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Multifaceted immune functions of human defensins and underlying mechanisms

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Abstract

Defensins have been long recognized as natural antimicrobial peptides, but they also possess diverse and versatile immune functions. Defensins can both induce inflammation and suppress inflammatory responses by acting on specific cells through distinct mechanisms. Defensins can also modulate the immune response by forming a complex with cellular molecules including proteins, nucleic acids, and carbohydrates. The mechanisms of defensin-mediated immune modulation appear to be cell-type and context specific. Because the levels of human defensins are often altered in response to infection or disease states, suggesting their clinical relevance, this review summarizes the complex immune functions of human defensins and their underlying mechanisms of action, which have implications for the development of new therapeutics.

Keywords

Defensins; Immune functions; Pathways

1. Introduction

Defensins are antimicrobial peptides known to protect the host through their direct or indirect activities on microbes [1–3], although recent studies have demonstrated their ability to promote viral infectivity [4,5], indicating a complex role of defensins in host defense in a microbe, defensin, cell-type specific manner. As major players at the front line of defense, there has been much discussion of the immunological activities of defensins and their role as alarmins in host defense [1,6–8]. Here, we focus on the immune functions of human defensins that are beneficial or detrimental to the host. We highlight advances in our understanding of the molecular mechanisms of immune modulatory activities of human defensins.

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Conflict of interests

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2. Overview of human defensins and their regulation

Human defensins are cationic peptides of approximately 30 amino acids; they are classified into two subfamilies, α-, and β- defensins, based on their disulfide bond linkages (Fig. 1, reviewed in [1,3,9]). Most human defensins within the same family have similar structure, and form dimers or oligomers [3,10,11]. However, dimers of human α defensins, e.g., HNP3 (human neutrophil peptide 3) and human β defensins, e.g., hBD2 (human β-defensin 2), are topologically distinct [12]. Human defensins are synthesized as a pre-pro-peptide, and are then processed intracellularly or extracellularly depending on the defensin and cell type (reviewed in [1]). In humans, there are 6 α-defensins: HNPs1–4 and human α-defensins 5 and 6 (HD5 and HD6). Six human β-defensins (hBD1–6) have been characterized [7,11,13], although gene-based analysis suggests that there are an additional 28 hBDs [14].

Human defensins are produced mainly by leukocytes and epithelial cells. HNPs1–3, which differ by single amino acid substitutions, are predominantly produced by neutrophils [15]. HNP4, comprising less than 2% of defensins in neutrophils, has a relatively distinct sequence but similar structure as HNPs1–3 [10,16]. While neutrophils produce the largest amounts of HNPs, these peptides are also found in other immune cells (reviewed in [17]). Cells can absorb and internalize HNPs [18–20], underlying the complexity of defining true HNP-producing cells. HNPs have been detected in placenta, spleen, thymus, intestinal mucosa, saliva, and cervical mucus plugs, and are released by neutrophils in response to chemokines, FCγ receptor cross-linking, phorbol myristate acetate (PMA), and bacterial products that trigger Toll-like receptors (TLRs, reviewed in [1,17]).

Although leukocyte α-defensins are conserved evolutionally and have been isolated from many species, mice do not express neutrophil α-defensins [1]. Mice do, however, express numerous cryptdins, which are enteric α-defensins, in intestinal Paneth cells [1,21]. Similarly, HD5 and HD6 are produced predominantly by intestinal Paneth cells in humans [22]. HD5-transgenic mice are markedly resistant to oral challenge with virulent *Salmonella typhimurium,* indicating that human and mouse enteric defensins have distinct functions [23]. HD5 can be found in other tissues such as the salivary glands, female genital tract, kidney, urinary tract, and inflamed colon (reviewed in [17,24]); intestinal HD6 expression is elevated in the presence of colon cancer [25,26]. NOD2 and Wnt signaling transcription factor TCF-4 proteins play a role in modulating HD5, but TCF4-mediated HD5 regulation does not depend on NOD2 [27,28]. HD5 is induced at the genital mucosa in patients with bacterial vaginosis, *Neisseria gonorrhoeae* (GC), and *Chlamydia trachomatis* infections [29,30]. Induction of HD5 and HD6 gene expression in GC-exposed cervicovaginal epithelial cells contributes to GC-mediated enhanced HIV infectivity [31]. HD6 gene expression is upregulated by atonal homolog 1 and β-catenin in colon cancer cell lines, DLD1 and SW480 [32].The TCF4 binding site and E-box site in the proximal promoter region are critical for HD6 gene expression. Expression of HD6 but not HD5 is down-regulated in non-inflamed jejunum of patients with Crohn's disease [32]. In aggregate, the evidence indicates that levels of defensins are often altered in response to infection, inflammation, or tissue damage (reviewed in [1,17,33]), indicating an immune regulatory role in disease pathogenesis.

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hBDs1–3 are mainly expressed by epithelial cells, but are also found in hematopoietic cells including peripheral blood mononuclear cells (PBMCs), monocytes, macrophages, plasmacytoid dendritic cells (pDCs), and monocyte-derived dendritic cells (MDDCs) (reviewed in [1,7,17]). Expression of hBDs4–6 is limited to specific tissues [13]. hBDs1–3 can be induced by cytokines, TLR activation, or viral exposure, but the underlying mechanisms of gene regulation of each hBD appear to be distinct [1,11,17,34]. Although hBD1 is often constitutively expressed, induction of hBD1 gene and protein expression has been observed in pDCs and monocytes in vitro and in lungs of mice in response to viral exposure [35]. hBD2 is induced by TLR2, TLR3, TLR4, TLR7, NOD1, and NOD2 signaling, important for sensing pathogens, in various epithelial cells and keratinocytes (reviewed in [17]). For example, TLR3 activation induces hBD1 and hBD2 expression in uterine epithelial cells and keratinocytes [35,36], and increases hBD2 and hBD3 expression in bronchial epithelial cells [37]. In oral epithelium, TLR2 and NOD1/2 ligands synergistically induce hBD2 expression through nuclear factor-κB (NF-KB) [38]. TLR2 or TLR4 activation induces hBD2 expression in keratinocytes and vaginal epithelial cells [39,40]. Cytokines including IL-17, IL-22, oncostatin M, TNFα, or IL-1α can induce hBD2 and hBD3 [41–44]. IL-17, important for maintaining mucosal homeostasis, upregulates hBD2 in primary human airway epithelium through JAK and NF-κB pathways [42]. However, NF-κB-dependent induction of hBD2 by IL-17A is mediated by the PI3K pathway and MAPK pathway in airway epithelial cells, whereas regulation of hBD2 by NF-κB is not dependent on the PI3K pathway in bronchial epithelial cells, [45–47], indicating that specific pathways involved in regulation of hBD2 are cell-type dependent. IL-22 can act together with IL-17 to induce hBD2 in keratinocytes in a synergistic or additive fashion [43]. TNF-α induces hBD2 but not hBD3 gene expression in a dose-dependent manner in human keratinocytes [48]. Unlike hBD-2, hBD-3 mRNA is preferentially stimulated by IFNγ rather than TNFα, indicating specific hBD regulation in response to specific cytokines. PMA, EGF, IFNγ, and IL-1β induce hBD3 gene expression in human oral epithelial cells and in TR146 oral cancer cells [44]. EGF-mediated hBD3 induction is blocked by inhibitors of MEK1/MEK2, p38 MAPK, PI3K, and PKC but not by JAK and STAT3 inhibitors [44]. Taken together, there are common pathways involved in induction of both hBD2 and hBD3 but some pathways are dependent on cell types and stimuli.

3. Human α-defensins

3.1. Inflammatory activities of human α-defensins

Neutrophils are often the first cells that are recruited to the site of an infection, where they release effector molecules such as HNPs [49]. Although HNPs exhibit direct and potent antimicrobial activities [3], these peptides also control infection through modulating various immune activities including chemotaxis, phagocytosis, and cytokine induction during acute infection. However, uncontrolled inflammation can lead to tissue damage and worsen disease progression. HNPs1–3 act as chemoattractants for various types of immune cells including monocytes, immature DCs, and naïve CD4+ T cells but not for memory CD4+ T cells or mature DCs [7,50–52]. HNP1 and HNP3 also induce the migration of macrophages and mast cells but not DCs [53]. HNP1 and HNP2 aggregate influenza virus A and bacteria including *Staphylococcus aureus* (Gram positive) and *E. coli* (Gram negative) and increase

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phagocytosis [54]. HNP1 induces TNFα in PBMCs [55]. HNPs also upregulate the expression of CC-chemokines and IL-8 in macrophages and epithelial cells, respectively [56,57]. HD5 but not the linear HD5Abu mutant, in which cysteine residues are replaced with L-α-aminobutyric acid, induces IL-8 in the intestinal epithelial Caco-2 cell line [58]. HD5 and TNFα have a synergistic effect on IL-8 production [58], indicating the role of defensins in modulating activities of cytokines.

Antimicrobial peptides can act on immune cells directly or can modulate immune responses by forming a complex with other molecules. The most well characterized example is the antimicrobial peptide LL37, which forms a complex with self-DNA to activate pDCs and monocytes through TLR9 [59,60]. LL37 also complexes with self-RNA to activate pDCs (through TLR7) and myeloid DCs (mDCs) through TLR8 [61]. In response to infection and inflammation, activated neutrophils release neutrophil extracellular traps (NETs), composed of HNPs and self-DNA [62]. NETs found in patients with systemic lupus erythematosus activate pDCs through TLR9 [63]. Although the complex of HNPs and DNA is not sufficient to induce IFNα production by pDCs, HNPs can promote pDC activation by suboptimal concentrations of LL37 through protecting DNA degradation [63]. HNPs can bind to other host proteins to modulate immune or metabolic functions [8]. HNPs bind to low-density lipoprotein receptor-related proteins and interact with protein kinase Cα and β, leading to decreased smooth muscle contraction in response to phenylephrine [64]. HNPs also interact with adrenocorticotrophic hormone receptors and heparan sulfate proteoglycan to modulate other biological activities [65,66]. HNP1 inhibits the activity of conventional PKC isoforms in a cell-free system [67]. This PKC inhibitory activity appears to be involved in HNP1-mediated inhibition of HIV replication in primary $CD4^+$ T cells [68], and is involved in suppression of influenza A virus in lung epithelial cells [69]. Although the role of PKC in defensin-mediated immune modulation has not been characterized extensively, the PKC inhibitor blocks the ability HNP1 and HD5 to desensitize hBD2 in human primary macrophages [53]. Additionally, HNP1 blocks the classical and lectin pathways of complement activation by binding to complement C1q and to mannose-binding lectin, respectively [70,71].

3.2. Anti-inflammatory activities of human α-defensins

Regulation of initial inflammation in response to infection is crucial for maintaining immune homeostasis. Although defensins are known to induce immune responses, their anti-inflammatory activity has also been documented. HNP1 and HNP4 suppress NK cell activity and production of IFNγ and IL-6 by PBMCs in response to phytohemagglutinin (PHA) or concanavalin Astimulation [72]. HNPs in conditioned media from TNFα-stimulated polymorphonuclear leukocytes (PMNs) may contribute to suppressed PMN migration [73]. Indeed, HNP1 suppressed PMN migration in response to formyl-methionyl-leucyl-phenylalanine, a potent chemotactic peptide for PMNs [73]. Lectin-like soluble factors such as HNPs from apoptotic and necrotic neutrophils inhibit LPS-mediated TNFα induction by monocyte-derived macrophages (MDMs) [74]. HNP1 potently induces TNFα production in MDMs, promotes phagocytic activity, and plays a major role in this anti-inflammatory effect in LPS-stimulated MDMs and in a murine peritonitis model [74]. Linear HNP1 does not have an anti-inflammatory effect. HNP1 dampens live or dead *Pseudomonas aeruginosa*-

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mediated cytokine production (TNFα, IL-1β, and IL-8) but does not reduce the bacterial burden [74]. HNP-1 also blocks IL-6 production byTLR7/8 or CD40L/IFNγ, mimicking T cell engagement [75]. Tertiary structure is required for the cytokine inhibitory effect as linearized HNP and the W26A mutation, which disrupts dimerization, abrogate the ability to block cytokine induction. Mechanistically, HNP1 is internalized and binds to mRNA in a sequence-independent manner to block translation [75].

Paneth cell defensin-deficient mice (MMP-7−/−) have a higher baseline level of intestinal IL-1β, and are more susceptible to dextran sulfate sodium-induced colitis [55]. HD5 reduces the Th1 inflammatory phenotype in NOD2−/− mice in response to bacterial infection [76]. Both HNP1 and HD5 blockATP-induced IL-1β release from LPS-activated monocytes. The blockade of IL-1β release is not mediated through inhibition of caspase 1 activity. Treatment with HNP-1 or HD5 leads to a reduction of newly synthesized cell-associated pro-IL-1β proteins in LPS-activated monocytes in response to ATP, suggesting that human α-defensins may destabilize pro-IL-1β [55].

3.3. Receptors and signaling involved in immune functions of human α-defensins

The chemotactic effect of HNPs on both T cells and dendritic cells is sensitive to pertussis toxin (PTX), suggesting the involvement of Gαi protein-coupled receptor [52]. Similarly, the induction of migration of macrophages and mast cells by HNP1, HNP3, and HD5 are sensitive to PTX and MAPK inhibitors PD098059 and SB203580, indicating that Gαi protein-coupled receptor and MAPK ERK1/2 and p38 play roles in the chemotactic activity of human α-defensins [53]. The specific receptors for the chemotactic effects of HNPs in specific immune cells remain to be identified. With respect to receptors for HNP-mediated cytokine induction, HNPs induce IL-8 through G-protein coupled nucleotide receptor P2Y6 in lung epithelial cells [77], and HNP-1 suppresses neutrophil apoptosis through the P2Y receptor pathway [78]. The purinergic P2 receptor but not the P2Y6 receptor is involved in HNP1-mediated IL-8 induction [79]. Activation of ERK1/2 and PI3K/Akt but not Src is required for HNP-mediated IL-8 induction; however, Src is required for IL-8 release in monocytic cell lines in response to HNPs [80]. Although HNP1 activates ERK1/2 and p38, ERK1/2 is involved in HNP1-mediated IL-8 induction through P2 receptors, but this is independent of P2Y6 receptors [52,79]. In pDCs, HNP-1 induces production of IFNα, IFNβ, and IL-6, and enhances TLR9 activation through NF-κB and IRF1 pathways [81], although the interplay between these two pathways in activation of pDCs remains to be determined. In the LPS-primed THP1 monocytic cell line, HNP-1 activates NLRP3 inflammasome and induces the release of IL-1β [82]. HNP-1 binds to the ATP-gated ion channel receptor, P2X7, and triggers P2X7- potassium (K^+) efflux-caspase-1 signaling pathway, leading to pyroptotic pore formation and enhancement of the inflammatory response [82]. Receptors and pathways involved in the immune functions of HNPs are summarized in Table 1.

4. Human β-defensins

The immune functions of hBDs, particularly hBD2 and hBD3, have been studied extensively. hBDs chemoattract immune cells, induce cytokines/chemokines, and modulate cellular functions and differentiation/activation markers. Although findings pertaining to the

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precise roles of receptors and cell signaling in the functions of hBDs have not been wholly consistent, Gαi protein-coupled receptors and MAPK signaling appear to play a key role in multiple immune functions of hBDs (Table 1).

4.1. hBD1

Similar to HNP-1, hBD1 chemoattracts MDDCs, upregulates costimulatory markers CD80, CD86, and CD40, upregulates maturation markers CD83 and HLA-DR, upregulates scavenger receptor CD91, induces production of TNFα, IL-6, and IL-12p70, and enhances DC-mediated T cell proliferation [83]. hBD1 also induces the migration of immature mast cell line HMC-1 in a dose-dependent manner [84]. hBD1 induces migration of HEK293 expressing CCR6, the receptor for chemokine ligand 20 (CCL20; also known as MIP-3α) [85]; however, the role of CCR6 in immune functions of hBD1 in primary cells is not well established.

hBD1 mRNAs are upregulated in pDCs and monocytes in response to infection by influenza, HSV-1, and Sendai virus [35]. However, hBD1 gene expression is suppressed in normal human bronchial epithelial cells in response to influenza virus infection and in human gingival epithelial cells in response to HSV-1 infection [35], indicating cell-type specific hBD1 gene regulation. In response to influenza virus challenge, murine β defensin 1, mBD1(−/−) mice have more severe pathological scores than C57BL/6 wild-type mice despite the fact that the virus titers in the lung are comparable and that there is an increase in inflammatory influx in the lung of infected-mBD(−/−) mice [35]. The elucidation of the details of the mechanism by which hBD1 or mBD1 in epithelial cells and pDCs controls viral infection will require further investigation.

4.2. hBD2/hBD3

hBD2 up-regulates IL-1β, IL-6, IL-8, IL-10, MCP-1, MIP-1β, and RANTES in PBMCs [86], and exhibits multiple activities on mast cells, including induction of cell migration, degranulation, and production of prostaglandin D_2 [87]. Studies on the role of hBDs in skin inflammation demonstrate the interplay between hBDs and inflammatory cytokines (reviewed in [6]). Elevated levels of IL-17A, IL-22, oncostatin M, TNFα, and IL-1α are found in psoriatic skin, and these cytokines can induce hBD2 and hBD3 in skin explants [41]. In atopic dermatitis, hBD2 enhances IL-22 and oncostatin M production [81]. hBD3 enhances IL-4, IL-13, and IL-31 production in CD3/28-stimulated T cells, suggesting it plays a role in Th2 responses [88]. Conversely, IL-22 and oncostatin M promote induction of hBD2 and hBD3 in keratinocytes. STAT3 and p38 MAP kinase pathways are involved in IL-22-induced hBD2/3 production. MEK is involved in oncostatin M-induced hBD2/3 induction, whereas the NF-κB pathway is involved in oncostatin M-mediated production of hBD2 but not of hBD3 [88]. The interplay between hBDs and cytokines in immune regulation as well as the control of their immune activation require further investigations.

4.2.1. Receptors involved in immune functions of hBD2/hBD3

4.2.1.1. CCR6.: hBD2 induces the migration of immature DCs and memory CD4+ T cells through CCR6 [85]. hBD2 binds to HEK293T cells expressing CCR6 but not CXCR4, CCR1, or CCR5. The chemotactic activity of hBD2 can be blocked by MIP-3α, anti-CCR6

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antibody, or pertussis toxin [85]. hBDs1–4 induce migration of MDMs, although hBD3 has a less potent effect [84]. In contrast to the finding by Yang et al. [85], Soruri and colleagues have shown that hBD2 and hBD3 have no chemotactic effect on memory cells, and have a weak effect on DCs. Additionally, hBD2 and hBD3 do not induce the migration of the CCR6 stably transfected cell line RBL-2H3 [84]. However, hBD2-mediated chemotaxis of MDMs is sensitive to PTX, and can be blocked by inhibitors of ERK, JNK, and p38 MAPK [84]. The lack of involvement of CCR6 in hBD2-mediated chemotaxis of MDMs may due to low levels of cell surface receptors. For example, hBD2 but not hBD1 induces the migration of neutrophils in response to TNFα, which induces CCR6 expression [89]. The chemotactic effect of hBD2 on TNFα-stimulated neutrophils is dependent on CCR6, and is sensitive to PTX and a phospholipase C (PLC) inhibitor, indicating the involvement of the Gαi protein-coupled PLC pathway in the action of hBD2 on activated neutrophils [89]. The Gαi protein-coupled PLC pathway is also involved in the chemotactic effect of hBD2 on mast cells [90].

CCR6 is also involved in hBD-mediated cytokine production and in apoptosis. Plasma levels of hBD1 and hBD3 but not hBD2 are elevated in asthmatic patients [91]. hBD3 induces IL-8 in human airway smooth muscle cells in a CCR6-dependent manner. hBD3 also induces apoptosis via an ERK1/2 MAPK dependent pathway. Mitochondrial ROS induction is involved in hBD3-mediated IL-8 induction and apoptosis [91]. hBD3 but not hBD1, 2, or 4 prevent spontaneous neutrophil apoptosis in a dose-dependent manner [92]. hBD-3, but not hBD1, hBD2, or hBD4 significantly suppresses activation of caspase 3. hBD3 down-regulates truncated Bid, but up-regulates Bcl-xL [92]. hBD3 suppresses membrane potential change in mitochondria. The suppression of neutrophil apoptosis by hBD3 is mediated through CCR6 [92]. hBDs play a role in intestinal homeostasis, and hBD2 is often down-regulated in inflamed intestines [93]. While hBD2 does not have an effect on the growth or death of the intestinal epithelial cell line HT29, it promotes wound healing of intestinal epithelial cells in vitro by induction of mucins Muc 2 and 3 [94]. hBD2 also induces cell migration via a CCR6 dependent mechanism, and blocks TRAIL-mediated apoptosis, suggesting that hBD2 contributes to the maintenance of epithelial barriers [94].

A role of CCR6 in the interplay between hBDs and mucosal immune mediators has been demonstrated [95]. TCR activation with anti-CD3/CD28 antibodies significantly induces CCL20, IL-17, and IL-22 in Th17 cells, and IL-17 further induces hBD2 but not hBD1 or hBD3 [95]. CCL20 and hBD2 increase the numbers of Th17 cells but not the numbers of Th1 or Th2 cells that adhere to inflamed endothelial cells in response to IL-1β and TNFα, both of which upregulate CD54 (ICAM-1). Although CCL20 often has a more potent chemotactic activity than hBD2, both CCL20 and hBD2 have comparable effects on the adherence of Th17 cells to inflamed endothelial cells or fibroblasts [95]. Adherence to Th17 cells is dependent on CCR6 but not CXCR4. Prolonged TCR activation down-regulates CCR6, which may restrict the migration of cells to inflamed sites.

4.2.1.2. CXCR4, CCR2.: In addition to CCR6, other chemokine receptors have been shown to play a role in the immune functions of hBD2/hBD3. hBD2 or hBD3 but not hBD1 down-regulates surface expression of CXCR4 on PBMCs [96]. hBD3 blocks CXCR4-dependent chemotaxis in a CD4+ T Jurket cell line and in primary activated CD4+ T cells induced by CXCL12/SDF1α. hBD3 induces internationalization of CXCR4 by the

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transformed CD4+ CEM T cell line [96,97]. The interaction between hBD3 and CXCR4 was further demonstrated by the ability of hBD3 to compete with SDF-1α [97]. Pre-incubation of hBD3 also blocks calcium mobilization induced by SDF-1α but not by RANTES in differentiated THP-1 cells; SDF-1α induces ERK1/2 phosphorylation in CEM and primary CD4+ T cells [97]. Interestingly, expression of hBD2 and 3 and their mouse orthologs mBD4 and mBD14 as C-terminal fusion proteins human IgG_1 Fc shows that defensin-IgFc fusion proteins bind to and induce migration of HEK293 cells expressing CCR2 but not CXCR4 [98]. The chemotactic activity of hBD2 and mBD4 Ig fusion proteins is apparent at concentrations as low as 1 ng/ml, and reaches the maximal effect at 100 ng/ml, whereas hBD3 and mBD14 IgFc fusion proteins require 10–100 ng/ml for chemotaxis of HEK293 cells expressing CCR2. Interestingly, hBD3 and mBD14 IgFc fusion proteins but not hBD2 or mBD4 IgFc fusion proteins at 1 ng/ml have chemotactic activity for peripheral monocytes. All defensins chemoattract murine peritoneal exudate cells that express CCR2 but not CCR6 [98]. It's apparent that the chemotactic activities of defensins are cell-type dependent. It remains to be determined whether the defensin-Ig fusion protein represents native defensins. For example, hBD2 is known to form higher order oligomers [12], although it's not clear whether the higher order structure is required for the binding to the receptor. hBD3 induces the expression of IL-1α, IL-6, IL-8, CCL18, and TNFα by MDMs, and recruits monocytes/macrophages through CCR2 [99]. Disruption of intrachain disulfide bonds in hBD3 abolishes its chemotactic but not its antimicrobial activities [100].

4.2.1.3. TLRs.: mBD2 can recruit bone-marrow-derived immature DCs through CCR6, and can induce DC maturation through TLR4 [101]. In humans, hBD3 induces expression of costimulatory markers on antigen presenting cells [102]. Stimulation of PBMCs with recombinant or synthetic hBD3 leads to increased levels of surface expression of CD80, CD86, and CD40 on myeloid dendritic cells and monocytes but not on pDCs or B cells [102]. Activation of monocytes by hBD3 is mediated through MyD88 and IRAK-1 activation. The JAK2 inhibitor (AG490) has no effect on blocking activation of monocytes by hBD3. Activation of NF-κB by hBD3 in transformed cell lines is mediated through TLR2 coupling with TLR1 but not withTLR6 [102]. Pre-incubation of PBMCs with anti-TLR1 and TLR2 antibodies suppresses induction of CD80 by hBD3, confirming a role of TLR1 and TLR2 in hBD3-mediated activation of antigen presenting cells [102]. Although both hBD3 and Pam_3CSK_4 (TLR1/2 agonist) induce IL-1β through TLR1/2 receptor signaling in monocytes, Pam_3CSK_4 but not hBD3 induces IL-10, resulting in down-regulation of CD86 on monocytes [103]. Of the MAP kinases, neither JNK1/2 nor p38 appears to be involved in the differential ability of hBD3 and Pam_3CSK_4 to induce IL-10, as both stimuli activate MAP kinases. However, Pam3CSK4 but not hBD3 activates non-canonical NF-κB pathway. IL-10 down-regulates hBD3-mediated CD86 induction in monocytes [103]. Pam_3CSK_4 and hBD3 synergistically induce IL-1β production in monocytes. However, hBD3 has no effect on IL-10 induction by Pam3CSK4, indicating that hBD3 does not complete for the binding of Pam_3CSK_4 to TLR1/2 receptors [103]. hBD3 induces expression of stimulatory receptor CD69 on NK cells through TLR1/TLR2, and this induction is facilitated by CCR2 [104]. hBD3 induces NK cell-mediated IFNγ secretion and promotes mDC-dependent NK cell cytotoxic activity [104]

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4.2.1.4. P2X7R.: hBD3 at high concentrations (greater than 5 μM) causes membrane damage, mediated by negatively charged phospholipids, in monocytes but not in B cells or T cells [105]. hBD3 induces membrane repair mechanisms as indicated by an increase in surface LAMP, a membrane repair marker. hBD3 activates monocytes by upregulating CD80 and CD86 [106]. Induction of expression of CD86 by hBD3 is mediated through P2X7R; however, hBD3 induces CD80 expression through P2X7R-independent pathways, and does not directly activate P2X7R ion channel function [106]. It was suggested that hBD3 activates P2X7R through autocrine ATP release from both live and lytic cells [106].

4.2.1.5. MrgX2.: Human mast cells express G protein-coupled receptor, Mas-related gene X2 (MrgX2), which is involved in hBD-mediated mast cell degranulation [107]. hBD2 and hBD3 induce Ca^{2+} mobilization and degranulation in mast cells [107]. In contrast to the chemotactic effect of hBDs on dendritic cells, T cells, and monocytes via CCR2, CCR6, and TLRs [85,98,102], the activity of hBD2 and hBD3 in mast cells is not sensitive to PTX. CCL2 does not induce Ca^{2+} mobilization, suggesting that neither CCR6 nor CCR2 is involved. Interestingly, PTX, La^{3+}, or 2-aminoethoxydiphenyl borate (2-APB, a dual inhibitor of inositol 1, 4,5, triphosphate receptor and transient receptor potential channels) blocks hBD-mediated degranulation in mast cells.

4.2.1.6. STAT signaling.: In activated CD4+ T cells, hBD3 does not induce ERK1/2 or p38 MAP kinases [108]. hBD3 but not hBD2 activates STAT1 phosphorylation, although the significance of hBD3-mediated STAT1 phosphorylation in T cell functions is not well defined [108]. The role of STAT signaling pathways in the crosstalk between defensins and cytokines remains to be determined. In keratinocytes, TNFα/IFNγ stimulation induces hBD2 and hBD3 by activating STAT1 and NF-κB signaling [109]. Activation of STAT6 and suppressors of cytokine signaling-1 and −3 inhibit TNFα/IFNγ mediated-induction of hBD2 and hBD3 by interfering with STAT1 and NF-κB signaling [109]. Further studies on the autocrine or paracrine effect of hBD involved in STAT pathways would offer a better understanding of the role of STAT signaling in regulation of hBDs and their functions.

4.2.2. Role of hBD2/hBD3 in modulation of TLR responses—hBD2 and hBD3 do not induce either TNFα or IL-6 production in macrophages; however, hBD3 can suppress LPS-mediated TNFα and IL-6 production in the monocytic THP-1 cell line, MDMs, murine transformed cell line RAW264.7, and murine BMDMs [110]. hBD3 also suppresses TNFα induction in BMDMs in response to IFNγ and CD40L, but does not have an effect on TLR1/2-mediated TNFα induction. Additionally, hBD3 reduces TNFα production in mice. Anti-inflammatory activity is not mediated through melanocortin receptors, nor through IL-10 or cAMP [110]. Inhibition of the TLR4 signaling pathway by hBD3 is dependent on MyD88 or TRIF pathways, indicating an anti-inflammatory role for hBD3 [111]. hBDs induce TLR3-mediated induction of TNFα, IFNβ, IL6, and IL-8 in BMDMs [112]. hBD3 promotes TLR3-mediated IFNβ production through the MDA5/MAVS pathway, but suppresses CXCL10 through TRIF signaling [112]. It is worth noting that pre-treatment of hBD2-IgFc or hBD3-IgFc fusion proteins enhance, rather than suppresses, TLR responses in macrophages through induction of extracellular ATP, which is followed by action of P2X7R [113].

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Both hBD2 and hBD3 promote IFNα production by pDCs by binding to self DNA or CpG oligonucleotides to form DNA complexes. hBD3 promotes TLR9-mediated activation of pDC and mDC in vitro and in mice, suggesting an adjuvant activity of hBD3 as well as involvement in skin inflammation [114]. In this regard, hBD2 and hBD3 are found in psoriatic skin lesions, and promote pDC activation [115]. Synthetic hBD3 but not hBD1 enhances TLR9-dependent IFNα induction by pDC in response to self-DNA. Although higher concentrations of hBD2 are required to activate pDCs in the presence of genomic DNAs, both hBD2 and hBD3 have comparable synergistic effects on LL37-mediated pDC activation. Similar to LL37-medated pDC activation in response to TLR9 [59,63], hBD2 and hBD3 aggregate DNA and protect host DNA from DNAse I degradation, allowing internalization into endosomal compartments for TLR9 activation [115]. hBD3 also promotes TLR9-mediated IFNα and IL-6 induction in murine Flt-3-induced DCs and PBMCs in response to bacterial DNAs [116]. The effect is more pronounced in cDCs (CD11c + B220-) than in pDCs (CD11c + B220high). hBD3 aggregated DNA and increased the uptake of DNA into murine DCs [116].

4.3. hBD6

Structural analysis indicates that hBD6 binds to a peptide derived from the extracellular domain of CCR2 [117]. hBD6 forms a complex with glycosaminoglycans (GAG) in a 2:1 stoichiometry [118]. The GAG binding site overlaps with CCR2 binding sites as indicated by the replacement of CCR2 sulfopeptides by increased amounts of GAG [118]. The role of CCR2 or GAG in the immune functions of hBD6 as well as putative competition between CCR2 and GAG in the hBD6 activity remain to be established.

4.4. The immune modulatory role of hBDs in carcinogenesis

Elevated CCR6 and CCR7 expression has been found in metastatic squamous cell carcinoma of the head and neck (SCCHN) [119], suggesting immune modulation of cancer cell migration. hBD3 induces CCR7 expression on primary SCCHN tumor cells and promotes tumor cell migration toward CCL19 in a NF-κB dependent manner [120]. hBD3 is internalized into tumor cells via endocytosis. The induction of CCR7 by hBD3 is not mediated through a G-protein coupled receptor pathway or by TLR signaling, shown by the fact that the induction is not inhibited by PTX or inhibitors of TLR signaling (MyD88 or TRIF-peptide inhibitor). hBD3 protects cisplatin-mediated apoptosis of SCCHN cells through the PI3/Akt pathway, indicating a role of hBD3 in promoting cancer cell survival [120].

hBDs play a role in oral tumorigenesis (reviewed in [121]). In normal oral epithelium, hBD1 and hBD2 are expressed in differentiated cells near the keratinized superficial layer, whereas hBD3 is expressed in the proliferative cells in the basal layers of oral epithelium [44,122]. In oral biopsy samples of moderate dysplasia, hBD3 expression is also found in dysplastic cells near the spinosum layer [44]. hBD1 and hBD2 are not found in the carcinoma in situ (CIS), whereas hBD3 is highly expressed in pre-malignant cells in CIS. The overexpression of hBD3 is associated with infiltration of macrophages into the site of the lesion, suggesting a role of hBD3 in progression of oral cancer [44]. In the nude mouse model, hBD3 is

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associated with macrophage recruitment to the lesions, and the induction of infiltration of tumor-associated macrophages by hBD3 is mediated through CCR2 [99].

5. Conclusions

Defensins have multifaceted immune functions that can be critical both for combating pathogens and for disease progression. Understanding the immune functions of defensins will continue to be an exciting and important area of research. To advance the field, caution should be taken to avoid contamination. Rigorous controls are needed when analyzing the activities of defensins in myeloid cells, which are highly sensitive to TLR agonists. Further delineation of the role of critical determinants and of the higher order structure of defensins with respect to their immune functions may help in the design of multi-purpose defensin analogs to combat pathogens through direct antimicrobial activities and indirect immune modulatory activities. Because the activity of defensins is cell-type specific, studies using complex models (e.g., explants in an air-liquid culture) will likely offer insight into mucosal immune functions of defensins. A more thorough comparative analysis of the properties of human defensins and their murine orthologs as well as appropriate animal models to validate the role of receptors and signaling pathways in defensin-mediated immune functions will help address the immune functions of defensins in complex models. Additionally, identification of attributes that direct inflammatory and anti-inflammatory functions of defensins will be crucial for their development as new immunotherapeutics.

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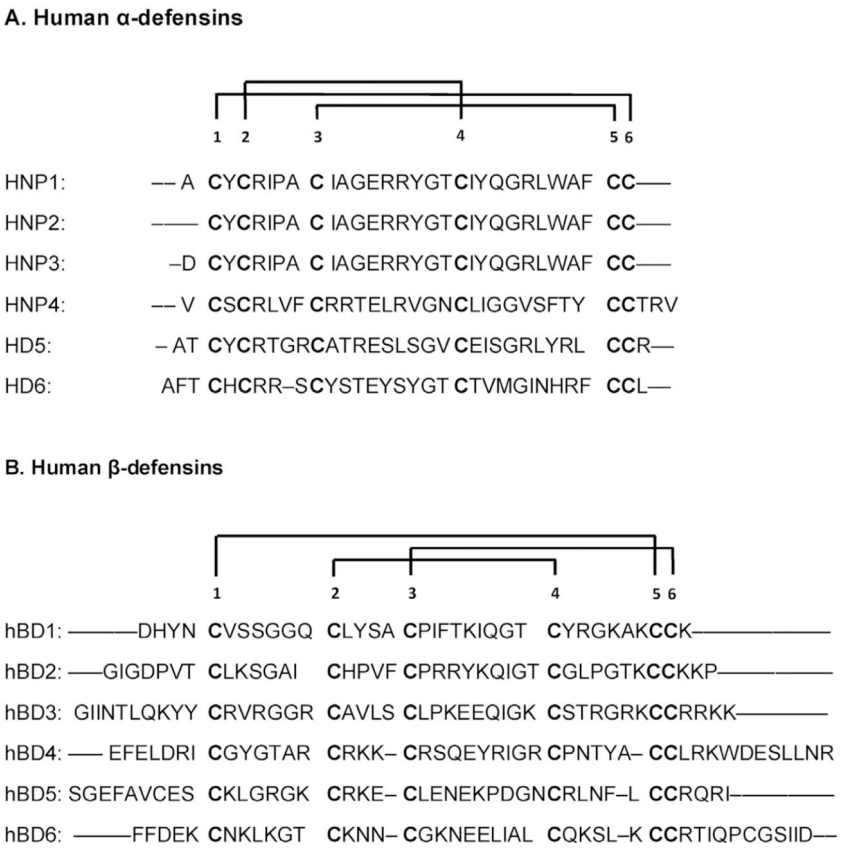

Fig. 1.

A. Human α-defensins, B. Human β-defensins.

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Table 1.

Receptors and pathways involved in immune functions of human defensins.

Defensins	Receptors/Signaling	Function	Cell Type	References
HNPs	Gαi protein-coupled receptor	Chemotaxis	naïve CD4+ T cells, CD8+ T cells, immature DCs	[52]
	P2Y6 receptor, ERK1/2, PI3K/Akt	Induce IL-8	lung epithelial cells	[77,80]
	Src	Induce IL-8	monocytic cell lines	[80]
HNP1	P2Y receptor	Block apoptosis	neutrophils	[78]
	P2 receptor(but not P2Y6), ERK1/2, p38	Induce IL-8	intestinal epithelial cell lines	[79]
	NF-κβ, IRF1	Induce IFNα, IFNβ, IL-6, enhance TLR9 activation	pDCs	[81]
	P2X7-potassium channel efflux-capsease-1 signaling pathway	Activate NLRP3 inflammasome, induce IL-1β	LPS-primed THP-1 monocytic cell line	[82]
HNP1, HNP3, HD5	Gαi protein-coupled receptor, MAPK ERK1/2, p38	Chemotaxis	macrophages, mast cells	[53]
hBD1	CCR6	Chemotaxis	HEK293 CCR6 expressing cells	[85]
hBD2	CCR6	Chemotaxis	memory CD4+ T cells, immature DCs	[85]
	CCR6	Cell migration (wound healing), block TRAIL-mediated apoptosis	intestinal epithelial cell lines	[94]
	CCR6	Adhere to inflamed endothelial cells	Th17cells	[95]
	ERK1/2, JNK, p38 MAPK	Chemotaxis	MDMs	[84]
	CCR6, Gαi protein-coupled PLC	Chemotaxis	TNFα-stimulated neutrophils	[89]
	Gαi protein-coupled PLC	Chemotaxis	mast cells	[90]
hBD3	CCR6, ERK1/2 MAPK	Induce IL-8, apoptosis	airway smooth muscle cells	[91]
	CCR6	Block apoptosis	Neutrophils	[92]
	CCR2	Induce IL-1α, IL-6, IL-8, CCL18, TNFα	MDMs	[99]
	CCR2	Recruitment	monocytes, macrophages (tumor-associated)	[99]
	CXCR4	Chemotaxis	activated CD4+ T cells, CD4+T cell line	[96,97]
	MyD88, IRAK-1 activation	Activation	monocytes	[102]
	TLR1/2	Activation	monocytes, mDCs	[102]
	TLR1/2	Induce IL-1β	monocytes	[103]
	TLR1/2, CCR2	Induce CD69	NK cells	[104]

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Defensins	Receptors/Signaling	Function	Cell Type	References
	TLR9	Activation	pDCs, mDCs	[114]
	TLR9	Induce IFNα, IL-6	murine DCs, PBMCs	[116]
	P2X7 receptor	Induce CD86	monocytes	[106]
	STAT1	N/A	activated CD4+ T cells	[108]
hBD2/hBD3	MrgX2	Degranulation	mast cells	[107]
hBD2-IgFc, hBD3-IgFc, mBD4-IgFc, mBD14-IgFc		Chemotaxis	HEK293 CCR2 expressing cells	[98]
hBD3-IgFc, mBD14-IgFc		Chemotaxis	monocytes	[98]
mBD2-IgFc	CCR6	Chemotaxis	bone marrow derived DCs	[101]
	TLR4	Maturation	Immature DCs	[101]