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Epithelial cell-derived antimicrobial peptides are multi-functional agents that bridge innate and adaptive immunity

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Mucosal barriers are not only physical; they are the source of potent antimicrobial peptides. These ancient compounds are important for the innate defense of an eukaryotic host. They function within a matter of hours, on a broad spectrum of bacteria, fungi, and encapsulated viruses (reviewed in (34, 94, 102)). The innate immune system works in conjunction with the adaptive immune system in mammals, by permitting the host to curb, delay, or avoid microbial growth shortly after an infection. For example, human β-defensins, specific epithelial cell-derived antimicrobial peptides (see below), have been shown to “cross-talk” with the adaptive immune system by interacting with specific chemokine and Toll-like receptors, resulting in modulation of immunocompetent cell responses of the host (6, 27, 29, 98). It is theorized therefore, that surveillance through epithelial cell-derived antimicrobial peptides functions to keep the natural flora of microorganisms in a steady state in different niches such as the skin, the intestines, and the mouth. This review will highlight recent findings, by our group and others, demonstrating that antimicrobial peptides are not just antimicrobial; they play an added role of cross-talking with a number of cell types in functions as diverse as regulating epithelial cell proliferation, enhancing wound healing, inhibiting/inducing pro-inflammatory cytokines, promoting/inhibiting angiogenesis, stimulating chemokine production, promoting chemotaxis of various leukocytes, degranulating mast cells or modulating host cell gene expression.

Small cationic antimicrobial peptides of epithelial cell origin

It is important to note, at the outset, that human epithelial cells, oral epithelial cells notwithstanding, are a rich source of antimicrobial peptides. While this review will focus principally on recent data highlighting the newly discovered regulatory functions of human β-defensins and LL-37 (see below), the field has yet to discover if other epithelial derived AMP's also harbor immunoregulatory functions. It stands to reason that the aforementioned peptides may work in concert with other epithelial cell-derived antimicrobial peptides. These include S100 proteins such as calprotectin (73) and psoriasin (S100A7) (25, 55), the cathelicidin LL-37 (28), adrenomedullin (3, 45), secretory leukocyte protease inhibitor (80), neutrophil gelatinase-associated lipocalin (10, 32), and the host-defense-related angiogenin, RNase 7 (37). Table I summarizes the antimicrobial peptides discussed in this article. We have intentionally omitted antimicrobial peptides of salivary gland origin such as histatins (69), as this review focuses on oral epithelial cell-derived antimicrobial peptides. S100 proteins regulate a number of epithelial cell functions including intracellular Ca^{2+} signaling, differentiation, cell-cycle progression, cytoskeletal membrane interactions, leukocyte

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chemotaxis (25, 40) and, in the case of psoriasin and calprotectin, they also contribute to innate host defense. Psoriasin, initially characterized as a psoriasis specific marker, is secreted by skin keratinocytes and is very effective in killing *Escherichia coli*, which could explain why skin is naturally resistant to *E. coli* colonization (31). Calprotectin exhibits biostatic activity against the oral fungal opportunist *Candida albicans* (17, 82). LL-37, LL-37 (named after the first two N-terminal residues and the total number of residues of the mature peptide) is a member of the cathelin family of antimicrobial peptides with a characteristic α-helix (101). While highly expressed in psoriatic lesions, LL-37 is decreased in lesions of atopic dermatitis, similar to what has been described for the inducible human β-defensins (66, 68). Decreased expression of human β-defensins and LL-37 appear therefore to predispose atopic dermatitis patients to skin infections; complications not encountered in psoriasis patients (66). Adrenomedullin is a pluripotent peptide that can be induced in human oral epithelial cells by oral bacteria (45). It demonstrates broad spectrum antimicrobial activity in low micromolar concentrations (3). Secretory leukocyte protease inhibitor, a member of the Kazal superfamily of serine protease inhibitors generally containing 3–7 tandem Kazal domains, structures originally named in reference to a pancreatic secretory trypsin inhibitor first isolated by Kazal et al (48), is highly expressed in respiratory secretions and saliva and exhibits anti-bacterial, anti-retroviral and anti-inflammatory properties (41, 92). Interestingly, salivary concentrations of secretory leukocyte protease inhibitor have been shown to decrease with advanced age (81). Neutrophil gelatinase-associated lipocalin, is a siderophore-binding protein that exerts a bacteriostatic effect by sequestering iron (10). It is found in human saliva and is secreted from oral epithelial cells (95). Interestingly, human β-defensin 3, secretory leukocyte protease inhibitor and neutrophil gelatinase-associated lipocalin are all regulated via the epidermal growth factor receptor by transforming growth factor-α and insulin-like growth factor-I (83). RNase 7, a member of the RNase A superfamily, exhibits broad-spectrum antimicrobial activity against gram-negative bacteria and the yeast *C. albicans* at low micromolar concentrations (37). It is expressed in various mucosal sites, including the oral cavity, and is induced by proinflammatory cytokines and bacteria (37).

β-defensins

The discoveries that β-defensins originate in mammalian mucosal epithelium (8, 23, 30, 35, 36, 38, 57, 67, 75, 103) has led to the hypothesis that these antimicrobial peptides function to protect the host against microbial pathogenesis at critical confrontational sites. We have extended this hypothesis to also encompass the oral epithelium (20, 51, 52, 94). This tissue, and cells derived from it, constitutively express human β-defensin 1 and can be induced to express human β-defensins 2 and 3 (24, 51, 52, 94). In addition to antimicrobial properties, β-defensins engage the CCR6 receptor on selected immune effector cells, such as immature dendritic cells and T cells and, in a chemokine manner, lead to recruitment of these cells to the site of interest (98). Moreover, we have demonstrated that human β-defensin 3 can down modulate the human immunodeficiency virus (HIV) co-receptor CXCR4, leading to antagonism of cellular activity (27). In addition, antigen presenting cells undergo maturation in the presence of human β-defensin 3 via Toll-like receptors 1 and 2 (29). With the identification of 28 new human β-defensin genes in five syntenic chromosomal regions (76), it is likely that new and well characterized beta defensins are playing a key role in mediating the complex interaction between diverse microorganisms in our environment, the innate host defense system and the acquired immune response necessary to protect hosts from foreign invaders.

β-defensins and the oral cavity

The first evidence of β-defensins in a mammalian oral cavity was described in 1995 by Schonwetter et al. (75). Since then, we and others have described the presence of β-defensins in the human oral cavity (9, 20, 24, 52, 56, 60, 74). In gingival tissue, mRNA for both human β-defensins 1 and 2 is localized in suprabasal stratified epithelium and the peptides are detected in upper epithelial layers consistent with the formation of the stratified epithelial barrier (20) (Fig. 1). β-defensins 1 and 2 are not detected in junctional epithelium that serves as the attachment to the tooth surface. Our investigations into the distribution of β-defensin 3 expression in oral epithelium suggests that while β-defensin 2 compartmentalizes to the more differentiated stratum granulosum and spinosum, β-defensin 3 is expressed in the less differentiated stratum basale (47) (Fig. 1); further suggesting "cross-talk" capacity between this peptide and resident immunocompetent cells. Most recently, β-defensin 3 has been shown to be overwhelmingly produced in premalignant epithelial cells in carcinoma *in situ* and that this correlates with recruitment and infiltration of monocytes/macrophages exclusively to the lesion site (47). We are currently assessing the ability of β-defensin 3 to promote chemotaxis of monocytes/macrophages via a novel G protein coupled receptor (unpublished data).

α-defensins are detected only in polymorphonuclear neutrophils that migrate through the junctional epithelium (20), a localization that persists during inflammation, when the junctional epithelium and surrounding tissue are highly infiltrated with polymorphonuclear neutrophils. Therefore, the undifferentiated junctional epithelium contains exogenously expressed α-defensins and the stratified epithelium contain endogenously expressed β-defensins. These results demonstrate that defensins are localized in specific sites in the gingiva, are synthesized in different cell types, and are likely to serve different roles in various regions of the periodontium.

The distribution of β-defensins in salivary glands also suggests a degree of specificity for these peptides. While human β-defensin 1 is ubiquitous to all salivary glands (74), human β-defensin 3 expression is rarely found in salivary glands (24), and human β-defensin 2 is expressed only in minor salivary glands (9, 56).

A notable difference between oral and most other epithelia is the expression of human β-defensins 2 and 3. These defensins are expressed only in the presence of infection or inflammation in most tissues, including skin, trachea and gut epithelium (4, 54, 67, 68, 93). However, both human β-defensins 2 and 3 are expressed in normal uninflamed gingival tissue (20). We are currently testing the hypothesis that the baseline level of human β-defensin 2 expression in oral epithelium is due to the chronic exposure of the tissue to specific oral commensal bacteria that induce its expression (unpublished data) (see Fig. 2).

Antimicrobial peptides as immune regulators

Prior to the year 2000, most reviews of antimicrobial peptides described these host derived peptides as the body's "natural antibiotics;" i.e., as microbicidal agents that can function rapidly against multiple microbial species at epithelial barriers or during phagocytosis. However, early pioneering work (86) demonstrated that neutrophil derived α-defensins were chemotactic towards human monocytes. This finding, however, could not be appreciated nor put into context until a number of years later when other laboratories started realizing that antimicrobial peptides indeed had additional properties related to directing adaptive immune responses (i.e., cross-talk).

As more information is gathered from such findings, it is anticipated that exploiting antimicrobial peptide immune regulatory strategies will become more commonplace as

translational options in bolstering the native host response, without incurring concerns of bacterial resistance. In fact, the first landmark *in vivo* report using an anti-infective peptide to selectively modulate the innate immune response, was published by Scott et al. (78). The authors described the utility of a 13 amino acid non-toxic peptide, innate defense-regulator peptide 1 (KSRIVPAIPVSLL-NH_2), in a mouse model of aggressive bacterial infection. Interestingly, while the peptide showed little antimicrobial activity, it was reported to attenuate pro-inflammatory cytokine production by microbial products, while promoting selective recruitment of monocytes over neutrophils and thereby enhancing and sustaining the levels of monocyte chemokines. While mechanisms for this selective activity still need to be elucidated, results are reminiscent of findings attributed to LL-37 and its anti-inflammatory capacity. Overall, this novel study demonstrated that inflammation can be attenuated *in vivo* through the use of anti-infective peptides.

It is important to state at the outset that works highlighted herein that focus on antimicrobial peptide capacity to regulate epithelial cell proliferation, enhanced wound healing, inhibition/induction of pro-inflammatory cytokines, angiogenesis/antiangiogenesis, stimulation of chemokine production, chemotaxis of various leukocytes, mast cell degranulation or modulation of host cell gene expression were determined in physiological conditions, not in media of low ionic strength that are often used to determine antimicrobial peptide antimicrobial activity. Therefore, positive outcomes in the presence of serum and physiological salts suggest that results obtained are actually relevant to *in vivo* functions and conditions.

Antimicrobial peptide neutralization of lipopolysaccharide

The ability of antimicrobial peptides, particularly LL-37, to neutralize endotoxin, was first believed to be due to their cationic and amphipathic capacities to interact with anionic lipopolysaccharide (58), as well as their ability to block lipopolysaccharide binding to lipopolysaccharide-binding protein, as an initial step in activating immune cells (79). Further investigation revealed that antimicrobial peptides can actually dampen pro-inflammatory responses induced by lipopolysaccharide. Lipopolysaccharide-induced genes in macrophages can be suppressed by LL-37, as it directly up-regulates macrophage gene expression, including certain anti-inflammatory genes (77). Importantly, these observations were reported in whole blood and in low micromolar concentrations of LL-37. These results suggest that LL-37 has anti-inflammatory properties. Interestingly, although LL-37 was able to inhibit tumor necrosis factor-α production in bacteria challenged macrophages (77), polymyxin B, another antimicrobial peptide that inhibits lipopolysaccharide binding to lipopolysaccharide-binding protein (79), could not, thereby suggesting specificity of LL-37 activity. Additionally, LL-37 was also found to induce expression of potent chemokines such as interleukin-8 (CXCL8) and monocyte chemoattractant protein-1 (CCL2) *in vitro*(77). One could speculate, therefore, that the action of LL-37 in the context of neutralizing endotoxin, may be part of a feedback mechanism intended to limit the induction of septic levels of pro-inflammatory cytokines. By re-balancing an obviously dangerous scenario, LL-37 and other antimicrobial peptides could then participate in recruiting cells intended to initiate healing and repair processes.

Antimicrobial peptide related chemotaxis activity and associated receptors

As stated above, the first non-microbicidal related activity attributed to antimicrobial peptides was that α-defensin human neutrophil peptide-1 and -2, but not -3, were chemotactic towards human monocytes. Subsequently, these peptides were found to also chemoattract naïve (CD4+/CD45RA+) CD4+ and CD8+ T cells, as well as immature dendritic cells, but not memory (CD4+/CD45RO+) T cells (97). Later, LL-37 was found to also chemoattract monocytes, T cells and neutrophils, but not dendritic cells, and that this

recruitment was dependent upon the G protein coupled receptor formyl peptide receptor-like 1 (16, 22, 99). In addition to formyl peptide receptor-like 1, LL-37 also utilizes the purinergic receptor $P2X_7$ to activate a number of cell types (26, 59, 104). Interestingly, Elssner et al. (26) showed that by trans-activating $P2X_7$, LL-37 promotes interleukin-1β processing and secretion; a result that may enhance inflammatory effectors through synergy between LL-37 and released interleukin-1β (100).

LL-37 chemoattracts mast cells, but apparently in an formyl peptide receptor-like 1 receptor independent manner (62), and promotes mast cell activation (15, 64). Human β-defensin 1, 2 and 3 were found to recruit memory T cells and immature dendritic cells via the G protein coupled receptor CCR6 (98). Human β-defensin 2 can also recruit mast cells (61) and induce mast cell degranulation, prostaglandin D_2 production and intracellular Ca^{2+} mobilization (64). Recently, human β-defensins 3 and 4 have been shown to induce mast cell degranulation, prostaglandin D_2 production, intracellular Ca^{2+} mobilization and promote chemotaxis (14). Moreover, human β-defensin 2 is chemotactic for human neutrophils via CCR6 (63). Interestingly, human β-defensin 3 has been shown to recruit monocytes in an isoform dependent manner; i.e., different disulfide bond motif forms of human β-defensin 3 chemoattract monocytes to varying degrees (96). This suggests that oxidative conditions in mucosae of chronic disease could impact conformational outcomes of antimicrobial peptides during folding, which could then impact their ability to recruit innate and adaptive immune cells.

The specificity of antimicrobial peptides for receptors and respective outcomes of these interactions is noteworthy, and best exemplified when comparing LL-37 and human β-defensin 3. As stated above, LL-37 recruits a number of peripheral blood mononuclear cells through interaction with the G protein coupled receptor formyl peptide receptor-like 1. However, we recently showed that human β-defensin 3 has no effect on formyl-met-leu-phe receptors, such as formyl peptide receptor-like 1 (27). Instead, human β-defensin 3 interacts with another G protein coupled receptor, CXCR4, resulting in antagonism of T cell migration, rather than promotion of chemotaxis (27). CXCR4 is an important co-receptor used by HIV-1 to allow cell fusion and replication of the virus in CD4+ T cells (72). We previously showed that human β-defensin 3 protects T cells from HIV-1 infection (72) by promoting CXCR4 internalization, without cellular activation (27). Since CXCR4 also plays an important role in hemopoiesis, neurogenesis, cardiogenesis and angiogenesis, human β-defensin 3 or its derivatives offer a new paradigm in immunoregulatory therapeutics and provide the opportunity to enhance future drug design.

Antimicrobial peptides can also direct chemotaxis, indirectly

Antimicrobial peptides have been shown to induce a variety of chemokines in epithelial cells, thereby enhancing their own chemotactic capacity and possibly prolonging chemotaxis overall. Interleukin-8 can be produced in epithelial cells upon challenge with either LL-37 or α-defensins (77, 89). Human β-defensin and LL-37 can induce chemokines such as monocyte chemotactic protein-1, macrophage inflammatory protein-3α (MIP-3α; CCL20) and interferon-γ inducible protein-10 (IP-10; CXCL10) in human epidermal keratinocytes (65) (Fig. 2).

These data, along with findings discussed in the previous section, collectively, indicate that antimicrobial peptides likely have a multifaceted role in controlling microbial infections. Aside from their direct antimicrobial activity, antimicrobial peptides are capable of initially promoting leukocyte migration to combat infection, as evidenced by up-regulation of interleukin-8 and monocyte chemotactic protein-1, and also have the capacity to, at a later point in the inflammatory process, act as feedback inhibitors to control inflammation by attenuating immune cell activation.

Antimicrobial peptide related epidermal growth factor receptor interactions

LL-37 can induce lung epithelial cell signaling by transactivating the epidermal growth factor receptor. This is apparently carried out in a multi-step fashion, where LL-37 activates membrane-bound metalloproteinases, which then cleave membrane-anchored epidermal growth factor receptor-ligands (87), which in turn activate the cell by binding epidermal growth factor receptor. Since neutrophils are the major source of LL-37, it is conceivable that infiltrating neutrophils, by releasing LL-37, could contribute to lung epithelial cell signaling. In addition, neutrophil derived matrix metalloproteinase-9 and matrix metalloproteinase-25 (44, 49) could aid in releasing epithelial membrane bound epidermal growth factor receptor ligands and thereby contribute to epidermal growth factor receptor activation and cell signaling. These intriguing results suggest that epithelial cell activation and cytokine release in the lungs, and possibly elsewhere, is the result of neutrophil derived LL-37. Furthermore, LL-37 can induce keratinocyte migration via heparin-binding-epidermal growth factormediated transactivation of epidermal growth factor receptor, and can also promote cell proliferation via epidermal growth factor receptor (12). Importantly, the first *in vivo* verification of an antimicrobial peptide promoting wound healing was recently demonstrated when adenoviral transfer of LL-37 to excisional wounds in mice promoted re-epithelialization and granulation tissue formation (12).

Clearly, it is probable that other antimicrobial peptides, in conjunction with LL-37, function collectively to promote wound healing. Evidence to support this include the following: (i), epidermal growth factor, when released in areas of infection, has been shown to induce epithelial cell proliferation and wound healing (85); (ii), Sorensen et al. (83) found that in addition to epidermal growth factor, additional epidermal growth factor receptor ligands, such as insulin growth factor-1 and transforming growth factor-α, induce expression of a host of epithelial cell derived antimicrobial peptides, including LL-37, human β-defensin 3, neutrophil gelatinase-associated lipocalin and secretory leukocyte protease inhibitor, suggesting a common epidermal growth factor receptor dependent mechanism for AMP induction; (iii), both LL-37 (88) and human β-defensin 3 (65) promote epithelial cell migration and proliferation; (iv), wound closure; i.e., epithelial cell migration, appears to require epidermal growth factor receptor activation and downstream signaling pathways (2); (v), alpha defensins from human neutrophils, which induce lung epithelial cell proliferation in an epidermal growth factor receptor independent fashion (1), promote the expression of MUC5B and MUC5AC, two mucins that contribute to regeneration of the epithelium (2). Therefore, collective AMP induction and activation may work in synergy to support the growth and antimicrobial potential of epithelial cells when endangered through microbial challenges and wounding.

Evidence and implications for antimicrobial peptide expression in wounds

LL-37 is (i) highly expressed in skin wounds *in vivo*, reaching its peak by 48 hrs post-injury and declining to its lowest level upon wound closure; (ii) detected in the inflammatory infiltrate and in epithelial cells migrating over the wound; (iii) blocked using specific antibodies which leads to inhibition of re-epithelialization in a concentration dependent manner (39). However, in chronic ulcers, LL-37 expression is very low and is not detected in ulcer edge epithelium (39). Since angiogenesis is an important component in tissue repair and wound healing, Koczulla et al. (50) investigated the neo-vascularization capacity of LL-37 in *in vitro* and *in vivo* models. They found that LL-37 caused endothelial cell activation and proliferation, resulting in the formation of vessel-like structures. Interestingly, mice deficient in cathelin-related antimicrobial peptide, the mouse orthologue of human cathelicidin LL-37, are deficient in wound neo-vascularization (50).

Differential expression of antimicrobial peptides in human synovial membranes is governed by specific diseases. Human β-defensin 3 and/or LL-37 are detected in synovial membrane samples from pyogenic arthritis, osteoarthritis or rheumatoid arthritis, while bactericidal permeability-increasing protein, HD5, HD6 and human β-defensin 2 are absent from all of these samples (70). Under inflammatory conditions, human β-defensin 3 is induced in pyogenic arthritis, LL-37 in rheumatoid arthritis and both in osteoarthritis (70). More recently, cytokines involved in the pathogenesis of osteoarthritis, tumor necrosis factor-α and interleukin-1, were shown to induce human β-defensin 3 in cultured chondrocytes and human β-defensin 3 was shown to mediate tissue remodeling in articular cartilage by increasing chondrocyte derived cartilage-degrading matrix- metalloproteases and reducing levels of their endogenous inhibitors (91). The authors concluded that human β-defensin 3 links host defense mechanisms and inflammation with tissue-remodeling processes in articular cartilage and suggest that human β-defensin 3 is a new factor in the pathogenesis of osteoarthritis.

Role of antimicrobial peptides in adaptive immunity

From a series of studies conducted over the last seven years, we can now point to the ability of antimicrobial peptides to modulate adaptive immune functions. A number of studies have reported that co-administering antimicrobial peptides with relatively benign antigens results in enhancement of the host's cell mediated and humoral immune responses to these antigens. Co-administering ovalbumin with α-defensins human neutrophil peptide-1-3 in mice leads to enhanced IgG antibody response to ovalbumin when compared to ovalbumin alone (53). Ovalbumin-specific CD4+ T cells were found to produce elevated cytokine levels as well (53). These data suggest that the human neutrophil peptides may act as adjuvants. Another study showed enhanced ovalbumin-specific IgG response in mice when ovalbumin was co-nasally administered with either 1 μg of either human neutrophil peptide-1, human β-defensin 1 or human β-defensin 2 (11) suggesting that β-defensins may also share the ability to modulate antigen presentation and direct the adaptive immune response. Furthermore, intraperitoneal administration of a B-cell lymphoma idiotype antigen combined with daily injections of human neutrophil peptides increased IgG levels to that antigen and augmented resistance to tumor challenge in mice (84). These findings strongly implicate α-defensins as immune adjuvants that promote T cell-dependent cellular immunity as well as antigen-specific immunoglobulin production.

Using a DNA-vaccine strategy, Biragyn et al. (7) immunized mice with constructs encoding murine β-defensins or various chemokines fused to non-immunogenic lymphoma antigens, and studied their capacity to deliver antigens to subsets of immune cells in order to elicit antitumor immunity. This elegant study demonstrated that DNA immunization, where the vaccine contained murine defensins or chemokines that chemoattract immature dendritic cells via CCR6; i.e., mβ-defensin-2, macrophage inflammatory protein-3α, but not mature dendritic cells, elicit humoral and protective immunity against lymphoma (7). The authors speculated that the targeting of immature dendritic cells by these specific defensins and chemokines via CCR6 (98), results in increased uptake of antigen and induces the expression of co-stimulatory molecules that have been reported by others to induce a robust immune response against weak immunogens (5, 13, 42, 71). Interestingly, this group showed in the murine model that mβ-defensin-2, which does not appear to have a human orthologue, can activate murine immature dendritic cells directly via Toll-like receptor 4 (6). More recently, we showed that human β-defensin 3 induces expression of costimulatory molecules CD40, CD80 and CD86 on human immature dendritic cellss and monocytes, and that human β-defensin-3 promotes expression of pro-inflammatory cytokines by antigen presenting cells (29). LL-37 has been shown to modulate dendritic cell differentiation by enhancing endocytic capacity, upregulating co-stimulatory molecule expression, enhancing secretion of

pro-inflammatory cytokines and promoting Th1 responses *in vitro* (21). Human α-defensins have also been shown to promote expression of costimulatory molecules on lymphocytes (53) as well as the production of proinflammatory cytokines (90). Chemokines, such as monocyte chemotactic protein-1, can promote interleukin-4 production (46) and induce Th2 polarization (33), while macrophage-derived chemokines (CCL22) selectively chemoattract Th2 cells toward antigen presenting cells (43).

While the mechanisms for these intriguing outcomes have not be established, we can speculate that antimicrobial peptides may be modulating lymphocyte responses, modifying cytokine expression during the antigen presenting cell encounter with the antigen, and possibly, as we recently reported with human β-defensin-3 (29), causing the maturation of immature dendritic cells by inducing co-stimulatory molecules, resulting in more effective antigen presentation and subsequent robust T cell activation. These collective observations lead us to conclude that specific defensin molecules and chemokines, or their active homologs, could one day be used as adjuvants to both target antigen to antigen presenting cells as well as selectively prime for humoral or cellular immune responses *in vivo*. Clearly, these and future studies will lead to an enhanced interest in antimicrobial peptides and their homologs as immuno-therapeutic candidates to bolster the host's immune response.

Antimicrobial peptides in oral cancer

New evidence is emerging that tumor cells produce innate response elements and bioactive peptides, other than chemokines, that alter the tumor micromilieu and contribute to tumor-related inflammatory processes including angiogenesis, recruitment and infiltration of leukocytes, and invasion of tumor cells (19). LL-37 was most recently shown to be produced by ovarian cancer cells, and has the capacity to recruit mesenchymal stem cells to the tumor site, resulting in increased production of pro-tumor cytokines, growth factors and enhanced vascularization (18). We recently reported that human β-defensin 3 is overexpressed in oral carcinoma *in situ* (Fig. 3) resulting in specific macrophage recruitment to the lesion site (47). Moreover, human β-defensin 3 over expression is also associated with increased tumor size and vascularization, and an investigation into the mechanism of human β-defensin 3 chemoattraction of tumor associated macrophages has revealed a novel receptor related to this activity (Jin et al, unpublished data). Collectively, these observations suggest, for the first time, that tumor cell-derived factors, other than chemokines, are associated with chemoattraction and activation of important cells that contribute to tumor-related inflammation and protection of tumors from immune surveillance.

Conclusion

Antimicrobial peptides cross-talk with numerous cell types and function diversely to regulate proliferation, wound healing, pro- and anti-inflammatory cytokine response, angiogenesis, chemokine production, mast cell degranulation and chemotaxis of various leukocytes. Therefore, antimicrobial peptides clearly function to modulate host cell response at the both the molecular and cellular level. This diverse class of regulatory peptides will likely be exploited to modulate immune regulatory strategies as a translational option to bolster the native host response, without incurring concerns of bacterial resistance. Clearly, current and future studies will lead to an enhanced interest in antimicrobial peptides and their homologs as immuno-therapeutic candidates.

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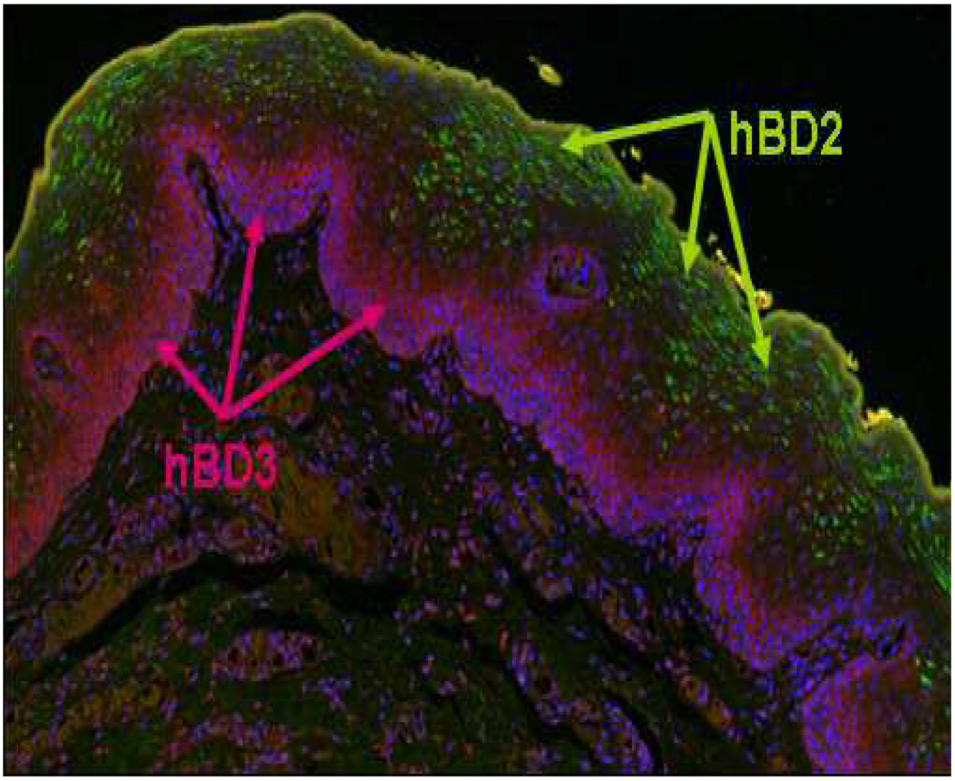

Figure 1. Immunofluorescence detection of human β-defensin (hBD) 2 and 3 in normal oral epithelium

Formalin fixed and parafin embedded normal human oral tissue was labeled with an anti-human β-defensin 2 specific primary antibody, and detected with AlexaFluor 488 (green) conjugated secondary antibody. Human β-defensin 3 was labeled with specific primary antibodies and detected using an AlexaFluor 594 (purple) conjugated donkey anti-rabbit. Note green fluorescence detection of β-defensin 2 localized to the stratum spinosum and straum granulosum, while pink/red fluorescence detects human β-defensin 3 exclusively to the stratum basale. β-defensin 1, not shown, is localized to the same regions as β-defensin 2 (20). (Courtesy of Dr. Ge Jin, Dept. Biological Sciences, Case Western Reserve Univ, School of Dental Medicine)

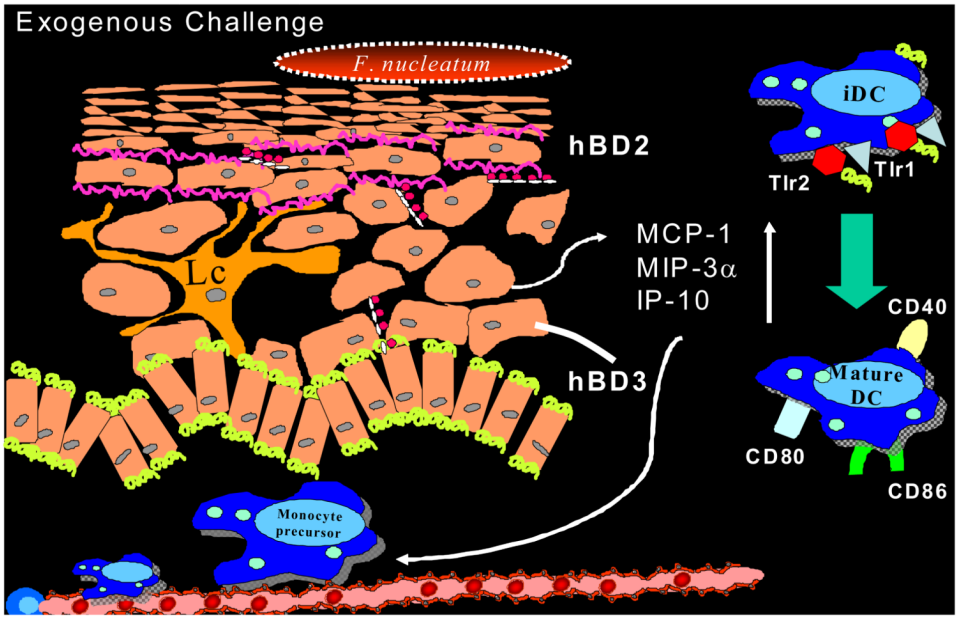

Figure 2. AMP's direct chemotaxis indirectly

Certain bacterial challenge (as depicted by *Fusobacterium nucleatum*) results in defensin upregulation and release from oral epithelial cells. Defensins in turn induce chemokine production (monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-3α (MIP-3α), interferon-γ inducible protein-10 (IP-10)) that elicit recruitment of monocyte precursors such as immature dendritic cells. Upon contact with defensins via Toll-like receptors, immature cells mature and express co-stimulatory molecules (see below). Lc, Langerhans cell.

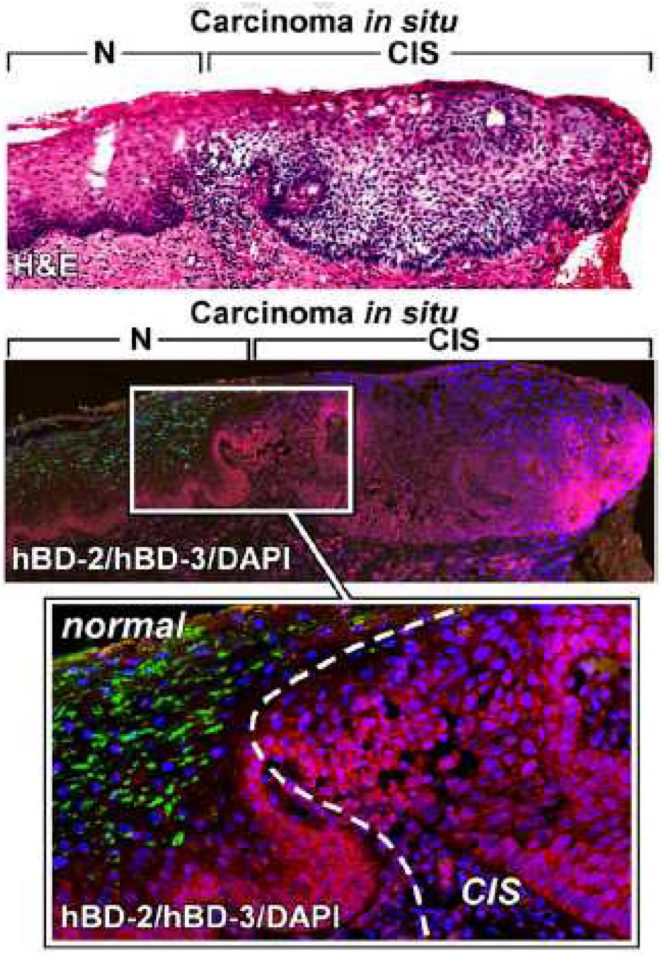

Figure 3. Expression of human β-defensin (hBD) 2 and human β-defensin 3 in carcinoma in situ

carcinoma *in situ* (CIS) biopsy sections were stained with hematoxilin & eosin stain (upper panel) and double immunofluorescence using antibodies to human β-defensin-2 (green) and human β-defensin-3 (red) (lower panel), respectively. The carcinoma *in situ* lesion and adjacent normal epithelium are separated by the dashed white line in the enlarged inset (lowest panel). (courtesy of Kawsar et al, 2009)

Table I
Epithelia-derived antimicrobial peptides discussed in this review

AMP	class/structure	origin	expression/source
LL-37	cathelicidin, α-helical peptide	human	neutrophils, monocytes/macrophages, epithelial cells of respiratory, gastrointestinal and urogenital tract, keratinocytes
hBD-3	β-defensin, β-sheet peptide	human	epithelial cells of respiratory and gastrointestinal tract, keratinocytes
calprotectin (MRP-8/14)	S100 calcium binding proteins	human	neutrophils, monocytes/macrophages, epithelial cells, keratinocytes, skeletal muscle
psoriasin	S100 calcium binding protein A7	human	keratinocytes, various carcinomatous tissues including bladder, stomach, breast
secretory leukocyte protease inhibitor (SLPI)	N-terminal transglutaminase domain substrate and two WFDC (whey acidic protein four disulfide core domains)	human	epithelial cells (various), activated macrophages
adrenomedullin	calcitonin gene-related peptide family	human	epithelial cells, endothelial cells, synovial cells, macrophages
neutrophil gelatinase-associated lipocalin (NGAL)	eight β-strands that form a β-barrel defining a calyx (lipocalin superfamily)	human	neutrophils, epithelial cells, hepatocytes
RNase7	ribonuclease	human	keratinocytes, various somatic tissues including the liver, kidney, skeletal muscle and heart