

## Human Defensins: Turning Defense into Offense?

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**Abstract:** Defensins are a family of antimicrobial cationic peptides that act as a rapid response force against microbial invasion in a wide range of organisms, including plants, insects, animals and humans. In humans, defensins are produced predominantly by leukocytes and epithelial cells and are an important factor of innate immunity. In addition to their major role as natural antibiotics, defensins are increasingly recognized as signaling molecules in adaptive immunity and aberrant defensin expression has been associated with infectious diseases. In this review, we discuss the role of human defensins in relation to infectious disease and the possibility of novel defensin-based therapeutic approaches.

**Keywords:** Defensins, infectious disease, therapeutics.

### INTRODUCTION

Antimicrobial peptides constitute an important component of innate immunity in many higher organisms, including multi-cellular plants and animals [1,2]. Despite great diversity in molecules that show antimicrobial activities, a shared characteristic are clusters of hydrophobic and cationic residues which are spatially organized in an "amphipatic design" in many of these peptides [2]. Their antimicrobial properties have evolved to exploit a fundamental difference in membrane composition between microbes and multi-cellular organisms. Due to their cationic nature, antimicrobial peptides preferentially target bacterial membranes which contain large amounts of negatively charged phospholipids. In contrast, the outer leaflet of the mammalian cell membrane is composed principally of neutral, zwitterionic phospholipids. Various models for the selective activity of antimicrobial peptides towards microorganisms have been proposed. These include the formation of solvent-permeable pore structures, covering the membrane like detergents or possibly by inhibiting metabolic processes of microorganisms through intracellular targeting (for a comprehensive review, see [3]). In addition to their antimicrobial activities, these peptides interact with the host itself and are increasingly recognized as potential anti-infective therapeutic molecules. This review will focus on one of two major families of antimicrobial peptides, termed defensins, in humans. We will focus on the abnormal expression of these peptides associated with disease as well as their potential as anti-infective agents.

### DEFENSINS

Defensins are cationic, cysteine-rich peptides with molecular masses ranging from 3 to 5 kDa that form characteristic intramolecular disulfide bonds [4-6]. In humans, two main defensin subfamilies,  $\alpha$ -defensins and  $\beta$ -defensins, have been identified differing in their disulfide bond pairing. Structural analyses of human defensins from both families have revealed a similar structure, despite variation in sequence and cysteine connectivity. Defensin tertiary structure shows a common fold of three-stranded anti-parallel  $\beta$ -sheets constrained by three intra-molecular disulfide bonds [7-11].  $\alpha$ -defensins are synthesized as "pre-propeptides", including a signal sequence. Cleavage of the signal peptide produces inactive "pro- $\alpha$ -defensins" which are processed further depending on their site of expression [12,13]. In  $\beta$ -defensins, the mature domain is separated from its signal peptide by only few amino acids.

To date, 6  $\alpha$ -defensins have been identified; 4 of them have been termed  $\alpha$ -defensin 1, 2, 3 and 4, but are more commonly referred to as human neutrophil peptides 1-4 (HNP1-4), after their cellular origin of expression [14]. These peptides are stored in azurophilic granules of neutrophils as fully processed, mature peptides. Two more  $\alpha$ -defensins, termed human defensin 5 and 6 (HD-5 and -6) are expressed in specialized epithelial cells termed Paneth cells in the small intestine as well as in epithelial cells of the female urogenital tract [15-17]. Unlike in neutrophils, where defensins are stored as fully processed mature peptides, defensins in Paneth cells are stored as propeptides [18]. Upregulation in the activity of  $\alpha$ -defensins seems to occur at the peptide level via increased peptide mobilization or increased secretion. HNPs in azurophilic granules fuse with endocytosed microbes and are not secreted into the outside environment. HNP1-3 are released by natural killer cells, but only upon activation of these cells by bacterial ligands such as flagellin or OmpA [19]. In contrast, HD-5 and HD-6 are constitutively secreted by Paneth cells and secretion is stimulated by various microbial antigens [20].

Four  $\beta$ -defensins have been identified in humans so far, termed human beta defensins 1-4 (HBD1-4), however, a recent computational screening has suggested the presence of five beta defensin gene clusters encoding as many as 30  $\beta$ -defensins [21]. HBD1-4 are widely expressed in epithelial tissues, including skin, lung, urinary and oral epithelium and in neutrophils [6]. In contrast to  $\alpha$ -defensins, which seem to be expressed constitutively,  $\beta$ -defensin expression can be induced. Often, expression of  $\beta$ -defensins is upregulated in response to recognition of microbial compounds via Toll-Like Receptors (TLRs) or TLR-mediated production of proinflammatory cytokines (for comprehensive reviews, see [6,22]).

In addition to their primary antimicrobial activities, human defensins play important roles as immune modulators in adaptive immunity. Human neutrophil  $\alpha$ -defensins were shown to chemoattract monocytes [23] as well as different subsets of T lymphocytes and dendritic cells [24,25], processes thought to be high-affinity receptor-mediated. Similar functions have been shown for human  $\beta$ -defensins, which selectively chemoattract memory T cells and immature dendritic cells [26,27]. HBD2 has been shown to act directly on immature dendritic cells as an endogenous ligand for Toll-like receptor 4 [28]. Chemotaxis of immature dendritic cells and memory T cells by this peptide resulted from direct binding and activation of the chemokine receptor CCR6 [26]. The role of these receptor interactions in defensin-mediated immunomodulatory activities underlines the versatility of defensin function. In the next paragraphs, we will focus on abnormal defensin expression and anti-infective activities in relation to infectious disease.

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**Table I. Infectious Diseases Associated with Abnormal Defensin Expression. Many Studies have Shown Increased Defensin Expression as a Result of Infection with Known Microbial Pathogens, These Studies are not Included in this Table.**

Defensin	Disease	Expression	Reference
HD-5	Ileal Crohn's disease	downregulated	[30]
HBD1	Cystic fibrosis*	inactivated	[72]
HBD1, HBD2	psoriasis	upregulated	[42]
HBD2	Atopic dermatitis	downregulated	[39]
HBD2, HBD3	Periodontal disease	Downregulated	[73]
HBD1, HBD2	Acne	Upregulated	[74]
HBD2, HBD3	Ulcerative colitis	Upregulated	[75]

HBD-1 in this case is inactivated by elevated salt levels in airway surface fluid from cystic fibrosis patients

### ABNORMAL DEFENSIN EXPRESSION RELATED TO INFECTIOUS DISEASE

The relationship between irregular defensin expression and disease has been recognized only very recently. Table 1 lists infectious diseases in which abnormal defensin expression has been described. As examples, we will discuss the role of defensins in inflammation of the gut and skin in more detail below.

#### Inflammatory Bowel Disease

There is increasing evidence that aberrant defensins expression is correlated to inflammation of the gastrointestinal tract. Initial studies revealed that HD-5 was expressed by so-called metaplastic Paneth cells in the colon in inflammatory bowel disease, whereas in healthy individuals expression is restricted to ileal Paneth cells exclusively [18,29]. More recently, a specific deficiency in HD-5 was observed in patients suffering from ileal Crohn's disease [30]. Interestingly, the HD-5 deficiency was more pronounced in patients carrying loss-of-function mutations in the cellular receptor NOD2. NOD2 is an intracellular receptor for the bacterial peptidoglycan component muramyl dipeptide and is expressed in Paneth cells [31]. Genetic polymorphisms in the encoding gene *NOD2/CARD15* have been identified to be tightly linked with susceptibility to Crohn's disease [32,33] and with decreased defensin expression [34]. Expression of  $\beta$ -defensins has also been linked to inflammatory bowel disease. HBD-2 was found to be induced in inflamed colonic tissue and appeared more prominent in ulcerative colitis than in Crohn's disease [35]. Similarly, expression of HBD-3 and HBD-4 was found to be upregulated in ulcerative colitis, but not in Crohn's disease of the colon [36]. Together, these studies have led to the suggestion that the underlying cause of intestinal inflammation may in part be explained by the altered defensin levels [37].

#### Atopic Dermatitis and Psoriasis

The skin is a source of a variety of peptides with antimicrobial activities, including defensins (reviewed in [38]). A significant reduced expression of HBD-2 has been demonstrated in patients suffering from atopic dermatitis [39]. More recently, reduced expression for HBD-3 was observed in these patients [40]. As a result of the decreased expression of defensins and other antimicrobial peptides, several microorganisms, including *Staphylococcus aureus*, are frequently detected in infected skin. In contrast, a decrease in several types of infection has been observed in patients suffering from psoriasis [41]. This notable difference is explained in part by the high expression levels of antimicrobial peptides in keratinocytes of psoriasis patients compared to atopic dermatitis patients [42].

Together, the studies on irregular defensin expression in relation to disease seems to point towards a cause-and-effect relationship, whereby the aberrant defensin expression may be the effect of varying underlying causes. These causes may include altered cell signaling processes (which may be exacerbated by genetic mutations as in the case of NOD2 described above), variation in cytokine and chemokine levels (for example IL-4 and IL-10 up-regulation in the case of atopic dermatitis [39,40]) or via corticosteroids [43,44].

### ANTI-INFECTIVE ACTIVITY OF DEFENSINS

Defensins act against a broad range of micro-organisms, including Gram-positive- and Gram-negative bacteria, fungi, viruses and protozoans [1,45-47]. Their anti-infective properties are perhaps best understood in the case of viruses (for a comprehensive review see [48]). Here, we will focus on the suggested role of defensins against HIV infection.

The inhibition of HIV replication was first reported for synthetic rodent  $\alpha$ -defensins [49], and more recently anti-HIV activities have been described for human  $\alpha$ - and  $\beta$ -defensins [50-54]. A number of mechanisms of anti-HIV activity by defensins have been proposed. The first mechanism suggests a direct interaction of defensins with the virus itself. HNP1, 2, and 3 have been shown to bind gp120, the HIV envelope glycoprotein [55]. HNP4 was reported to be a more effective inhibitor than HNP1, 2, and 3, but interestingly does not bind to gp120 [50,53]. HBD2 and HBD3 were shown to interact with HIV virions [52]. These interactions were reported to occur in the absence of serum only. In the presence of serum, the binding between HNP and gp120 was greatly reduced.

Secondly, a number of mechanisms of anti-HIV activity of defensins suggest that rather than binding to the virus, they interact with target host cells. Binding of defensins is suggested to block virus-host cell interactions in a number of distinct ways. First, directly by competing for binding the cellular receptors CXCR4 and CCR5 used by the virus as has been shown in the case of HBD2 and HBD3 [56,57]. Second,  $\alpha$ -defensins have been shown to stimulate the expression of  $\beta$ -chemokines such as MIP1 $\alpha$  and RANTES [58,59], which is suggested to result indirectly in competition for receptor binding with the virus. Thirdly, HNP1 was shown to affect protein kinase C signaling in primary CD4+ T cells associated with HIV inhibition in the presence of serum [60]. Recently, defensins were shown not only to bind to CD4, a primary receptor for HIV, but also to downregulate the expression of this receptor [61]. Some of the above mentioned inhibitory effects of defensins, such as host cell receptor modulation and blocking fusion of the viral membrane, are not as yet fully resolved and await further study.

Although the inhibitory effects of defensins on HIV infection have been shown *in vitro*, the *in vivo* effects are less well established. In one study, the levels of HNP1, HNP2 and HNP3 appeared elevated in T cells from HIV patients and seronegative HIV-exposed individuals compared to controls [62], however a direct association needs to be established further. Recently, various studies have reported on HBD1 gene polymorphisms associated with infectious diseases, including HIV (Table 2). In the case of HIV, single nucleotide polymorphisms in the DEFB1 gene, encoding HBD1, were associated with HIV infection [63,64]. In both studies, the SNPs were found in the 5' untranslated region of the DEFB1 gene, suggesting altered gene translation and a possible use of this region as a marker for HIV infection.

**Table II. Gene and single nucleotide polymorphisms in the DEFB1 gene, encoding human  $\beta$ -defensin 1, associated with infectious diseases. Gene copy number polymorphisms have been reported for human defensins also [76,77], but as yet have not been associated with disease and are not included in this table.**

Disease	Reference
HIV	[63,64]
Atopic dermatitis	[78]
Asthma	[79,80]
<i>Candida albicans</i> carriage in Type I diabetes	[81]
Periodontal disease	[82]
Cystic fibrosis	[83]

#### DEFENSIN AS FUTURE "OFFENSINS"

Many studies have reported on the antimicrobial, antiviral and chemoattractive properties of defensins. This in turn has prompted increasing interest in their putative use as molecular therapeutics. Since defensin primary function is antimicrobial, the focus has been on their development as non-toxic peptide antibiotics and this area of research is perhaps the most promising to date. It is beyond the scope of this review to describe the potential antimicrobial use of defensins. Instead, we will focus on two recent findings that may be of particular interest.

In addition to their bacterial membrane permeabilizing capacity, defensins have been shown to neutralize bacterial invasion by directly binding to bacterial toxins. HNP1 has been shown to neutralize the *Bacillus anthrax* lethal toxin and was furthermore shown to act protective *in vivo* in mice [65]. Similar properties have been described for retrocyclins, a class of circular defensins found in non-human primates, which were shown to bind to the anthrax lethal factor with high affinity [66]. Recently, these neutralizing properties of HNP1 have been extended to members of the mono-ADP-ribosyltransferase family of bacterial toxins, including diphtheria toxin and *Pseudomonas* exotoxin A [67].

Based on their cationicity, the antimicrobial properties of defensins involve electrostatic interactions [3]. Consistent with this, their antibacterial activity is inhibited at high ionic strength. The discovery of cyclic defensins, briefly mentioned above, has shown that these peptides retain activity even at high salt conditions [68,69]. This may be of particular interest in cases where defensin activity is inhibited by elevated ionic strength, as for example the inhibition of  $\beta$ -defensins in the airway of cystic fibrosis patients

(Table 1). Interestingly, anti-HIV properties have also been reported for these cyclic defensins [50]. One possible drawback for the use of defensins, and cyclic defensins in particular, is efficient large scale synthesis. Recent progress has been made in this area and both  $\alpha$ - and  $\beta$ -defensins have been functionally over-expressed in bacterial expression systems [70,71].

Defensins have long been recognized as natural antibacterial peptides. This primary function is to date best supported *in vivo*. In addition, defensins act antifungal, antiviral, anti-protozoan and furthermore link innate to adaptive immunity by virtue of their immunomodulatory properties. These functions are mainly supported by results obtained *in vitro*. Nevertheless, defensins offer great potential to exploit these properties for the development of therapeutics. First and foremost, the development of defensins as an alternative to classical antibiotics in cases where toxicity or microbial resistance occurs is an important objective. The use of defensins in preventing or treating infectious diseases is equally as important, but is more of a challenge since less is known of the precise role defensins play in these processes. For example, while treatment of atopic dermatitis with defensins may prove to be beneficial, a reduction of defensin levels in psoriasis by targeting underlying signaling events may be more helpful. Many advances have been made in understanding the mechanisms that control defensin expression and secretion. Also, there is increasing emerging evidence that defensins may play a more prominent role in infectious diseases than previously recognized. A combination of fundamental research, improving defensin production and the development of appropriate delivery systems may be necessary ingredients to turn defensins into therapeutics.

#### Acknowledgments

The authors recognize that the field of defensin research has expanded greatly and apologize for not citing many publications on defensins in this review. This work is supported by the National Institutes of Health.

#### ABBREVIATIONS

HD	=	Human defensin
HNP	=	Human neutrophil peptide
HBD	=	Human $\beta$ -defensin
TLR	=	Toll like receptor
IL	=	Interleukin

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