Richard M. Epand *Editor*

Host Defense Peptides and Their Potential as Therapeutic Agents

Springer

Chapter 12
Host Defense Peptides and Their Advancements in Translational *Staphylococcus aureus* Research

Sarah C. Mansour, Robert E.W. Hancock and Michael Otto

Abstract *Staphylococcus aureus* is responsible for a multitude of infections ranging from skin and soft tissue infections to more severe invasive diseases. In response to *S. aureus*, host defense peptides (HDPs) are produced as nature's own sentinel effector molecules. HDPs are small, often cationic, molecules that possess numerous biological activities, such as antimicrobial activity, cellular recruitment, anti-inflammatory properties, and wound healing, all of which play a role in controlling *S. aureus* infections. In hopes of capitalizing on the powerful anti-infective functions of HDPs, there has been a considerable amount of interest in deriving HDP-based therapeutics. Here, we highlight current advancements in HDP research, constraints to commercial development, and solutions for safer and more feasible HDP-based therapies against *S. aureus*.

12.1 Staphylococcal Species and Colonization

Staphylococci are the most abundant bacterial inhabitants of the human skin microbiome. Humans are generally colonized with many different Staphylococcal species, with *S. epidermidis*, a coagulase-negative *Staphylococcus* (CoNS), being the most universal and dominant colonizer. The skin is colonized with many other CoNS species including *S. haemolyticus*, *S. saprophyticus*, *S. capitis*, *S. hominis*, *S. warneri*, *S. cohnii*, and *S. simulans* (Coates et al. 2014). Staphylococcal species differ in their ability to cause disease. Most CoNS are commensal, causing opportunistic infections in immune-compromised individuals (Otto 2010).

S.C. Mansour · R.E.W. Hancock
Centre for Microbial Diseases and Immunity Research, Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada

S.C. Mansour · M. Otto (✉)
Pathogen Molecular Genetics Section, Laboratory of Bacteriology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA
e-mail: motto@niaid.nih.gov

R.M. Epand (ed.), *Host Defense Peptides and Their Potential as Therapeutic Agents*, DOI 10.1007/978-3-319-32949-9_12

Coagulase-positive *S. aureus*, however, has received considerable attention for its ability to cause disease even in healthy individuals. Colonization is known to be a risk factor for *S. aureus* infection and 20 % of the population is persistently colonized while 30 % carry *S. aureus* transiently. *S. aureus* causes a wide spectrum of diseases ranging from mild skin and soft tissue infections (boils, abscess, impetigo) to life-threatening diseases, such as severe sepsis, pneumonia, toxic shock syndrome, and endocarditis. *S. aureus* is also capable of forming multicellular communities or recalcitrant biofilms, resulting in a high incidence of indwelling device and catheter-related infections.

Adding to its severity, *S. aureus* is a highly adaptable human pathogen. In fact, resistance to methicillin was reported in 1961, only 1 year after it was first introduced. Initially confined to hospitals and other health care facilities, methicillin-resistant *Staphylococcus aureus* (MRSA) affected mainly immune-compromised individuals. Since the mid 1990s, however, there has been an explosion in the number of MRSA cases reported in the community, affecting healthy individuals that may have not been previously exposed to the healthcare environment (Herold et al. 1998, David and Daum 2010). Moreover, the incidence of vancomycin-intermediate and vancomycin-resistant *S. aureus* (VISA and VRSA, respectively) have increased (Tiwari and Sen 2006). Alarmingly, resistance to almost all clinically available antibiotics has emerged (Waldvogel 1999; Levin et al. 2005; Gu et al. 2013).

During the past half-century, very few new antibiotic classes have been developed that can effectively target *S. aureus*. To highlight this urgency, MRSA infections have claimed more lives than human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) in the western world (Klevens et al. 2007). With the antibiotic pipeline running dry and multi-drug resistance on the rise, there has been considerable interest to exploit HDPs for the treatment of *S. aureus* infections. In this chapter, we will explore advancements in anti-*S. aureus* HDP research and HDP-directed translational breakthroughs.

12.2 Important Anti-staphylococcal HDPs

Host defense peptides (HDPs; also often referred to as antimicrobial peptides, AMPs) are an essential part of the innate defense response against *S. aureus*. HDPs are generally cationic and range in size from 12 to 50 amino acids, roughly 50 % of which are hydrophobic resulting in an overall amphipathic structure. In vitro, the bactericidal activity exhibited by HDPs is mediated by integration within the cytoplasmic membrane, resulting in pore formation and subsequent lysis.

HDPs are produced by a variety of cell types, either constitutively as a constant line of defense, or inducibly upon infection. Generally speaking, two major classes of HDPs, cathelicidins and defensins, have been described. However, other HDPs

such as RNase7 and dermcidin are important members of the constitutive cutaneous defense mechanism protecting us against initial *S. aureus* colonization.

12.2.1 Defensins

Humans express α- and β-defensins, small cysteine-rich amphipathic peptides that are 18-45 amino acid residues long and stabilized by three disulfide bonds. All defensins are initially synthesized as prepropeptides and become propeptides after cleavage of a signal peptide. Proper folding and activation of the defensin requires proteolytic cleavage of the anionic propiece, a segment of the peptide considered important for maintaining charge balance and minimizing host toxicity.

Four α-defensins (HNP1-4) are produced in the azurophilic granules of human neutrophils as well as epithelial and certain hematopoietic cells (natural killer cells and monocytes). Despite accounting for almost half of the degranulating neutrophil proteins, α-defensins do not effectively kill *S. aureus* (Lehrer 2007). Instead, certain HNPs have shown to neutralize *S. aureus* toxins. For example, HNP3 was shown to bind to both Panton–Valentine leukocidin subunits, LukS-PV, and Luk-PV, partially reducing pore formation and neutrophil lysis (Cardot-Martin et al. 2015).

In humans, four types of β-defensins have been characterized (hβD1-4), and are subcategorized based on the number and location of their disulfide bridges. hβD1 was first isolated from human plasma; and hβD2 and hβD3 were subsequently isolated from psoriatic scale extracts (Harder et al. 1997, 2001). hβ4has been identified in lung tissue and is produced by bronchial and bronchiolar epithelium (Yanagi et al. 2005). β-defensins are expressed broadly by epithelial cells such as keratinocytes as well as certain leukocytes. In most tissues, hβD1 is expressed constitutively, while expression of hβD2 and hβD3 is induced upon pro-inflammatory stimuli with cytokines, various microorganisms and microbial products as well as upon tissue injury (Menzies and Kenoyer 2006). Of all the β-defensins, hβD-3 represents the most potent anti-staphylococcal peptide, retaining the highest antimicrobial activity in vivo (Kisich et al. 2007).

β-defensin expression is mediated by recognition of pathogen-associated molecular patterns (PAMPs) by toll like receptors (TLRs). TLR2 recognizes common cell wall constituents from *S. aureus* such as peptidoglycan, lipopeptides and lipotechoic acid, and instigates a number of pro-inflammatory intracellular signaling events. Specifically, *S. aureus* activates the TLR2-mediated p38 MAPK signaling cascade, which regulates the expression of a number of inflammatory mediators (through AP-1 transcription factors) such as cytokines, chemokines and certain HDPs such as hβD2 and hβD3 (Menzies and Kenoyer 2006). Moreover, internalized *S. aureus* has shown to activate the nucleotide-binding oligomerization domain 2 (NOD-2), an intracellular receptor that recognizes muramyl dipeptide in

peptidoglycan, and may by responsible for hβD2 production by keratinocytes (Voss et al. 2006). Lastly, wounding of human skin results in the activation of the epidermal growth factor receptor, which leads to the enhanced production of hβD3 (Sorensen et al. 2006).

12.2.2 Cathelicidin

The human cathelicidin (hCAP-18) is an α-helical amphipathic cationic peptide constitutively expressed in the phagosomes of neutrophils, and inducibly in the mucosal epithelia and keratinocytes. Recent studies by Zhang et al. have shown that dermal adipocytes are also an important source of cathelicidin during cutaneous *S. aureus* infection, as mice defective in adipogenesis produced lower levels of this HDP (Zhang et al. 2015).

Upon release, hCAP-18 becomes proteolytically cleaved by proteinase 3, releasing the inactive N-terminal cathelin domain and generating the active peptide LL-37. LL-37 is 37 amino acid residues in length and is produced in a vitamin D-dependent manner. Moreover, two other cleavage products, RK-31 and KS-30 have been identified, and demonstrates even greater antimicrobial activity compared to LL-37 (Murakami et al. 2002).

12.2.3 RNAse 7

In 2002, RNase7 was first characterized in human skin as a peptide that exhibits broad-spectrum antimicrobial activity (Harder and Schroder 2002). RNAse7 is constitutively expressed in epidermis and in the stratum corneum of healthy skin, however, can be further induced upon bacterial challenge as well as stimulation by IL-1β and IFN-γ (Harder and Schroder 2002). Importantly, RNAse7 is considered an important part of the constitutive host defense, preventing colonization and infection. For example, after 2 h of *S. aureus* bacterial challenge, RNAse7 levels in the stratum corneum of human skin explants are significantly up-regulated at levels high enough to prevent *S. aureus* colonization (Simanski et al. 2010).

12.2.4 Dermcidin

Dermcidin is expressed constitutively in eccrine sweat glands and secreted into sweat onto the epidermal surface, preventing bacterial colonization and serving as an integral cutaneous defense mechanism (Schittek et al. 2001). Post-secretory

proteolytic processing of dermcidin protein (110 amino acid long) in sweat, results in the 47-aa peptide (DCD-1), 48-aa peptide (DCD-IL) or truncated peptide SSL-46, which all display broad-spectrum activity against *Escherichia coli*, *Enterococcus faecalis*, *Candida albicans* and *S. aureus* (Flad et al. 2002). Within human sweat, DCD-1 is found at concentrations (1–10 μg/mL) sufficiently high to kill microorganisms, and its activity is retained in pH and salt conditions that are characteristic of sweat (Schittek et al. 2001). Interestingly, dermcidin does not possess any homology to known HDPs. Notably, in contrast to most AMPs, it possesses a net negative charge. This suggests that the mode of action of dermcidin might be different from other HDPs that rely on electrostatic interactions. While the mode of action of dermcidin remained obscure for a long time, it has recently been shown to form pores (Song et al. 2013).

12.3 HDP-Related Diseases

A growing body of evidence has demonstrated that deficiency of certain HDPs predisposes individuals to *S. aureus* infection. For example, lower induction of hβD-3 in *S. aureus*-infected human skin explants, has been correlated to more severe *S. aureus* skin infection as well as greater susceptibility to reoccurring infection (Zanger et al. 2010). Moreover, lesions taken from individuals with atopic dermatitis (AD), a skin disease that is correlated to increased susceptibility to *S. aureus*, produced significantly lower levels of hβD-2, hβD-3 and LL-37 (Ong et al. 2002). Furthermore, reduced production of dermcidin has been shown to contribute to the propensity of AD patients to recurrent bacterial skin infections (Rieg et al. 2005). This deficiency of dermcidin in sweat is correlated to reduced antimicrobial activity against *S. aureus* in vivo (Flad et al. 2002). Interestingly, A/J mice, which are more susceptible to *S. aureus* bacteremia compared to C57BL6 mice, have polymorphisms in their defensin genes (Ahn et al. 2010). Furthermore, mice deficient of cathelicidin-related antimicrobial peptide (CRAMP) produced larger lesions when injected with Group A *Streptococcus* compared to normal littermates (Nizet et al. 2001).

Conversely, elevated levels of certain HDPs have been linked with increased resistance to *S. aureus*. For example, elevated levels of hβD-2 and LL-37 found in psoriatic lesions prevent *S. aureus* colonization (Ong et al. 2002). Moreover, high baseline levels of RNAse7 expression in the healthy skin confer protection against *S. aureus* skin infections (Zanger et al. 2009).

HDP deficiency can be attributed to an impairment of particular T cell responses. T cell responses, specifically those associated with Th17 cells, are especially important for HDP production. Th17 cytokines IL-17A and IL-22 were shown to up-regulate antimicrobial peptide expression namely, hβD-2, hβD-3 and cathelicidin, in keratinocytes (Liang et al. 2006). Further evidence that supports a role of

Th17 cells in cutaneous immunity is derived from studies, in which mice deficient in IL-17-producing epidermal γδT cells demonstrated higher susceptibility to *S. aureus* skin infection (Cho et al. 2010). In patients with autosomal dominant hyper-IgE syndrome, impaired Th17 cell differentiation, caused by a mutation in signal transducer and activator of transcription 3 (STAT3) (Renner et al. 2008), was linked to a variety of recurrent bacterial including *S. aureus* infections (Milner et al. 2008).

Furthermore, a predominance of Th2 cytokines can specifically impair the production of hβD-2 and hβD-3 (Nomura et al. 2003). Elevated levels of Th2 cytokines in skin lesions from AD patients are thought to promote *S. aureus* colonization by enhancing *S. aureus* binding to fibronectin and fibrinogen (Cho et al. 2001). Moreover, specific *S. aureus*-secreted super antigens such as enterotoxins A and B as well as toxic shock syndrome toxin-1 (TSST-1) elicit dermal infiltration of eosinophils and mononuclear cells, which skews the immune response toward a Th2 environment, exacerbating *S. aureus*-infected skin lesions in AD patients (Laouini et al. 2003).

12.4 Immune-Regulatory Roles of HDPs

Over the years, the immune-regulatory roles of HDPs have become more appreciated. HDPs exert a broad range of activities that refine host defenses to respond to infection, such as chemoattraction, suppression of pro-inflammatory mediators, and wound healing (Mansour et al. 2014). As the antimicrobial activities of many HDPs are significantly dampened at physiological conditions, some would argue that the immune-modulatory activities of HDPs are comparatively more relevant.

In fact, many of the studies highlighting antimicrobial activity are based on in vitro experiments using purified HDP extracts. Moreover, many experiments are performed under conditions (i.e., low ionic strength, neutral pH) that allow for optimal killing, while conditions that would better represent the physiological situation, such as by inclusion of divalent cations, anions, serum components, glycosaminoglycans, mucin and 150 mM NaCl, antagonize peptide activity. For example, when minimal inhibitory concentrations (MIC) assays are conducted on LL-37 under low salt conditions (often ≤20 mM NaCl), the MIC against a number of common bacteria is between 1 and 30 μg/ml. However, in the presence of more relevant ionic conditions (100 mM NaCl), the antimicrobial activity of LL-37 is 2–8 fold lower (Turner et al. 1998) with essentially no activity against *S. aureus* at concentrations as high as 100 μg/ml. Moreover, under these same conditions, the antimicrobial activity of hβD-1 and hβD2 is completely lost. However, the importance of HDPs has been validated in the aforementioned human and animal studies (See Sect. 12.3). These findings have led many to believe that the most

important function of HDPs is to refine the host responses. Here we will highlight the diverse immune-modulatory roles of HDPs that enhance host defenses against *S. aureus*.

12.4.1 Chemoattraction

Interestingly, certain HDPs and chemokines share similar structure as they are both amphipathic and cationic. In fact, an evolutionary relationship between chemokines and HDPs have been inferred based on their strong involvement in the host innate immune response (Yount and Yeaman 2006). Specifically, HDPs were shown to possess a number of chemoattractant capabilities, assisting in the recruitment of immune cells that are important for resolving infection. When produced at sufficiently high concentrations, HDPs can directly act as chemokines, whereas at lower concentrations, they can promote the release of cytokines from other leukocytes (Mansour et al. 2014). For instance, if induced at high enough concentrations, LL-37 can directly attract neutrophils and eosinophils via interactions with formyl-peptide receptors (Tjabringa et al. 2006). However, since these activities are only promoted at LL-37 concentrations above physiological conditions, it is unlikely they possess this direct activity in vivo. On the other hand, LL-37 can indirectly promote chemoattraction by stimulating epithelial cells to release IL-8, an important chemokine for neutrophils and monocytes. Similarly, when human peripheral blood mononuclear cells (PBMCs) are stimulated by LL-37 in culture, they produce neutrophil chemokine IL-8, as well as monocyte chemoattractant protein-1 (MCP-1) and MCP-3 (Davidson et al. 2004). In mice, injection of cathelicidin-related antimicrobial peptide (CRAMP) results in the recruitment of neutrophils and monocytes (Kurosaka et al. 2005). This enhanced chemokine production may have important therapeutic implications, as local application of MCP-1 has been shown to reduce *S. aureus* infection in an osteomyelitis rat model by increasing the number of neutrophils, the first responders to *S. aureus* infection (Li et al. 2010).

Human defensins display a variety of chemotactic roles inducing the migration of immature dendritic cells and lymphocytes, promoting adaptive immunity. For example, hβD2 is capable of enhancing mobility of immature dendritic and memory T cells via interactions with chemokine CCL-20 receptor CCR6 (Yang et al. 1999). Along with CCR6, β-defensins bind to chemokine receptor CCR2 expressed on monocytes, dendritic cells, and certain macrophage subsets. Importantly, CCR2-mediated recruitment of monocytes is essential for innate immune defense and clearance of bacteria in vivo (Kurihara et al. 1997; Jia et al. 2008). Moreover, HNP1 and HNP2 have been shown to serve as a chemotactic for CD4 and CD8 T cells as well as immature dendritic cells via unidentified receptors (Yang et al. 2000).

12.4.2 Wound Healing

LL-37 plays a role in the re-epithelialization of skin wounds and wound closure. Upon skin injury, high levels of LL-37 precursor protein, hCAP18, is produced in the wound bed and it has been suggested that LL-37 plays a role in epithelial cell proliferation. Similarly, low levels of LL-37 precursor protein hCAP18, have been correlated with delayed wound closure and chronic ulcers. Moreover, treatment of anti-LL-37 antibodies inhibited epithelial healing in an ex vivo wound healing model in a dose-dependent manner (Heilborn et al. 2010). Furthermore, hβD3 accelerated wound healing in an *S. aureus*-infected porcine diabetic wound.

12.4.3 Anti-inflammatory

Certain HDPs are important for protecting the host from a harmful cytokine storm that is imposed during bacterial infection. For example, LL-37 has shown to attenuate the production of pro-inflammatory mediators (IL-1β, IL-6, IL-8, and TNF-α) in neutrophils when subjected to *S. aureus* (Alalwani et al. 2010). Likewise, in PBMCs, production of anti-inflammatory IL-10 is up-regulated. This effect on cytokine release is mediated by LL-37 binding directly to an internal signaling molecule, GAPDH, impairing p38 MAPK signaling and subsequent transcription of chemokines and cytokines (Mookherjee et al. 2009). As inflammation has shown to exacerbate conditions such as pneumonia and early stage sepsis, anti-inflammatory activities of HDPs can be considered an asset provided bacterial clearance is not compromised. Fortunately, certain HDPs such as LL-37 enhance neutrophil-killing capacity by increasing *S. aureus* phagocytosis and enhancing ROS production (Alalwani et al. 2010). Similarly, mice deficient in cathelicidin-related antimicrobial peptide (CRAMP) or murine cathelicidin showed significantly less bactericidal activity against *S. aureus*.

12.5 *S. aureus* HDP Evasion Techniques

Despite the numerous anti-infective roles of HDPs, *S. aureus* can tolerate relatively high concentrations of these peptides (Peschel et al. 1999). As with other innate and adaptive immune responses, *S. aureus* has developed multiple HDP evasion mechanisms (summarized in Fig. 12.1). It is thought that cationic HDPs and HDP-resistance mechanisms have co-evolved to allow *S. aureus* to quickly adapt to these integral and ancient host defense components (Peschel et al. 1999).

S. aureus can recognize challenges by various HDPs using the antimicrobial peptide sensor (Aps) system, which induces several resistance mechanisms (Li et al. 2007a, b). One major Aps-regulated mechanism is the activation of the *dltABCD*

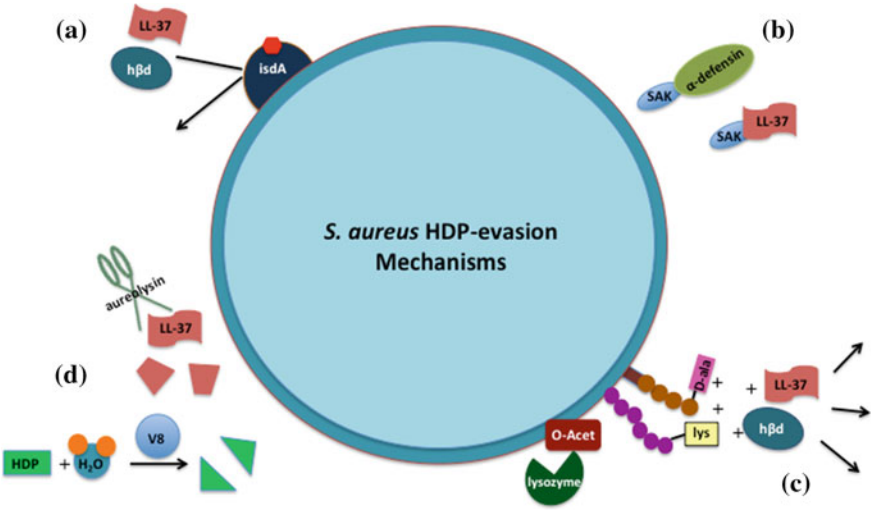

Fig. 12.1 ***S. aureus*** **HDP immune evasion mechanisms**. **a. HDP repulsion via hydrophobic surface molecules**. Cell-wall bound proteins such as IsdA, decrease bacterial hydrophobicity, resulting in increased resistance to certain HDPs. **b**. **HDP inactivity via staphylokinase (SAK) interference**. SAK has shown to form a complex with alpha-defensins, abrogating their activity. Also, SAK binds directly to cathelicidin, enhancing SAK-dependent plasminogen activation, fibrinolysis and bacterial dissemination. **c**. **Cell surface modifications repel HDPs**. Activation of the *dltABCD* operon and expression of MprF, incorporate positively charged amino acids to cell surface constituents, causing repulsion of positively charged HDPs. Steric hindrance is created via theO-acetylation of peptidoglycan muramic acid preventing lysozyme from targeting *S. aureus*. **d. Degradation of HDPs by secreted enzymes**. Aureolysin and V8 protease are two major *S. aureus*-secreted proteases that cleave and inactivate HDPs

operon, which incorporates positively charged D-alanine onto *S. aureus* anionic teichoic acids, conferring a greater positive charge and ultimately repelling HDPs such as defensins and LL-37 (Peschel et al. 1999). Moreover, to reduce anionic charge, *S. aureus* expresses multiple peptide resistance factor, MprF, which transfers lysine to anionic membrane lipid phosphatidylglycerol (PG), and translocates the lysyl-PG to the outer membrane leaflet conferring a net +1 charge and resulting in α-defensins and LL-37 repulsion (Peschel et al. 2001). Indeed, MprF contributes to clinical failures of daptomycin, the only currently available AMP therapy used to combat *S. aureus* infections (Jones et al. 2008). Interestingly, MRSA strains are more resistant to LL-37 compared to less positively net-charged methicillin-susceptible *S. aureus* (MSSA) isolates, verifying electrostatic repulsion as a major resistance mechanism (Ouhara et al. 2008).

S. aureus secretes an array of factors that contribute to resistance against the innate immune system and HDPs. LL-37 is susceptible to proteolytic degradation by two major *S. aureus*-secreted proteases, aureolysin and V8 protease. Specifically, V8 protease is involved in hydrolyzing particular peptide bonds, while aureolysin cleaves LL-37, abolishing its antimicrobial activity (Sieprawska-Lupa et al. 2004).

Furthermore, the exoprotein staphylokinase (SAK) has been shown to form a complex with alpha-defensins, abrogating the effect of these peptides in vitro and in vivo (Jin et al. 2004). *S. aureus* also exploits the production of cathelicidin as it binds directly to staphylokinase, which enhances SAK-dependent plasminogen activation and fibrinolysis, resulting in bacterial dissemination and enhanced virulence (Braff et al. 2007). Dermcidin has also been shown to induce global regulatory changes, resulting in the expression of dermcidin-degrading proteases in *S. epidermidis* and *S. aureus* (Lai et al. 2007). Furthermore, *S. aureus* expresses IsdA, a cell-wall bound heme-binding protein that decreases bacterial hydrophobicity, resulting in increased resistance to bactericidal human skin fatty acids as well as cathelicidins and β-defensins (Clarke et al. 2007).

Growth states have also been associated with increased resistance to HDPs. For example, *S. aureus* small colony variants (SCVs), which have been associated with slow growth and complex physiological and metabolic changes, are less susceptible to RNase 7, hβD2, hβD3, and LL-37 (Glaser et al. 2014).

Finally, peptidoglycan O-acetyltransferase OatO, is a major determinant of lysozyme resistance in *S. aureus*. The enzymatic activity of OatA results in the O-acetylation of the C6 hydroxyl group of muramic acid found in peptidoglycan, creating steric hindrance and preventing lysozyme binding to peptidoglycan (Bera et al. 2005).

12.6 Therapeutics

HDPs demonstrate modest direct antimicrobial activity with concomitant immune-modulatory activities, making them ideal starting points for deriving new therapies to treat multi-drug resistant *S. aureus* infections. Furthermore, while antibiotics typically impair a specific and essential bacterial process, HDPs target multiple hydrophobic and anionic bacterial targets, making it more difficult for bacteria to develop resistance. Much work has been performed to exploit the powerful activities of natural HDPs through the creation of synthetic mimetics. This has led to the creation of peptides with improved anti-infective and anti-inflammatory activities. For example, innate defense regulator-1 (IDR-1) was derived conceptually from the bovine AMP, bactenecin. In an invasive peritonitis model, IDR-1 was protective against MRSA by enhancing levels of monocyte chemokines, thereby recruiting monocytes and macrophages to combat infection. Moreover, IDR-1 reduced levels of harmful pro-inflammatory cytokine responses, which also aided in resolving the infection (Scott et al. 2007). High-throughput screening of bactenecin derivatives led to the identification of IDR-1002, a peptide that demonstrated enhanced chemokine induction and greater protection compared to IDR-1 in an *S. aureus* peritonitis murine infection model (Nijnik et al. 2010). This heightened anti-infective activity was attributed to increased recruitment of neutrophils and monocytes to the site of the infection. Synergistic therapy combining two peptides has also demonstrated efficacy in live infection models. For instance, combining

LL-37 and IDR-1ameliorated MRSA-induced pneumonia by significantly attenuating anti-inflammatory cytokine release (mainly TNF-α and IL-6) in bronchoalveolar lavage fluid, reducing pulmonary tissue damage. Furthermore, another 12-amino acid bactenecin derivative, IDR-1018, possessed very potent immune-modulatory properties through the induction of chemokine and suppression of pro-inflammatory responses. Along with these activities, IDR-1018 demonstrated accelerated wound healing in an *S. aureus*-infected porcine wound model compared to LL-37 (Steinstraesser et al. 2012). Interestingly, this activity was not conserved in an immune-compromised infection model, demonstrating the importance of a functional immune state for peptide activity. Furthermore, in an orthopedic implant *S. aureus* murine infection model, IDR-1018 accelerated the clearance of *S. aureus* by enhancing the recruitment of macrophages (Choe et al. 2015). In this model, IDR-1018 also enhanced osseointegration, which is typically impaired with *S. aureus* bone infection.

12.7 Constraints to HDP Therapeutic Development

Their numerous anti-infective roles make HDPs intriguing candidates for *S. aureus* infections. Nevertheless, there are many hurdles that must be overcome before they may become commercially available. Here, we will highlight these issues, which include toxicity, immunogenicity, susceptibility to proteolytic degradation, and cost.

12.7.1 Toxicity

Unfortunately, a major impediment for the pharmaceutical exploitation of HDPs is their toxicity. This toxicity can be due to many reasons, one of which is the potential of HDPs to stimulate immune responses, which can have unforeseen consequences. In fact, high levels of HDPs have been previously linked to diseases. For example, in humans, abnormally high levels of cathelicidin in facial skin have been linked to rosacea (Yamasaki et al. 2007). In mice, injection of LL-37 in mouse skin induced inflammatory hallmarks of rosacea, such as erythema and vascular dilatation (Yamasaki et al. 2007). This coincided with an increase in IL-8 production and neutrophil infiltration. Moreover, significantly elevated levels of LL-37 have been identified in psoriatic lesional skin (Morizane et al. 2012), where heightened levels of LL-37 were shown to increase the expression of TLR9 on keratinocytes. The enhanced TLR9 expression sensitizes keratinocytes to their ligands, CpG or genomic DNA, resulting in the increased production of type I IFNs, which exacerbate psoriasis (Morizane et al. 2012). Furthermore, LL-37 and hβD2 were shown to stimulate histamine release from mast cells (Niyonsaba et al. 2001).

Interestingly, LL-37-induced mast cell activation results in the release of β-tryptase, a LL-37 degrading enzyme (Duplantier and van Hoek 2013).

Hydrophobic cores of HDPs and synthetic mimetics have been shown to self-associate, aggregate, integrate into zwitterionic membranes, and cause hemolysis (Yin et al. 2012). Although not much is known about whether aggregation is responsible for the observed immune-modulatory effects displayed by HDPs, hydrophobicity (within a certain window) is required for bacterial membrane insertion. Therefore, these properties must be tampered with carefully, preventing peptide inactivation or increase of toxicity (Bahar and Ren 2013).

It is thought that since HDPs have been conserved throughout evolution, they should represent feasible anti-infective strategies (Mansour et al. 2014). In addition, HDPs can be altered to reduce toxicity. For instance, judicious formulation such as nanoparticle encapsulations can alter charge distribution and minimize toxic aggregation. Peptide lengths can be adjusted to minimize toxicity. For example, a shortened 15-amino acid derivative of melittin proved to be 300 times less toxic than melittin (Subbalakshmi et al. 1999). Simple high-throughput screening methodologies that measure cytolytic properties can be used to select for new peptide candidates with reduced toxicity (Haney et al. 2015). Moreover, after HDP targets are identified, computer-aided system biology approaches can be used to predict molecular pathways that may be interrupted, preventing undesirable off-targets of new HDP therapies.

12.7.2 Degradation

Another potential constraint that would reduce efficacy of HDP therapy is the risk of proteolytic degradation. As previously indicated, HDPs are inactivated and degraded by *S. aureus*-secreted enzymes and proteases (See Sect. 12.5). Furthermore, endogenous host enzymes readily degrade HDPs. Notably, digestive enzymes such as trypsin and chemotrypsin cleave peptides at basic and hydrophobic residues altering important structural and functional features of HDPs (Kim et al. 2014).

Many measures can be taken to tackle degradation issues of HDP therapy. Since host proteases cleave peptide bonds between naturally occurring residues, incorporating non-proteinogenic amino acids such as ornithine and β-didehydrophenylalanine into HDP structures can potentially improve metabolic stability (Bahar and Ren 2013). Moreover, cyclization of linear peptides has been shown to increase protease resistance of HDPs (Rozek et al. 2003). As host proteases only cleave peptides with L-enantiomeric backbones, the incorporation of D-amino acids in peptides can drastically reduce degradation, as has been shown with LL-37 (Wang et al. 2014). Presumably due to increased stability, D-amino acid peptides demonstrated greater protective activity compared to their L-counterparts against lethal multi-drug resistant bacteria (de la Fuente-Nunez et al. 2015). However, little is known regarding the pharmacokinetics and toxicity surrounding D-peptide

therapies and whether such manipulations would affect immune-modulatory targets. Furthermore, such modifications in peptide structure and composition are likely to increase the costs of peptide synthesis. Kim et al., described a more economical approach to designing peptides with residues that are systemically arranged to avoid protease-targeted sites (Kim et al. 2014). By using this method and maintaining the structural features that are important for HDP activity (amphipathy, cationic character, helicity, etc.), peptides with retained antimicrobial activity but heightened stability against host proteases could be derived.

12.7.3 Immunogenicity

As mentioned, HDPs are capable of interacting with cells of both the innate and adaptive arms of the immune system, eliciting a number of immunomodulatory roles. While these characteristics may serve as the basis for their protection, peptides may also prove to be immunogenic, stimulating an undesirable humoral response.

Immunogenicity, specifically through the development of anti-therapeutic antibodies, has been reported in a number of current FDA-approved antibody and protein-based therapies (Baker et al. 2010). Initially, these events can be triggered when the foreign peptide is recognized by an antigen-presenting cell (APC). For example, scavenging dendritic cells can phagocytose and present the foreign peptides via the exogenous MHC class II processing pathway, ultimately priming T cell responses. Capturing of antigen by dendritic cells is typically mediated by the FcR receptors that can interact with peptide-immune complexes. Indeed, LL-37 and certain defensins have also been shown to interact with TLRs on the surface of dendritic cells. These interactions can further activate the cells, resulting in the maturation and expression of lymphocyte co-stimulatory receptors initiating a T-cell dependent anti-peptide response (Yang et al. 2009). T-cell independent anti-peptide responses are speculated to be triggered by aggregated peptides that effectively cross-link B cell receptors, enabling the activation of B cells. Such responses can result in the generation of neutralizing antibodies or in some cases, antibodies that cross-react with endogenous proteins. Overall, immunogenicity may result in ineffective peptides or occasionally, autoimmune diseases.

Some proof of concept methods for reducing immunogenicity of protein therapies has involved the use of immunosuppressive therapies (Reding 2006) or slowly inducing tolerance to therapies. Furthermore, removal of T cell epitopes through rational sequence design and targeted amino acid substitutions can lower the development of immunogenicity of protein therapies (Jones et al. 2009). Immunogenicity of peptide derivatives can be assayed using various in vitro T cell assays.

12.7.4 Cost

Another constraint that has hindered mass production of HDPs for therapeutic use is the high cost of synthesis. Peptide synthesis using classic fluorenylmethoxycarbonyl (F-moc) chemistry can range between $50 and $400 per gram of peptide, making widespread clinical usage unrealistic. As peptides are antagonized by physiological conditions and degraded by host or bacterial proteases, relatively high doses may be required to attain the desired therapeutic effects. Moreover, deriving large peptide libraries (>100 peptides) to screen for optimized peptide candidates can become exorbitantly expensive.

Although cost of peptide synthesis has remained one of the major barriers to use of HDPs, several solutions have been proposed in the literature. For example, deriving synthetic HDP mimetics with shorter sequences can tackle the high cost of production. For example, efforts to re-engineer GF-17, a short and active fragment of LL-37, has led to the development of 17BIPHE2, a peptide with enhanced activity against *S. aureus*, which has also shown anti-biofilm properties in a mouse model of catheter-related *S. aureus* infection (Wang et al. 2014). Importantly, peptides must contain a minimum of 7–8 amino acids to maintain amphipathic structures, and for α-helical AMPs, 22 amino acids are required to completely span the bacterial lipid bilayer (Bahar and Ren 2013). Furthermore, exceptional anti-infective activity of synthetic mimetics can potentially lower dosage and thus cost of peptide therapy. The classical approach to optimize peptide activity is through rational design of large synthetic peptide libraries via systematic single amino acid substitutions of short HDP backbone templates (Haney et al. 2015). High-throughput screening methodologies can then select peptides that are optimized for various biological properties. For example, screening for anti-*S. aureus* biofilm activity, chemokine induction and endotoxin suppression, led to the selection of the optimized peptide IDR-2009 (Haney et al. 2015). Lastly, exploiting biological expression systems to derive recombinant fusion peptides serves as a scalable and low cost alternative for peptide synthesis (Li 2009). However, purification of peptides from prokaryotic systems must be carried out carefully to prevent endotoxin and bacterial contamination.

12.8 The Future of HDP Therapy

12.8.1 Synergistic Cocktails

Many of the aforementioned issues hindering widespread clinical use of HDPs can be tackled by administrating synergistic drug cocktails. Indeed, HDPs have previously shown to act synergistically with each other as well as with conventional antibiotics. For example, studies have demonstrated that when β-defensins are combined at sub-effective concentrations with human cathelicidin (LL-37) and

lysozyme, they exhibit additive or synergistic activity, which may be important for retaining in vivo activity (Midorikawa et al. 2003; Chen et al. 2005). Likewise, HDPs and synthetic mimetics display strong synergistic interactions with a number of conventional antibiotics (Reffuveille et al. 2014). Presumably, synergistic combination therapy would drastically lower the dosage of peptide and antibiotic required, reducing the overall cost of peptide therapy and toxicity.

12.8.2 Stimulating Natural HDP Production

Enhancing nature's own arsenal may represent a safer and more feasible method for HDP-directed therapies. Studies have demonstrated that inducing LL-37 by stimulating keratinocytes with both butyrate and vitamin D3 increases antimicrobial activity of keratinocytes against *S. aureus* (Schauber et al. 2008). Furthermore, topical treatment with the vitamin D analog, calcipotriol, following acute skin infection enhanced levels of hCAP18/LL-37 in human skin (Heilborn et al. 2010). As LL-37 has been shown to promote re-epithelialization and tissue repair, therapies that promote LL-37 production may serve as effective wound treatments. However, as previously noted (Sect. 12.7.1), LL-37 displays a wide variety of immune-regulatory activities and levels must be carefully tuned to avoid any adverse consequences.

12.8.3 Impairing CAMP Resistance Mechanisms

An interesting and unexplored avenue for HDP-targeted therapy is to inactivate CAMP resistance mechanisms to sensitize *S. aureus* to HDPs. Studies by Li et al. have shown that mutants of the Aps HDP-sensing system are less virulent in an *S. aureus* murine infection model (Li et al. 2007a, b). Likewise, inactivating Dlt reduces the D-alanine addition to teichoic acids, thereby increasing *S. aureus* susceptibility to HDPs (Peschel et al. 1999). Consistent with these findings, mutants lacking *dltA* and *mprF* are more susceptible to HDPs and have attenuated virulence in vivo (Peschel et al. 1999; Collins et al. 2002; Kristian et al. 2003; Weidenmaier et al. 2005). Moreover, targeting secreted proteases that degrade HDPs may help to enhance the activity of HDPs.

12.9 Conclusions

HDPs serve as one of the first lines of defense against *S. aureus* infections. Specifically, defensins, cathelicidins, dermcidins, and RNAse 7 display potent anti-staphylococcal activity along with a number of immune-modulatory roles.

Despite these various biological activities, *S. aureus* has developed numerous HDP evasion techniques that function for example by repelling positively charged HDPs and abrogating peptide activity. Nevertheless, many HDPs as well as synthetic peptidomimetics have demonstrated exceptional anti-*S. aureus* activity. Scalable and commercial use of HDPs or their synthetic counterparts is, however, constrained by the cost of production, toxicities, immunogenicity and degradation. The future of HDP therapeutic development relies on re-engineering peptides with greater biological activity and reduced toxicities, deriving synergistic cocktails, identifying new HDP-stimulants and sensitizing bacteria to HDPs.

References

Ahn SH, Deshmukh H, Johnson N, Cowell LG, Rude TH, Scott WK, Nelson CL, Zaas AK, Marchuk DA, Keum S, Lamlertthon S, Sharma-Kuinkel BK, Sempowski GD, Fowler VG Jr (2010) Two genes on A/J chromosome 18 are associated with susceptibility to Staphylococcus aureus infection by combined microarray and QTL analyses. PLoS Pathog 6(9):e1001088

Alalwani SM, Sierigk J, Herr C, Pinkenburg O, Gallo R, Vogelmeier C, Bals R (2010) The antimicrobial peptide LL-37 modulates the inflammatory and host defense response of human neutrophils. Eur J Immunol 40(4):1118–1126

Bahar AA, Ren D (2013) Antimicrobial peptides. Pharmaceuticals (Basel) 6(12):1543–1575

Baker MP, Reynolds HM, Lumicisi B, Bryson CJ (2010) Immunogenicity of protein therapeutics: the key causes, consequences and challenges. Self Nonself 1(4):314–322

Bera A, Herbert S, Jakob A, Vollmer W, Gotz F (2005) Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan O-acetyltransferase OatA is the major determinant for lysozyme resistance of Staphylococcus aureus. Mol Microbiol 55(3):778–787

Braff MH, Jones AL, Skerrett SJ, Rubens CE (2007) Staphylococcus aureus exploits cathelicidin antimicrobial peptides produced during early pneumonia to promote staphylokinase-dependent fibrinolysis. J Infect Dis 195(9):1365–1372

Cardot-Martin E, Casalegno JS, Badiou C, Dauwalder O, Keller D, Prevost G, Rieg S, Kern WV, Cuerq C, Etienne J, Vandenesch F, Lina G, Dumitrescu O (2015) alpha-Defensins partially protect human neutrophils against Panton-Valentine leukocidin produced by Staphylococcus aureus. Lett Appl Microbiol 61(2):158–164

Chen X, Niyonsaba F, Ushio H, Okuda D, Nagaoka I, Ikeda S, Okumura K, Ogawa H (2005) Synergistic effect of antibacterial agents human beta-defensins, cathelicidin LL-37 and lysozyme against Staphylococcus aureus and Escherichia coli. J Dermatol Sci 40(2):123–132

Cho JS, Pietras EM, Garcia NC, Ramos RI, Farzam DM, Monroe HR, Magorien JE, Blauvelt A, Kolls JK, Cheung AL, Cheng G, Modlin RL, Miller LS (2010) IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. J Clin Invest 120(5):1762–1773

Cho SH, Strickland I, Tomkinson A, Fehringer AP, Gelfand EW, Leung DY (2001) Preferential binding of Staphylococcus aureus to skin sites of Th2-mediated inflammation in a murine model. J Invest Dermatol 116(5):658–663

Choe H, Narayanan AS, Gandhi DA, Weinberg A, Marcus RE, Lee Z, Bonomo RA, Greenfield EM (2015) Immunomodulatory peptide IDR-1018 decreases implant infection and preserves osseointegration. Clin Orthop Relat Res 473(9):2898–2907

Clarke SR, Mohamed R, Bian L, Routh AF, Kokai-Kun JF, Mond JJ, Tarkowski A, Foster SJ (2007) The Staphylococcus aureus surface protein IsdA mediates resistance to innate defenses of human skin. Cell Host Microbe 1(3):199–212

Coates R, Moran J, Horsburgh MJ (2014) Staphylococci: colonizers and pathogens of human skin. Future Microbiol 9(1):75–91

Collins LV, Kristian SA, Weidenmaier C, Faigle M, Van Kessel KP, Van Strijp JA, Gotz F, Neumeister B, Peschel A (2002) Staphylococcus aureus strains lacking D-alanine modifications of teichoic acids are highly susceptible to human neutrophil killing and are virulence attenuated in mice. J Infect Dis 186(2):214–219

David MZ, Daum RS (2010) Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 23 (3):616–687

Davidson DJ, Currie AJ, Reid GS, Bowdish DM, MacDonald KL, Ma RC, Hancock RE, Speert DP (2004) The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. J Immunol 172(2):1146–1156

de la Fuente-Nunez C, Reffuveille F, Mansour SC, Reckseidler-Zenteno SL, Hernandez D, Brackman G, Coenye T, Hancock RE (2015) D-enantiomeric peptides that eradicate wild-type and multidrug-resistant biofilms and protect against lethal Pseudomonas aeruginosa infections. Chem Biol 22(2):196–205

Duplantier AJ, van Hoek ML (2013) The human cathelicidin antimicrobial peptide LL-37 as a potential treatment for polymicrobial infected wounds. Front Immunol 4:143

Flad T, Bogumil R, Tolson J, Schittek B, Garbe C, Deeg M, Mueller CA, Kalbacher H (2002) Detection of dermcidin-derived peptides in sweat by ProteinChip technology. J Immunol Methods 270(1):53–62

Glaser R, Becker K, von Eiff C, Meyer-Hoffert U, Harder J (2014) Decreased susceptibility of Staphylococcus aureus small-colony variants toward human antimicrobial peptides. J Invest Dermatol 134(9):2347–2350

Gu B, Kelesidis T, Tsiodras S, Hindler J, Humphries RM (2013) The emerging problem of linezolid-resistant Staphylococcus. J Antimicrob Chemother 68(1):4–11

Haney EF, Mansour SC, Hilchie AL, de la Fuente-Nunez C, Hancock RE (2015) High throughput screening methods for assessing antibiofilm and immunomodulatory activities of synthetic peptides. Peptides

Harder J, Schroder JM (2002) RNase 7, a novel innate immune defense antimicrobial protein of healthy human skin. J Biol Chem 277(48):46779–46784

Harder J, Bartels J, Christophers E, Schroder JM (1997) A peptide antibiotic from human skin. Nature 387(6636):861

Harder J, Bartels J, Christophers E, Schroder JM (2001) Isolation and characterization of human beta -defensin-3, a novel human inducible peptide antibiotic. J Biol Chem 276(8):5707–5713

Heilborn JD, Weber G, Gronberg A, Dieterich C, Stahle M (2010) Topical treatment with the vitamin D analogue calcipotriol enhances the upregulation of the antimicrobial protein hCAP18/LL-37 during wounding in human skin in vivo. Exp Dermatol 19(4):332–338

Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, Leitch CD, Daum RS (1998) Community-acquired methicillin-resistant Staphylococcus aureus in children with no identified predisposing risk. JAMA 279(8):593–598

Jia T, Serbina NV, Brandl K, Zhong MX, Leiner IM, Charo IF, Pamer EG (2008) Additive roles for MCP-1 and MCP-3 in CCR2-mediated recruitment of inflammatory monocytes during Listeria monocytogenes infection. J Immunol 180(10):6846–6853

Jin T, Bokarewa M, Foster T, Mitchell J, Higgins J, Tarkowski A (2004) Staphylococcus aureus resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. J Immunol 172(2):1169–1176

Jones T, Yeaman MR, Sakoulas G, Yang SJ, Proctor RA, Sahl HG, Schrenzel J, Xiong YQ, Bayer AS (2008) Failures in clinical treatment of Staphylococcus aureus Infection with daptomycin are associated with alterations in surface charge, membrane phospholipid asymmetry, and drug binding. Antimicrob Agents Chemother 52(1):269–278

Jones TD, Crompton LJ, Carr FJ, Baker MP (2009) Deimmunization of monoclonal antibodies. Methods Mol Biol 525:405–423, xiv

Kim H, Jang JH, Kim SC, Cho JH (2014) De novo generation of short antimicrobial peptides with enhanced stability and cell specificity. J Antimicrob Chemother 69(1):121–132

Kisich KO, Howell MD, Boguniewicz M, Heizer HR, Watson NU, Leung DY (2007) The constitutive capacity of human keratinocytes to kill Staphylococcus aureus is dependent on beta-defensin 3. J Invest Dermatol 127(10):2368–2380

Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridkin SK, M.I. Active Bacterial Core surveillance (2007) Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA 298(15):1763–1771

Kristian SA, Lauth X, Nizet V, Goetz F, Neumeister B, Peschel A, Landmann R (2003) Alanylation of teichoic acids protects Staphylococcus aureus against Toll-like receptor 2-dependent host defense in a mouse tissue cage infection model. J Infect Dis 188(3):414–423

Kurihara T, Warr G, Loy J, Bravo R (1997) Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. J Exp Med 186(10):1757–1762

Kurosaka K, Chen Q, Yarovinsky F, Oppenheim JJ, Yang D (2005) Mouse cathelin-related antimicrobial peptide chemoattracts leukocytes using formyl peptide receptor-like 1/mouse formyl peptide receptor-like 2 as the receptor and acts as an immune adjuvant. J Immunol 174 (10):6257–6265

Lai Y, Villaruz AE, Li M, Cha DJ, Sturdevant DE, Otto M (2007) The human anionic antimicrobial peptide dermcidin induces proteolytic defence mechanisms in staphylococci. Mol Microbiol 63(2):497–506

Laouini D, Kawamoto S, Yalcindag A, Bryce P, Mizoguchi E, Oettgen H, Geha RS (2003) Epicutaneous sensitization with superantigen induces allergic skin inflammation. J Allergy Clin Immunol 112(5):981–987

Lehrer RI (2007) Multispecific myeloid defensins. Curr Opin Hematol 14(1):16–21

Levin TP, Suh B, Axelrod P, Truant AL, Fekete T (2005) Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant Staphylococcus aureus: report of a clinical failure. Antimicrob Agents Chemother 49(3):1222–1224

Li M, Cha DJ, Lai Y, Villaruz AE, Sturdevant DE, Otto M (2007a) The antimicrobial peptide-sensing system aps of Staphylococcus aureus. Mol Microbiol 66(5):1136–1147

Li M, Lai Y, Villaruz AE, Cha DJ, Sturdevant DE, Otto M (2007b) Gram-positive three-component antimicrobial peptide-sensing system. Proc Natl Acad Sci USA 104 (22):9469–9474

Li Y (2009) Carrier proteins for fusion expression of antimicrobial peptides in Escherichia coli. Biotechnol Appl Biochem 54(1):1–9

Li B, Jiang B, Dietz MJ, Smith ES, Clovis NB, Rao KM (2010) Evaluation of local MCP-1 and IL-12 nanocoatings for infection prevention in open fractures. J Orthop Res 28(1):48–54

Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA (2006) Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med 203(10):2271–2279

Mansour SC, Pena OM, Hancock RE (2014) Host defense peptides: front-line immunomodulators. Trends Immunol 35(9):443–450

Menzies BE, Kenoyer A (2006) Signal transduction and nuclear responses in Staphylococcus aureus-induced expression of human beta-defensin 3 in skin keratinocytes. Infect Immun 74 (12):6847–6854

Midorikawa K, Ouhara K, Komatsuzawa H, Kawai T, Yamada S, Fujiwara T, Yamazaki K, Sayama K, Taubman MA, Kurihara H, Hashimoto K, Sugai M (2003) Staphylococcus aureus susceptibility to innate antimicrobial peptides, beta-defensins and CAP18, expressed by human keratinocytes. Infect Immun 71(7):3730–3739

Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, Kanno Y, Spalding C, Elloumi HZ, Paulson ML, Davis J, Hsu A, Asher AI, O'Shea J, Holland SM, Paul WE, Douek DC (2008) Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 452(7188):773–776

Mookherjee N, Lippert DN, Hamill P, Falsafi R, Nijnik A, Kindrachuk J, Pistolic J, Gardy J, Miri P, Naseer M, Foster LJ, Hancock RE (2009) Intracellular receptor for human host defense peptide LL-37 in monocytes. J Immunol 183(4):2688–2696

Morizane S, Yamasaki K, Muhleisen B, Kotol PF, Murakami M, Aoyama Y, Iwatsuki K, Hata T, Gallo RL (2012) Cathelicidin antimicrobial peptide LL-37 in psoriasis enables keratinocyte reactivity against TLR9 ligands. J Invest Dermatol 132(1):135–143

Murakami M, Ohtake T, Dorschner RA, Schittek B, Garbe C, Gallo RL (2002) Cathelicidin anti-microbial peptide expression in sweat, an innate defense system for the skin. J Invest Dermatol 119(5):1090–1095

Nijnik A, Madera L, Ma S, Waldbrook M, Elliott MR, Easton DM, Mayer ML, Mullaly SC, Kindrachuk J, Jenssen H, Hancock RE (2010) Synthetic cationic peptide IDR-1002 provides protection against bacterial infections through chemokine induction and enhanced leukocyte recruitment. J Immunol 184(5):2539–2550

Niyonsaba F, Someya A, Hirata M, Ogawa H, Nagaoka I (2001) Evaluation of the effects of peptide antibiotics human beta-defensins-1/-2 and LL-37 on histamine release and prostaglandin D(2) production from mast cells. Eur J Immunol 31(4):1066–1075

Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, Pestonjamasp V, Piraino J, Huttner K, Gallo RL (2001) Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature 414(6862):454–457

Nomura I, Goleva E, Howell MD, Hamid QA, Ong PY, Hall CF, Darst MA, Gao B, Boguniewicz M, Travers JB, Leung DY (2003) Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. J Immunol 171(6):3262–3269

Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, Gallo RL, Leung DY (2002) Endogenous antimicrobial peptides and skin infections in atopic dermatitis. N Engl J Med 347 (15):1151–1160

Otto M (2010) Staphylococcus colonization of the skin and antimicrobial peptides. Expert Rev Dermatol 5(2):183–195

Ouhara K, Komatsuzawa H, Kawai T, Nishi H, Fujiwara T, Fujiue Y, Kuwabara M, Sayama K, Hashimoto K, Sugai M (2008) Increased resistance to cationic antimicrobial peptide LL-37 in methicillin-resistant strains of Staphylococcus aureus. J Antimicrob Chemother 61(6):1266–1269

Peschel A, Jack RW, Otto M, Collins LV, Staubitz P, Nicholson G, Kalbacher H, Nieuwenhuizen WF, Jung G, Tarkowski A, van Kessel KP, van Strijp JA (2001) Staphylococcus aureus resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with l-lysine. J Exp Med 193(9):1067–1076

Peschel A, Otto M, Jack RW, Kalbacher H, Jung G, Gotz F (1999) Inactivation of the dlt operon in Staphylococcus aureus confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J Biol Chem 274(13):8405–8410

Reding MT (2006) Immunological aspects of inhibitor development. Haemophilia 12(6):30–35; discussion 35–36

Reffuveille F, de la Fuente-Nunez C, Mansour S, Hancock RE (2014) A broad-spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms. Antimicrob Agents Chemother 58(9):5363–5371

Renner ED, Rylaarsdam S, Anover-Sombke S, Rack AL, Reichenbach J, Carey JC, Zhu Q, Jansson AF, Barboza J, Schimke LF, Leppert MF, Getz MM, Seger RA, Hill HR, Belohradsky BH, Torgerson TR, Ochs HD (2008) Novel signal transducer and activator of transcription 3 (STAT3) mutations, reduced T(H)17 cell numbers, and variably defective STAT3 phosphorylation in hyper-IgE syndrome. J Allergy Clin Immunol 122(1):181–187

Rieg S, Steffen H, Seeber S, Humeny A, Kalbacher H, Dietz K, Garbe C, Schittek B (2005) Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo. J Immunol 174 (12):8003–8010

Rozek A, Powers JP, Friedrich CL, Hancock RE (2003) Structure-based design of an indolicidin peptide analogue with increased protease stability. Biochemistry 42(48):14130–14138

Schauber J, Oda Y, Buchau AS, Yun QC, Steinmeyer A, Zugel U, Bikle DD, Gallo RL (2008) Histone acetylation in keratinocytes enables control of the expression of cathelicidin and CD14 by 1,25-dihydroxyvitamin D3. J Invest Dermatol 128(4):816–824

Schittek B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, Schirle M, Schroeder K, Blin N, Meier F, Rassner G, Garbe C (2001) Dermcidin: a novel human antibiotic peptide secreted by sweat glands. Nat Immunol 2(12):1133–1137

Scott MG, Dullaghan E, Mookherjee N, Glavas N, Waldbrook M, Thompson A, Wang A, Lee K, Doria S, Hamill P, Yu JJ, Li Y, Donini O, Guarna MM, Finlay BB, North JR, Hancock RE (2007) An anti-infective peptide that selectively modulates the innate immune response. Nat Biotechnol 25(4):465–472

Sieprawska-Lupa M, Mydel P, Krawczyk K, Wojcik K, Puklo M, Lupa B, Suder P, Silberring J, Reed M, Pohl J, Shafer W, McAleese F, Foster T, Travis J, Potempa J (2004) Degradation of human antimicrobial peptide LL-37 by Staphylococcus aureus-derived proteinases. Antimicrob Agents Chemother 48(12):4673–4679

Simanski M, Dressel S, Glaser R, Harder J (2010) RNase 7 protects healthy skin from Staphylococcus aureus colonization. J Invest Dermatol 130(12):2836–2838

Song C, Weichbrodt C, Salnikov ES, Dynowski M, Forsberg BO, Bechinger B, Steinem C, de Groot BL, Zachariae U, Zeth K (2013) Crystal structure and functional mechanism of a human antimicrobial membrane channel. Proc Natl Acad Sci USA 110(12):4586–4591

Sorensen OE, Thapa DR, Roupe KM, Valore EV, Sjobring U, Roberts AA, Schmidtchen A, Ganz T (2006) Injury-induced innate immune response in human skin mediated by transactivation of the epidermal growth factor receptor. J Clin Invest 116(7):1878–1885

Steinstraesser L, Hirsch T, Schulte M, Kueckelhaus M, Jacobsen F, Mersch EA, Stricker I, Afacan N, Jenssen H, Hancock RE, Kindrachuk J (2012) Innate defense regulator peptide 1018 in wound healing and wound infection. PLoS One 7(8):e39373

Subbalakshmi C, Nagaraj R, Sitaram N (1999) Biological activities of C-terminal 15-residue synthetic fragment of melittin: design of an analog with improved antibacterial activity. FEBS Lett 448(1):62–66

Tiwari HK, Sen MR (2006) Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infect Dis 6:156

Tjabringa GS, Ninaber DK, Drijfhout JW, Rabe KF, Hiemstra PS (2006) Human cathelicidin LL-37 is a chemoattractant for eosinophils and neutrophils that acts via formyl-peptide receptors. Int Arch Allergy Immunol 140(2):103–112

Turner J, Cho Y, Dinh NN, Waring AJ, Lehrer RI (1998) Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. Antimicrob Agents Chemother 42(9):2206–2214

Voss E, Wehkamp J, Wehkamp K, Stange EF, Schroder JM, Harder J (2006) NOD2/CARD15 mediates induction of the antimicrobial peptide human beta-defensin-2. J Biol Chem 281 (4):2005–2011

Waldvogel FA (1999) New resistance in Staphylococcus aureus. N Engl J Med 340(7):556–557

Wang G, Hanke ML, Mishra B, Lushnikova T, Heim CE, Chittezham Thomas V, Bayles KW, Kielian T (2014) Transformation of human cathelicidin LL-37 into selective, stable, and potent antimicrobial compounds. ACS Chem Biol 9(9):1997–2002

Weidenmaier C, Peschel A, Kempf VA, Lucindo N, Yeaman MR, Bayer AS (2005) DltABCD- and MprF-mediated cell envelope modifications of Staphylococcus aureus confer resistance to platelet microbicidal proteins and contribute to virulence in a rabbit endocarditis model. Infect Immun 73(12):8033–8038

Yamasaki K, Di Nardo A, Bardan A, Murakami M, Ohtake T, Coda A, Dorschner RA, Bonnart C, Descargues P, Hovnanian A, Morhenn VB, Gallo RL (2007) Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. Nat Med 13(8):975–980

Yanagi S, Ashitani J, Ishimoto H, Date Y, Mukae H, Chino N, Nakazato M (2005) Isolation of human beta-defensin-4 in lung tissue and its increase in lower respiratory tract infection. Respir Res 6:130

Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schroder JM, Wang JM, Howard OM, Oppenheim JJ (1999) Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286(5439):525–528

Yang D, Chen Q, Chertov O, Oppenheim JJ (2000) Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells. J Leukoc Biol 68(1):9–14

Yang D, de la Rosa G, Tewary P, Oppenheim JJ (2009) Alarmins link neutrophils and dendritic cells. Trends Immunol 30(11):531–537

Yin LM, Edwards MA, Li J, Yip CM, Deber CM (2012) Roles of hydrophobicity and charge distribution of cationic antimicrobial peptides in peptide-membrane interactions. J Biol Chem 287(10):7738–7745

Yount NY, Yeaman MR (2006) Structural congruence among membrane-active host defense polypeptides of diverse phylogeny. Biochim Biophys Acta 1758(9):1373–1386

Zanger P, Holzer J, Schleucher R, Scherbaum H, Schittek B, Gabrysch S (2010) Severity of Staphylococcus aureus infection of the skin is associated with inducibility of human beta-defensin 3 but not human beta-defensin 2. Infect Immun 78(7):3112–3117

Zanger P, Holzer J, Schleucher R, Steffen H, Schittek B, Gabrysch S (2009) Constitutive expression of the antimicrobial peptide RNase 7 is associated with Staphylococcus aureus infection of the skin. J Infect Dis 200(12):1907–1915

Zhang LJ, Guerrero-Juarez CF, Hata T, Bapat SP, Ramos R, Plikus MV, Gallo RL (2015) Innate immunity. Dermal adipocytes protect against invasive Staphylococcus aureus skin infection. Science 347(6217):67–71