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INVITED MEDICAL REVIEW

Beta-defensins: what are they REALLY doing in the oral cavity?

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Initially identified as broad-spectrum antimicrobial peptides, the members of the β-defensin family have increasingly been observed to exhibit numerous other activities, both *in vitro* and *in vivo*, that do not always relate directly to host defense. Much research has been carried out in the oral cavity, where the presence of commensal bacteria further complicates the definition of their role. In addition to direct antimicrobial activity, β-defensins exhibit potent chemotactic activity for a variety of innate immune cells, as well as stimulating other cells to secrete cytokines. They can also inhibit the inflammatory response, however, by the specific binding of microbe-associated molecular patterns. These patterns are also able to induce the expression of β-defensins in gingival epithelial cells, although significant differences are observed between different species of bacteria. Together these results suggest a complex model of a host-defense related function in maintenance of bacterial homeostasis and response to pathogens. This model is complicated, however, by numerous other observations of β-defensin involvement in cell proliferation, wound healing and cancer. Together, the *in vitro*, *in vivo* and human studies suggest that these peptides are important in the biology of the oral cavity; exactly how is still subject to speculation.

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Introduction

In the 1980s, research began in earnest to identify oxygen-independent antimicrobial agents that played a role in the killing of phagocytosed microbes. This led to the characterization of a class of cysteine-rich peptides, found at high concentrations in the granules of neutrophils and macrophages (Selsted *et al*, 1984, 1985). As a result of their potent, *in vitro* antibacterial and antiviral activities, they were assumed to contribute in this fashion to innate host defense, and were thus named defensins. Subsequently, examination of a similar innate immune role played by mucosal epithelial cells led to the discovery of similarly structured peptides expressed in both the bovine airway (Diamond *et al*, 1991) and the mouse (Ouellette and Lualdi, 1990) and human intestinal epithelium (Jones and Bevins, 1992). The intestinal peptides were closely related to the phagocytic defensins, but the bovine airway peptides [and homologues found in bovine neutrophils (Selsted *et al*, 1993)] were sufficiently different to require the reclassification into two families: the alpha-defensins, primarily found in neutrophils and in the intestine; and the beta-defensins, primarily found in other epithelia. In humans, three β-defensins are predominantly expressed in epithelial cells (hBD-1, 2, and 3), although expression in some myeloid cells has been observed (Duits *et al*, 2002; Ryan *et al*, 2003). In addition, other, recently discovered β-defensins, such as hBD-9, have been seen in some epithelial cells, including gingival epithelium (Premratanachai *et al*, 2004).

Examination of the genes that encoded both family members suggested that α-defensin activity was mostly regulated post-translationally (Harwig *et al*, 1992; Valore and Ganz, 1992; Ganz *et al*, 1993; Valore *et al*, 1996), while β-defensin gene expression was regulated at the transcriptional level by interaction with pathogens (Diamond *et al*, 2000; Beckloff and Diamond, 2008). Thus was born the convention that β-defensins were an integral member of the innate immune defense against microbial pathogens, especially at the epithelial interface. Initially identified in airway epithelium (Diamond *et al*, 1991), subsequent analysis demonstrated that these β-defensins were expressed almost uniformly in other epithelial surfaces as well, including the oral cavity, suggesting the β-defensins were important in killing microbes at the epithelial surface [reviewed in (Diamond *et al*, 2004)]. However, the stark differences

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between the almost sterile environment of the airway and the microbe-laden oral cavity suggested a more complex role played by these peptides. This realization led to a broad examination of the activities of β-defensins, both *in vitro* and *in vivo*, to determine their actual function (or functions).

Antimicrobial activity

As stated above, β-defensins were first isolated and characterized based on their antimicrobial activity. *In vitro* assays demonstrate broad-spectrum activity of all β-defensins against Gram-positive and -negative bacteria, viruses (predominantly enveloped), and fungi, with minimal inhibitory concentrations in the $\mu g\ ml^{-1}$ range. With respect to oral infectious agents, activity was observed against periodontal pathogens including *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, as well as other oral pathogens, including *Streptococcus mutans*, *Candida albicans* and other non-albicans Candida species (Joly *et al*, 2004; Feng *et al*, 2005; Ji *et al*, 2007; Song *et al*, 2009). However, in contrast to other tissues, where activity against pathogens was almost universal, there were wide variabilities in susceptibility of periodontal pathogens to the different β-defensins. The broad spectrum of activity, together with the observation of some resistance, suggested to most that β-defensins function as a first line of defense against microbial colonization in the oral cavity. Furthermore, some periodontal pathogens such as *Porphyromonas gingivalis*, and *Treponema denticola* have developed resistance to killing by β-defensins (Devine *et al*, 1999; Brissette and Lukehart, 2002; Shelburne *et al*, 2005), which enhances their pathogenicity. A recent study to support the antibacterial activity of β-defensins demonstrates the intracellular killing of *F. nucleatum* in gingival epithelial cells (GEC) by hBD-2 and 3 (Ji *et al*, 2010). Antiviral activity of β-defensins against HIV and HSV is also observed [(Weinberg *et al*, 2006; Quinones-Mateu *et al*, 2003), Ryan LK, Dai J, Yin Z, Megjugorac N, Uhlhorn V, Yim S, Schwartz KD, Abrahams JM, Diamond G, Fitzgerald-Bocarsly P, unpublished data]. In the oral cavity, it was recently demonstrated that adult gingival epithelial cells, which express hBD-2 and 3, inactivate HIV, whereas fetal cells not expressing those β-defensins allow for viral infectivity (Tugizov *et al*, 2010). Inactivation of the β-defensins in the adult cells restored the infectivity of the HIV, suggesting that they play a direct role in antimicrobial defense of the oral cavity.

To further support the hypothesis that β-defensins act as part of the innate antimicrobial defense in the oral mucosa, numerous studies were carried out demonstrating the microbe-mediated regulation of β-defensin gene expression [reviewed in (Diamond *et al*, 2009)]. Expression of human β-defensins 1–3 has been demonstrated in gingival epithelium, buccal epithelium, dental pulp and salivary gland tissue reviewed in (Diamond *et al*, 2008). More specifically, hBD-1, as with other epithelial tissues, is expressed at a low level, with little regulation in response to infection or other stimuli. The inducible β-defensins, hBD-2 and -3, are generally expressed at low levels *in vivo* under normal conditions, and then induced in response to microbial colonization and inflammation. Specifically, these β-defensins are induced by Candidal infections in buccal epithelium (Sawaki *et al*, 2002); in gingivitis (Offenbacher *et al*, 2009); and in periodontal disease (Lu *et al*, 2004). *In vitro* studies using both cell lines and primary cultures confirm this induction and have identified numerous signal transduction pathways responsible for the induction. In general, it is observed that hBD-2 and 3 are induced *in vitro* in gingival and buccal epithelial cells in response to most microbial pathogens [reviewed in (Diamond *et al*, 2008)]. These genes are similarly regulated in odontoblasts (Veerayutthwilai *et al*, 2007). *In vivo*, this can be also observed in rat gingival epithelium in response to *A. actinomycetemcomitans* colonization (Kurland *et al*, 2006). Interesting to note is that some bacterial species, such as *P. gingivalis*, do not induce β-defensin expression *in vitro* (Krisanaprakornkit *et al*, 2000), and *T. denticola* is able to suppress the induction through interaction with the signal transduction pathway (Brissette *et al*, 2008; Shin and Choi, 2010 Shin *et al*, 2010). Moreover, in contrast to the bacterial induction of β-defensins in other epithelial cells, *F. nucleatum* and *A. actinomycetemcomitans* do not induce *via* binding of lipopolysaccharide (LPS) to toll-like receptor (TLR) 4, but rather through other bacterial products and receptors. This demonstrates an important development in the differentiation of mucosal epithelial tissues in the body with respect to their natural microbiota. This is not limited to the oral cavity, as a recent study similarly showed the differential induction of β-defensin genes in the skin by commensals and pathogens (Wanke *et al*, 2010).

As direct stimulation of β-defensin expression by microbial pathogens supports a defensive role of the peptide, so too does their induction by cytokines that themselves are also stimulated through innate immune pathways. β-defensin expression is induced in epithelial cells by pro-inflammatory cytokines, including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and Il-17, and the IL-1β-mediated induction in keratinocytes can be significantly enhanced by IL-12, -23, and -27 [reviewed in (Diamond *et al*, 2008, 2009)]. *In vivo* studies support a role for IL-17-mediated induction of β-defensins in defense against Candida colonization (Conti *et al*, 2009).

Together, the results that β-defensins are directly antimicrobial, and that microbial pathogens and pathogen-induced cytokines stimulate their expression in oral epithelial cells, strongly supports the hypothesis that they function as direct antibiotic agents to maintain a homeostasis of microbes in the oral cavity, and that they can naturally function to prevent colonization of pathogens. Furthermore, some pathogens have developed either resistance to the antimicrobial activity, or have evolved evasive mechanisms to not induce, or to suppress induction.

Taken together, the numerous published results described above strongly support a defensive role of β-defensins in the oral cavity, to maintain homeostatic

levels of commensal bacteria, and to protect against colonization by pathogenic microbes, both under constitutive circumstances, and in response to the interaction of the pathogen with the epithelial cell.

Chemotaxis and innate immune signaling

As can be seen from Table 1, β-defensins, like most antimicrobial peptides, exhibit broad-spectrum antimicrobial activity in the $\mu g\ ml^{-1}$ range. However, it was soon discovered that they also exhibit numerous other activities at much lower concentrations, suggesting either that the direct antimicrobial activity is attributed to non-physiologically correct concentrations, or that β-defensins have multiple roles in host defense. As with other cationic peptides, binding to LPS was observed, affecting the ability of LPS to stimulate an innate immune response (Scott *et al*, 2000; Semple *et al*, 2010). Subsequently, however, it was recognized that β-defensins bind numerous bacterial and viral antigens, independent of their microbicidal activity [reviewed in (Kohlgraf *et al*, 2010b)]. Therefore, while *P. gingivalis* appears to have developed two different mechanisms to evade β-defensin-mediated defense, by resistance to direct killing and by the lack of induction of β-defensin gene expression, another interesting observation has been made to suggest a different role of β-defensins in host defense against this periodontal pathogen. Specifically, hBD-3 was found to bind to *P. gingivalis* hemagglutinin B (hagB) (Dietrich *et al*, 2008; Pingel *et al*, 2008). This adherence can prevent the binding of the bacterium to keratinocytes and dendritic cells (DCs). Such an inhibition, observed as a reduction in cytokine stimulation (Pingel *et al*, 2008; Kohlgraf *et al*, 2010a) can suppress what could be a detrimental inflammatory response. This could be an essential function to suppress inflammation in the microbe-laden environment of the oral cavity. It can also lead to an enhanced adaptive immune response (see below), by increasing the endocytosis into antigen-presenting cells such as DCs.

As members of the defensin family exhibit structural similarity to cytokines (Cole *et al*, 2001), and they were released upon stimulation by pathogens, they were examined for chemotactic activity. Surprisingly, β-defensins were observed to exhibit potent chemotactic activity for a variety of immune cells, at concentrations at least 2–3 logs below that of their antimicrobial activity [reviewed in (Yang *et al*, 2002)]. Specifically, hBD-1-3 can all recruit memory T cells and immature dendritic cells (Yang *et al*, 1999). hBD-2 and 3 can recruit neutrophils and mast cells (Niyonsaba *et al*, 2002, 2004), and induce mast cell degranulation as well as other effects on these cells (Niyonsaba *et al*, 2001; Chen *et al*, 2007). hBD-3 is also chemotactic for monocytes. Surprisingly this occurs differentially, based on the disulfide structure, and may vary *in vivo* depending on the inflammatory state (Wu *et al*, 2003). This chemotaxis may also be enhanced by the binding of β-defensins to glycosaminoglycans (Seo *et al*, 2010).

One additional activity of hBD-3 suggests a possible role in defense against HIV infection, which could support an important function in the oral cavity. This β-defensin binds to the chemokine receptor CXCR4 on T cells, which can lead to internalization of the receptor, protecting the cell from HIV-1 infection (Feng *et al*, 2006).

Table 1 Some activities of β-defensins in the oral cavity

Activity	*Target*	*Method*	*Reference*
Direct antimicrobial	Periopathogenic bacteria	Lytic	(Joly *et al*, 2004; Ouhara *et al*, 2005; Ji *et al*, 2007)
	Cariogenic bacteria	Lytic	(Joly *et al*, 2004; Ouhara *et al*, 2005)
	Candida albicans	Lytic	(Feng *et al*, 2005)
	Root canal pathogens	Lytic	(Song *et al*, 2009)
	Viruses	Inhibition of binding, replication; immune modulation	(Weinberg *et al*, 2006)
Chemotactic	Memory T cells/DCs	Binding of CCR6	(Yang *et al*, 1999)
	Neutrophils/mast cells	Binding of CCR6	(Niyonsaba *et al*, 2002, 2004)
	Monocytes	Biding of CCR2	(Wu *et al*, 2003; Rohrl *et al*, 2010)
Cell signaling			
Induction of chemokines	Keratinocytes	–	(Niyonsaba *et al*, 2007)
	Mast cells	–	(Niyonsaba *et al*, 2010)
Inhibition of cytokines	Macrophages	Binding to LPS?	(Semple *et al*, 2010)
	Keratinocytes	Binding to microbial antigens	(Kohlgraf *et al*, 2010a)
Adaptive immunity			
Enhanced immune response	iDCs	Binding to CCR6	(Biragyn *et al*, 2001)
	–	Binding to microbial antigens	(Kohlgraf *et al*, 2010b)
	DCs	Induction of co-stimulatory molecules	(Funderburg *et al*, 2007)
Wound healing			
Promotion of cell proliferation	Keratinocyte	Phosphorylation of EGFR, STATs	(Niyonsaba *et al*, 2007)
Cancer	Recruitment of tumor-associated macrophages	Binding to CCR2	(Jin *et al*, 2010)
	Enhancement of angiogenesis	Binding to vitronectin receptor	(Baroni *et al*, 2009)
Venom	Multiple systems	Pharmacologic	(Whittington *et al*, 2008a,b)

LPS, lipopolysaccharide; DC, dendritic cell; EGFR, endothelial growth factor receptor; CCR6 CC-chemokine receptor 6.

In addition to their own direct chemotactic activity, β-defensins can also induce cells to express chemokines. Treatment of keratinocytes with hBD-3 leads to the induction of monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-3α (MIP-3α; also known as the chemokine CCL20) and interferon-γ inducible protein-10 (IP-10; CXCL10) (Niyonsaba *et al*, 2007). Mast cells are also induced by β-defensins to secrete IL-31 and other cytokines and growth factors (Niyonsaba *et al*, 2010). One very important study in the oral cavity that appears to tie these results together demonstrated that β-defensins are stimulated differentially by different oral bacteria in both GEC and dendritic cells (Yin *et al*, 2010). Additionally, the IL-β stimulated in the DCs by the bacteria induces expression of β-defensins in the GEC, leading to a model of cross-talk between immune cells such as DCs and the epithelium in the β-defensin response to periodontal pathogens.

While the activities described above could be considered pro-inflammatory, some β-defensins have also been observed to exhibit immunosuppressive activities, by inhibiting the production of TNF-α and IL-6 (Kohlgraf *et al*, 2010a; Semple *et al*, 2010). Thus, it appears that β-defensins can contribute to host defense and innate immunity in multiple ways, often in opposite directions.

β-defensins in adaptive immunity

It is now evident that in addition to their potential role in innate immunity, β-defensins can also enhance the adaptive immune response [reviewed in (Yang *et al*, 2004)]. What is known is that β-defensins (along with other antimicrobial peptides) can act as potent adjuvants that enhance the adaptive immune response [reviewed in (Kohlgraf *et al*, 2010b)]. When co-introduced intranasally into mice with ovalbumin (OVA), hBD-1 and 2 enhanced the OVA-specific IgG response (Brogden *et al*, 2003). When DNA constructs that encoded mouse β-defensins 2 and 3 fused to non-immunogenic antigens were introduced into mice, the antigens became immunogenic. This induction of an adaptive immune response is attributed to the binding of the β-defensin to CCR6 on immature DCs (Biragyn *et al*, 2001). The mechanisms of β-defensin's role in adaptive immunity may involve the induction of DC maturation and induction of co-stimulatory molecules (Funderburg *et al*, 2007).

Wound healing

As part of host defense independent of antimicrobial activity, β-defensins are also associated with the promotion of wound healing. Keratinocytes secrete hBD-3 at wound sites, which can be induced in these cells in response to growth factors such as insulin growth factor (IGF)-1, TGF-α and epidermal growth factor (EGF) (Sorensen *et al*, 2003). hBD-2 is significantly induced by EGF, although only when co-stimulated with IL-1α (Johnston *et al*, 2010). While this could be considered to act at a wound site in an antibacterial capacity to prevent infection, it is interesting to recognize that hBD-3 can promote the proliferation and migration of keratinocytes, by the phosphorylation of EGF receptor and STAT proteins (Niyonsaba *et al*, 2007).

Cancer

β-defensins appear to play specific roles in regulating tumor growth and metastasis by manipulating the tumor microenvironment to favor tumor development in oral carcinomas, acting as tumor suppressor genes or exhibiting direct cytotoxic activity toward cancer cells. In addition, β-defensins link innate immune responses to adaptive immune responses and may activate anti-tumor immunity. All of these observations suggest that dysregulation of β-defensins may be associated with tumor development. Abiko *et al* first observed this alteration of the pattern of hBDs in oral tumors (Abiko *et al*, 1999) and subsequent studies from this group confirmed this observation (Yoshimoto *et al*, 2003).

Recent studies have examined the role of β-defensin involvement in oral cancer in more depth. In normal, undiseased oral epithelium, hBD-3 is produced in dividing cells in the basal layers, whereas hBD-1 and hBD-2 are coexpressed in the differentiated spinosum and granulosum layers (Kawsar *et al*, 2009). Precancerous cells overexpress hBD-3 *in situ* and correlate with the specific recruitment and infiltration of macrophages, while hBD-1 and hBD-2 were not found (Kawsar *et al*, 2009). EGF-induced hBD-3 expression in both human oral epithelial cells and in TR146 oral cancer cells *via* the involvement of EGF signaling mediators such as activated AKt40, JNK, JUN41, and activated MEK kinase 1 (Kawsar *et al*, 2009). Pharmacologic inhibitors of intracellular kinases inhibited EGF-induced hBD-3 expression, indicating that the activation of these EGF signaling mediators is also involved in the pathway of EGF-induced hBD-3 expression (Kawsar *et al*, 2009).

In another study of 45 oral squamous cell carcinoma (OSCC) mucosa samples, hBD-3 was localized to epithelial cells, lymphocytes, plasma cells, fibroblasts and differentiated fibrocytes and their surrounding stroma, but no significant dependence of hBD-3 mRNA levels correlating with tumor size, histological differentiation, cervical lymph node status or stage of the cancers was found. However, a significantly higher expression of hBD-3 was detected in the corresponding healthy mucosa of T3/T4 tumors compared with healthy mucosa of T1/T2 tumors. In addition, fluorescent staining of hBD-3 was much higher in intensity in OSCC mucosa compared with healthy mucosa, which showed scattered expression in the spinous and granular cell layer (Kesting *et al*, 2009).

Jin *et al* (2010) showed that oral carcinomas produce hBD-3 *in situ*, but not MCP-1 or hBD-2. hBD-3 chemoattracts monocytes/macrophages by binding to CCR2 (Rohrl *et al*, 2010). Pretreatment with MCP-1, which also binds to CCR2, or the CCR2-specific inhibitor, RS102895, desensitized hBD-3-induced migration of monocytes (Jin *et al*, 2010). Tumor-associated macrophages have long been associated with the

inflammatory microenvironment of tumors that progress and constitute a poor prognosis for the patient (Pollard, 2004; Allavena *et al*, 2008). hBD-3 also stimulated the expression of tumor-promoting cytokines and chemokines, including IL-1α, IL-6, IL-8, CCL18, and TNF-α in peripheral blood monocytic cell-derived macrophages. Therefore, hBD-3 is important in establishing a tumor-associated inflammatory microenvironment by recruiting monocytes from the peripheral blood, which become tumor-associated macrophages that support growth and progression of tumors (Jin *et al*, 2010).

Kawsar *et al* (2010) compared the expression of hBD-2 in biopsy specimens of undiseased oral mucosa, OSCC and Kaposi's sarcoma (KS) lesions using CD34 as a biomarker for endothelial cells (Kawsar *et al*, 2010). They found that hBD-2 was localized in the terminally differentiated OSCC lesions adjacent to keratin pearls, while the vascular endothelia in the stromal region extending from the normal tissue adjacent to the OSCC lesion did not express hBD-2. Expression of hBD-2 was also observed in cancer cells of the OSCC lesion. In KS, CD34 expressing spindle-shaped endothelial cells and a collection of irregular endothelium produced hBD-2. They were also able to show that TGF-β1 and IL-1β induced a significant increase in hBD-2 peptide in human vascular endothelial cell lysates and that hBD-2 was diffusely distributed as granules throughout the cytoplasm of these endothelial cells. The cells stimulated with TGF-β1 or IL-1β had in higher amounts of hBD-2 in the granules compared with unstimulated cells.

As Baroni *et al* (2009) demonstrated that hBD-2 can independently induce migration of endothelial cells *via* the $\alpha_v\beta_3$ vitronectin receptor, indicating that hBD-2 is able to modulate angiogenic processes through mechanisms that are independent of vascular endothelial growth factor, hBD-2 expression may play a role in enhancing tumor angiogenesis and cancer metastasis (Baroni *et al*, 2009). In addition, the hBD-2 expressed from endothelial cells in the OSCC microenvironment could also recruit immature dendritic cells to the tumor site, as hBD-2 has been shown to recruit DC and memory T cells (Yang *et al*, 1999). Once at the site, other human β-defensins could theoretically induce the maturation and activation of the dendritic cells, as shown in a mouse model, where mBD-2 caused the activation of immature dendritic cells *via* TLR4 (Biragyn *et al*, 2002).

Human BD-2 was also localized in epidermoid and intermediate cells of oral mucoepidermoid cells, along with the epithelial hyperplasia regions adjacent to the tumor tissue (Mizukawa *et al*, 2001).

In a study of five OSCC tumors, gene expression of hBD-1 in OSCC dropped 50-fold compared with healthy gingival tissue levels of hBD-1 mRNA (Wenghoefer *et al*, 2008b). Irritation fibromas and leukoplakia lesions did not have the precipitous drop in gene expression of hBD-1, but hBD-1 mRNA levels decreased five-fold and two-fold, respectively. Another study corroborated this observation in OSCC cell lines compared with healthy gingival kerotinocyte cell lines (Joly *et al*, 2009). The ability of hBD-1, -2, and -3 to be induced by IL-1β, TNF-α, IFN-γ, IL-1β combined with IFN-γ, IL-1β combined with TNF-α, and IFN-γ combined with TNF-α was significantly reduced in the OSCC cell lines. Four hBD-1 single nucleotide polymorphisms (SNP) were differentially distributed between cancer and control populations in this study. In addition, genotype distribution at the hBD-1 locus suggested loss of heterozygosity in OSCC. Combined with the rise in hBD-3 in the tumors and the rise of hBD-2 in the endothelium of the tumor microenvironment, this suggests that hBD-1 loss may also contribute to the progression of OSCC.

Two other studies also showed that hBD-1 was modulated in oral tumors of the salivary glands. In one study, hBD-1 was detected in healthy salivary gland tissue and in benign and malignant tumors in the salivary gland, but in all seven of the malignant tumors, hBD-1 was located only in the nucleus, whereas in the non-malignant cells, hBD-1 was located in the cytoplasm (Wenghoefer *et al*, 2008a). In the other study, hBD-1 was downregulated in pleomorphic adenomas of the salivary glands (Pantelis *et al*, 2009). Like the other study, the hBD-1 translocated to the nucleus. The results suggest that hBD-1 may accumulate in the nucleus and downregulate its own promotor. Thus, hBD-1 may be acting as a tumor suppressor gene and may play a role in the oncogenesis of these tumors of the oral cavity.

Conclusions

The studies described above clearly demonstrate that the role(s) of β-defensins in the oral cavity in particular, and in the body in general are anything but well-defined. Most of the studies, however, were either *in vitro* experiments, or descriptive analyses of β-defensin expression in different conditions. More definitive studies would require *in vivo* experimentation. Unfortunately, genetic deletion of only one β-defensin (BD-1) has been described (Morrison *et al*, 2002; Moser *et al*, 2002), to allow for such *in vivo* studies. Only a few studies with these mice have been performed. Infection of these mBD-1 knockouts with airway pathogens has demonstrated some reduction in defense against bacterial colonization in the first 24 h (Morrison *et al*, 2002; Moser *et al*, 2002), and a reduction of viral pathogenesis early in infection with influenza virus with an increased morbidity and mortality (Ryan and Diamond, 2010). As these β-defensins are closely linked on the same chromosome, production of knockout mice for multiple β-defensins is not possible.

Human studies have been somewhat more productive, with the association between several SNPs in the hBD-1 gene and several infectious diseases, including periodontal disease (Schaefer *et al*, 2010), dental caries (Ozturk *et al*, 2010) and colonization by *C. albicans* (Jurevic *et al*, 2003). Variability in the expression of hBD-2 and 3 has also been associated with susceptibility to inflammatory bowel disease (Zilbauer *et al*, 2010), which would suggest a role in inflammation.

Evolutionary studies have suggested that defensins, including the human β-defensins, are extraordinarily ancient, having evolved from sequences in myxobacteria (Zhu, 2007). Further analysis has identified homologous β-defensins in a wide variety of bird (van Dijk *et al*, 2008) and fish species (Wong *et al*, 2007), as well as reptiles (Stegemann *et al*, 2009). In addition, there are homologous structures in platypus venom and reptile venoms (Whittington *et al*, 2008a,b), suggesting that evolution has used the basic structure of the β-defensin to address different functions in the oral cavity, depending on the species. In light of these studies, together with the widely variable *in vitro* activities described above it is interesting to speculate on the numerous actual functions that may be played by these multifaceted molecules.

Author contributions

Gill Diamond and Lisa K. Ryan contributed to the writing of the manuscript.

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