Chapter 3
Antiviral Host Defence Peptides

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Abstract The ongoing global mortality and morbidity associated with viral pathogens highlights the need for the continued development of effective, novel antiviral molecules. The antiviral activity of cationic host defence peptides is of significant interest as novel therapeutics for treating viral infection and predominantly due to their broad spectrum antiviral activity. These peptides also display powerful immunomodulatory activity and are key mediators of inflammation. Therefore, they offer a significant opportunity to inform the development of novel therapeutics for treating viral infections by either directly targeting the pathogen or by enhancing the innate immune response. In this chapter, we review the antiviral activity of cathelicidins and defensins, and examine the potential for these peptides to be used as novel antiviral agents.

3.1 Introduction

The ongoing mortality and disease associated with circulating viral infections of humans and animals, together with the ongoing threat of emerging viral strains underscores the requirement for the development of novel antiviral molecules. While vaccination against common viral pathogens is effective and desirable, direct antiviral therapeutics play a key role in treating diseases caused by viral pathogens that have no vaccine, that lack a global vaccination programme, or those with vaccines of limited efficacy. In addition, as the resistance of viral pathogens to common antiviral drugs increases, new classes of antiviral molecules could provide a strategy for treatment of both existing and emerging infections for decades to come.

The antiviral activity of Cationic Host Defence Peptides (CHDP; also known as antimicrobial peptides) is of increasing interest for informing the development of novel antiviral therapeutics. Due to their broad spectrum activity, CHDP play a key
role in the innate immune response to both bacterial and viral pathogens. In
addition, CHDP have substantial immunomodulatory activities that can contribute
to the rapid clearance of infections. Therefore, a better understanding of the
antiviral and immunomodulatory activities of CHDP in the context of viral infec-
tion will be of great importance in the race to develop new treatments that are
broadly effective against viral pathogens.

CHDP are small, evolutionarily conserved peptides with a positive charge that
have broad spectrum activity against a range of pathogens, both in vitro and in vivo.
In humans, there are two major families of CHDP; the cathelicidins and the defen-
sins, however, more families exist in mammals, birds, fish, reptiles, arthropods and
plants, each showing antimicrobial potential. While the antiviral activity of many
peptides has been established in a significant number of studies, a comprehensive
understanding of the mechanism(s) of action involved remains elusive. Of particular
interest are the immunomodulatory and inflammammodulatory activities of CHDP,
from gaining a more fundamental understanding of innate responses to infection in
addition to informing development of novel therapeutics. CHDP have been shown to
have the capacity to modulate cell death pathways in infected cells, assist in the
recruitment of immune cells to sites of infection, promote angiogenesis, alter
immune cell differentiation and to mediate production of pro- and anti-inflammatory
cytokines.

In this chapter, we review the antiviral activities of both cathelicidins and
defensins, and also highlight key CHDP from other species that demonstrate
antiviral potential, either through direct antiviral activity or by modulation of the
immune response to the infection.

3.2 The Antiviral Activity of Cathelicidins

Cathelicidins range from 12–88 amino acids in length and are characterised by the
presence of an N-terminal signal sequence which directs the newly synthesised
protein towards the secretory pathway, a conserved cathelin-like domain which has
a high sequence homology with the porcine cysteine protease inhibitor, cathelin and
a variable C-terminal antimicrobial domain which becomes the mature functional
peptide upon proteolytic cleavage.

Cathelicidins were first identified in bovine neutrophils and are widely dis-
tributed in mammals including humans, rhesus monkeys, rats, mice, guinea pigs,
rabbits, sheep, cows, horses and dogs and also in non-mammalian species including
chicken, rainbow trout and hagfish (Zanetti et al. 1993). In humans, only one
cathelicidin has been described—the cationic antimicrobial peptide of 18kDa
(hCAP18), which can be found at high concentrations in the specific granules of
neutrophils and can be expressed by epithelial cells of skin and mucosa of the
respiratory, urogenital and gastrointestinal tracts (De et al. 2000). hCAP18 is
cleaved extracellularly by proteinase-3 to generate its active form LL-37, a linear 37
aminoacids peptide with two leucine residues at the N-terminal and an amphipathic
α-helical structure (Sorensen et al. 2001). The peptide is known to be expressed by macrophages, eosinophils, lymphocytes, mast cells and NK, T- and B-cells (Agerberth et al. 2000).

Mice express the cathelicidin mCRAMP (murine cathelin-related antimicrobial peptide), which has high sequence identity with hCAP18 and the porcine cathelicidin PR-39. mCRAMP shows similar expression and function patterns to its human ortholog (Gallo et al. 1997) that is stored in neutrophil granules and is expressed by epithelial cells and leukocytes. mCRAMP knock-out mice have a high susceptibility to infections when compared to wild type mice (Huang et al. 2007; Iimura et al. 2005; Kovach et al. 2012).

In contrast to humans and mice, which only express one cathelicidin, pigs express a variety of cathelicidins which differ in activity and structure. The porcine cathelicidins include five different protegrins (PGs), three α-helical peptides (PMAP-23, -36, -37), two prophenins (PF-1, -2) and the PR-39 peptide (Zhang et al. 2000). PGs are produced and stored by porcine neutrophils as inactive propeptides but are proteolytically cleaved into their active forms by neutrophil elastase in the extracellular environment. Their expression is enhanced by bacterial LPS, IL-6, retinoic acid and salmonella infections (Wu et al. 2000) and PG-1 has been shown to have the broadest antimicrobial activity spectrum (Yasin et al. 1996a, b). Cathelicidins have also been characterized in many other species, such as sheep, monkeys, horses and cows. In sheep, eight cathelin-associated peptides have been identified and SMAP-29 (sheep myeloid antimicrobial peptide 29) is one of the most potent cationic host defence peptide known in terms of antimicrobial activity, having a wide spectrum of activity against bacteria, fungi and virus (Tomasinsig and Zanetti 2005).

Cathelicidins play key roles in host defence via direct antimicrobial activity (Putsep et al. 2002), by acting as critical immunomodulatory molecules and in the control of inflammation. Recently, a number of studies conducted in humans, mice and other in vitro models have highlighted the potent antiviral activity of cathelicidins. Viruses that are most susceptible to cathelicidins include enveloped DNA and RNA viruses, and while all of the antiviral mechanisms remain to be elucidated, there does appear to be a direct effect on the viral envelope. However, non-enveloped viruses such as adenovirus can also be inactivated by cathelicidins (Gordon et al. 2005; Barlow et al. 2014). Therefore, the antiviral activity of these peptides likely comprises complex array of mechanisms that cannot all be explained by a direct effect on the virus particles.

### 3.3 RNA Viruses

#### 3.3.1 Influenza Virus (IAV)

Influenza virus (IAV) is an enveloped virus from the Orthomyxoviridae family. Viral influenza has caused the death of more people in short periods of time than any other infectious disease (Taubenberger and Morens 2006). There is a well-established
global vaccination programme in place for preventing influenza infection on an annual basis, but this is limited to protecting against the prevalent circulating strains, and therefore emerging new strains can potentially lead to a global pandemic outbreak. Antiviral treatments are available, although the emerging resistance of circulating strains to neuraminidase inhibitors, one of the current front-line treatment, is of serious concern (Nitsch-Osuch and Brydak 2014; Hurt 2014).

Studies have demonstrated the antiviral potential of several cathelicidins against influenza virus. A study by Barlow et al. 2011 demonstrated the antiviral properties of human and murine cathelicidins in vivo and in vitro (Barlow et al. 2011). mCRAMP and LL-37, but not porcine cathelicidin PG-1, showed antiviral properties when pre-incubated with IAV in vitro. Tripathi et al. (2013) further demonstrated that pre-incubation of LL-37 peptide with IAV is necessary for optimal inhibition of IAV, although a host cell pre-treatment or a delayed treatment with exogenous LL-37 also inhibits IAV replication to an extent (Tripathi et al. 2013). This reveals that LL-37 peptide has the ability to interact with epithelial cells although the most potent activity of the peptide involves direct interactions with the virus particles.

In in vivo studies, murine models receiving LL-37 or mCRAMP treatment showed a significant increase in survival compared to saline treated mice. Mice treated with PG-1 showed no significant alterations compared to the control group (Barlow et al. 2011). This demonstrates that antiviral activities of cathelicidins are species-specific, and it was proposed that LL-37 protects against IAV infection through modulation of inflammatory response in the lungs. Mice infected with IAV exhibited a pronounced up-regulation of numerous pro-inflammatory cytokines, although this was attenuated in LL-37-treated mice, suggesting that LL-37 modulates the inflammatory response by inhibiting excessive inflammation (Barlow et al. 2011).

In order to determine the effects of LL-37 on the cellular uptake and replication of IAV, qPCR was used to quantify viral infection and replication. The results showed that LL-37 did not reduce the number of virus particles associated with cells after 45 min of infection, although at 24 h post-infection, LL-37 caused a significant reduction in the amount of virus present in cells and in the cell culture supernatant (Tripathi et al. 2013). Electron microscope images revealed that LL-37 had induced disruption of viral membranes, which may be one possible mechanism of antiviral activity.

Interestingly, pandemic IAV strains have shown to be more resistant to innate inhibitors of seasonal IAV strains, such as human and murine cathelicidins. A recent study compared the antiviral activities of LL-37 and derived fragments from LL-37 against seasonal and pandemic strains of influenza virus and revealed that the central fragment of LL-37 showed greater activity against the pandemic IAV strain than LL-37 in vitro (Tripathi et al. 2015). This finding suggests the possibility that synthetic derivatives of LL-37 with more potent antiviral activity could be used as a potential therapeutic for this infection.
3.3.1.1 Human Immunodeficiency Virus (HIV)

HIV is an enveloped lentivirus of family Retroviridae that causes HIV infection and acquired immune deficiency syndrome (AIDS). An estimate of 34.2 million people worldwide live with HIV and around 2.5 million new infections and 1.7 million deaths were detected in 2011 only (Piot and Quinn 2013). The hallmark of this infection is the gradual loss of CD4+ T-cells which leads to an acquired immune deficiency syndrome or AIDS (Simon et al. 2006). The production of up to $10^{10}$ viral particles per day together with low fidelity of reverse transcription and recombination generate viral quasi-species in chronically infected subjects makes lifelong treatment with a combination of highly active antiretroviral drugs (HAART) the only option capable of keeping the infection controlled.

Indolicidin, a cathelicidin isolated from large granules of bovine neutrophils, was the first to show anti-HIV activity (Robinson et al. 1998). It is thought that the potent antimicrobial properties of indolicidin are related to their ability to disrupt pathogen membranes. Experimentally, indolicidin directly inactivated the HIV-1 virus particles, an observation that was attributed to a membrane-mediated antiviral mechanism.

A in vitro study by Bergman et al. (2007) showed that LL-37 could inhibit HIV-1 replication in peripheral blood mononuclear cells (PBMC), including primary CD4+ T-cells. This was shown to be independent of formyl peptide receptor-like-1 (FPRL-1) signalling, a receptor which is thought to be partially responsible for mediating some of the chemotactic and immunomodulatory effects of LL-37 (Bergman et al. 2007; Barlow et al. 2006). Another study examined the anti-HIV effects of LL-37 and its derived fragments, together with BMAP-18, a fragment derived from bovine cathelicidin BMAP-27, in vitro (Wang et al. 2008). The peptide sequence order, aromatic residues and helical structures were examined and were shown to play an important role in HIV inhibition. Again, a central fragment of LL-37 (known as GI-20), and BMAP-18, were the most active against HIV-1 compared to LL-37 and BMAP-27. This study essentially provides new antimicrobial templates that could be used to develop novel anti-HIV therapies.

A more recent study examined the effects of LL-37 and its derived fragments on HIV-1 reverse transcriptase, HIV-1 integrase and HIV-1 protease (Wong et al. 2011). It was shown that all peptides tested lacked the ability to inhibit translocation of HIV-1 integrase, an enzyme essential for HIV replication. The most potent inhibitory effects of the peptides were seen on HIV-1 reverse transcriptase, and the central peptide, LL13-37, was revealed to have the strongest inhibitory activity.

hCAP-18 has been shown to be strongly expressed in the epithelium of the epididymis, which suggests an important role of hCAP-18 in the antimicrobial protection of the reproductive male system (Malm et al. 2000). It has also been shown that vaginal fluid of healthy women has intrinsic anti-HIV-1 properties and these were conferred by cationic polypeptides. A depletion of cationic polypeptides caused a reduction of the intrinsic anti-HIV-1 activity (Venkataraman et al. 2005) and, in addition to this, cervicovaginal secretions (CVS) of Kenyan women in HIV-serodiscordant relationships contained HIV neutralizing activity and CVS
which revealed no intrinsic anti-HIV activity could be enhanced by the addition of recombinant LL-37 (Levinson et al. 2012). Further studies have revealed that high concentrations of LL-37 were found in cervicovaginal secretions (CVS) collected from Kenyan sex workers with bacterial transmitted infections, which are associated with increased HIV acquisition (Levinson et al. 2009). This finding does not seem to correlate with previous studies, where CVS contain HIV neutralizing activity conferred by cationic polypeptides. Therefore, there is a pressing need to determine the in vivo significance of LL-37 peptide in HIV infections.

### 3.3.1.2 Junin Virus (JV)

Junin virus (JV) is an enveloped virus of the Arenaviridae family, which causes Argentine haemorrhagic fever (AHF). A study by Albiol Matanic and Castilla (2004) assessed the antiviral effects of indolicidin against junin virus, although it was determined that this cathelicidin was not able to induce substantial rates of viral inactivation (Albiol Matanic and Castilla 2004). However, the relatively low virucidal action of indolicidin against junin virus that was observed was thought to be due to direct inactivation of the virus particles, similar to the antiviral mechanisms proposed for the action of cathelicidins against HIV-1 (Robinson et al. 1998).

### 3.3.1.3 Dengue Virus (DENV)

Dengue virus (DENV) is an enveloped member of the Flaviviridae family which causes dengue fever—a mosquito-borne tropical disease. An important target for antiviral therapies against dengue virus is NS2B/NS3 serine protease, as disruption of NS2B/NS3 serine protease functions inhibit virus replication. A study by Tambunan and Alamudi (2010) demonstrated that cationic cyclic peptides have a high potential to inhibit NS2B/NS3 serine protease activities of dengue virus (Tambunan and Alamudi 2010). A more recent study proposed that protegrin-1 (PG-1), a cationic cyclic peptide which was originally isolated from porcine blood cells, is able to inhibit NS2B/NS3 serine protease activity, thus translating to reduced viral replication in vitro (Rothan et al. 2012).

### 3.3.1.4 Human Respiratory Syncytial Virus (RSV)

RSV is an enveloped virus and a member of the Paramyxoviridae family, and has been shown to be responsible for significant numbers of respiratory tract infections. It is the major cause of viral bronchiolitis in young children (Nair et al. 2010). A recent study demonstrated that human cathelicidin displays concentration-dependent antiviral activity against RSV in vitro at physiologically relevant concentrations of 25 µg/ml (Currie et al. 2013). LL-37 inhibited RSV replication and decreased the spread of infection, and these effects were highest when the peptide
was pre-incubated with RSV or added to cells simultaneously. This study demonstrated that LL-37 mediates direct effects against the virus particles, similar to the action of LL-37 against influenza A virus (Tripathi et al. 2013). It was also demonstrated that a delayed LL-37 exposure, taking place 2 h after infection, resulted in the loss of antiviral effects which revealed the inability of the peptide to rescue infected cells. However, pre-treating cells with LL-37 prior to infection resulted in a reduction of infectivity, suggesting that the peptide is retained by the epithelial cells exerting a protective antiviral state. These results indicate that LL-37 can protect epithelial cells from viral infection through a mechanism distinct from direct antiviral activity. In addition, LL-37 was also shown to actively protect RSV-infected epithelial cells from cell death.

3.3.2 DNA Viruses

3.3.2.1 Vaccinia Virus (VV)

Vaccinia virus (VV) is an enveloped poxvirus, of the family Poxviridae, which is the active constituent of the vaccine that eradicated smallpox. It has been demonstrated that individuals with atopic dermatitis (AD) have a predisposition to develop eczema vaccinatum in response to the vaccine, and that, in these individuals, the expression of hCAP18 is reduced (Howell et al. 2006). It has been shown that in normal skin biopsies, the expression of LL-37 was induced by vaccinia virus, but this was not observed in AD skin. Furthermore, a study by Howell et al. (2004) demonstrated that both LL-37 and the murine cathelicidin, mCRAMP, have antiviral activity against vaccinia virus, and the antiviral mechanism by which LL-37 exerts its effect involves the removal of the outer membrane of vaccinia virus, thus causing envelope damage (Howell et al. 2004; Dean et al. 2010).

3.3.2.2 Herpes Simplex Virus (HSV)

Herpes simplex virus 1 and 2 (HSV-1 and -2) are enveloped viruses of the family Herpesviridae, that are widely found in humans and are particularly infectious. While they can be suppressed by some antiviral drugs, they are not normally susceptible to complete eradication from a host. A study by Yasin et al. (2000) screened 20 host defence peptides to test their antiviral activity against HSV type 1 and 2 (Yasin et al. 2000). LL-37 was shown to have very little capacity to induce viral inactivation. However, the bovine cathelicidin, indolicidin, displayed potent antiviral activity against both HSV types.

Other peptides which were investigated for anti-HSV activity include BMAP-27 and -28 (acronym of “bovine myeloid antimicrobial peptides”), which are cathelicidins found in bovine neutrophils. BMAP-27 and -28, and their synthetic 1–18 fragments, were analysed for their in vitro antiviral activity against HSV-1. Only
BMAP-28 was shown to provide some protection in vitro against HSV-1 whereas all other peptides were ineffective at non-cytotoxic concentrations (Benincasa et al. 2003). Another study examined the underlying mechanism for the potent antiviral activity of indocilidin observed against HSV type -1 and -2 in vitro, and suggested that the mechanism underlying the antiviral activity was related to the ability of the peptide to disrupt the viral envelope, thus inactivating the virus particles (Albiol Matanic and Castilla 2004). However, contrary to previous reports, Gordon et al. 2005 reported a potent antiviral activity of LL-37 against HSV-1 in vitro (Gordon et al. 2005).

It is known that a subgroup of patients with atopic dermatitis (AD) will develop eczema herpeticum (ADEH) due to a disseminated infection with HSV (Wollenberg et al. 2003). Patients with AD have decreased expression of host defence peptides, and it has been suggested that a deficiency of LL-37 may cause patients with AD to be more susceptible to ADEH (Ong et al. 2002). A study by Howell et al. (2006) showed that LL-37 exhibited direct antiviral activity against HSV-2 in vitro. In addition, a particularly physiologically relevant model was employed whereby human keratinocytes cells were pre-incubated with HSV for a period of 6 h before treatment with LL-37 for 18 h to assess whether intracellular viral replication could be inhibited with physiologic concentrations of LL-37 (Howell et al. 2006). This study showed that the peptide was able to significantly reduce the levels of HSV gene expression in infected keratinocytes. In vivo studies using mice deficient in mCRAMP revealed higher rates of HSV replication compared to the wild type mice, indicating an important role for host defence peptides in controlling HSV in skin infection.

Interestingly, a recent study tested and compared two different approaches to fight HSV-1 corneal infection. A sustained release of LL-37 delivered through nanoparticles incorporated within corneal implants was compared with a cell-based delivery of LL-37 cDNA transfected into HCECs (human corneal epithelial cells). LL-37 released from implants showed an ability to inhibit HSV-1 activity, but did not clear HSV-1 from infected cells. HCEC producing LL-37 also showed direct anti-HSV-1 activity, although none of these approaches were able to completely eliminate the virus infection (Lee et al. 2014).

### 3.3.2.3 Adenovirus (Ad)

Adenovirus is non-enveloped virus that is part of the *Adenoviridae* family. Adenoviruses are a major cause of conjunctivitis and keratoconjunctivitis, but can also cause upper and lower respiratory and gut infections. Although usually self-limiting, human adenovirus (HAdV) infections are quite contagious and put immunocompromised individuals at serious risk of severe and recurrent pulmonary infections, with mortality rates that reach up to 55 % (Lion 2014). Treatment for an infection is largely supportive therapy rather than direct antiviral therapeutics.

A study by Gordon et al. (2005) investigated the antiviral activity of LL-37 against different adenovirus serotypes (Ad19, Ad8, Ad5 and Ad3) (Gordon et al. 2005). LL-37 demonstrated a significant reduction of Ad19 titer in vitro (2 log...
| Cathelicidin                      | Structure | Source | Virus | Genome | Mechanism                                                                 | References                                |
|----------------------------------|-----------|--------|-------|--------|---------------------------------------------------------------------------|-------------------------------------------|
| LL-37                            | α-helix   | Human  | IAV   | RNA    | Viral envelope damage; cellular target; modulation of inflammatory response in vivo; | Tripathi et al. (2013), Bardow et al. (2011) |
|                                  |           |        | HIV   | RNA    | Inhibition of HIV-1 reverse transcriptase                                  | Wongs et al. (2011)                      |
|                                  |           |        | RSV   | RNA    | Direct effects on virus particles; cellular target;                       | Currie et al. (2010)                     |
|                                  |           |        | HSV   | DNA    | Viral envelope damage; virus inactivation;                               | Dean et al. (2010)                       |
|                                  |           |        | VV    | DNA    | Viral envelope damage; modulation of inflammatory response in vivo;      | Howell et al. (2006)                     |
|                                  |           |        | HSV   | DNA    | Unknown                                                                   | Gordon et al. (2005)                     |
|                                  |           |        | Ad    | DNA    | Unknown                                                                   | Howell et al. (2006)                     |
|                                  |           |        | IAV   | DNA    | Unknown                                                                   | Howell et al. (2006)                     |
| mCRAMP                           | α-helix   | Mouse  | IAV   | DNA    | Viral inhibition                                                           | Howell et al. (2006)                     |
|                                  |           |        | HIV   | RNA    | Viral inhibition                                                           | Benincasa et al. (2003)                  |
| Indocilidin                      | Extended  | Bovine | JV    | RNA    | Viral inhibition                                                           | Wang et al. (2008)                       |
| BMAP-18 (fragment derived from BMAP-27) | α-helix   | Bovine | HIV   | RNA    | Viral inhibition                                                           | Benincasa et al. (2003)                  |
| BMAP-28                          | α-helix   | Bovine | HSV   | DNA    | Viral inhibition                                                           | Wang et al. (2008)                       |
| Protegrin-1                      | β-sheet   | Porcine| DENV  | RNA    | Inhibit NS2B/NS3 serine protease activity; viral inhibition                | Wongs et al. (2012a, b)                  |
reduction over 4 h). This study also provided some insight on a potential antiviral mechanism of cathelicidins, based on the comparison of HSV and Ad19 time-kill assays. The data demonstrated a rapid killing of HSV-1 which suggested a disruption of the viral lipid membrane as a possible mechanism. Several other studies have also proposed similar mechanisms for other cathelicidins (Robinson et al. 1998; Albiol Matanic and Castilla 2004; Dean et al. 2010). Interestingly, for the Ad19 strain, LL-37 produced a much slower progressive reduction in virus titers. As adenovirus lacks a viral envelope, this suggests that the direct antiviral mechanism of cathelicidins does not involve membrane disruption. Alternative mechanisms have been proposed such as disruption of the adenovirus particles (detergent-effect) and/or blockage of viral entry into the cell.

3.3.3 Summary—The Antiviral Activity of Cathelicidins

In summary, a review of the current literature shows that cathelicidins have antiviral properties against a broad spectrum of viruses; the underlying mechanisms likely involve a direct effect on viral particles as well as the capacity to modulate host immune responses that may contribute in the clearance of infection. A better understanding of how cathelicidins interact with virus particles directly, in addition to their effects on infected host cells, remains to be established. However, it is clear that cathelicidins are ideal targetable components of the innate immune system that can be used to inform the development of novel therapeutics with broad activity targeting a number of viruses (Table 3.1).

3.4 The Antiviral Activity of Defensins

Defensins are small, cysteine-rich cationic peptides that act as important effectors of the innate immune system (Ding et al. 2009; Ganz 2003). Human defensins are classified according to their structure, α and β-defensins differing in their disulphide bond pairing, and θ-defensins, being present in Old World monkeys, displaying a circular structure (Lehrer 2004). Of note, despite RNA transcripts for θ-defensins are found in human bone marrow cells, a premature stop codon prevents protein translation (Nguyen et al. 2010).

Neutrophil α-defensins (HNPs 1–4) are mainly synthesized as prepropeptides in promyelocytes in the bone marrow, and the mature peptide is stored in the granules of neutrophils. These peptides are found in lower concentrations in NK-cells, B-cells, γδ T-cells, monocytes, macrophages and immature dendritic cells (Rehaume and Hancock 2008). In contrast, human alpha defensins 5 and 6 (HD5 and HD6) are constitutively expressed and secreted as a propeptide in Paneth cells, salivary glands and also in genital mucosa (Ouellette 2006). Human β defensins (HBDs) 1-3 are mainly expressed in epithelial cells, but can also be found in
immune cells, mainly monocyte/macrophages and dendritic cells, whereas 3 θ-defensins (RTD1-3) have been found in rhesus macaque leukocytes (Ganz 2003; Tang et al. 1999). Retrocyclin, an artificial peptide based on the human θ defensins pseudogene has been also shown to possess antimicrobial capacity (Tran et al. 2008; Yasin et al. 2004).

Defensins have broad antimicrobial activities, including the capacity to inhibit viral infections. Despite structural and physical similarities, such as overall size and positive charge, each defensin has variable antiviral activity. The differences in activity are likely due to the mechanism of action exhibited by each peptide towards a particular DNA or RNA virus.

### 3.4.1 DNA Viruses

#### 3.4.1.1 Herpes Simplex Virus

One of the first viruses which was shown to be inhibited by α-defensins was HSV-1, which was inactivated after incubation with HNP-1, -2 and -3, an effect that was abrogated by serum addition (Daher et al. 1986). Further work has shown that one β defensin (hBD3) and all six α-defensins inhibited HSV-2 infection (Hazrati et al. 2006). Interestingly, while HNP1-3 and HD5 interacted with the viral glycoprotein Gb2 present on the viral envelope, HNP-4 and HD6 bound to heparan sulfate and heparin, cellular receptors used for HSV-2 to gain entry to cells. All of these interactions have been shown to result in diminished viral entry. In contrast, the same study demonstrated that β-defensin 1 and 2 (hBD1 and hBD2) which lack anti-HSV-2 effects, do not bind to neither Gb2 nor heparan sulfate.

Similar antiviral effects against HSV are displayed by θ defensins, as both rhesus θ defensins 3 (RTD3) and retrocyclins 1 and 2 (RC1 and RC2) have been demonstrated to inhibit HSV viral entry to cells (Yasin et al. 2004). Of these peptides, retrocyclin 2 was shown to interact with the viral glycoprotein Gb2, thus interfering with viral entry without causing significant cytotoxicity to target cells. Defensins often display an antiviral activity that spans different steps on the target virus cycle. In this regard, HNP-1 and HD5 are able to block HSV viral gene expression even when added after the virus infection, indicating that defensins are also able to block post-entry events in the HSV cycle.

#### 3.4.1.2 Human Papilloma Virus (HPV)

HNP-1-3 and HD5 but not hBD1, hBD2 or HD6 have been shown to exhibit antiviral activity against HPV, which is the primary cause of cervical cancer in sexually transmitted infections (Buck et al. 2006). HNP-1-3 and HD5 block HPV infection by impairing virion escape from endocytic vesicles. A recent study also suggested that HD5 blocks viral entry by interacting with viral particles and
blocking L2 cleavage, a necessary step for successful viral entry and post-entry events (Wiens and Smith 2015). Detailed studies on HD5 and HPV interactions suggest that hydrophobic residues, in particular, Arg-28, are very important for the antiviral activity of HD5 against HPV and other non-enveloped viruses (Gounder et al. 2012; Tenge et al. 2014).

3.4.1.3 Vaccinia Virus (VV)

Several defensins have been shown to have varying potential to neutralize VV infection. Incubating VV for 24 h with hBD3 was shown to reduce the number of viral plaques formed on BSC-1 green monkey kidney cells. In addition, hBD3 reduced the expression of viral DNA-dependent RNA polymerase (Howell et al. 2007) during viral infection. However, HNP-1, hBD1 and hBD2 showed no activity against this enveloped virus (Howell et al. 2004).

3.4.1.4 JC Polyomavirus (JCPyV) and BK Virus

JC polyomavirus (also known as John Cunningham virus) is a member of the family *polyoviridae*, and chronically infects between 70–90 % of the human population. This pathogen only tends to cause clinical symptoms in immunocompromised individuals, where infection spreads to the central nervous system (Shackelton et al. 2006; Wollebo et al. 2015). In experimental studies, a panel of α and β-defensins were tested for their capacity to neutralize JCPyV infection, resulting in HD5 and hBD3 being able to block the infection when incubated at 100 μg/ml with the virus for 1 h before infecting SVG-A human foetal glial cells (Zins et al. 2014). Interestingly, hBD3 showed significant cytotoxicity on cells at this concentration, whereas HD5 neutralized JCPyV in a dose-dependent manner (Zins et al. 2014; Dugan et al. 2008). Of note, HNP1-3 and hBD1, 2 and 4 showed no anti JCPyV activity. Further experiments also showed that HD5 was able to interact with JCPyV virions, stabilizing the viral capsid and thus preventing the viral genome release (Zins et al. 2014), a novel mechanism of action for a peptide that is generally thought to employ detergent-like modes of antiviral activity.

BK virus shares 75 % of its genome with JCPyV, and was first isolated from the urine of a renal transplant individual (Gardner et al. 1971). Around 80 % of healthy individuals in England have been shown to display antibodies against the virus, generally in the absence of symptoms (Gardner 1973). If symptoms are noted, these can consist of fever and nonspecific upper respiratory infection which might lead to kidney manifestations such as cystitis or nephritis in immunocompromised individuals or those receiving transplants (Reploeg et al. 2001).

HNP1 and HD5 were shown to reduce viral V-protein expression in VERO cells when incubated directly with the virus at 20 or 50 μg/ml for 1 h, while hBD2 only was effective at 50 μg/ml. In contrast, hBD1 showed no inhibitory effect (Dugan et al. 2008). When the direct antiviral effect of defensins was studied further, HD5
was shown to interact directly with BK virus particles, inducing aggregation, and thus reducing viral attachment to cell membranes. Of note, similar antiviral effects of HD5 were also observed against other related polyomaviruses such as simian virus-40 (SV40). It was also shown that both HNP-1 and HD5 were also able to block adenovirus escape from endosomes.

3.4.1.5 Adenovirus (Ad)

It has been shown that hBD-1 and HD-5, when expressed on eukaryotic cell lines, showed potential to protect those cells against adenoviral infection, in particular from Av1CF2 (Gropp et al. 1999). Different HAdV species show variable susceptibility to defensin actions. In this regard, HNP-1 inhibited HAdV A, B and C infection, while increased HAdV D, E and F infectivity (Smith et al. 2010). HD5 showed similar actions, blocking HAdV A, B, C and E while increasing D and F infectivity. Interestingly, and similar to other non-enveloped viruses, HD5 interacts with adenoviral capsids preventing viral uncoating and avoiding its release from endocytic vesicles (Nguyen et al. 2010; Smith et al. 2010; Smith and Nemerow 2008). Further studies confirmed that Arg-28 residues of HD5 are critical for the antiviral effects seen against adenovirus (Gounder et al. 2012). Much less is known about other defensins effects on adenovirus. However, HNP-1 was shown to be effective at a concentration of 50 μg/ml in blocking adenovirus-type 5 infection of 293 cells, whereas hBD-2 showed a reduced effect (Bastian and Schäfer 2001).

3.4.1.6 Cytomegalovirus (CMV)

CMV is an enveloped virus that belongs to the Herpesviridae family, and is the most common congenital infection, affecting up to 0.2–2.2 % of all live births (Huygens et al. 2014). Foetal or perinatal infections can have devastating neurological consequences for the baby. However, post-natal CMV infections are usually asymptomatic, establishing lifelong infections without severe consequences on immune competent individuals.

In the context of CHDP activity against this virus, super physiological concentrations (100 and 200 μg/ml) of HNP-1 peptide were shown to directly inhibit CMV viral particles reducing the PFU/ml by 0.29 and 0.81 log10, respectively (Daher et al. 1986). However, this virus notably showed less susceptibility to HNP-1 compared to HSV-1, which was also assessed in the same study.

3.4.1.7 Baculovirus

Baculovirus naturally infects insect larvae hosts, usually Lepidoptera (Butterflies and moths), with no known diseases caused by this virus in organisms others than arthropods (Airenne et al. 2013). Due to its efficient reproduction cycle and ability
to carry large DNA inserts, baculovirus are extensively used as tools for gene delivery and recombinant production of proteins in insect cells. Baculovirus gp64 protein allows viral fusion in an acidified endosome, an event seen upon infection of *Spodoptera frugiperda* insect cells, resulting in gp64 expression and the formation of cell syncytia (Leikina et al. 2005). However, it has been shown that RC-2 peptide is capable of blocking baculovirus fusion with host cells, inhibiting the virus-mediated syncytium formation.

### 3.4.2 RNA Viruses

#### 3.4.2.1 Human Immunodeficiency Virus (HIV)

It has been shown that a variety of defensin peptides can have an impact on HIV infection. HNP-1 is clearly deleterious for viral dissemination, having a direct effect against a low multiplicity of infection (MOI) of viral particles in the absence of serum, whereas in the presence of serum HNP-1 acts on host cells (Chang et al. 2005). One of the suggested mechanisms for such cellular effects is the HNP-1 mediated inhibition of the PKC signalling pathway, which is required for viral uncoating (Fields et al. 1988). Importantly, this HNP-1 effect did not affect the expression of viral receptors CD4, CXCR4 or CCR5 (Chang et al. 2003). Interestingly, as a part of its direct effects on HIV viral particles, HNP1-3 peptides were shown to interact with gp120, a viral glycoprotein, thus impairing viral attachment to cell membranes. This effect was further potentiated by HNP-1-3 interactions with cellular CD4. Both antiviral effects were reduced, but not completely abolished by the presence of serum (Demirkhanyan et al. 2012; Furci et al. 2006; Wang et al. 2004). A closely related peptide, HNP-4 was also able to bind gp120 and CD4 (Wu et al. 2005). A more indirect but also effective anti-HIV effect of HNP1-3 peptides is their capacity to increase the secretion of the C-C chemokines MIP-1α, MIP-1β and RANTES, which can bind to CCR5 to act as antagonists for viral R5 strains that use CCR5 as a co-receptor (Guo et al. 2004).

In stark contrast to HNP1-3, HD5 and HD6 seem to increase viral infectivity without any blockage of post-entry viral events (Klotman et al. 2008). Further assessment of this unexpected effect suggested that HD5 and HD6 actually enhance viral attachment to the cells (Rapista et al. 2011). Another study, however, showed HD5 to inhibit HIV infection by blocking gp120–CD4 interaction (Furci et al. 2012). Further work is needed to clarify HD5 effects on the HIV infection process, particularly when considering sexually transmitted infections may increase HD5 and HD6 secretions in the genital mucosa prior to a possible HIV infection (Klotman et al. 2008).

Given the importance of HIV interaction with mucosal surfaces, β-defensin impact on HIV infection has also been investigated. HIV was shown to upregulate hBD2 and hBD3 release by human oral epithelial cells, and incubation of viral particles with these peptides protected cells from infection (Quiñones-Mateu et al. 70 F.H. Sousa et al.
Interestingly, hBD1 was neither upregulated nor protective in this context. However, hBD2 and hBD3 did appear to act both directly on viral particles, and also on the host cell, by downregulating CXCR4 expression. Accordingly, these peptides were found to be more effective against X4 viral isolates. A later study found a similar anti-HIV activity of hBD2 and hBD3 to both X4 and R5 strains, indicating that defensin treatment inhibited early products of reverse transcription (Sun et al. 2005).

Given the interest in the broad antiviral effects of defensins, rhesus macaque \( \theta \)-defensins (RTD) and synthetic retrocyclins (RC) have been also tested for their anti-HIV activity. RC-1 and RC-2 were shown to bind glycosylated gp120 and CD4, thus explaining the previously observed protective activity (Cole et al. 2002; Wang et al. 2003). Interestingly, RTD peptides also showed anti-HIV activity (Wang et al. 2004). Further work demonstrated that RC-1 was able to block HIV-1 fusion with target cells (Gallo et al. 2006). Finally, rhesus macaque \( \alpha \)-defensins 4 (RMAD 4) also showed anti-HIV activity at 150 \( \mu \)g/ml by blocking viral entry (Tanabe et al. 2004). Interestingly, the same study showed that cryptidin-3 (a mouse derived \( \alpha \)-defensin) actually increased HIV-1 replication. In this regard, guinea pig, rabbit and rat \( \alpha \)-defensins have also been tested for anti-HIV activity, demonstrating an ability to inhibit infection of T-cell lines (Nakashima et al. 1993).

### 3.4.2.2 Hepatitis C (HCV)

Hepatitis C infection primarily affects the liver, being a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Maheshwari et al. 2008). HCV infection is estimated to affect 170 million people worldwide, is frequently asymptomatic, and spontaneous clearance is seen in a third of infected individuals. Chronic infection (with viral replication detected for more than 6 months) leads to hepatic complications. Little is known about the capacity of defensins to impact viral hepatitis. However, it has been shown that HCV core protein have been shown to activate the transcription of alpha-defensin genes, indicating that they may play a role in the innate immune response to the virus (Aceti et al. 2006).

### 3.4.2.3 Influenza A Virus (IAV)

Early studies have reported HNP-1-3 activity against the A/WSN (H1N1) strain of IAV (Daher et al. 1986). More detailed work has shown that HNP-1 affects influenza replication by blocking the PKC signalling pathway on host cells, needed for viral endosomal trafficking (Salvatore et al. 2007). It was shown that HNP-1 did not have a direct impact on viral particles, but did increase viral clearance by neutrophils (Tecle et al. 2007). A similar effect was also observed with HNP-2, HD5 and to a lesser extent with hBD2 but not with HNP-3 or hBD1. Another \( \beta \)-defensin, hBD3, was also shown to block IAV infection by blocking hemagglutinin-mediated fusion and immobilizing membrane proteins (Leikina et al. 2003).
Interestingly, RC2 also showed similar effects to hBD3. Of note, murine β-defensin 2 and 3 (mBD2 and mBD3) protected mice from infection with the A/PR/8 (H1N1) IAV strain, and in similarity to their human analogues, their activity was attributed to impaired viral entry (Gong et al. 2010; Jiang et al. 2012).

3.4.2.4 Respiratory Syncytial Virus (RSV)

Defensins are expressed at high concentrations in the inflamed lung, and are usually upregulated during viral infections of the airway epithelia. In this context, it has been demonstrated that RSV infection increased hBD2 secretion by alveolar A549 cells, which was subsequently able to block further viral entry by directly disrupting the viral envelope (Kota et al. 2008). Interestingly RSV did not induce the expression of hBD1, as this is constitutive, but hBD1 lacked direct anti-RSV activity. Although alveolar macrophages are permissive to RSV infection, they do not tend to influence the later development of disease (Pribul et al. 2008). However, this interaction can result in increased secretion of pro-inflammatory cytokines that can impact on β-defensin expression in the lungs (Becker et al. 1950).

3.4.2.5 Rhinovirus (HRV)

Rhinovirus is a major cause of common cold and it is associated with asthma exacerbations (Jartti and Korppi 2011). It has been demonstrated that RV16 infection of primary bronchial epithelial cells results in the induction of hBD2 and hBD3 mRNA, whereas hBD1 remains unaltered (Duits et al. 2003). It was also demonstrated that the same serotype also upregulated hBD2 mRNA and protein in A549 alveolar epithelial cells. Interestingly this effect was conserved in other major group serotype HRV-14 or in 2 minor group serotypes such as HRV-2 and HRV-1A.

Further work has investigated the inoculation of human subjects divided into non-smokers, smokers and COPD groups with RV-16 (Proud et al. 1950). Interestingly, a larger proportion of smokers (78.6 %) were successfully inoculated with the virus, compared to those with COPD (66.7 %) or non-smokers (58 %). RV16 was shown to increase α-defensin secretion in the sputum 9 days post-infection compared to baseline, but this was only observed in the COPD group (Mallia et al. 2012). RV-16 infection also resulted in increased neutrophil elastase, which may subsequently degrade secreted host defence peptides.

3.4.2.6 Echovirus and Reovirus

The Enteric Cytopathic Human Orphan (ECHO) virus, belongs to the Picornaviridae family. Enteroviruses are mainly found in the gastrointestinal tract, and can cause opportunistic infections mainly in children by indirect faecal–oral
transmission, causing febrile illness (Sherris Medical Microbiology 2004). HNP-1 peptide was tested for its antiviral activity against this non-enveloped RNA virus without showing any direct antiviral effect at concentrations of peptide known to inhibit HSV-1 (Daher et al. 1986).

In similarity to Echovirus, HNP-1 showed no direct activity against this dsRNA, non-enveloped virus, which generally causes a mild and limited upper respiratory and gastrointestinal tract infection which can spread across individuals, and also across species (Daher et al. 1986). Interestingly, the use of reovirus is being contemplated in cancer therapy due to its oncolytic activity in melanoma lines and xenografts (Galanis et al. 2012).

3.4.2.7 Vesicular Stomatitis Indiana Virus (VSIV)

VSIV belongs to the Rhabdoviridae family and is an arthropod-borne virus primarily affecting rodents, cattle, swine and horses. Infected livestock or sand flies can be a source of infection for humans, which develops as mild flu-like symptoms (Kuzmin et al. 2009). This enveloped RNA virus showed an intermediate susceptibility to direct inactivation by HNP-1 peptide, reducing its PFU/ml by 0.74 and 0.84 log_{10} with 50 or 100 μg/ml of peptide, respectively (Daher et al. 1986).

3.4.2.8 SARS Coronavirus (SARS-CoV)

Severe acute respiratory syndrome (SARS) affected over 8000 individuals in 2002–2003, causing almost 800 deaths, underscoring the importance of testing antiviral agents (Peiris et al. 2003). It has been shown that intranasal injections of ~125 μg RTD-1 peptide prior to virus inoculation protected BALB/c mice against a mouse-adapted strain of SARS-CoV (Wohlford-Lenane et al. 2009). Of note, untreated animals exhibited around 75 % mortality rate, whereas treated ones showed a 100 % survival. RTD-1 treatment altered lung cytokine responses to the virus, suggesting immunomodulatory effects where at least in part, behind the protective action of RTD-1. Interestingly DEFA-3 AND DEFA-4 genes (coding for HNP-3 and -4) were upregulated in blood samples of patients suffering acute SARS coronavirus infection (Lee et al. 2005).

3.4.2.9 Dengue Virus (DENV)

Recombinant RC-1 peptide has shown to reduce DENV-2 viral replication in VERO cells when incubated directly with the viral particles, but also showed a moderate effect when pre-treating or treating cells post-viral entry (Rothan et al. 2012). This study suggested that RC-1 impacts DENV-2 replication by inhibiting the activity of viral NS2B-NS3 serine protease. Interestingly further studies showed
that human skin fibroblasts release HD5 and hBD2 upon infection with DENV-2 (Bustos-Arriaga et al. 2011).

### 3.4.2.10 Sindbis Virus (SINV)

SINV, a member of the *Togaviridae* family, is transmitted by a mosquito vector, and causes sindbis fever which results in arthralgia, malaise and rash. It has been shown that both RC2 and hBD3 peptide were able to block the virus fusion with target cells (Leikina et al. 2005) (Table 3.2).

### 3.5 Other CHDP with Antiviral Activity

In addition to the well-characterized activity of cathelicidins and defensins against a wide variety of viral pathogens, a number of CHDP from other non-human organisms have been the focus of a number of studies attempting to understand whether these peptides, or synthetic derivatives, could be used to inform the design of novel therapeutics against viral infections specific to humans.

A substantial diversity in CHDP exists across a number of other species that possess activity against human pathogens. The Antimicrobial Peptide Database (http://aps.unmc.edu/AP) now contains in excess of 2,500 peptides with demonstrable antimicrobial activity isolated from six kingdoms, and includes peptides from bacteria, fungi, plant, amphibians, fish, reptiles and birds. Interestingly, approximately 170 of these peptides (which include cathelicidins and defensins) demonstrate antiviral activity against a broad range of viral pathogens.

#### 3.5.1 Cecropin

A family of CHDP with particularly potent activity against human viral pathogens is the cecropins. These peptides, identified initially in the Cecropia moth (*Hyalophora cecropia*) form part of the immune response to infection in a number of insect orders including Diptera and Lepidoptera, and are typically 30–39 amino acids in size (Boman 1991). Cecropin and cecropin-like peptides are a highly conserved, predominantly alpha helical group of peptides that have broad spectrum antibacterial and antifungal activity (Bulet and Stocklin 2005).

A recent study revealed that the mature form of an induced cecropin-like peptide found in the salivary glands of the female mosquito, *Aedes aegypti*, had substantial antiviral activity against Dengue virus (Luptetlop et al. 2011). The virus utilizes *A. aegypti* as a vector for transmission to humans and induces an innate immune response, characterized by the expression of a cecropin-like peptide, produced by up-regulation of the AAEL000598 gene. The peptide is subsequently cleaved from
| Defensin      | Structure | Source  | Virus | Genome | Antiviral mechanism                                                                 | REF                                                                 |
|--------------|-----------|---------|-------|--------|------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| HNP-1-3      | α-defensin| Human   | HSV-2 | dsDNA  | Interacts with viral gb2; prevents binding to target cell; also blocks post-entry events | Yasin et al. (2004), Hazrati et al. (2006)                             |
| HPV          |           |         | dsDNA |        | Blocks virus escape from endocytic vesicles                                          | Buck et al. (2006)                                                   |
| VV           |           |         | dsDNA |        | No antiviral effect                                                                  | Howell et al. (2004)                                                 |
| JCPyV        |           |         | dsDNA |        | No antiviral effect                                                                  | Zins et al. (2014)                                                   |
| BK virus     |           |         | dsDNA |        | Inhibits infection; mechanism unknown                                                | Dugan et al. (2008)                                                 |
| HAdV A, B1,B2,C |         |         | dsDNA |        | Inhibits infection; direct interaction with viral particles preventing uncoating    | Smith et al. (2010), Bastian and Schäfer (2001)                       |
| CMV          |           |         | dsDNA |        | Modest direct antiviral effect                                                      | Daher et al. (1986)                                                 |
| HIV-1        |           |         | ssRNA |        | Direct effect on virion; blocks viral gp120–cellular CD4 interaction; blocks host PKC signalling, affecting reverse transcription and integration; increased secretion of C–C chemokines | Chang et al. (2003), Demirkhanyan et al. (2012), Furci (2006), Wang et al. (2004), Guo (2004) |
| VSIV         |           |         | (−)ssRNA |       | Direct effect on virus, inhibits infection. mechanism unknown                      | Daher et al. (1986)                                                 |
| HCV          |           |         | (+)ssRNA |     | HCV core protein activates HNP transcription (antiviral activity untested)         | Aceti et al. (2006)                                                 |
| Echovirus    |           |         | (+)ssRNA |       | Lack of direct effect on virion                                                     | Daher et al. (1986)                                                 |
| Reovirus (type 3) |       |         | dsRNA |        | Lack of direct effect on virion                                                    | Daher et al. (1986)                                                 |
| IAV          |           |         | (−)ssRNA |       | Inhibition of PKC pathway in host cells; blocks viral replication and protein synthesis; increased uptake and clearance by neutrophils | Salvatore et al. (2007), Tecle et al. (2007)                           |

(continued)
| Defensin | Structure | Source | Virus | Genome | Antiviral mechanism | REF |
|----------|-----------|--------|-------|--------|---------------------|-----|
| HNP-4 α-defensin Human HSV-2 dsDNA | Interacts with heparan sulfate on host cells; prevents viral entry | Hazrati et al. (2006) |
| | | JCPyV dsDNA | No antiviral effect | Zins et al. (2014) |
| | | HIV-1 ssRNA | Lectin independent antiviral effect; weak interaction with gp120 and CD4 | Wu et al. (2005) |
| RMAD3 Rhesus Macaque α-defensin 3 Rhesus HIV-1 ssRNA | Moderate direct antiviral effect | Tanabe et al. (2004) |
| RMAD4 Rhesus Macaque α-defensin 4 Rhesus HIV-1 ssRNA | Blocks viral entry | Tanabe et al. (2004) |
| HD5 α-defensin Human HSV-2 dsDNA | Interacts with viral gb2; prevents binding to target cell; blocks post-entry events | Hazrati et al. (2006) |
| | HPV dsDNA | Blocks virus escape from endocytic vesicles; blocks virus unfolding (host mediated capsid L2 cleavage) | Yasin et al. (2004), Hazrati et al. (2006), Buck et al. (2006), Gounder et al. (2012) |
| | JCPyV dsDNA | Binds to viral particles inhibiting genome release | Zins et al. (2014), Dugan et al. (2008) |
| | BK virus dsDNA | Causes aggregation of viral particles, reducing viral attachment to cells | Zins et al. (2014), Dugan et al. (2008) |
| | HAdV A,B1,B2,C,E dsDNA | Inhibits infection by direct interaction with viral particles preventing uncoating | Gounder et al. (2012), Smith et al. (2010) |
| | HIV-1 ssRNA | Enhances HIV-1 infection by increasing viral attachment; effects independent on CD4 or viral co-receptors in target cells | Klotman et al. (2008), Rapista et al. (2011) |
| Defensin | Structure | Source | Virus | Genome | Antiviral mechanism | REF |
|----------|-----------|--------|-------|--------|---------------------|-----|
| IAV      | (−)ssRNA  | Increased neutrophil uptake and viral clearance | Tecle et al. (2007) |
| HD6      | α-defensin | Human  | HSV-2 | dsDNA  | Interacts with heparansulfate on host cells; prevents viral entry | Hazrati et al. (2006) |
|          | HPV       | dsDNA  | No antiviral effect | Buck et al. (2006) |
|          | HIV-1     | ssRNA  | Enhances HIV-1 infection by increasing viral attachment; effects independent on CD4 or viral co-receptors In target cells | Klotman et al. (2008), Rapista et al. (2011) |
| NP-1     | α-defensin | Rabbit | HSV-1 | dsDNA  | Direct effect on viral particles; prevents viral entry and VP16 translocation to the nucleus | Sinha et al. (2003) |
|          | HIV-1     | ssRNA  | Inhibits HIV infection in transformed cell lines | Nakashima et al. (1993) |
| α-defensins | α-defensin | Rat    | HIV-1 | ssRNA  | Inhibits HIV infection in transformed cell lines | Nakashima et al. (1993) |
| α-defensins | α-defensin | Guinea pig | HIV-1 | ssRNA  | Inhibits HIV infection in transformed cell lines | Nakashima et al. (1993) |
| Crp 3    | α-defensin | Mouse  | HIV-1 | ssRNA  | Moderate increase in HIV infectivity | Tanabe et al. (2004) |
| Crp 4    | α-defensin | Mouse  | HIV-1 | ssRNA  | Moderate antiviral activity | Tanabe et al. (2004) |
| hBD1     | β-defensin | Human  | HSV-2 | dsDNA  | No antiviral effect | Hazrati et al. (2006) |
|          | HPV       | dsDNA  | No antiviral effect | Buck et al. (2006) |
|          | VV        | dsDNA  | No antiviral effect | Howell et al. (2004) |

(continued)
Table 3.2 (continued)

| Defensin | Structure | Source | Virus | Genome | Antiviral mechanism | REF |
|----------|-----------|--------|-------|--------|---------------------|-----|
|          |           |        | JCPyV | dsDNA  | No antiviral effect | Zins et al. (2014) |
|          |           |        | BK virus | dsDNA | No antiviral effect | Dugan et al. (2008) |
|          | HAdV (Av1CF2) | dsDNA | Inhibits infection; mechanism unknown | Gropp et al. (1999) |
|          | HIV-1     | ssRNA  | Increased virus uptake by neutrophils | Queñones-Mateu (2003) |
|          | RSV       | ssRNA  | Increased secretion; direct effect on viral particles; disruption of the viral envelope | Kota et al. (2008) |
| hBD2     | β-defensin| Human | HRV | (+)ssRNA | Not induced by virus | Duits et al. (2003) |
|          | HPV       | dsDNA  | No antiviral effect | Buck et al. (2006) |
|          | VV        | dsDNA  | No antiviral effect | Howell et al. (2004) |
|          | JCPyV     | dsDNA  | No antiviral effect | Zins et al. (2014) |
|          | BK virus  | dsDNA  | Moderate inhibitory effect; mechanism unknown | Dugan et al. (2008) |
|          | HAdV (5)  | dsDNA  | Moderate inhibitory effect; mechanism unknown | Bastian and Schäfer (2001) |
|          | HIV-1     | ssRNA  | Inhibits both X4 and R5 infection; downregulates CXC4; inhibits early products of reverse transcription | Queñones-Mateu et al. (2003), Sun et al. (2005) |
|          | IAV       | ssRNA  | Increased uptake by neutrophils | Tecle et al. (2007) |
|          | RSV       | ssRNA  | Increased secretion; direct effect on viral particles; disruption of the viral envelope | Kota et al. (2008) |
|          | HRV       | (+)ssRNA | Induction of expression by virus | Duits et al. (2003) |
| Defensin | Structure | Source | Virus | Genome | Antiviral mechanism                                                                                   | REF                          |
|----------|-----------|--------|-------|--------|-----------------------------------------------------------------------------------------------------|------------------------------|
| mBD2     | β-defensin | Mouse  | IAV (PR8) | (−)ssRNA | Blocks viral entry to target cells                                                                  | Gong et al. (2010)           |
|          |           |        | DENV-2 | (+)ssRNA | Induction of expression                                                                               | Bustos-Arriaga et al. (2011) |
| hBD3     | β-defensin | Human  | HSV-2 | dsDNA  | Interacts with heparansulfate on host cells and also viral gb2; prevents viral binding                  | Hazrati et al. (2006)        |
|          |           |        | VV    | dsDNA  | Reduced viral DNA-dependent RNA pol expression and plaque formation                                     | Howell et al. (2004)         |
|          |           |        | JCPyV | dsDNA  | Inhibits infection; cytotoxicity towards host cells                                                    | Zins et al. (2014)           |
| HIV-1    | ssRNA     | Human  | HSV-2 | dsDNA  | Inhibits both X4 and R5 infection; downregulates CXC4; inhibits early products of reverse transcription | Quiñones-Mateu (2003), Sun et al. (2005) |
|          |           |        | IAV   | (−)ssRNA | Blockshemagglutinin-mediated viral fusion to host cells                                                | Leikina et al. (2005)        |
| mBD3     | β-defensin | Mouse  | IAV (PR8) | (−)ssRNA | Blocks viral entry to target cells                                                                  | Jiang et al. (2012)          |
| RC1      | Inferred from human θ defensin genes | Human  | HSV-2 | dsDNA  | Blocks viral attachment; reduced nuclear translocation of VP16                                        | Yasin et al. (2004)          |
|          |           |        | HSV-1 | dsDNA  | Direct effect on viral particles; mechanism unknown                                                      | Yasin et al. (2004)          |
|          |           |        | HIV-1 | ssRNA  | Blocks viral fusion; binds to viral gp41 and glycosylated gp120 and cellular CD4                       | Cole et al. (2002), Gallo et al. (2006), Wang et al. (2004) |
|          |           |        | HIV-2 | ssRNA  | Modest inhibition of viral fusion                                                                       | Gallo et al. (2006)          |

(continued)
| Defensin | Source | Structure | Virus Type | Genome | Antiviral mechanism | REF |
|----------|--------|-----------|------------|---------|---------------------|-----|
| RC2      | Human  | Inferred from human θ defensin genes | SIV       | ssRNA   | Reduces replication by inhibiting viral NS2B-NS3 serine protease | Gallo et al. (2006) |
|          | Human  | d dsDNA   | DENV-2     | (+)ssRNA| Blocks viral attachment; reduced nuclear translocation of VP16 | Rothan et al. (2012) |
|          | Human  | d dsDNA   | HSV-2      | dsDNA   | Direct effect on viral particles; mechanism unknown | Yasin et al. (2004) |
|          | Human  | d dsDNA   | HSV-1      | dsDNA   | Blocks viral fusion with target cells, avoids syncytium formation | Yasin et al. (2004) |
|          | Human  | d ssRNA   | HIV-1      | ssRNA   | Blocks viral fusion; binds to viral glycosylated gp120 and cellular CD4 and cellular CD8 | Wang et al. (2004), Cole et al. (2002) |
|          | Human  | d (+ssRNA)| IAV        | (+ssRNA)| Prevents hemagglutinin-mediated viral fusion with target cells | Leikina et al. (2005) |
|          | Human  | d ssDNA   | SINV       | (+)ssRNA| Prevents viral fusion with target cells | Leikina et al. (2005) |
|          | Human  | d ssDNA   | RTD1       | ssRNA   | No antiviral effect | Yasin et al. (2004) |
|          | Rhesus | d dsDNA   | Rhesus 0   | dsDNA   | No antiviral effect | Wang et al. (2004) |
|          | Rhesus | d (+ssRNA)| HIV-1 (various isolates) | ssRNA   | Moderate direct antiviral effect; mechanism unknown | Wohlford-Lehane et al. (2009) |

Immunomodulatory effect; reduction in lung cytokine secretion

(continued)
| Defensin | Source | Virus | Genome | Antiviral mechanism | REF |
|----------|--------|-------|--------|---------------------|-----|
| RTD2     | Rhesus | HSV-2 | dsDNA  | Moderate antiviral effect; mechanism unknown | Yasin et al. (2004) |
|          |        | HSV-1 | dsDNA  | No antiviral effect | Yasin et al. (2004) |
|          |        | HIV-1 (various isolates) | ssRNA | Moderate direct antiviral effect; mechanism unknown | Wang et al. (2004) |
| RTD3     | Rhesus | HSV-2 | dsDNA  | Direct effect on viral particles; cytotoxicity towards host cell | Yasin et al. (2004) |
|          |        | HIV-1 (various isolates) | ssRNA | Direct effect on viral particles; mechanism unknown | Wang et al. (2004) |
an immature form into a mature active form. Interestingly the immature (MK) form of the cecropin-like peptide was more active against all four strains of Dengue virus tested (Dengue-1, -2, -3 and -4) than the mature (GK) cleaved form. The authors also established that both the GK and MK forms of the peptide were active against Chikungunya virus, a pathogen also transmitted by the *A. aegypti* mosquito that can cause fever and severe long lasting joint pain. Another study revealed that scorpine, a hybrid peptide with structural similarities to cecropins and defensins derived from the venom of the scorpion *Pandinus imperator*, also exhibited antiviral activity against dengue virus by inhibiting the replication of Dengue-2 virus (Carballar-Lejarazu et al. 2008).

Cecropin A is reported to inhibit HIV production by infected T-cells and fibroblasts in a dose-dependent manner (Wachinger et al. 1998). The mechanism responsible for this inhibition was found to be reduced viral gene expression and synthesis of viral products, indicating that Cecropin has an antiviral role beyond that of direct interaction with the HIV virion.

Cecropins have also been shown to have antiviral activity at early and late points in the viral infection and replication cycle. For example, both cecropin P1 and cecropin D have been shown to have antiviral activity at several stages in infection with porcine reproductive and respiratory syndrome virus (PRRSV), an infection which can have substantial financial impact in the pig industry. Cecropin P1 is a peptide initially thought to be produced in the porcine intestine (Lee et al. 1989), but was later revealed to have been produced by the nematode, *Ascarissuum*, which survives in the porcine gut (Andersson et al. 2003). This peptide was shown to block attachment and replication of the virus in kidney and alveolar macrophage cell lines, and also reduced the number of infectious viral particles produced after infection in vitro (Guo et al. 2014). The peptide was also shown to have immunomodulatory properties by preventing the onset of PRRSV-induced apoptosis. Similarly, cecropin D was recently shown to inhibit PRRSV attachment and replication, and also attenuated apoptosis induced by the virus (Liu et al. 2015).

The broad spectrum antiviral activity of cecropins is further evidenced by a study demonstrating that cecropin A could inhibit replication of Junin virus, the cause of Argentine hemorrhagic fever (Albiol Matanic and Castilla 2004). The peptide inhibited Junin virus-related protein production in host cells, but the authors suggested that the antiviral effects were predominantly limited to later stages in the virus replication cycle, since the peptide did not appear to alter viral infectivity. Interestingly, the same study also demonstrated that cecropin A did not exhibit any inhibitory activity against herpes simplex virus types 1 and 2, suggesting that any antiviral activity exhibited by these peptides is pathogen specific. It should be noted that all HSV-1 and HSV-2 contain a DNA genome, in contrast to HIV-1, Junin, PRRSV and Dengue, which are RNA viruses, and thus the activity of cecropins against other DNA viruses remains to be understood.
3.5.2 Dermaseptin

There have been a substantial number (~1000) Host Defence Peptides identified from amphibian skin, of which Dermaseptins are a superfamily. These host defence peptides have a cationic charge due to an abundance of lysine residues, and tend to be between 27–34 amino acids in size (Amiche et al. 1994). They have been found in several species of frogs, including the Hylidae (tree frogs) and the Ranidae (true frogs) (Nicolas and El Amri 2009). Dermaseptins have been demonstrated to have antimicrobial activity against a wide range of bacterial pathogens involved in human diseases including Pseudomonas, Salmonella, Staphylococcus, Escherichia and Enterobacter species. Interestingly, the peptide Dermaseptin S1, a 34-residue peptide isolated from the frog genus Phyllomedusa, was shown to have immunomodulatory activity via an ability to stimulate the production of reactive oxygen species and myeloperoxidase by primary human neutrophils (Ammar et al. 1998). Dermaseptin S2 has also been touted as a potential therapeutic molecule in the treatment of cancer in a recent study demonstrating antitumor and angiostatic activity in a range of tumour cell types, likely due to the induction of necrosis (van Zoggel et al. 2012).

In terms of their antiviral activity, dermaseptins have been shown to be highly effective against a broad range of viral pathogens, with activity that affects several steps in the infection and replication process. Dermaseptin-1 was initially identified to have antiviral activity against two viruses that are known to infect ectothermic animals; Frog virus 3 from the family Iridoviridae, and Channel catfish virus, a member of the Herpesviridae family (Chinchar et al. 2004). This study provided indication that dermaseptin peptides possessed antiviral activity and subsequent studies were extended to examine the activity against human viral pathogens.

Dermaseptins S1-S5 shown to have direct and varying antiviral activity against Herpes Simplex Virus-1 in vitro (Belaid et al. 2002). This study identified Dermaseptin S4 as having the most potent antiviral activity, but only at very early stages in the viral infection process as experiments suggested that it exerted an effect when exposed to the virus prior to infection or during viral attachment. A later study further evaluated the activity of Dermaseptin S1 and derivatives against Herpes Simplex Virus, demonstrating that the antiviral activity of the parent peptide could be increased by alteration of the original sequence (Savoia et al. 2010). Interestingly, the same study also showed that some of the derivatives also exhibited activity against Papillomavirus Psv-16 in vitro, but with very low cytotoxicity towards the host cells. A recent study by Bergouï et al. also demonstrated that Dermaseptin S4 and synthetic derivatives exhibited activity against Herpes Simplex Virus-2 with reduced cytotoxicity, although in vivo efficacy remains to be determined (Bergouï et al. 2013).

Other studies have revealed a possible role for Dermaseptins in the treatment of HIV-1 infection. Dermaseptins have been shown to partially inhibit HIV virus infection of T-cells although cytotoxicity was observed at higher concentrations (VanCompernolle et al. 2005). Another study by Lorin et al. identified Dermaseptin
S4 as having potential inhibitory activity against HIV-1 by inhibiting viral infection of human primary T-lymphocytes (Lorin et al. 2005). The authors attributed this activity to a direct disruption of the virion and, while the parent peptide elicited cytotoxicity at higher concentrations, were able to reduce host cell cytotoxicity by reducing the positive charge of the native peptide through amino acid deletion or substitution. Notably, the modified peptides were also able to reduce HIV-1 binding to endometrial cells together with inhibition of capture and transmission of virus from dendritic cells to CD4+ T-cells. Dermaseptin S9 was also identified as having weak activity against HIV-1, but a mutant S9 peptide, where three lysine residues were replaced with arginines, exhibited potent inhibitory activity against HIV-1 although the mechanism underlying this observation remains unclear (Wang et al. 2010).

### 3.5.3 Magainin

Magainins are cationic host defence peptides that were originally identified in the skin and granular secretions of *Xenopus laevis* (the African clawed frog) (Zasloff 1987; Giovannini et al. 1987), but have also been identified as inducible peptides in other species in the *Xenopus* genus; *X. borealis*, *X. clivi*, *X. muelleri*, *X. petersii*, *X. amieti* and *X. andrei* (Conlon et al. 2012). These peptides are 23–34 amino acids in length and the native peptides, together with synthetic derivatives, have been demonstrated to have broad antimicrobial activity against a range of bacterial pathogens (Zairi et al. 2009; Chen et al. 1988). There is, however, a limited amount of information on the activity of magainins against viral pathogens. One study has assessed the activity of a number of synthetic magainin derivatives against Herpes Simplex-1 virus, establishing that several peptide derivatives have the capacity to reduce viral plaque formation in vitro assays (Egal et al. 1999). Further evidence of antiviral activity of magainins was also revealed in a study by Chinchar et al. (2004) which showed magainin II, but not magainin I, was able to reduce the infectivity of channel catfish virus (CCV) (Chinchar et al. 2004). However, both magainins exhibited less activity against frog virus 3, indicating that the activity was likely virus specific.

### 3.5.4 Melittin

Melittin is an alpha helical peptide that is 26 amino acids in length and was initially identified in bee venom (Habermann and Jentsch 1967) and later characterized as an amphipathic peptide with broad ranging antimicrobial activity (Terwilliger and Eisenberg 1982; Wade et al. 1992). The native melittin peptide and synthetic analogues have been investigated in a number of studies as potential novel antimicrobial therapeutics, but their well-characterized antiviral activity against a number of pathogens is of particular interest.
One of the first studies to assess the antiviral activity of melittin was published by Wachinger et al. (1992) which identified that melittin and six derivatives reduced HIV-1 replication in host cells (Wachinger et al. 1992). A subsequent study revealed that the mechanism underlying this inhibition was that melittin was capable of specifically reducing HIV-associated gene expression in the host cell while not affecting the overall gene expression profile (Wachinger et al. 1998). This suggests an important immunomodulatory role for this peptide, rather than direct antiviral activity, as potentially contributing to the treatment of HIV infection.

Several studies have also characterized the influence of melittin, and synthetic derivatives upon the establishment and progression of Herpes Simplex Virus infection. Baghian et al. (1997) synthesized a number of derivatives based upon the original melittin structure to assess their antiviral activity against HSV-1 virus. One such peptide was called Hecate, which has an altered amino sequence that changes the distribution of charged residues within the peptide without altering the overall amphipathic α-helical structure (Baghian et al. 1997). The authors determined that Hecate prevented HSV-1 plaque formation and prevented virus spread in in vitro models while some synthetic melittin analogues were unable to do so, suggesting that sequence played an important role in the antiviral activity of the peptide. A later study screened a number of known CHDP against HSV-1 and HSV-2 and established that many of the α-helical peptides screened (including magainins, cecropins and cathelicidin) did not display activity against the viruses (Yasin et al. 2000). However, melittin did have substantial activity against both HSV-1 and HSV-2, although the mechanism underlying this observation was not fully described.

More recently, it has been demonstrated that the native melittin peptide showed antiviral activity towards Junin virus (Albiol Matanic and Castilla 2004), and that synthetic melittin analogues showed activity towards Tobacco Mosaic Virus (Marcos et al. 1995). Interestingly, a recent study by Falco et al. (2013) used melittin-loaded liposomes to specifically target fish viral hemorrhagic septicemia rhabdovirus (VHSV) (Falco et al. 2013). The authors coated the immunoliposomes with antibodies targeting the surface G glycoprotein of VHSV, and showed a reduction in infectivity of greater than 95%. This approach provides an exciting avenue for the targeted delivery of antiviral host defence peptides whilst minimizing cytotoxic damage to host cells.

### 3.5.5 Tachyplesin and Polyphemusin

Tachyplesin is a 17 amino acid cationic β-sheet peptide that was originally identified in the hemocytes of *Tachylepus tridentatus* (Horseshoe crab) and was demonstrated to have broad spectrum antimicrobial activity (Nakamura et al. 1988). Polyphemusin I is a 18 amino acid peptide identified in the hemocytes of the American horseshoe crab, *Limulus polyphemus*. Both peptides are thought to play a key role in the innate immune system of the crab, and have been shown to have potent LPS binding and neutralization activity (Nakamura et al. 1988; Powers et al. 2006).
The antiviral activity of Tachyplesin against HIV was investigated by Morimoto et al. (1991) who showed that the peptide could reduce virus-mediated cytopathic effects by more than 70% in in vitro models (Morimoto et al. 1991). The peptide also reduced the infectivity of the virus, an effect that was shown to be independent on the reverse transcriptase activity of HIV. Interestingly, subsequent studies by Nakashima et al. (1992), Murakami et al. (1997) and Xu et al. (1999) characterized the activities of the isopeptide T22 (Tyr^5,12, Lys^7-polyphemusin II), and synthetic analogues against HIV, and determined that the antiviral activity was due to ability of the peptide to bind to CXCR4 (Xu et al. 1999; Murakami et al. 1997; Nakashima et al. 1992). CXCR4 is a chemokine receptor used by T-cell tropic strains of HIV to infect host cells. Thus, the host cell mediated mechanism of action of these peptides against HIV contrasts to the direct antiviral activity exhibited by other CHDP.

Tachplesin peptides have also been demonstrated to have activity against other viruses that affect humans. It was shown that Tachyplesin peptides were able to inactivate Vesicular stomatitis virus (VSV; also known as vesicular stomatitis Indiana virus), a zoonotic member of the family Rhabdoviridae that can infect cattle and cause disease in humans (Murakami et al. 1991). The same study also identified that Influenza A (H1N1) was also moderately susceptible, although HSV-1 and -2, adenovirus-1, reovirus-2 and poliovirus-1 were not susceptible to the antiviral activities of the peptide. However, a subsequent study, which examined the antiviral activity of tachyplesin against HSV-1 and HSV-2 using an in vitro cytotoxicity model, did suggest that the peptide offered a moderate degree of protection against both virus strains (Yasin et al. 2000).

### 3.6 Conclusion

Collectively, the experimental studies presented here highlight the crucial role that CHDP play in the innate immune response across a wide variety of cell types and species. These peptides possess powerful antiviral activity, and can modulate the cellular immune response to provide a key defence mechanism infection and pathology associated with a myriad of viruses.

While we are beginning to understand some of the underlying mechanisms through which cathelicidins, defensins and other CHDP mediate their antiviral effects; research into the activities of these peptides is still in relative infancy. It is clear, however, that CHDP have huge therapeutic potential as exogenous peptides, and for the development of powerful synthetic analogues that can be directed towards specific viral pathogens.

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