptides have even broader activity against both enveloped and non-enveloped viruses and include DNA, RNA, and retroviruses. The mechanisms of viral inactivation also vary and include not only direct binding of the virus to the peptide, but also indirect methods of inactivation *via* intracellular modulation of the viral replication, modulation of signaling pathways necessary for antiviral effects, and recruitment of immune cells that contribute to antiviral activity *in vivo*. Thus, both oral and respiratory epithelial cells are capable of influencing antiviral immunity *via* the induction of antimicrobial peptides by either bacteria or viruses.

Early studies showed that a human neutrophil α-defensin, HNP-1, exhibited *in vitro* inactivation of the enveloped viruses, Herpes Simplex virus (HSV)-1, HSV-2, influenza virus, cytomegalovirus, vesticular stomatitis virus, and Sendai virus, whereas the non-enveloped viruses, echovirus type 11 and reovirus type 3, were not inactivated (Daher *et al.*, 1986). In this study, HSV-1, which was most susceptible, bound directly to HNP-1 in a temperature-dependent manner. To support the selectivity against enveloped viruses, HNP-1, hBD-1, and hBD-2 did not inhibit viral replication of vaccinia virus, a non-enveloped virus, despite strong activity against *E. coli* (Howell *et al.*, 2004). In contrast to defensins, the human cathelicidin LL-37 effectively kills vaccinia virus, a non-enveloped virus, *in vitro* and *in vivo* (Howell *et al.*, 2004). However, subsequent studies showed that interaction with the viral envelope is only one mechanism of defensin antiviral activity, and that antimicrobial peptide activity against viruses depends on the type of virus and the type of peptide involved.

Various mechanisms of defensin activity have been demonstrated by studies on an enveloped RNA virus that infects respiratory epithelium, influenza virus. In addition to the direct inhibition of viral plaques mentioned above by HNP-1, retrocyclin 2, hBD-3, and another innate immune molecule, mannan-binding lectin, all inhibited influenza virus hemagglutinin fusion with the cellular membrane of respiratory epithelial cells (Leikina *et al.*, 2005) at concentrations similar to those reported for hBD-3 inhibition of HIV-1 infection–from 10 to 20 μg/mL (Quinones-Mateu *et al.*, 2003). Other indirect defensin mechanisms against influenza virus also appear to be at work, suggesting that inhibiting membrane fusion with the virus is only one method of antiviral activity. Recently, it was demonstrated that HNP-1 inhibited the replication and protein synthesis of influenza virus by affecting host cell signal transduction pathways, inhibiting protein kinase C activation in several target cells, including A549, a human lung alveolar type II cell line derived from an adenocarcinoma (Salvatore *et al.*, 2007). In addition, HNP-1 and hBD-2 can induce aggregation of influenza virus particles and increase their uptake by neutrophils. In addition, these peptides can also interact with surfactant protein D, a collectin present in alveolar epithelium that also binds influenza virus and increases respiratory burst in neutrophils (Hartshorn *et al.*, 2006; Tecle *et al.*, 2007; White *et al.*, 2007).

Defense against HSV-1, an enveloped DNA virus that infects oral epithelium, may also involve mechanisms other than the direct binding of the virus to the peptide. HSV-1 infection of CaSki cells, a cell line derived from human cervical epithelial cells, is blocked by two α-defensins, HD-5 and HNP-1, at the stage of viral gene expression 4 hrs after viral entry. Coupled with the observation that these α-defensins accumulate intracellularly inside the epithelial cells (the source being from other cells such as neutrophils) and the fact that they are cationic, this result suggested that some of the post-entry anti-HSV-1 activity may be mediated by interactions of defensins with viral DNA (Hazrati *et al.*, 2006). Both synthetic and rhesus-monkey-derived retrocyclins, which are theta-defensins, also have effects against both HSV-1 and HSV-2 at the point of viral entry and inhibition of HSV-2 VP-16 protein translocation. However, not all retrocyclins are effective against both HSV-1 and HSV-2, and each retrocyclin can have different mechanisms of inhibition of HSV-2 (Yasin *et al.*, 2004), suggesting that mechanisms of defensin inhibition of viral neutralization in the Herpes family can vary. The role of defensins in HSV-1 infection of oral epithelium has yet to be studied, and the variable mechanisms among the different defensins against HSV-2 show that these specific mechanisms cannot be extrapolated from these studies to infer mechanisms of HSV-1 in oral epithelium.

The mechanism of defensin activity against HIV-1 infection is more complex and also involves different mechanisms. Defensins, both natural (Nakashima *et al.*, 1993; Zhang *et al.*, 2002, 2004; Chang *et al.*, 2003; Mackewicz *et al.*, 2003; Tanabe *et al.*, 2004) and synthetic retrocyclin, a theta-defensin (Cole *et al.*, 2002a), exhibit direct *in vitro* activity against human immunodeficiency virus (HIV). LL-37 has also been demonstrated to inhibit HIV replication in numerous isolates, although the mechanism has not been elucidated (Bergman *et al.*, 2007). In addition to the direct interaction with the HIV envelope, defensins can participate in antiviral defense using indirect mechanisms by modulating either the CXCR4 (β-defensins) (Quinones-Mateu *et al.*, 2003; Feng *et al.*, 2006) or the CD4 (α-defensins) (Furci *et al.*, 2007) HIV co-receptors on peripheral blood mononuclear cells. In the oral epithelium, HIV-1 can induce the gene expression of hBD-2 and hBD-3, which then can modulate HIV entry into T-lymphocytes (Quinones-Mateu *et al.*, 2003).

Other mechanisms, not involving the interaction between defensins and the envelope, are also apparent. Defensins are also active against non-enveloped viruses. Human α-defensins HNP 1-3 and HD-5 are active against human papillomavirus by blocking viral escape from endocytotic vesicles, but not by viral binding and internalization (Buck *et al.*, 2006). Overexpression of defensins in cell cultures also provided the cultures with increased resistance to infections with both bacteria and adenovirus (Gropp *et al.*, 1999). Both α- and β-defensins

inhibit the infectivity of adenovirus (Bastian and Schafer, 2001) and adeno-associated virus *in vitro* (Virella-Lowell *et al.*, 2000). Unlike with enveloped viruses, the mechanism for neutralization of multiple human adenovirus serotypes by the human α-defensins HNP-1 and HD-5 is not restriction of virus-binding to cells *via* its primary receptor, CD 46. Instead, HD-5 and HNP-1 use a later step in viral entry—the direct interaction with the virus capsid, blocking the dissociation of the capsid vertex region (containing pVI) in the endosome and preventing pVI-mediated endosomalysis. This interaction prevents viral escape from the endosome, leading to prolonged residency in the endosome, with subsequent accumulation in the lysosomes, thus resulting in failure of the virus to reach the nuclear membrane (Smith and Nemerow, 2008).

In addition to the antiviral activity of defensins, the attraction of cells important to viral immunity to the site of infection by human β-defensins further supports the hypothesis that β-defensins also play a role in the innate immune response to viruses. For example, cells producing hBD-1 or hBD-2 could influence chemotaxis, since both defensins have been shown to bind to HEK293 cells expressing the chemokine receptor CCR6, and hBD-2 attracted immature monocyte-derived dendritic cells and memory T-cells *via* CCR6 *in vitro* (Yang *et al.*, 1999). There are a few clinical examples where the presence or infection of epithelial cells with virus leads to the induction of β-defensins, which then could function by attracting immune cells to the local area of infection. For example, a high level of gene expression of hBD-1, -2, and -3 was detected in papillomavirus-induced epithelial lesions compared with normal oral epithelial cells (Chong *et al.*, 2006). Another example shows the correlation with increased levels of hBD-2 mRNA and peptide by airway epithelium infected with rhinovirus-16 in healthy human volunteers (Proud *et al.*, 2004). Decreased viral titers correlated with the increased β-defensin levels detected following infection. The mechanisms of β-defensin antiviral activity are not well-understood.

One cell that has recently become recognized as being very important in innate immunity against viruses is the plasmacytoid dendritic cell (PDC) (Siegal *et al.*, 1999, reviewed in Fitzgerald-Bocarsly and Feng, 2007; Fitzgerald-Bocarsly *et al.*, 2008). Once known as “natural interferon producing cell”, “plasmacytoid T-cells”, or “plasmacytoid monocytes”, these cells can produce extraordinary amounts of IFN-α—as much as 1-2 IU or 3-10 pg of IFN-α *per* cell—upon stimulation with enveloped viruses (Cederblad and Alm, 1990; Howell *et al.*, 1994). Enveloped viruses that stimulate PDC to produce IFN-α include HSV, influenza virus, HIV, and Sendai virus. PDCs are found both in the lung (Lommatzsch *et al.*, 2007; Ryan *et al.*, unpublished observations) and in the oral cavity (Santoro *et al.*, 2005; Kajita *et al.*, 2007) in numerous pathological conditions. These cells express both hBD-1 mRNA and peptide (Ryan *et al.*, 2003), which is induced upon stimulation with HSV-1, influenza, and Sendai virus (Ryan *et al.*, submitted). Since hBD-1 is not reported to exhibit antiviral activity *in vitro* (Quinones-Mateu *et al.*, 2003; Sun *et al.*, 2005; Hazrati *et al.*, 2006), possibly due to its unstable nature outside the cell, its role may be chemotactic for the recruitment of other antiviral cells, or it has intracellular antiviral effects. In our laboratory, HSV-1 plaque formation on Vero cells was inhibited by hBD-1 (at concentrations similar to the direct antiviral activity reported in other studies), but this antiviral activity was observed only with freshly reconstituted recombinant hBD-1 incubated with virus in serum-free medium prior to addition of the virus to the target cell line (Ryan *et al.*, unpublished observations). The importance of the native peptide state for direct inactivation of HSV-1 has been shown with HNP-1 (Daher *et al.*, 1986), and the relative inactivity of the β-defensins may simply reflect the loss of the active peptide state.

Other Roles of HDPs in Innate Immune Defenses

Besides their ability to permeate membranes, several other properties of HDPs have been discovered. The amphiphilic cationic design facilitates maximum interactions in biological

systems. Once inside the bacterial cell, HDPs have been shown to exhibit microbicidal behaviors, inhibiting protein, cell wall, and nucleic acid synthesis (Zasloff, 2002). Along with the bovine peptide Indolicidin, they accumulate in the cytoplasm, binding both DNA and RNA (Hsu *et al.*, 2005; Jenssen *et al.*, 2006). Many classes of HDPs can bind the anionic-binding pocket of aminoglycoside-modifying enzymes, thus inhibiting their activity (Jenssen *et al.*, 2006).

In addition to their direct antimicrobial activity, defensins, both α- and β-, exhibit numerous other biological activities (reviewed in Yang *et al.*, 2004). Primary among these is selective chemotactic activity for a variety of host defense cells. Specifically, HNP1-3 and hBD1-3 are chemotactic for immature dendritic cells (Yang *et al.*, 1999, 2000b); hBD2 exhibits chemotactic activity for mast cells (Niyonsaba *et al.*, 2002); and hBD2 and HNPs for selective T-lymphocytes (Yang *et al.*, 1999, 2000b). Together, the defensins can provide a potent inflammatory response, in that products of mast cell degranulation [which can be induced by both α- and β-defensins (Yamashita and Saito, 1989; Befus *et al.*, 1999; Niyonsaba *et al.*, 2001) and CXCL8 (which is induced on bronchial epithelial cells by HNPs (Van Wetering *et al.*, 1997)] are neutrophil chemotactic agents (Echtenacher *et al.*, 1996; Malaviya *et al.*, 1996; Baggiolini, 1998). Other pro-inflammatory cytokines, such as TNF-α and IL-1β, are induced by HNPs as well (Chaly *et al.*, 2000).

While these and other studies demonstrate a role for defensins as a link between innate and adaptive immunity, there are also examples of defensin effects directly on the adaptive immune response. HNPs can enhance the antigen-specific immune response to ovalbumin when introduced intranasally, increasing the production of ovalbumin-specific IgG and CD4+ T-cells (Lillard *et al.*, 1999). β-defensins were also shown to enhance adaptive immunity, using a fusion vector containing a sequence encoding a mouse β-defensin fused to that of a B-cell lymphoma-specific epitope. The resultant DNA vaccine provided enhanced antitumor activity (Biragyn *et al.*, 1999, 2002).

Most recently, a β-defensin was identified as a regulator of coat color in dogs (Candille *et al.*, 2007), primarily due to its binding to a melanocortin receptor. This suggests that they may participate in other functions in the cells as well.

While initially isolated as an antimicrobial peptide, LL-37 has been discovered to exhibit numerous other activities. Since its expression in epithelium is induced in response to infection and inflammation (Dorschner *et al.*, 2001), it was proposed to play additional roles in inflammation besides antimicrobial. Indeed, LL-37 demonstrates chemotactic activity for neutrophils, monocytes, and some T-cells (Yang *et al.*, 2000a), and induces IL-8 secretion from epithelial cell lines (Tjabringa *et al.*, 2003). Furthermore, LL-37 affects dendritic cell (DC) maturation, and can act synergistically with the DC-maturation cytokine GM-CSF to activate signal transduction pathways in monocytes (Scott *et al.*, 2002). Together, these multiple activities of LL-37 (as reviewed in Bowdish *et al.*, 2005) suggest that it plays an important, multifunctional role in host defense.

Expression of HDPs in Mucosal Epithelia

HDP Expression in the Airway Epithelium

The respiratory tract is continuously exposed to a variety of micro-organisms that can adhere and potentially cause infection. Epithelial cells lining the mammalian trachea release numerous antimicrobial factors, including β-defensins and LL-37, forming a crucial site in the host defense against airborne microbial pathogens (reviewed in Diamond *et al.*, 2000b). Deficiencies in these defenses, or conditions where they are inactive, may result in recurrent airway infections such as those seen in cystic fibrosis (CF) (Smith *et al.*, 1996).

In several systems, expression of β-defensin genes responds to the presence of pathogens (reviewed in Kaiser and Diamond, 2000). For example, in primary cultures of bovine and human tracheal epithelial cells (TEC), significant increases in the steady-state mRNA levels of the bovine β-defensin tracheal antimicrobial peptide (TAP) and its human homologue β-defensin-2 (hBD-2) were noted upon incubation with *Pseudomonas aeruginosa* and *Escherichia coli* lipopolysaccharides (LPS) (Diamond *et al.*, 1996; Becker *et al.*, 2000). *P. aeruginosa* can also induce hBD-3 and -4 in human lung A549 cells (Harder *et al.*, 2000; Garcia *et al.*, 2001b). A list of peptides and their regulation can be found in Table 2.

In vivo studies showing the induction of β-defensin gene expression further support the hypothesis that defensins play a role in antimicrobial defense. Intratracheal instillation of *Mannheimia* (*Pasteurella*) *haemolytica* into a single lobe of a cow lung caused an increase in β-defensin expression in the airway epithelium localized to the site of infection (Stolzenberg *et al.*, 1997). Similarly, in the mouse, intratracheal instillation of *P. aeruginosa* caused an increased expression of mouse β-defensin-3 (mBD-3), the murine homologue for hBD-2, in the tracheal epithelium (Bals *et al.*, 1999).

LL-37 expression is observed in the respiratory epithelium, and its mRNA and protein can be induced *in vitro* by physiological concentrations of Vitamin D (Yim *et al.*, 2007) and *in vivo* by injury (Dorschner *et al.*, 2001). Thus, both classes of peptides appear to be regulated as part of an innate immune response in the airway.

HDP Expression in the Oral Mucosa

In contrast to the predominantly sterile airway, the oral cavity is host to a magnitude of microbial organisms, which necessitates a protective mechanism to prevent the onset of disease, yet without a sensitive response to the commensal organisms. HDPs are found both in saliva (Edgerton and Koshlukova, 2000; reviewed in Abiko and Saitoh, 2007) and in crevicular fluid (Diamond *et al.*, 2001; Dale *et al.*, 2006), suggesting that they play a role in the maintenance of microbial homeostasis. In human gingival tissue, hBD-1 and hBD-2 are both expressed in normal, uninflamed tissue, at the highest levels at the gingival margin near the site of plaque formation, and within the sulcular epithelium during states of inflammation (Dale *et al.*, 2001). In contrast, hBD3 expression was observed primarily in the basal layer, as well as in Merkel and Langerhans cells in healthy tissue. However, the expression changed in persons with periodontitis, with hBD3 expression extending to the superficial spinous layers (Lu *et al.*, 2005). Another study demonstrated that hBD2 mRNA levels were increased in tissue biopsies from persons with gingivitis, and both hBD2 and hBD3 mRNA levels were increased in biopsies from periodontitis tissue (Dommisch *et al.*, 2005). Furthermore, immunohistochemical analysis demonstrated the expression of hBD2 in the buccal epithelium in tissue from persons infected with *C. albicans*, but not from uninfected individuals (Sawaki *et al.*, 2002).

In vitro studies have demonstrated that *P. gingivalis* induces the expression of hBD1 (Vankeerberghen *et al.*, 2005) and hBD2 (Taguchi and Imai, 2006) in cultured human gingival epithelial cells (HGEC), while *Fusobacterium nucleatum* and *A. actinomycetemcomitans* stimulate the production of hBD-2 and -3 (Feucht *et al.*, 2003; Vankeerberghen *et al.*, 2005). The response to *A. actinomycetemcomitans* is both strain-specific and variable between individuals (Feucht *et al.*, 2003; Vankeerberghen *et al.*, 2005), with a reduced response observed in cells from a person with Localized Aggressive Periodontitis (Laube *et al.*, 2008). In all cases, however, the induction was not mediated by LPS, suggesting that regulation of the induction of these mediators of innate immunity in the oral mucosa is controlled by more complex mechanisms of innate immunity. In the case of *P. gingivalis*, it was demonstrated that one of its secreted proteases, RgpB, could stimulate hBD2 expression *via* a protease-activated receptor (Chung *et al.*, 2004; Dommisch *et al.*, 2007). With *A. actinomycetemcomitans*, the

induction can be attributed at least in part to the outer membrane protein OMP100 (Ouhara *et al.*, 2006).

The variability in the responses of oral epithelial cells to different bacteria, and the variable sensitivity of oral flora to the peptides (Ji *et al.*, 2007a), led to the hypothesis that commensal bacteria with increased resistance to killing by the peptides would induce the expression of peptides to kill the more sensitive pathogens (Weinberg *et al.*, 1998; Dale and Fredericks, 2005). Early studies, which demonstrated that *F. nucleatum* induced hBD2 expression in gingival epithelial cells, while *P. gingivalis* did not (Krisanaprakornkit *et al.*, 2000; Vankeerberghen *et al.*, 2005), seemed to support this. A more recent study comparing the responses of bacteria associated with periodontal disease with those of other oral bacteria not associated with periodontal disease demonstrated that there was a wide range of responses in cultured HOK-16B cells (Ji *et al.*, 2007b). Specifically, *Prevotella intermedia* induced all peptide genes (hBD1, 2, and 3, as well as LL-37); *F. nucleatum* induced hBD2 and 3; and *P. gingivalis* induced hBD2. Other species associated with periodontal disease, such as *Tannerella forsythia* and *Treponema denticola*, either did not induce expression, or led to a reduction in steady-state mRNA levels. LL-37 expression in an oral epithelial cell line was similarly complex, with *P. gingivalis* demonstrating no induction, while *A. actinomycetemcomitans* and *F. nucleatum* induced significant amounts of both mRNA and peptide (Hosokawa *et al.*, 2006). Finally, a study using biofilm cultures showed that early stages of biofilm formation could induce β-defensin expression, but later, more mature, biofilms did not (Eberhard *et al.*, 2008). These results suggest that the response may be more complicated than previously hypothesized, and that it will be difficult to develop an accurate model of the innate immune response based on *in vitro* studies.

Few studies have been carried out in animal models. Dixon *et al.* examined several innate immune mediators in gingival tissue from conventionally reared mice in comparison with germ-free mice. Their results indicated significant differences in IL-1β levels (higher protein in conventionally reared mice, yet lower mRNA levels) (Dixon *et al.*, 2004), suggesting that commensal micro-organisms may influence innate immune gene expression. Our recently published results demonstrated that introduction of both pathogenic bacteria and commensal bacteria can transiently induce β-defensin gene expression in rat gingival tissue (Kurland *et al.*, 2006).

In the oral cavity, LL-37 was initially identified in infiltrating neutrophils (Dale *et al.*, 2001), but was subsequently observed in salivary glands as well, in both human (Woo *et al.*, 2003) and murine oral tissue (Murakami *et al.*, 2002). Furthermore, the LL-37 gene was inducible in gingival epithelial cells by bacteria, including *A. actinomycetemcomitans* (Hosokawa *et al.*, 2006), suggesting a role for LL-37 in the natural defense against colonization by periodontal pathogens, such as *A. actinomycetemcomitans*.

Regulation of HDP Gene Expression

In response to microbes, microbial components, or pro-inflammatory cytokines, a typical general result is gene induction mediated by the activation of transcription factors. In the airway, LPS induces both TAP and hBD-2 gene expression through its binding to epithelial-cell-expressed CD14. This stimulates a signal transduction pathway, which results in the activation of NF-κB, which binds to an NF-κB consensus sequence upstream from the TAP and hBD-2 genes (Becker *et al.*, 2000; Diamond *et al.*, 2000a). Other microbe-associated molecular patterns known to activate the NF-κB pathway also stimulate β-defensin gene expression in airway epithelial cells, including Pam3CSK4 (Klein-Patel *et al.*, 2006), flagellin (Takahashi *et al.*, 2001), poly IC (Duits *et al.*, 2003), and CpG DNA (Platz *et al.*, 2004). In gingival epithelial cells, however, the mechanism is somewhat different. We and others have

shown that gingival epithelial cells appear to be insensitive to LPS (Krisanaprakornkit *et al.*, 2002; Laube *et al.*, 2008). However, they are extraordinarily sensitive to cell wall extract from the commensal bacterium *F. nucleatum*, which induces hBD2 expression (Krisanaprakornkit *et al.*, 2000). Similarly, live pathogenic bacteria, both Gram-positive and -negative, can induce this response in oral epithelial cells (Chung and Dale, 2004; Laube *et al.*, 2008). However, there are apparently two different pathways leading to increased hBD2 levels. Cell wall extract from *F. nucleatum* induces expression primarily through activation of p38 and JNK pathways (Krisanaprakornkit *et al.*, 2002), while induction by pathogens proceeds through the NF-κB pathway (Chung and Dale, 2004). A more recent study further defines this response, demonstrating that pathogens such as *P. gingivalis* and *A. actinomycetemcomitans* utilize an alternative pathway leading to NF-κB activation, *via* the IKKα/TRAF3 pathway (Chung and Dale, 2008). When gingival epithelial cells were stimulated with *F. nucleatum*, however, the response induced hBD2, but not through any NF-κB-stimulating pathway. This suggests that there is a unique response of gingival cells leading to the regulation of β-defensin gene expression, in contrast to that of airway cells. A model showing known microbe-associated molecular patterns and their respective pathways that stimulate HDP gene expression in the two tissues is shown in Fig. 2.

Diseases Associated with HDPs

Since HDPs are gene-encoded, it follows that mutations in these genes should lead to increased susceptibility to infection. Such phenotypes associated with HDP mutations have been rarely observed, possibly due to the redundancy of peptide-based antimicrobial defense systems, with numerous peptides expressed in the same tissues or cells. Supporting this hypothesis was the observation that a deletion of the entire region encoding both α- and β-defensins ($8p^-$ syndrome) results in recurrent airway infections (Ostergaard and Tommerup, 1989). In the airway, it was first observed that the airway surface fluid from persons with CF exhibited characteristics different from that of non-CF individuals. In a report by Smith *et al.* (Smith *et al.*, 1996), it was determined that the salt concentration was elevated in CF airway surface fluid, and β-defensin activity was inhibited at such a high salt concentration. Subsequently, other investigators have identified HDPs in the airway with both salt-dependent (Bals *et al.*, 1998a; Garcia *et al.*, 2001b) and salt-independent activities (Turner *et al.*, 1998; Bals *et al.*, 2001; Garcia *et al.*, 2001a), and further hypothesized that the difference between CF and non-CF airway surface fluid lies in characteristics besides salt concentration (Matsui *et al.*, 1998; Bals *et al.*, 2001). Nonetheless, it appears that numerous differences in the ability of HDPs to kill airway pathogens appear in CF airways compared with normal airways.

In the oral cavity, associations between polymorphisms in some defensin genes and infections has been observed. Jurevic *et al.* demonstrated that individuals, both diabetic and non-diabetic, with a SNP in the 5′ UTR of the hBD1 sequence are more likely to exhibit lower levels of Candida carriage (Jurevic *et al.*, 2003). Furthermore, Tao *et al.* (2005) observed an association between lower levels of salivary α-defensins HNP1-3 and the presence of dental caries.

A rare disorder, infantile congenital agranulocytosis, also known as morbus Kostmann (MK), is associated with a complete absence of LL-37, and is characterized by, among other symptoms, chronic periodontitis and overgrowth with *A. actinomycetemcomitans* (Putsep *et al.*, 2002; Carlsson *et al.*, 2006). Individuals with another genetic disorder, Papillon-Lefèvre Syndrome, demonstrate a deficiency in LL-37 and exhibit severe periodontitis. This may be due to a deficiency in the serine proteinases that process hCAP-18 to the mature, active LL-37 peptide (de Haar *et al.*, 2006). Together, these studies suggest a role for LL-37 in the natural defense against colonization by periodontal pathogens. Surprisingly, neither of these conditions apparently exhibits increased airway infections, suggesting that LL-37 plays a different role in the oral cavity than in the airway. Since MK is a disorder characterized by a

lack of mature neutrophils, it can be treated with the hematopoietic growth factor G-CSF, which leads to normal neutrophil counts. However, even with these normal neutrophil counts, persons with MK still exhibit a deficiency in LL-37 and have severe periodontal disease (Defraia and Marinelli, 2001). This suggests that this particular peptide plays an important role in defense against colonization by *A. actinomycetemcomitans*. This supports a more crucial role of the neutrophil in the containment of periodontal infections compared with lung infections. Variability in the inducibility of β-defensins in oral epithelial cells by oral pathogens can also lead to reduced levels of these peptides (Krisanaprakornkit *et al.*, 2000; Vankeerberghen *et al.*, 2005; Ji *et al.*, 2007b; Laube *et al.*, 2008).

In addition to inherent deficiencies in HDP activity leading to increased infections, exogenous factors that inhibit their activity have been proposed to reduce their defensive capacity. Bacterial virulence factors can suppress the innate immune response that naturally leads to the up-regulation of HDP expression. A type III secretion factor from the airway pathogen, *Bordetella bronchiseptica*, inhibits the activation of NF-κB in airway epithelial cells, leading to a reduced ability to induce β-defensin gene expression (Legarda *et al.*, 2005). Similarly, a penicillin-binding protein from Group B streptococcus provides protection against killing in rat lungs (Jones *et al.*, 2007). Environmental factors, such as components of inhaled air pollutant particulates, can also inhibit this response, predisposing individuals in highly polluted areas to bacterial infections (Klein-Patel *et al.*, 2006). In the oral cavity, bacterial virulence factors can have similar activities. Arginine- and lysine-specific proteases secreted by *P. gingivalis* can cleave to and inactivate the cationic HDPs (Devine *et al.*, 1999), and the *A. actinomycetemcomitans* leukotoxin can suppress the phagocytic capability of neutrophils, resulting in an indirect inhibition of the host defense activity of the α-defensins found in such high concentrations in those cells.

Therapeutic Potential of HDPs

Use of Peptides and Peptide Genes

Since HDPs exhibit broad-spectrum antimicrobial activity, and demonstrate a decreased ability to develop resistance (Zasloff, 2002), they represent an ideal potential therapeutic agent in any tissue or system that is a site for microbial infection, including the lung and oral cavity. After their initial discovery, numerous HDPs were examined for such therapeutic use. Unfortunately, several issues stood in the way of their development (reviewed in Marr *et al.*, 2006). These include: difficulty and expense of manufacturing; short half-lives due to proteolytic degradation; and inhibition by host molecules, such as those found in serum as well as physiological salt concentrations in some cases.

Since it was observed that certain HDPs, including β-defensins and cathelicidin, are regulated at the transcriptional level, it has been suggested that, rather than direct application of the peptide, exogenous modifiers of HDP expression could be used (Laube *et al.*, 2006). Unfortunately, the earliest discovered modifiers of HDP expression included microbial factors such as LPS, and inflammatory mediators, such as TNF-α and IL-1β. The inflammatory response that would be induced by these factors would outweigh the potential therapeutic value of increasing HDP levels. Recently, however, it was discovered that expression of the gene encoding LL-37 could be induced by a less toxic agent, the biologically active form of vitamin D, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]. This was initially observed in a variety of cell lines (Wang *et al.*, 2004; Gombart *et al.*, 2005), but it has also been found in primary cultures of airway epithelial cells (Yim *et al.*, 2007) as well as gingival epithelial cells (Diamond *et al.*, unpublished observations). This suggests a potential for the therapeutic modulation of host defense peptide genes to address infections in both sites.

Peptide Mimetics

One recently developed method to address the problems associated with HDP therapeutics is the use of non-peptidic analogues of AMPs. These molecules have many advantages over peptides because of their small size, which increases stability and enhances tissue distribution, and their ability to fine-tune their physical properties for optimization of potency and safety. As a first step in this direction, various laboratories have designed antimicrobial peptides by idealizing the amphiphilic alpha-helical arrangement of the side-chains observed in the natural structures, leading to a large number of potent and selective antimicrobial compounds (reviewed in Zasloff, 2002). More recently, peptidomimetic approaches that mimic the peptide primary structure have been pursued with amide bond isosteres or modifications of the peptide backbone by chain extension or hetero-atom incorporation (Merrifield *et al.*, 1994). Several groups have designed β-peptides, peptides with an additional methylene group in their backbone, which maintained this amphiphilic architecture (DeGrado *et al.*, 1982; Barron and Zuckermann, 1999). A systematic analysis of β-peptide structure and activity revealed that facial amphiphilicity as well as a precise ratio of charged to hydrophobic residues was essential for potent and selective activity, similar to what has been described for the antimicrobial β-peptides (Barron and Zuckermann, 1999). Helical, cationic, facially amphiphilic peptoid mimics (poly-N-substituted glycine) of magainin-2 have also been produced that show selective and potent antibacterial activity against Gram-positive and Gram-negative bacteria. Activity is length-dependent (12-17 mers) and, for several of the peptoids, is comparable with the antibacterial and hemolytic activities of synthetic magainin analogues and the antibacterial β-peptides (Stigers *et al.*, 1999). Significantly, the β-peptides and peptoids are resistant to proteases, providing important advantages over alpha-peptides. However, β-peptides and peptoids are relatively difficult and expensive to synthesize in large quantities. Therefore, the *de novo* design of inexpensive oligomers and polymers that adopt amphiphilic secondary structures and exhibit potent and selective antimicrobial activity was pursued (DeGrado *et al.*, 1982).

A third approach to the discovery of synthetic antimicrobial compounds sought to transfer the physiochemical compounds of AMP to arylamide backbones. These molecules, containing amide bonds similar to those of AMP, mimic the essential architecture for amphipathicity without the helical structure (Tew *et al.*, 2006). Composed of a hydrocarbon polyphenylethylene backbone, these biologically active molecules can be enhanced, through the addition of side-chains, tuning their molecular weight (MW) (Ilker *et al.*, 2004). These non-peptidic analogues have many advantages over peptides, including small size, increased stability, and enhancement of physical properties for optimization of potency and safety (Rennie *et al.*, 2005). Several variations have been produced using similar hydrocarbon backbone (Ilker *et al.*, 2004; Tew *et al.*, 2006).

One such compound, named mPE, has shown improved activity and selectivity over its first-generation counterparts (Tew *et al.*, 2006). It displays a high level of selectivity against erythrocytes (88 μg/mL), while maintaining excellent activity against many clinical isolates, including antibiotic-resistant bacteria, and better than the magainin derivative oligomers mentioned previously (Rennie *et al.*, 2005). Vibrational spectroscopic evidence by Tew *et al.* showed that the ability of mPE to disrupt cellular membranes with great efficiency is the result of its ability to insert itself perpendicularly into a bacterial membrane (Chen *et al.*, 2006). Analysis of the data also showed no development of resistance carried through 17 passages, as compared with two other fluoroquinolone antibiotics (ciprofloxacin and norfloxacin), whose resistance levels quadrupled against *S. aureus* (Rennie *et al.*, 2005). Using *in vitro* antimicrobial assays, we have demonstrated the potent activity of mPE against oral pathogens, both Gram-positive and -negative bacteria, as well as several Candida species (Beckloff *et al.*, 2007). This molecule is active against both planktonic and biofilm cultures,

suggesting that it can be developed as an antimicrobial therapeutic agent for use in the oral cavity.

Conclusions

Antimicrobial peptides and the genes that encode them have been studied extensively in both the oral cavity and the airway. While they exhibit potent, broad-spectrum antimicrobial activity *in vitro*, the evidence that their most important role in either of these locations is directly antimicrobial is still circumstantial. Regardless, gene expression studies demonstrate that the predominantly sterile airway exhibits a sensitive, but broad, response to microbial challenge. In contrast, the oral epithelium exhibits a complex regulation of AMP genes, suggesting that they participate in the maintenance of the homeostasis of the oral flora. More recent studies on their immunomodulatory activities suggest that their development as therapeutic antibiotics may pose problems in either site, with the complexity of the oral flora, which includes both commensal and pathogenic species, being particularly problematic. Further examination of their role in the host defense of these mucosal epithelia, especially the factors that control AMP gene expression, is essential if their potential is to be understood.

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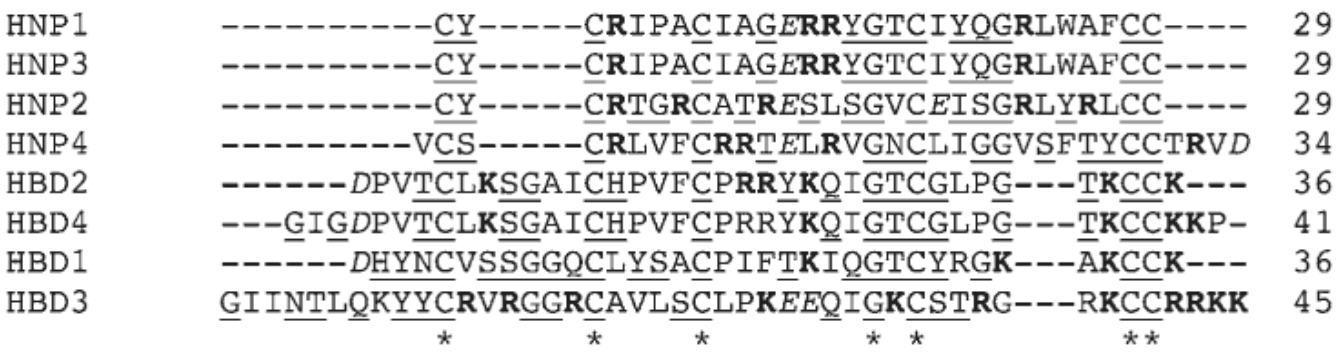

Figure 1.

ClustalW alignment of human α- and β-defensins. The mature peptide sequences of α- and β-defensins are aligned with the number of amino acids listed on the right. Amino acids are annotated based on the ClustalW scheme for physical characteristics: uppercase for small and hydrophobic, italic for acidic, boldface for basic, and underlined for hydroxyl, amide, and basic. Highly conserved residues in each sequence are denoted by an asterisk.

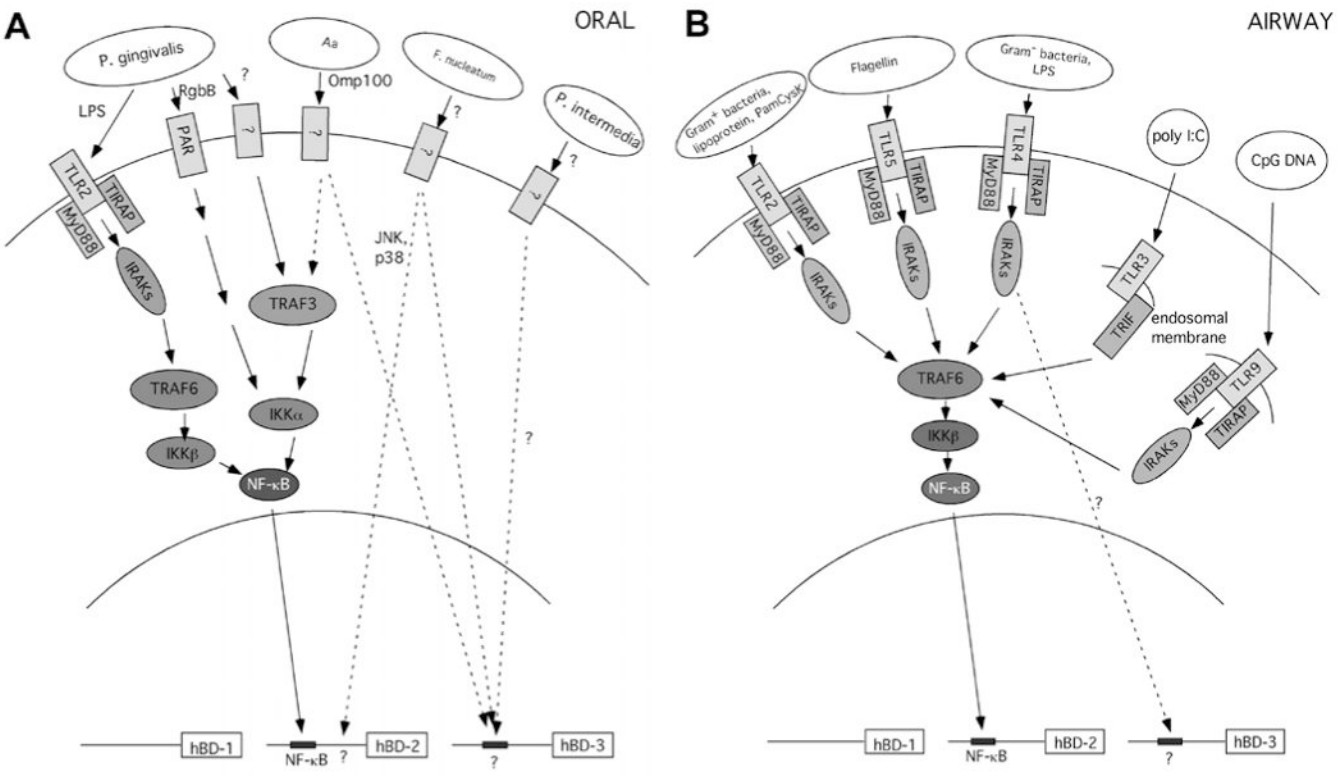

Figure 2.

Pathways stimulated by microbes and their associated molecular patterns in oral **(A)** and airway **(B)** epithelial cells leading to β-defensin expression. These models are based solely on published studies demonstrating the role of the respective receptors, adapter molecules, and transcription factors. In some cases, *e.g.*, stimulation of hBD-2 by *P. intermedia*, specific stimulatory molecule, receptor, pathway, and/or transcription factors have not been identified, and are shown as a question mark.

Table 1

Susceptibilities of Oral and Airway Pathogens to HDPs

		Minimal Inhibitory Concentration, μg/mL					
Species	**Strain**	**HNP1-3**	**hBD1**	**hBD2**	**hBD3**	**LL-37**	**Reference**
S. sanguinis	MPC-1				31.3	62.5	Ji *et al.*, 2007a
	66×49				62.5	37.2	Ji *et al.*, 2007a
A. actinomycetemcomitans	Y4			>250	47.9		Joly *et al.*, 2004
					49.6	37.8	Ji *et al.*, 2007a
			50		200	200	Ouhara *et al.*, 2005
		>500					Miyasaki and Lehrer, 1998
P. gingivalis	ATCC33277			34.6	5.7		Joly *et al.*, 2004
					31.3	>125	Ji *et al.*, 2007a
	W50			>250	>250		Joly *et al.*, 2004
			50		200	100	Ouhara *et al.*, 2005
	FDC381	nd					Miyasaki and Lehrer, 1998
T. denticola	ATCC33521				15.7	39.4	Ji *et al.*, 2007a
			>100	>100			Brissette and Lukehart, 2002
F. nucleatum	ATCC10953				7.8	4.9	Ji *et al.*, 2007a
	1908			>250	13.2		Joly *et al.*, 2004
	ATCC49256			10.3	4.5		Joly *et al.*, 2004
	ATCC25586	>100					Miyasaki and Lehrer, 1998
	21		20		12.5	12.5	Ouhara *et al.*, 2005
P. aeruginosa	NCTC 6750	1					Varkey and Nagaraj, 2005
	MR3007	>250				4.7	Turner *et al.*, 1998
	(not specified)			10			Harder *et al.*, 1997
	clinical					64	Saiman *et al.*, 2001
	PA01				26.5		Garcia *et al.*, 2001a
	ATCC27853		100	75	25		Huang *et al.*, 2007
20 mM NaCl						16	Bals *et al.*, 1998b
155 mM NaCl						250	Bals *et al.*, 1998b
300 mM NaCl						300	Bals *et al.*, 1998b
S. aureus	29213			9.5	210	20	Kisich *et al.*, 2007
	COL		>50	10	5	5	Midorikawa *et al.*, 2003

Species	Strain	Minimal Inhibitory Concentration, μg/mL					Reference
		HNP1-3	hBD1	hBD2	hBD3	LL-37	
	NCTC8530	0.8					Varkey and Nagaraj, 2005
	(not specified)			100			Harder *et al.*, 1997
	67395	7.9				2.9	Turner *et al.*, 1998
B. cepacia	ATCC25416	>250				79.1	Turner *et al.*, 1998
	ATCC17770				6.6		Garcia *et al.*, 2001a

Table 2

Expression of HDP Genes in Oral and Airway Epithelium

Oral Cavity			
Peptide	**Site of Expression**	**Regulation**	**Stimulant**
hBD-1	Gingival epithelium	Inducible	*P. gingivalis*
hBD-2	Salivary glands, epithelium	Inducible	F. nucleatum, *A. actinomycetemcomitans*, IL-1β
hBD-3	Keratinocytes	Inducible	*F. nucleatum*, *A. actinomycetemcomitans*
LL-37	Gingival Epithelium	Inducible	Vitamin D
	Neutrophils	Constitutive	-
Histatins	Salivary glands	Constitutive	-
Airway			
Peptide	Site of Expression	Regulation	Stimulant
hBD-1	Ciliated epithelium	Constitutive	-
	Plasmacytoid DCs	Inducible	Enveloped viruses
hBD-2	Ciliated epithelium	Inducible	Bacteria, TLR agonists, cytokines
hBD-3	Ciliated epithelium	Inducible	Bacteria, TLR agonists, cytokines
LL-37	Ciliated epithelium	Inducible	Vitamin D
	Neutrophils	Constitutive	-