Defensins in Viral Infections

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Abstract

Defensins are antimicrobial peptides important to innate host defense. In addition to their direct antimicrobial effect, defensins modulate immune responses. Increasing evidence indicates that defensins exhibit complex functions by positively or negatively modulating infections of both enveloped and non-enveloped viruses. The effects of defensins on viral infections appear to be specific to the defensin, virus and target cell. Regulation of viral infection by defensins is achieved by multiple mechanisms. This review focuses on the interplay between defensins and viral infections, the mechanisms of action of defensins and the in vivo studies of the role of defensins in viral infections.

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Introduction

The innate immune system provides the first line of defense for rapidly clearing microbes before the development of an adaptive immune response. In addition to the innate pathogen-recognition systems in immune cells using pattern recognition receptors, antimicrobial peptides such as defensins and cathelicidins play a significant role

in protecting the host from the invasion of pathogens. Although the anti-viral activity of defensins was first reported in 1986 [1], the underlying mechanisms are only now beginning to be established. It had been thought that defensins primarily target enveloped viruses by disrupting the envelope membrane in a manner similar to their antibacterial activities. The recent progress in our knowledge of human defensins in viral infection demonstrates that defensins exhibit complex functions by positively or negatively modulating infection of both enveloped and non-enveloped viruses and by regulating immune responses. This review mainly focuses on the role of human defensins in viral pathogenesis and transmission. A comprehensive overview of defensins can be found in several excellent reviews [2–6].

Overview of Human Defensins

Human defensins are classified into two subfamilies, α and β , which differ in their three disulfide bond pairing [reviewed in 3, 6]. Neutrophil α -defensins (HNPs 1–4) are mainly synthesized as prepropeptides in promyelocytes, neutrophil precursor cells in the bone marrow, and the mature peptide is stored in primary granules of neutrophils [3]. HNPs 1–3 can also be found in other immune cells, including natural killer cells, B cells, $\gamma\delta$ T cells, monocytes/macrophages and immature dendritic cells [4]. HNP release can be induced by chemokines, FC γ re-

ceptor cross linking, phorbol myristate acetate and bacterial components that activate Toll-like receptors (TLR) 2 and 5 [reviewed in 3, 4]. Cells can absorb and internalize HNPs [7–9], underlining the complexity in defining true HNP-producing cells versus cells that up-take defensins.

Two additional human α -defensins, human defensins 5 and 6 (HD5 and HD6), are constitutively expressed in intestinal Paneth cells but also found in the genital mucosa [reviewed in 2]. Unlike HNPs, HD5 is released as a propeptide that is processed extracellularly [2]. Although leukocyte α -defensins are evolutionarily conserved and have been isolated from many species – including human, rabbits, rats, guinea pigs and hamsters – mice do not express neutrophil α -defensin [3]. Mice express many enteric α -defensins known as cryptdins in intestinal Paneth cells [3, 5]. However, HD5 but not cryptdins can protect mice from virulent *Salmonella typhimurium* [10], indicating their distinct functions between species.

Human β -defensins (HBDs) 1–3 are expressed by epithelial cells and immune cells including monocytes, macrophages and monocyte-derived dendritic cells [3, 6]. While HBD1 is often constitutively expressed, expression of HBD2 and HBD3 can be induced by viruses, bacteria, microbial products, Toll-like receptor (TLR) ligands, EGF and pro-inflammatory cytokines, such as TNF- α and IL-1 [reviewed in 2, 3, 6].

An additional class of mammalian defensins is the θ -defensin with a circular structure originally found in rhesus monkeys [5]. Three θ -defensins have been found in leukocytes of rhesus macaques: rhesus θ -defensin (RTD)-1, RTD2 and RTD3 [11, 12]. Although RNA transcripts homologous to the RTD gene (DEFT for defensin theta) are found in human bone marrow, these transcripts contain a premature stop codon, which abolishes subsequent translation [13]. Retrocyclin, an artificially made circular peptide based on the sequence of the human θ -defensin pseudogene, displays antiviral activity in vitro [14].

Defensins have a wide range of functions in regulating both innate and adaptive immunity [6]. Both HNPs and HBDs exhibit chemotactic activity for T cells, monocytes and immature dendritic cells and induce production of cytokines and chemokines [6]. HNP1 also regulates the release of IL-1 β and enhances phagocytosis [15, 16]. HBDs and murine β -defensin-2 interact with CCR6 or TLR to modulate immune responses by recruiting and activating immune cells and inducing production of mediators such as cytokines, chemokines and prostaglandin D₂ [6, 17, 18].

Induction of Defensin Expression in Response to Viral Infection

Defensins are frequently induced in response to viral infection. In mice infected with influenza A virus (IAV), gene expression of murine β -defensin 3 and 4 (orthologs of HBD2) is induced in upper and lower airways [19], although the in vitro effect of murine β -defensins on IAV infection is not known. HIV-1 induces mRNA expression of HBD2 and HBD3 but not HBD1 in normal human oral epithelium, even in the absence of HIV-1 replication [20]. Similarly, expression of HBD2 and HBD3 but not HBD1 is induced in bronchial epithelial cells exposed to human rhinovirus [21, 22]. In contrast to HIV-mediated HBD gene induction, active replication of rhinovirus is required for HBD gene induction. Induction of HBD2 and HBD3 in response to human rhinovirus infection is mediated by NF- κ B activation but is independent of IL-1 [22].

Induction of HBD2 plays a role in innate antiviral response against human respiratory syncytial virus in lung epithelial cells [23]. This virus-mediated HBD2 induction is involved in TNF- α -mediated NF- κ B activation but is type I interferon independent. Productive respiratory syncytial virus infection activates NF- κ B and induces TNF- α , resulting in induction of HBD2 that is required for TNF- α -mediated anti-respiratory syncytial virus activity. Similar to infection with IAV in mice, respiratory syncytial virus infection induces expression of murine β -defensins 3 and 4 in the lung.

Effect of Defensins on Viral Infection

HNP1 was originally reported to have a direct effect on several enveloped viruses but not on non-enveloped viruses [1]. In studies of enveloped viruses, HNP1 has been shown to have a potent direct inhibitory effect on herpes simplex viruses (HSV) 1 and 2, a moderate direct effect on vesicular stomatitis virus and IAV, and little effect on cytomegalovirus [1]. Recent evidence indicates that defensins modulate viral infection through multiple mechanisms. The effect of defensins on viral infection is specific to the defensin, virus and target cell. Furthermore, defensins can inhibit or enhance viral infection. This effect is achieved through direct interaction with viral envelopes or through interactions with potential target cells. Table 1 summarizes the activities of defensins on viral replication.

The in vitro functions of defensins appear to be affected by factors such as serum and salt concentration

that may determine defensin functions depending on the sites (e.g. mucosal surfaces vs. blood). Serum and salt conditions alter the direct effect of HNPs and HBDs on the virion [1, 20, 24] but are not required for the chemotactic effects of defensins. Some defensins (e.g. HNPs but not HD5 or HD6) are known to cause cytotoxicity at high concentrations in the absence of serum, possibly through membrane permeabilization that can be abolished by the presence of serum [3]. Therefore, defensin-mediated cytotoxicity may partially account for the antiviral effect.

Human Immunodeficiency Virus

In vitro Studies. Recent studies indicate that, in contrast to the traditional role of defensins in host defense against pathogens, specific defensins can inhibit or enhance HIV infection. HNPs 1–3 block HIV infection through multiple mechanisms [25, 26]. HNPs 1–3 inhibit HIV-1 replication by a direct interaction with the virus as well as by affecting multiple steps of the HIV life cycle [8, 24, 25, 27, 28]. In the absence of serum, HNP1 can directly inactivate the virus prior to infection of a cell [24]. HNPs also block HIV-mediated cell-cell fusion and the early steps of HIV infection by interacting with HIV gp120 and CD4 through their lectin-like properties [25]. In the presence of serum and at non-cytotoxic concentrations (low dose), HNP1 acts on primary CD4+ T cells and blocks HIV-1 infection at the steps of nuclear import and transcription by interfering with protein kinase C signaling [24]. In the presence of serum, HNP1 did not affect expression of cell-surface CD4 and HIV coreceptors on primary CD4+ T cells [24], whereas HNP2 down-regulates CD4 expression in the absence of serum [25]. In macrophages, HNP1 and HNP2 up-regulate the expression of CC-chemokines, which may contribute to inhibition of HIV through competition for receptors [29]. In contrast to HNPs 1–3, HNP4 acts in a lectin-independent manner and binds to CD4 or HIV gp120 with low affinity [28, 30]. However, HNP4 inhibits HIV replication more effectively than HNPs 1–3 [30].

Other α -defensins, including HD5 and HD6, mouse Paneth cell cryptdin-3 and cryptdin-4, rhesus macaque myeloid α -defensins 3 and 4, guinea-pig, rabbit and rat α -defensins have been tested for their effect on HIV infection [31–33]. Guinea-pig, rabbit and rat α -defensins block HIV infection in transformed T cell lines [33]. At high concentrations associated with cytotoxicity, rhesus macaque myeloid α -defensin-4 blocks HIV replication, whereas HD5, HD6, and cryptdin-3 enhance viral replication [31, 32]. The enhancing effect of HD5 and HD6 was more pronounced with R5 virus compared with X4

virus, indicating a potential role of mucosal transmission of HIV, as R5 virus is preferentially transmitted during primary infection.

HBD2 and HBD3 inhibit HIV infection through multiple mechanisms [20, 34]. HBD2 does not affect viral fusion but inhibits the formation of early reverse transcribed HIV DNA products [34]. Sun et al. [34] demonstrated that HBD1 and HBD2 do not modulate cell-surface HIV co-receptor expression by primary CD4+ T cells, whereas Quinones-Mateu et al. [20] showed that HBD2 and HBD3 down-regulated surface CXCR4 but not CCR5 expression by peripheral blood mononuclear cells in the absence of serum. Interestingly, HBD2 is constitutively expressed in healthy adult oral mucosa but the level seems to be diminished in HIV-infected individuals [34].

Retrocyclins and RTDs 1, 2 and 3 act as lectins and inhibit HIV entry [14, 28, 35, 36]. Retrocyclin and RTDs 1, 2 and 3 inhibit several HIV-1 X4 and R5 viruses including primary isolates [28, 35, 36]. Retrocyclin binds to HIV gp120 and CD4 through interactions with their O-linked and N-linked sugars [35, 36]. Retrocyclin-1 binds directly to the C-terminal deptad repeat of HIV envelope protein gp41, blocking formation of the 6 helix bundle required for fusion [37].

In vivo Studies. HNPs were found in the media of stimulated CD8+ T cells from normal healthy controls and from long-term nonprogressors, but not from HIV progressors [26]. However, subsequent studies revealed that monocytes and residual granulocytes of allogeneic normal donor irradiated peripheral blood mononuclear cells as feeder cells were likely the main source of HNPs [8, 9]. Using similar co-culture systems, higher levels of HNPs were found in CD8+ T cells from HIV-exposed seronegative (ESN) individuals and HIV patients compared to normal controls [38].

HNPs levels have been shown to correlate with HIV RNA copy number in breast milk, which is a strong predictor of transmission [39]. However, after adjusting for breast milk HIV copy number, higher levels of HNPs in breast milk were associated with a decreased incidence of intrapartum or postnatal HIV transmission [39]. Bosire et al. [40] also demonstrated that women at one month postpartum with detectable HNPs had significantly higher mean HIV-1 RNA levels in breast milk than women with undetectable HNPs, although HNPs levels were not associated with vertical transmission.

Cationic peptides, including defensins, are required for *in vitro* anti-HIV activity of vaginal fluid from healthy women [41]. While it is well established that STIs significantly increase the likelihood of HIV transmission [re-

Table 1. Activities of defensins on viral infections

Virus	Defensins	Effect	Mechanism	References
<i>Enveloped viruses</i>				
HIV	HNP1	inhibit	inactivates virion	8, 24
	HNP1, HNP2	inhibit	up-regulate CC-chemokine production by macrophages	29
	HNP1, HNP2	inhibit	bind to gp120 and CD4, block fusion	25, 28
	HNP1	inhibit	blocks viral nuclear import and transcription	24
	HNP4	inhibit	binds to gp120 and CD4 (lectin independent)	28, 30
	HD5, HD6	enhance	enhance viral entry	32
	cryptdin-3	enhance	N/A	31
	HBD2	inhibit	blocks early RT product formation	34
	HBD2, HBD3	inhibit	down-regulate CXCR4 expression	20
	retrocyclin	inhibit	blocks viral entry	14, 35
	retrocyclin	inhibit	binds to gp120 and CD4	14, 28, 35, 36
	retrocyclin1	inhibit	blocks viral fusion	37
	RTD 1–3	inhibit	bind to gp120 and CD4	28
HSV1	HNP1	inhibit	inactivates virion	1
	rabbit NP-1	inhibit	blocks viral fusion, entry and post-entry steps	54
HSV2	HNP1	inhibit	inactivates virion	1
	HNPs 1–3	inhibit	inhibit viral entry	55
	HNPs 1–3, HD5	inhibit	inhibit post-entry steps	56
	HNP4, HD6, HBD3	inhibit	inhibit viral attachment and entry	56
	rabbit NP-1	inhibit	blocks viral fusion, entry and post-entry steps	54
IAV	retrocyclin-2	inhibit	inhibits viral attachment and entry	55
	HNP1	inhibit	inactivates virion (weak activity)	1, 57
	HNP1, HNP2, HD5	inhibit	aggregate virus, enhance viral clearance by neutrophils	15, 58
	HNP1	inhibit	interferes with cell signaling	57
	HBD3, retrocyclin-2	inhibit	inhibit viral fusion	59
RSV	HBD2	inhibit	inhibits viral entry, disrupts viral envelope	23
PIV3	sheep BD1	inhibit	N/A	60
	HBD6	enhance	N/A	61
Vaccinia virus	HBD3	inhibit	N/A	62, 63
VSV	HNP1	inhibit	inactivates virion	1
CMV	HNP1	inhibit	inactivates virion (weak activity)	1
<i>Non-enveloped viruses</i>				
BKV	HNP1, HD5	inhibit	inhibit early events of the virus lifecycle	64
HAdV	HNP1, HD5	inhibit	stabilize virus capsids, prevent uncoating	65–68
	HBD1	inhibit	N/A	67
Papillomavirus	HNP1, HD5	inhibit	restrain virus in endosomes	69

BKV = BK virus; CMV = cytomegalovirus; HAdV = human adenovirus; HBD = human β -defensin; HIV = human immunodeficiency virus; HNP = human neutrophil peptide; HSV = herpes simplex virus; IAV = influenza A virus; NP1 = neutrophil peptide 1; PIV = parainfluenza virus; RSV = Respiratory syncytial virus; RT = reverse transcription; RTD = rhesus θ -defensin; sheep BD = sheep beta defensins; VSV = vesicular stomatitis virus.

viewed in 42] and that levels of defensins, including HNPs, HBDs and HD5, in the genital fluid are elevated in patients with STIs [43–47], the role of defensins in STI-mediated HIV transmission is not well characterized. A recent study demonstrated the association between an increase in levels of HNPs and LL-37, which exhibited anti-HIV activity in vitro, in the IgA-depleted cervicovaginal

secretions from women with bacterial STIs and increased HIV acquisition [48], suggesting that defensins may cause immune activation, leading to enhanced HIV transmission, despite their direct antiviral effects.

Depending on the specific single-nucleotide polymorphism, variations in the *DEFB1* gene (coding for HBD1) have been associated with either a risk of perinatal HIV

transmission [49, 50] or protection against HIV infection [51, 52]. Although HBD1 has no effect on HIV infection in vitro [20, 34], the presence of single-nucleotide polymorphisms may modulate the overall immune response by regulation of HBD1.

The role of defensins in protection against HIV infection has been studied in HIV-ESN individuals. ESN expressed significantly greater mRNA copy numbers of HBD2 and 3 in oral mucosa than healthy controls, while no difference in mRNA copy numbers of HBDs1–3 in vaginal/endocervical mucosa was observed between ESN and controls [53]. In addition, homozygosity for the A692G polymorphism is significantly more frequent in ESN than in seropositive individuals [53].

Herpes Simplex Virus

Several defensins, including HNPs 1–4, HD5, HD6, HBD3, θ defensins (RTD and retrocyclin) and α rabbit defensin (NP1) have anti-HSV activity [54–56], whereas HBD1 and HBD2 do not exhibit anti-HSV2 activity [56]. NP1 exhibits a direct inhibitory effect on HSV1 and HSV2 virions and blocks HSV fusion and entry as well as post-entry steps [54]. HNP1 has a direct effect on HSV virions in the absence of serum [1]. HNPs 1–3 and retrocyclin 2 was first reported to inhibit HSV2 attachment and entry but not steps following entry [55], although the subsequent study showed that HNPs 1–3 also blocked the post-entry events [56]. HNP4, HD6 and HBD3 prevent HSV2 binding and entry, whereas HD5 inhibits post-entry events [56]. With the exception of HNP4, α -defensins and θ -defensins interact with the O- and N-linked glycans of HSV2, indicating that defensins may act as lectins to prevent HSV-2 glycoprotein B (gB) from interacting with its receptor HSPGs [55]. HNPs 1–3 and HD5 bind HSV gB with high affinity, but not heparan sulfate, the HSV2 attachment receptor [56]. In contrast, HNP-4 and HD6 bind heparan sulfate, but not gB. HBD3 binds both gB and heparan sulfate, whereas HBD1 and HBD2 do not bind to HSV gB or heparan sulfate.

Influenza Virus

HNPs 1–3 inhibit IAV through multiple mechanisms. While the direct effect of HNPs on the IAV particles is moderate [1], HNPs block various strains of IAV by acting on the target cells through interference with cell signaling [57] or by aggregating virus particles to promote viral clearance by neutrophils [15, 58]. HNP1, HNP2 and HD5, but not HBD2 and HBD3, enhance the uptake of IAV by neutrophils [15]. HNPs also modulate anti-IAV

activities of other innate effectors such as surfactant protein D by binding to it, resulting in interference with its hemagglutination-inhibiting activity [58] and reduction of neutrophil H_2O_2 production in response to surfactant protein D-treated IAV [15].

Retrocyclin-2 blocks the step of viral fusion mediated by the viral hemagglutinin proteins [59]. It also inhibits fusion mediated by other viral proteins such as baculovirus gp64 and Sindbis (Alphavirus) E1 proteins. Retrocyclin-2 acting as a lectin interferes with viral-mediated fusion by cross linking and immobilizing cell membrane glycoproteins. Pretreatment of either hemagglutinin-expressing cells or target cells with retrocyclin-2 inhibits fusion. Similar to retrocyclin-2, HBD3 has an inhibitory effect on hemagglutinin-mediated fusion and membrane protein mobility. These results indicate that a common pathway of membrane fusion is utilized for a broad range of activity of the innate immune response against different viruses.

Paramyxoviruses

Respiratory syncytial virus, as well as parainfluenza virus types 1–4, members of the Paramyxoviridae family, are major causes of respiratory diseases, particularly in young children and the elderly. HBD2 but not HBD1 inhibits the entry of respiratory syncytial virus and disrupts its envelope [23]. In vivo, induction of sheep β -defensin-1 and SP-A and SP-D expression correlates with a decrease in parainfluenza virus 3 viral replication in neonatal lambs [60]. Adenovirus-mediated HBD6 expression increases neutrophil recruitment and inflammation in the lungs of neonatal lambs [61]. Interestingly, parainfluenza virus 3 infection of neonatal lambs is enhanced during the treatment with adenovirus-mediated gene therapy, and HBD6 expression further exacerbates the infection.

Vaccinia Virus

HBD3, but not HBD1 and HBD2, exhibit anti-viral activity against vaccinia virus [62, 63], although the mechanism is not clear. Expression of HBD3 is induced in primary keratinocytes in response to vaccinia virus infection. Importantly, IL-4 and IL-13, frequently induced in patients with atopic dermatitis who are excluded from smallpox vaccination, down-regulate vaccinia virus-mediated HBD3 induction, suggesting that a deficiency in HBD3 may increase the susceptibility of patients with atopic dermatitis to vaccinia viral infection after smallpox vaccination [62].

Non-Enveloped Viruses

Increasing evidence indicates that defensins can block infection by non-enveloped viruses via multiple mechanisms. HNP1 and HD5, but not HBD1 and HBD2, inhibit infection of BK virus, a polyomavirus, by targeting an early event in the viral lifecycle [64]. HD5 inhibits BK virus by acting on the virion as HD5 treatment of BK virus, but not the target cell, reduces viral attachment to cells. HD5 binds to BK virus and colocalizes with BK virus in infected cells. Transmission electron microscopy analysis reveals HD5-induced aggregation of virions. HD5 also inhibits infection of cells by other related polyomaviruses including SV40 and JC virus.

HNP1, HD5 and HBD1 inhibit human adenovirus infection in lung and conjunctival epithelial cells [65–68]. Similar to anti-BKV activity of defensins, HD5 inhibits an early step in virus entry [68]. HNP1 and HD5 block human adenovirus infection by stabilizing the virus capsid, thereby preventing uncoating and virus-mediated endosome penetration.

HNPs do not have a direct effect on the virions of several non-enveloped viruses, including echovirus and reovirus [1]. HBD2 does not directly inactivate rhinovirus [22]. Using pseudoviruses carrying a green fluorescent protein, HNP-1 and HD5 inhibit various papillomavirus types [69]. These defensins do not affect initial binding of the viron and endocytosis but block virion escape from endosomes.

Conclusions

The in vitro effect of defensins on viral infection appears to be specific to the defensin, virus and target cell. In addition to the direct effect on the virus and target cell, defensins act as immune modulators that may play a role in viral transmission and disease progression in vivo. While aberrant defensin expression has been associated with diseases, the complex diversity of defensins among different animal species as well as apparent differences in mechanisms of action present a challenge to those investigating the role of defensins in viral pathogenesis in humans. Studies using knockout or over-expression in mice and further epidemiological or clinical studies in humans are a significant priority to gain a better understanding of the role of defensins in viral infection. The immunomodulatory role of defensins in viral infection requires further delineation. The negative feedback mechanism of down-regulation of defensins remains to be explored. Further studies focused on the contribution of the structure of defensins to their various effects on viral infections as well as standardization of sample collection methods and assays used to assess their biologic function could reveal some unifying principles and will contribute to the development of defensins as novel drugs for the prevention of infection.

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