# Chapter 2
# Antimicrobial Peptides as Endogenous Antibacterials and Antivirals at the Ocular Surface

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**Abstract** Ocular surface infections are a significant cause of blindness worldwide particularly in developing countries and are mainly caused by bacteria, fungal species, and parasites as well as by viruses. *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pneumonia*, and *Pseudomonas aeruginosa* are reported to be the most common bacteria associated with keratitis where the most frequent viruses leading to ocular surface infection are herpes simplex, adenovirus, and vaccinia virus. The eye and its adnexa have evolved a bulk of defense strategies to prevent microbial invasion. These include important components of the innate defense system in form of classical antimicrobial compounds as well as members of the cationic antimicrobial peptide family, distinct surfactant proteins, and potential new candidate molecules contributing to antimicrobial protection. Several of them are studied at the ocular surface not only for their antimicrobial properties, but based on easy topical application possibilities for their potential therapeutic effects. As several reviews already summarized the current knowledge with regard to the eye, this chapter will only briefly recapitulate the current knowledge of antimicrobial compound expression in the eye and then will focus on infectious keratitis resulting from bacterial and viral infection. In addition, the potential of using such peptides as therapeutics for treating bacterial and viral ocular surface infections will be elucidated.

## 2.1 Introduction

The ocular surface and its adnexal structures comprise the cornea, conjunctiva with the bulbar, the fornical, and palpebral parts, the main lacrimal gland, the

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glands of the eyelids (including the meibomian glands, the glands of Moll, and accessory lacrimal glands), as well as the nasolacrimal system (or efferent tear ducts with the upper and lower puncta, the paired lacrimal canaliculi, the lacrimal sac, and the nasolacrimal duct). The nasolacrimal system collects the tear fluid from the ocular surface and conveys it into the nasal cavity. All the other structures contribute to formation of the preocular tear film. The tear film together with the glands and cells that produce it (lacrimal glands, accessory lacrimal glands, ocular surface epithelia, meibomian glands, glands of Moll, and the sensory and motor nerves connecting these) has been assigned the general term lacrimal functional unit (LFU) (Stern et al. 1998). Disease in or damage to any component of the LFU can destabilize the tear film and lead to dry eye disease (DED), a condition expressed by signs and/or symptoms of ocular irritation, redness, and foreign body sensation. DED afflicts millions of people around the world and makes the ocular surface more vulnerable to infection (Gayton 2009).

## 2.2 Tear Film Composition

The tear film coats the cornea and conjunctiva and is a complex structure composed of an inner mucous layer that is part of the glycocalyx and is attached to the epithelial cells (composed mainly of membrane-bound mucins), an aqueous component consisting, beside water (99 %) of ions, soluble mucins and a wide range of different proteins and peptides (such as trefoil factor family peptides that function as linker molecules for mucins to form gels), as well as an outer, anterior-most lipid component that prevents evaporation. Ocular mucins and trefoil factor family (TFF) peptides take a variety of different functions which provide protection at the ocular surface. Mucins are a family of high molecular weight, heavily glycosylated hydrophilic proteins. Membrane-anchored mucins, MUC1, MUC4, und MUC16, are part of the epithelial glycocalyx and provide a continuous anatomic barrier across the ocular surface which prevents pathogen penetration. The membrane-spanning molecules also have signaling capabilities that influence epithelial activity. The negative charge of the mucins (glycocalyx) occurs in an accumulation of positive-charged tear components, like lysozyme, SLPI, as well as AMPs (see below), which enhance the local concentration of antimicrobial agents at the ocular surface. The high molecular secreted mucins are distinguished in gel-forming mucins (mainly MUC5AC from conjunctival goblet cells and MUC5B from the lacrimal gland) and small soluble mucins (MUC7 from the lacrimal gland). The secreted mucins as well as TFFs are responsible for the rheological properties of the tear film, enabling movement and spreading of the tear film. TFF peptides have many other physiological functions in addition to their rheological properties, such as promotion of epithelial cell migration, antiapoptotic properties, induction of cell scattering, epithelial restitution, and neuropeptide functions (Paulsen and Berry 2006).

### 2.2.1 *Antimicrobial Compounds at the Ocular Surface, in the Lacrimal Apparatus, and in the Tear Film*

Like other epithelia, also the ocular surface is constantly in contact with various microorganism and pathogen-associated molecular patterns (PAMPs). The ocular surface epithelia and associated structures of the lacrimal apparatus are effective in counteracting invasion and colonization of microorganisms. They produce the tear film that contains up to 1500 different proteins (Zhou et al. 2012). More than 90 % of the total amount of tear proteins are four antimicrobial compounds: lysozyme, lactoferrin, tear lipocalin and secreted immunoglobulin A (sIgA). The concentration of these four proteins in the tear fluid is in the mg/ml range. These tear compounds are well known and show broad antimicrobial activity. Lysozyme cleaves the peptidoglycan backbone of bacterial cell walls and is also able to digest fungal cell walls. Lactoferrin binds free iron at the ocular surface and inhibits bacterial and fungal growth. It has also been shown to have iron-independent anti-HIV activity. Tear lipocalin binds microbial siderophores (iron chelating compounds) and thus has a bacteriostatic effect. Lysozyme, lactoferrin, and tear lipocalin are built mainly by the acinar cells of the main lacrimal gland as well as by accessory lacrimal glands in the eyelids (*glandulae conjunctivales* [glands of Krause and Wolfring]). In contrast, sIgA is produced as a dimer by subepithelial plasma cells, transported via transcytosis, bound to a protein termed "secretory component," through the epithelium and then is secreted into the tear film (Mcdermott 2013; Tiffany 2008).

In addition to these principal antimicrobial factors, tear fluid contains a great bulk of other tear compounds, several of them with antimicrobial activity. The concentration of these compounds is in the range of µg/ml to pg/ml. Beside their antimicrobial activity, several of these compounds have no antimicrobial activity as main biological function and several are with yet unknown function (Mcdermott 2013). Among those constituents with antimicrobial activity is secretory phospholipase A2 (sPLA2). It binds to anionic bacterial membranes of Gram-positive bacteria due to its cationic domains and kills bacteria by its enzymatic activity. sPLA2 shows activity against Gram-positive bacteria in the physiological tear environment (Qu and Lehrer 1998). Antimicrobial activity and anti-inflammatory properties were shown for members of the whey acidic protein (WAP) family like secretory leukocyte protease inhibitor (SLPI) as well as elafin, which are characterized by a cysteine-rich region with four intramolecular disulfide bonds. SLPI was identified by its antiprotease activity and shows also activity against Gram-positive and Gram-negative bacteria, fungi, and HIV (Sallenave 2010). High amounts could be detected in closed-eye tears. SLPI may inhibit neutrophil elastase derived from immigrating neutrophilic granulocytes and protects the ocular surface from enzymatic degradation effects during the sleep (Sathe et al. 1998). Furthermore, bactericidal/permeability-increasing protein (BPI) was initially identified in neutrophils, but it is also expressed by ocular surface epithelial cells and could be detected in tears. BPI binds lipopolysaccharides (LPS) liberated from Gram-negative bacteria (Peuravuori et al. 2006). Clinical trials with recombinant modified BPI molecule (rBPI21)

showed reduced mortality of Gram-negative bacteria-induced sepsis (Domingues et al. 2009). The amino acid derivate β-lysin (3,6-diaminohexanoic acid) produced by platelets was also found in tears and showed antimicrobial peptide-like characteristics. β-Lysin interacts with bacterial cell membranes of Gram-positive bacteria, inhibits bacterial enzymes, and enhances phagocytosis (Yeaman 2010).

### 2.2.2 Surfactant Proteins

During recent years, the well-known lung surfactant proteins (SPs) have also been described in other mucosae such as in the ocular system (Schicht et al. 2010). Here, they have been associated with surface tension regulating activities of the tear film as well as with immunological functions. Tear SP-D has been shown to protect the ocular surface from infection with Gram-negative bacteria, in particular *Pseudomonas aeruginosa* (Mcdermott 2013). With regard to virus infection, SP-A and SP-D are able to bind and agglutinate viruses (such as influenza A virus, adenovirus, hepatitis B virus [HBV], and herpes simplex virus [HSV]) and they have been shown to initiate phagocytosis by macrophages (Harrod et al. 1999; Kishore et al. 2006; Levine et al. 2001). In chronic herpes virus infection, a protein misfolding shall lead to functional loss of SP-C with losing of the surface regulatory properties (Lawson et al. 2008). Also, the recently discovered surfactant protein H (*SFTA3*) has been demonstrated to occur at the ocular surface (Schicht 2012). Also, this surfactant protein has immunological functions by mediating activation of macrophages (Diler et al. 2014). However, so far, there exist no data demonstrating any function of surfactant proteins with regard to viral eye infection.

### 2.2.3 Antimicrobial Peptides (AMPs)

AMPs are defined as small endogenous proteins displaying direct antimicrobial activity. In general, AMPs have no specific consensus amino acid sequence but most of them have an amphipathic character and are positively charged under physiological pH conditions. AMPs have been detected across a wide spectrum of different species. In February 2015, the Antimicrobial Peptide Database (APD; http://aps.unmc.edu/AP/main.php) lists 2493 AMPs: 244 from bacteria (bacteriocins), 2 from archaea, 7 from protists, 13 from fungi, 312 from plants, and 1874 from animals. In human, 108 different AMPs have been described to date (Wang 2014). According to their secondary structure, AMPs can be classified into four groups: (i) AMPs with linear α-helical domains (e.g., human cathelicidin LL-37), (ii) AMPs with β-sheets and disulfide bonds (e.g., α- and β-defensins), (iii) AMPs with extended structures (e.g., indolicidin), and (iv) AMPs with loop structures (cyclic defensins, bactenecin) (Lai and Gallo 2009).

Various different human AMPs with activity against bacteria, fungi, and viruses have been described and characterized (Wang 2014). Figure 2.1 provides an overview about known AMPs at the ocular surface and lacrimal structures.

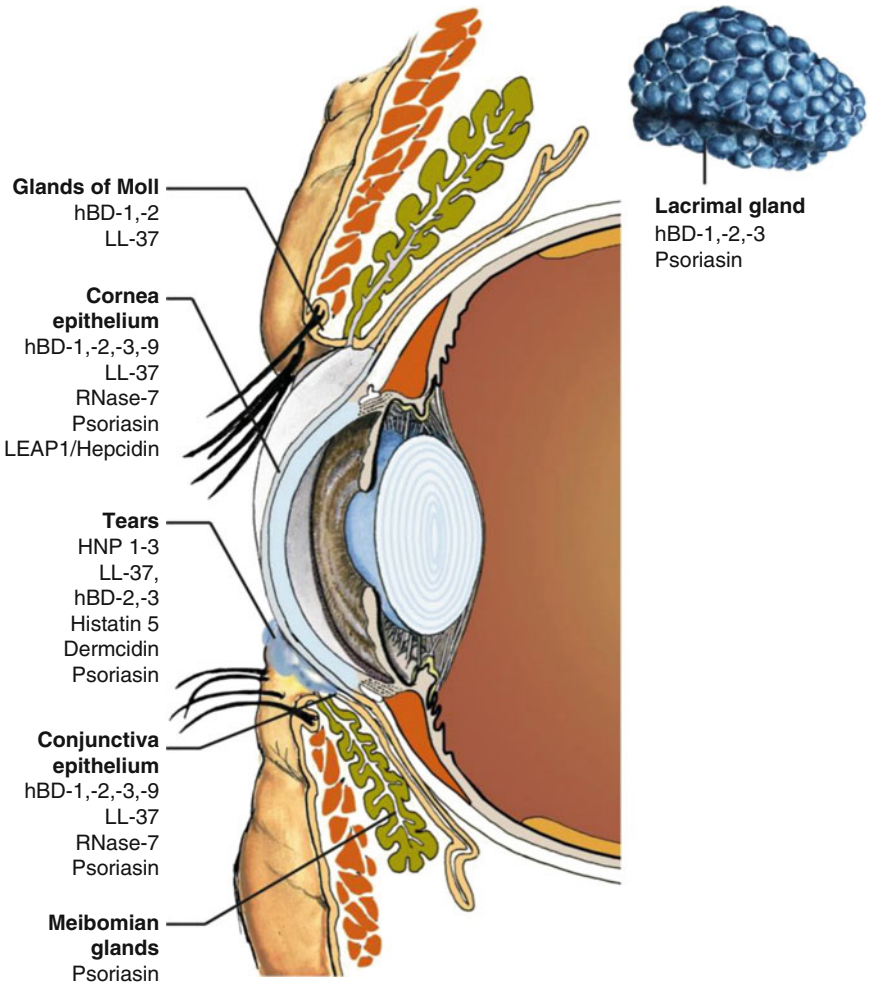


**Fig. 2.1** Expression of antimicrobial peptides with antibacterial and antiviral activity at the ocular surface structures, in the lacrimal gland, and in tears (Partly modified from Kolar and Mcdermott (2011))

#### 2.2.3.1 Human Defensins

Human defensins are small in size (29–45 amino acids), are cationic, and are characterized by the presence of six conserved cysteine residues. Based on the distribution of the cysteines and the linkage of disulfide bonds, defensins are classified into three subfamilies, referred to α-, β-, and θ-defensins (theta or minidefensins). Humans express six α-defensins and up to 31 β-defensins. α-Defensins and β-defensins differ by their organization of the three disulfide bonds (Hazlett and Wu 2011). θ-Defensins are not translated in humans due to premature stop codons preventing their expression (Lehrer et al. 2012). Interestingly, pseudogenes of

θ-defensins, named retrocyclins, show antimicrobial activity against the gut bacterium *E. coli* as well as against HIV-1 (Tran et al. 2008; Wang et al. 2004). Human α-defensins have been divided into human neutrophil peptides (HNP) 1–4 and human defensin (HD)-5 and human defensin-6. HNP1–HNP4 were identified in particularly high concentrations in azurophil granules of neutrophils but also in other immune cells. In contrast, HD-5 and HD-6 are expressed by Paneth cells in the small intestine as well as different epithelial cells (Lehrer et al. 2012). Human β-defensins (hBD) 1–3 are expressed by epithelial cells and also in different immune cells (Semple and Dorin 2012).

At the ocular surface, α-defensins and hBD-1 are constitutively expressed whereas hBD-2 and hBD-3 show a low basal expression at the ocular surface but enhanced expression after stimulation with different pro-inflammatory stimuli, including cytokines and bacterial compounds (Garreis et al. 2010). In addition, hBD-9 (*DEFB109*) has been identified at the ocular surface. Interestingly, hBD-9 shows a reduced *ex vivo* gene expression in patients with keratitis and also a down-regulation in human corneal limbal epithelial cells after cocultivation with *Acanthamoeba* spp. and bacteria from keratitis patients (Abedin et al. 2008; Otri et al. 2012).

Both α- and β-defensins have broad activity against different microbes, including various bacteria and viruses *in vitro* (Gwyer Findlay et al. 2013; Klotman and Chang 2006; Wilson et al. 2013). *In vivo*, only α-defensin concentrations are generally within the range of direct antimicrobial activity whereas the concentrations of constitutively expressed β-defensins often do not reach levels sufficient for a direct antimicrobial activity (Wilson et al. 2013). Interestingly, various studies indicate that the local β-defensin concentration can be increased markedly by various inflammatory and (patho)physiological processes. HBD-1 becomes a potent antimicrobial peptide after reduction of disulfide bridges against gut pathogens (Schroeder et al. 2011). In addition, all human defensins have also several immunomodulatory properties (see below).

#### 2.2.3.2 Human Cathelicidin LL-37

In humans, only one member of the cathelicidin family is functionally expressed: human cationic antimicrobial peptide-18 (hCAP-18). This 18 kDa peptide is encoded by the CAMP gene and cleaved by proteinase 3 into the 37 amino acid long-active peptide LL-37 (starts with two leucines) and the N-terminal cathelin domain. Human LL-37 is characterized as cationic, amphipathic peptide with an α-helical structure and a broad-spectrum antimicrobial activity against various pathogens (Vandamme et al. 2012). It is stored in neutrophils, inducible in most epithelial cells and leukocytes, and secreted into various body fluids, including tears. Human LL-37 and its derivatives are also able to inhibit and destroy biofilms and have antifungal and antiviral activity (Vandamme et al. 2012). Moreover, LL-37 modulates the adaptive immune system, stimulates angiogenesis, and supports reepithelialization (Vandamme et al. 2012).

#### 2.2.3.3 Other AMPs

Beside defensins and cathelicidins, also other AMPs and other antimicrobial compounds have been demonstrated to show antibacterial and antiviral activity at the ocular surface. Thus, a group of human inhibitors of neutrophil serine proteases known as SLPI (secretory leukocyte protease inhibitor) and elafin (also known as peptidase inhibitor 3 or skin-derived antileucoprotease [SKALP]) have been associated with anti-HIV activity of vaginal fluid (Ma et al. 2004; Pillay et al. 2001). The antiviral activity does not depend on inhibition of serine proteases. It is associated with binding of host cell membrane-associated proteins such as scramblase and/or annexin II (Ma et al. 2004; Tseng and Tseng 2000). The antifungal peptide histatins (histidine-rich cationic peptides) are present in high amounts in both human saliva and tears and are also discussed for their antiviral activity. A histatin-5 derivate affects HIV-1 replication by promoting host cell entry (Groot et al. 2006). Psoriasin (S100A7), a highly potent AMP against *Escherichia coli*, was detected in elevated concentrations in human tears and in tissues of the ocular surface and lacrimal apparatus (Garreis et al. 2011). Two RNases from eosinophils, eosinophil-derived neurotoxin (RNase 2/EDN), and eosinophil-cationic protein (RNase 3/ECP) have been shown to inhibit respiratory syncytial viruses (RSV) and HIV infection *in vitro* (Domachowske et al. 1998). RNase 2 antiviral activity depends on ribonucleolytic activity. In addition, RNase 2 functions as chemoattractant and can activate immune cells via Toll-like receptor (TLR)-2 and myeloid differentiation factor 88 (Myd88) signaling. RNase 3 is able to permeabilize microbial membranes and stimulate histamine release from mast cells (Wiesner and Vilcinskas 2010). In addition, also RNase 5 (angiogenin) acts antivirally against HIV with a yet unsolved mechanism (Cocchi et al. 2012). In contrast to RNases 2 and 3, which have not yet been analyzed at the ocular surface and in tears, tear fluid contains very high amounts of RNase 5 (Sack et al. 2005). However, neither the cellular source and whether and how it is functionally active at the ocular surface is unclear yet. A further RNase has been described at the ocular surface: RNase 7. However, RNase 7 has not yet been associated with antiviral activity (Boix and Nogues 2007). It plays an important role as antibacterial factor in cutaneous defense (Simanski et al. 2012) and its expression in corneal epithelial cells, which is regulated by tears and specific microRNAs, suggests that RNase 7 may also participate to protect the ocular surface against bacterial infection (Mun et al. 2013). The liver-expressed antimicrobial peptide (LEAP), 1/hepcidin, is expressed at the ocular surface and shows increased expression in viral keratitis (Mohammed et al. 2011).

## 2.3 Antimicrobial Activity of AMPs

AMPs are an essential part of the innate immune defense at the ocular surface, with both direct antimicrobial activity and immunomodulatory functions. AMPs demonstrate direct antimicrobial activity against a broad spectrum of ocular pathogens, including Gram-positive and Gram-negative bacteria, fungi, and various viruses.

### 2.3.1 Antibacterial Mechanism

Direct antimicrobial activity is based on the charge-dependent interaction of positively charged AMPs with the negatively charged surface of microorganisms. The microbial cell membrane contains a huge proportion of acidic phospholipids contributing to a negative charge of the surface. In contrast, eukaryotic cell membranes have much fewer negatively charged phospholipids and contain high amounts of cholesterol. This shall be responsible for the selective antimicrobial activity as well as a low cytotoxic effect of AMPs on eukaryotic cell membranes. Aggregation and integration of cationic AMPs into the lipid bilayer lead to expansion of the outer leaflet and to a local membrane thinning. The detailed mechanism of the electrostatic interaction, however, is still being under discussion yet. All models so far available show a permeabilization of the microbial cell membrane, which leads to loss of essential intracellular components and finally to cell death (Wiesner and Vilcinskas 2010). Interestingly, transfer and/or interaction with peptidoglycan in the bacterial cell wall are largely unknown. Furthermore, charge-independent mechanisms of AMP activity have also been described, which contain partly specific interactions with membrane receptors or intracellular molecules (Wiesner and Vilcinskas 2010). For example, buforin, a histone H2A-derived antimicrobial peptide, binds nucleic acids of bacteria (Jang et al. 2012). Further studies showed the direct interaction of AMPs with the cell cycle proteins as well as heat shock proteins resulting in bacterial and fungal growth (Kragol et al. 2001; Lobo et al. 2007). Recent studies have shown the ability of various AMPs and optimized derivatives to act against various stages of microbial biofilm formation including multidrug-resistant strains in biofilms (Di Luca et al. 2014).

### 2.3.2 Antiviral Mechanisms

Several AMPs have an antiviral activity against different viruses including human immunodeficiency virus (HIV), herpes simplex virus (HSV), influenza A virus (IAV), and non-enveloped viruses, like adenovirus (AV) and human papillomavirus (HPV). Figure 2.2 shows various antiviral mechanisms by AMPs. These include:

- Direct interaction (“virolysis”)
- Blockage of host cell surface receptors
- Inhibition of viral fusion to host cells
- Aggregation of viruses
- Inhibition of viral replication
- Activation of adaptive immune response

Most of the antiviral properties of AMPs are based on direct interaction between cationic AMPs and different components of the virus. The major compound of enveloped viruses is the negatively charged lipid bilayer. Cationic AMPs interact

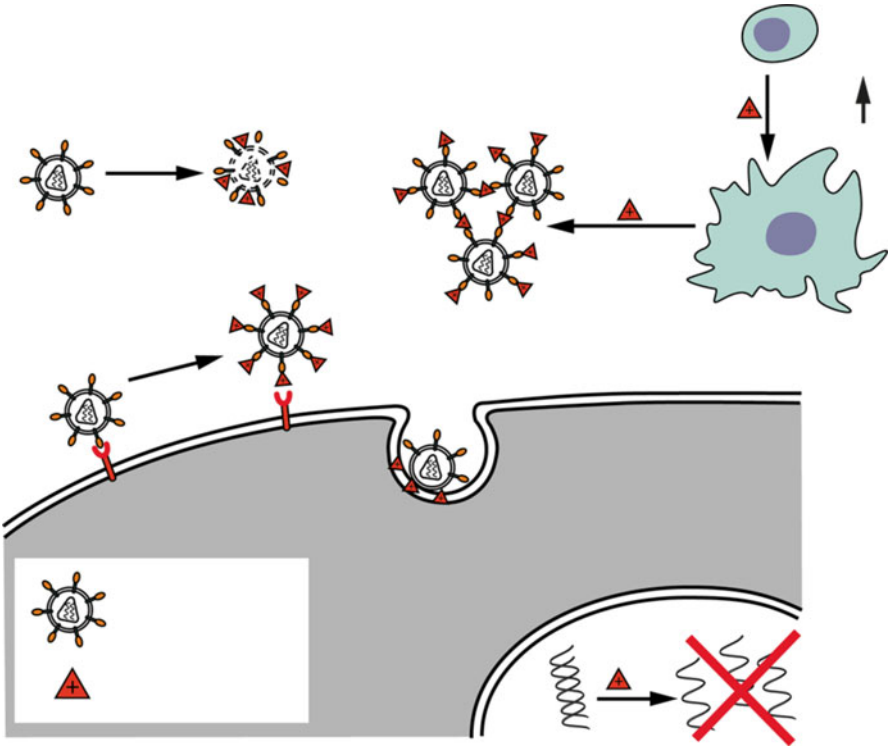

**Fig. 2.2** Overview of the main antiviral mechanisms of antimicrobial peptides (AMPs). AMPs show activity against various cell surface targets (virus and/or host cell) as well as intracellular targets. *In vitro*, cationic AMPs have been shown to decrease viral infection by following mechanism: *1* direct interaction with envelope virus ("virolysis"), *2* blocking of viral entry into host cell by binding of viral and/or host cell receptors, *3* suppressing viral fusion with host cell, *4* extracellular aggregation of viruses, *5* activation of adaptive immune response, and *6* inhibition of viral replication. *Arrow* indicates blockage of viral transcription

with negatively charged phospholipids of the viral envelope. This leads to a destabilization and neutralization of the virus ("virolysis") and additionally to an inhibition of viral fusion with host cells (Wilson et al. 2013). Furthermore, AMPs interact with viral attachment proteins (glycoproteins as well as capsid proteins) and endogenous host cell receptors. These blocking mechanisms inhibit the viral binding to host cells and inhibit virus infection (Wilson et al. 2013). A critical step in virus infection is the penetration of the virus (genome) into the host cell. Enveloped viruses have to fuse their lipid bilayer with the cell membrane of the host cell. Non-enveloped viruses penetrate cell membranes by specific viral capsid proteins. Several studies demonstrate inhibition of the viral fusion process by AMPs through preventing penetration of host cell membranes (Wilson et al. 2013). In addition, AMPs initiate accumulation and aggregation of viruses *in vitro*. It has not been

clarified yet whether AMP-mediated aggregation inhibits virus infection *in vivo*. Aggregation could affect binding to host cells and enhance opsonization by macrophages (Wilson et al. 2013). Furthermore, AMPs block viral infection by inhibiting intracellular viral reproduction through blockage of viral transcription, protein production, assembly, and release of new virus particles. This antiviral activity is based on the opportunity of AMPs to interact with viral and/or host cell compounds. At last, AMPs, especially at physiological concentrations, activate the adaptive immune system and demonstrate immunomodulatory properties. AMPs induce expression of pro-inflammatory molecules as well as chemokines, enhance phagocytosis, and act as chemotaxins for various immune cells (Choi et al. 2012). This immunomodulatory properties link the innate to the adaptive (cellular) immune defense.

## 2.4 Keratitis

Microbial keratitis is an infectious disease of the cornea that is characterized by inflammation and infiltration by leukocytes. A range of microorganisms, including fungi, bacteria, protozoa, and viruses, have been identified to induce microbial keratitis. Moreover, the use of contact lens and corneal trauma are common risk factors. Keratitis can progress rapidly with corneal destruction as well as pathological wound healing and requires immediate medical treatment.

### *2.4.1 Bacterial Keratitis*

Various studies have investigated AMP activity at the ocular surface related to bacterial infection. *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pneumonia*, and *Pseudomonas aeruginosa* are reported to be the most common pathogens associated with bacterial keratitis (Karsten et al. 2012). *P. aeruginosa* infection is the most common cause for bacterial keratitis in contact lens wearers. As described before, human defensins and LL-37 are expressed at the ocular surface and show varying levels of antibacterial activity against common ocular bacteria *in vitro*. Antimicrobial activity occurs in a low micromolar range, and AMPs show large variability in their efficiency in killing specific pathogens (McDermott 2013). Generally, the concentrations of AMPs in tears are too low for direct antibacterial activity. Furthermore, some AMPs show a reduced antibacterial activity in the presence of tear-specific salt concentration as well as in contact with ocular mucins. However, studies have also shown an elevated secretion of AMPs under inflammatory conditions or after bacterial infection. In addition, synergistic and/or additive interactions between different AMPs and other antimicrobial compounds of the tear film have been described. These effects may compensate the low AMP concentration in tears (McDermott 2013). Animal models of bacterial keratitis have demonstrated that CRAMP (mouse homolog of LL-37) and mouse beta-defensins 2 and

3 (putative orthologs of hBD-2) are important to prevent *P. aeruginosa* infection *in vivo* (Huang et al. 2007; Wu et al. 2009). Moreover, various studies reveal that human defensins and LL-37 also stimulate migration and proliferation of corneal epithelial cells and therefore also contributing to wound healing processes. In addition, defensins and LL-37 recruit and activate various immune cells by chemotaxis and cytokine production. This contributes to an activation of the adaptive immune defense at the ocular surface. For further information, the reader is referred to the review of A. McDermott (McDermott 2013).

### 2.4.2 *Herpes Simplex Virus Keratitis*

Herpes simplex virus type 1 (HSV-1) is the most common cause of viral keratitis (Fig. 2.3) and also the most common cause of irreversible cornea-derived blindness in developed nations (Karsten et al. 2012). Worldwide, an estimated ten million persons suffer from HSV keratitis, with about two million individuals left with impaired vision (Rowe et al. 2013). The incidence lies between 5.9 and 20.7/$10^5$ of the population per year and with a prevalence of 149/$10^5$ in the developed countries (for review, see Kaye and Choudhary 2006). The initial (not necessarily primary) sites of herpetic eye involvement usually manifest as a blepharitis (infection of the lid rim), conjunctivitis, or corneal epithelial keratitis. More often, a younger age group is infected and tends to be more severely infected, especially in the developing world, where malnutrition and several diseases as well as the lack of access to treatment may be present.

As already mentioned, defensins are beside LL-37 the most abundant group of AMPs in human. Their antiviral activity already was defined in the mid-1980s. Studies of AMPs interacting with HSV-1 revealed direct inactivation of the virus by HNP1 produced by neutrophils (Daher et al. 1986). Since that time, the antiviral activity of various AMPs against HSV infection has been followed up by an

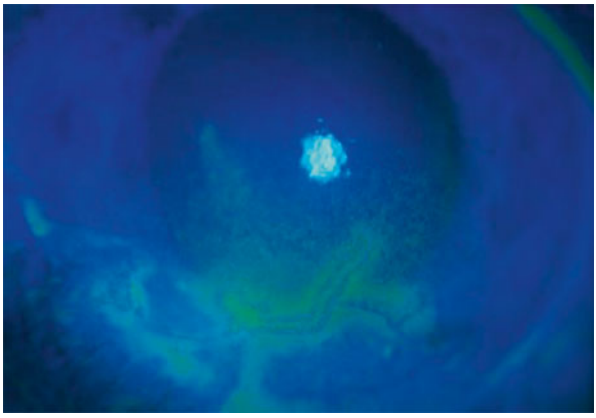

**Fig. 2.3** Herpes simplex virus (HSV) keratitis. Fluorescein staining of the cornea shows typical HSV lesions consisting of a linear branching corneal ulcer (dendritic ulcer). With kind permission of Prof. Gerd Geerling (Department of Ophthalmology, University Hospital Düsseldorf, Heinrich Heine University of Düsseldorf, Düsseldorf, Germany)

increasing level of research interest. Thus, α-defensins have been demonstrated to prevent HSV penetration through blocking viral glycoproteins as well as by binding of host cell receptors. HNP1–HNP3 prevent HSV infection through blockage of HSV surface glycoprotein B (gB) and in addition show postinfection effects, suggesting unknown effects with regard to viral replication (Hazrati et al. 2006). In contrast, HNP4 reduces HSV infection but do not bind to viral gB. This α-defensin shows high affinity against heparan sulfate, which is a preferred host cell receptor for attachment. Only hBD-3 inhibits HSV-1 infection by similar mechanisms. HBD-1 and HBD-2 show low affinity to gB and cellular glycosaminoglycans and are not able to reduce HSV infection (Hazrati et al. 2006). In cervical epithelium of the human uterus, θ-defensins from rhesus monkeys and modified derivates (retrocyclins) protect the epithelial cells from HSV infection by inhibiting viral adhesion and entry. Retrocyclin (RC)-2 has no direct HSV activity but decreases viral infection by cross-linking viral glycoproteins (Yasin et al. 2004). A prophylactic application of RC2 that was applied in a murine HSV-mediated keratitis model demonstrated reduced viral titers, reduced symptoms of blepharitis, corneal vascularization, and stromal disease. However, RC2 had no effect if it was applied after HSV infection (Brandt et al. 2007).

### *2.4.3 AMPs and Other Viral Keratitis Forms*

With regard to other frequent viral ocular surface infections such as adenovirus or vaccinia virus infection and in addition to other viruses, no data exist in a context with AMPs.

### *2.4.4 AMPs for Treating Ocular Surface Infections*

Here, the reader is referred to a very recent review by Curtis R. Brandt (Brandt 2014). This review presents an overview about antimicrobial drugs available for the treatment of ocular surface infections. In that review, it is also discussed that given the nature of peptides, topical applications are the most likely use to be successful for treating keratitis. Such peptides would be effective against drug-resistant pathogens and might act synergistically if used in combination therapy. Although hundreds of peptides with antimicrobial properties have been isolated or synthesized, only a handful have been tested so far against ocular pathogens and even fewer have been tested in animal models. The review summarizes the currently available information on the use of these peptides to treat keratitis, outlines some of the problems that have been identified, and discusses future studies that will be needed. As outlined in the review, most of the peptides that have been tested so far have shown activity at concentrations that do not warrant further development, but nevertheless, there are promising candidates such as, for example, RC2, as mentioned above, or

the coupling of acyclovir (ACV) to the 3-OS heparan sulfate (a co-receptor for HSV-1 glycoprotein) binding G2 (a M13 phage peptide) raising the possibility that peptides and peptide-drug conjugates can be developed to treat viral keratitis.

A further strategy to prevent microbial keratitis is the attachment of modified AMPs to contact lenses or contact lens cases. Contact lens-mediated microbial keratitis, in particular by *P. aeruginosa*, *Fusarium* spp., and *Acanthamoeba* (an ocular parasite), is associated with microbial adherence and biofilm formation. In the last decade, various antimicrobial strategies, such as cationic metals and peptides, selenium, quorum-sensing inhibitors, and various biocidal and non-biocidal agents, have been developed and tested with promising results in various animal models (Dutta and Willcox 2014). Covalent binding of cationic selenium to contact lenses revealed inhibition of bacterial colonization as well as reduction of acute red eye formation and bacterial ulceration in a rabbit model (Mathews et al. 2006). Fimbrolide-coated lenses (a quorum-sensing inhibitor) reduced bacterial and *Acanthamoeba* adhesion without negative ocular responses in a 1-month animal model and in an overnight human trial (Zhu et al. 2008). Moreover, contact lenses coated with melamine, a synthetic cationic hybrid AMP prepared by combining active regions of the AMPs protamine (from salmon sperm) and melittin (from bee venom), reduced adhesion to contact lenses and prevented colonization against a broad spectrum of ocular pathogens (Dutta et al. 2013). In addition, melamine-coated contact lenses showed reduced ocular inflammation and corneal infiltrates and less epithelial defects in some animal keratitis models (Cole et al. 2010).

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