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Antimicrobial Peptidesas an Alternative Approach to Treat Bacterial Infections

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Abstract

The spread of antibiotic resistance genes amongst microbes, the emergence of multi-drug resistant bacterial pathogens and the paucity of antibiotics in development have caused a major health care crisis. With few options available to treat multi-drug resistant bacteria, it is critical to develop alternative therapies to conventional antibiotics. An auspicious alternative strategy stems from antimicrobial peptides (AMPs), which are the host's own "endogenous antibiotics". AMPs are produced at mucosal surfaces, where they exert both bactericidal and immunomodulatory activities making them important components of the innate immune system. To date, the development of AMP-based therapies has focused on developing synthetic peptides with tailored activity and boosting endogenous AMP-expression. These therapies may be confounded by the multiple bacterial AMP-resistance mechanisms that have arisen during the co-evolution of bacteria with their hosts' innate immune system. Therefore, approaches that counteract bacterial AMP-resistance mechanisms can be added to the arsenal of novel therapies. This review provides an overview of human AMPs and summarizes the current strategies used to develop AMP-based therapies with particular focus on a novel strategy that aims to boost AMP activity by inhibiting bacterial AMP-resistance mechanisms.

Keywords: Antibiotic resistance; Infectious diseases; Innate immunity; Antimicrobial peptides; Cathelicidins; Defensins

Introduction

The rise and spread of antibiotic resistance among bacterial pathogens has created a major health care crisis. Among Gram-positive bacteria, the incidence of methicillin-resistant *Staphylococcus aureus* exceeds 50% in most countries [1]. Vancomycin-resistant enterococci have spread over the last 20 years to become a major cause of nosocomial infections. In Gram-negative pathogens, production of extended spectrum β-lactamasesis reported in 5-8% of *Escherichia coli* and *Klebsiella pneumoniae* isolated in North America and can reach more than 30% in some countries [2]. Until recently, carbapenem was the antibiotic of choice to treat these infections. However, carbapenem resistance mediated by carbapenemases, such as the New Delhi metallo-β-lactamase 1, arose and spread internationally [3]. The therapeutic options to treat these infections are limited to very few antibiotics such as colistin and tigecycline, which are not necessarily optimal therapies because of toxicity or tissue penetration issues. Since there are very few new antibiotics in the drug development pipeline, especially for the treatment of Gram-negative infections, it becomes critical to develop alternatives to classical antibiotics and identify new approaches to treat infectious diseases.

Antimicrobial peptides (AMPs) are critical components of the host innate immune system that serve as "endogenous antibiotics" [4,5]. Most organisms produce AMPs, including bacteria, fungi, plants, insects and vertebrates. They are multifunctional molecules with antimicrobial activity against bacteria, fungi, viruses and protozoan parasites; they also have numerous immunomodulatory functions. Some AMPs also exhibit antitoxic activity; they neutralize bacterial toxins, including lipid A of lipopolysaccharide (LPS) [6,7]. AMPs are also able to prevent biofilm formation and act on pre-formed biofilms [8]. Mature AMPs are small (<50 amino acid residues), positively charged (+1 to +11) and have amphipathic properties. They are diverse in amino acid sequence and adopt various secondary structures. In mammals, there are two major groups of AMPs, the α-helical cathelicidins and the β-sheet defensins. Both cathelicidins and defensins are found in large amounts in neutrophil granules and are expressed at mucosal surfaces, where they are synthesized as inactive prepropeptides that are processed into biologically active peptides by host proteases [9,10].

The Cathelicidins

The number of cathelicidin genes varies between mammalian species. Humans and mice have a single cathelicidin gene (human *CAMP* and murine *Camp*). Human *CAMP* encodes hCAP18, an 18 kDa inactive precursor, which upon secretion is processed into the N-terminal cathelin prodomain and the C-terminal LL-37 active peptide [11]. LL-37, a 37 amino acid peptide with two leucines at the N-terminus, adopts an amphipathic α-helical structure. Murine *Camp* encodes the precursor of the murine cathelicidin-related antimicrobial peptide (mCRAMP) [12]. Mature mCRAMP consists of a 34 amino acid peptide that also adopts an amphipathic α-helical structure [13]. Several host proteases are responsible for cathelicidin maturation in a tissue-specific manner. For example, LL-37 is cleaved from its precursor hCAP-18 by proteinase-3 in neutrophils and proteases of the kallikrein family in skin [14,15]. Further proteolytic processing of LL-37 by various proteases, including bacterial proteases, may lead to peptide fragments with different biological functions [16]. The key role of human and murine cathelicidins in the outcome of bacterial infections *in vivo* is supported by a number of studies and was facilitated by the generation of *Camp*^{-/-} mice [17-19]. Several external signals affect cathelicidin gene expression. For example, vitamin D_3 induces the expression of LL-37 in several cell types by interacting with its receptor that activates the

vitamin D3-responsive elements (VRE) of the *CAMP* promoter [20,21]. The level of induction of *CAMP* expression by vitamin D_3 is partially dependent on the inflammatory status of the tissue [22]. Conversely, the promoter of the murine cathelicidin gene lacks VREs and its expression is not regulated by vitamin D_3 [20]. Butyrate is a short fatty acid produced in the colon by the microbiota through fermentation of dietary fibers. Butyrate and its derivative phenylbutyrate induce cathelicidin expression and were shown to improve outcome in a rabbit model of *Shigella* infection [23,24].

The Defensins

Mature defensins are characterized by the presence of six cysteines forming three intramolecular disulfide bonds. Based on the connectivity of these disulfide bonds, defensins are further divided into α- and β-defensins. In contrast to cathelicidins, there are many defensin genes in humans and mice. In humans, five *DEFA* genes code for α-defensins, the human neutrophil peptides 1-4 (HNP-1 through-4) that are present in the azurophilic granules of neutrophils and human defensins 5 and 6 (HD-5 and -6) that are secreted in the small intestine by Paneth cells [25]. Although there are close to forty β-defensin genes in humans, only four β-defensin peptides have been well characterized (hBD-1 through -4). These genes show extensive copy-number polymorphism [26]. Human β-defensins are found at most mucosal epithelia and tissues. While hBD-1 is constitutively expressed in most tissues, the expression of hBD-2 and hBD-3 is induced by the presence of infectious stimuli and/or cytokines [27-29]. Similar to the LL-37 promoter, VREs are also found in the hBD-2 gene promoter region, but hBD-2 induction by vitamin D_3 is more modest than for LL-37 [30]. Several studies have highlighted the important role played by defensins *in vivo*. Transgenic mice expressing the human α-defensin HD-5 showed enhanced resistance to intestinal infection with *Salmonella enterica*, providing strong evidence that HD-5 restricts bacterial growth *in vivo* [31]. In addition, HD-5 was also shown to shape the composition of the small intestine microbiota [32].

Mechanisms of Action of AMPs

The mechanisms of action of AMPs are diverse and in some cases AMP specific. AMPs have both direct bactericidal and immunomodulatory activities. Most AMPs appear to exert their bactericidal activity by interacting with the negatively charged bacterial membrane through electrostatic interactions and then forming pores into the cytoplasmic membrane, which leads to bacterial cell lysis. Several studies have suggested alternative targets such as intracellular proteins or lipid II and the cell-wall biosynthesis pathway [33,34]. The *in vivo* bactericidal activity of AMPs has been questioned due to the facts that AMP-mediated killing is salt sensitive and that the AMP concentration at most sites is below the minimum inhibitory concentration. Nonetheless, it is most likely that the abundance of α-defensins inside phagocytes and in crypts of the small intestine allows direct bacterial killing. Some AMPs like hBD-1 and HD-6 are known for their weak bactericidal activity. A recent study has shown that the bactericidal activity of hBD-1 is enhanced upon reduction of the peptide, a process that may occur in vivo due to the thioredoxin system [35]. Another recent study revealed that HD-6 affords protection against invasion by enteric bacterial pathogens by binding to bacterial surface proteins and forming fibrils that entangle bacteria [36].

In addition to their direct antibacterial activity, AMPs participate in multiple aspects of immunity [37-39]. In respect to their immunological functions, AMPs are also known as host-defense peptides [40]. By interacting with a variety of host cell receptors, AMPs promote the recruitment of leukocytes to the site of infection through both direct chemotactic activity and stimulation of chemokine production by leukocytes, epithelial cells and other cell types [41,42]. AMPs also modulate host responses to microbial compounds, inducing both pro- and anti-inflammatory signals; for example LL-37 inhibits TNFα production and other host responses to LPS [43]. AMPs can impact the adaptive immune response by influencing antigen presentation, cell recruitment, and by modulating B- and T-cell responses [41,44-46]. Finally, some AMPs also play a role in angiogenesis and wound healing [47,48].

Bacterial Resistance to Host AMPs

During the co-evolution of hosts and microbes, bacteria have developed several strategies to resist and survive the activities of AMPs [49-51]. Since bacterial AMP-resistance could potentially confound therapeutic approaches using AMPs, learning more about the mechanisms at play becomes important, particularly in pathogens of importance to human health. Both Gram-positive and negative bacteria are able to sense the presence of AMPs in the environment through two-component regulatory systems. For example, *Salmonella enterica* and *Streptococcus pyogenes* use the PhoPQ and CsrRS two-component systems, respectively, to sense LL-37 [52,53]. Activation of these signaling pathways by AMPs results in the upregulation of genes associated with AMP resistance. These AMP resistance mechanisms can be grouped according to their mode of action into shielding structures, proteases, surface charge alterations, ABC transporters, and modulation of AMP gene expression.

Shielding of the bacterial cell surface

Surface structures external to the bacterial cell envelope, such as capsule polysaccharides (CPS), play a role in AMP resistance. They are proposed to act as a protective shield binding AMPs and reducing the amount of AMPs that reaches the bacterial membrane [54-57]. Specifically, anionic CPS of both Gram-positive and negative bacteria bind cationic AMPs to promote resistance [55]. CPS are not the only bacterial shielding structures, curli fimbriae from uropathogenic *Escherichia coli* and the M1 protein from group A Streptococci bind LL-37 to promote resistance [58,59].

Proteolytic degradation of AMPs

Both Gram-positive and negative bacteria produce membrane-bound and/or secreted proteases that can degrade and inactivate AMPs. For example, the outer-membrane protease OmpT of enterohemorrhagic *E. coli* (EHEC) cleaves and inactivates the α-helical LL-37 but cannot cleave HNP-1, which is stabilized by disulfide bonds [60,61]. Secreted proteases, such as the Zn^{++}-metalloprotease ZapA from *Proteus mirabilis* degrades LL-37 and hBD-1, whereas aureolysin from *S. aureus* inactivates LL-37, indicating specific substrate preference for AMPs [62,63].

Charge alterations

Bacteria minimize their interaction with AMPs by reducing the net negative charge of their membrane. Due to the basic physiological differences in the composition of the Gram-positive and negative bacterial cell walls, they use different mechanisms to reduce their net negative charge. In Gram-positive bacteria, the net negative charge of the cell wall is decreased by the addition of D-alanine to the phosphates of teichoic acids using a process that occurs nearly ubiquitously among species [49]. In Gram-negative bacteria, the phosphate groups present on the lipid A and core moieties of LPS are responsible for the net

negative charge of the outer-membrane. To neutralize the net negative charge of LPS, the lipid A and core are covalently modified with positively charged moieties that mask the phosphate groups, thereby preventing AMP binding [64,65].

ABC transporters

ABC transporters are general transport systems that are used to import or export a variety of substrates across the membranes of both Gram-positive and negative bacteria. In Gram-positive bacteria, these transporters have developed a unique relationship with two-component signal transduction systems to promote greater AMP resistance [66]. In Gram-negative bacteria, the import and/or export activities of ABC transporters are used by various species to resist killing by AMPs. For example, the MtrC export-type transporter is used by *Neisseria gonorrhoeae* and *Haemophilus ducreyi* to resist hBDs and LL-37, whereas the SapA import-type transporter is used by *Haemophilus influenzae* to deliver AMPs into the cytosol where they are degraded by proteases and recycled as nutrients [67,68].

Downregulation of AMP gene expression

Some pathogens actively suppress expression of AMP genes by host cells. For example, *Shigella flexneri* inhibits the expression of LL-37 in intestinal epithelial cells [69,70]. Similarly, *Helicobacter pylori* selectively inhibits the expression of hBD-3, which is particularly active against *H. pylori*, through a mechanism involving the virulence factor CagA that interferes with cell signaling upon translocation into host cells [71].

Therapeutic Approaches

Inhibition of bacterial resistance to AMPs

A novel approach to maximize the effects of AMPs would be to sensitize microbes to endogenous AMPs by inhibiting bacterial AMP resistance to promote the antimicrobial activities of AMPs. The main challenge is to identify the specific AMP resistance mechanisms used by a given pathogen in its infectious niche. For example, the closely related intestinal pathogens EHEC and enteropathogenic *E. coli* (EPEC) that colonize the large and small intestines, respectively, use different mechanisms to resist the AMPs present in their niches [60,61]. EHEC expresses high levels of the OmpT protease that cleaves and inactivates LL-37 produced in the colonic epithelium [60]. In contrast, EPEC produces CPS to shield its surface from the bactericidal activity of the α-defensin HD-5, the most abundant AMP present in the small intestine [Unpublished data]. The OmpT protease and related proteases of the omptin family are targets of choice to test this novel therapeutic approach. The enzyme active site faces the environment and is readily accessible to drugs. In addition, omptins have a unique catalytic mechanism and no specific inhibitors are currently available. Therefore, inhibition of omptins is unlikely to interfere with host protease activity. Inhibition of omptins is likely to result in increased levels of LL-37, which will enhance both the bactericidal and immunomodulatory activities of this AMP. However, it is unclear whether inhibition of a single target like OmpT will be sufficient to sensitize the pathogen to AMPs. Given the variety of resistance mechanisms, it may be necessary to inhibit several bacterial targets at once to give the host an advantage. The simultaneous inhibition of several AMP-resistance mechanisms can also be achieved by targeting the two-component signal transduction systems that regulate AMP resistance gene expression. For example, small molecules could turn off two-component systems that detect AMPs and, in turn, decrease expression of AMP-resistance

mechanisms. For this strategy to be successful, more study into the complex regulatory networks controlling expression of AMP-resistance mechanisms is required to select the most appropriate target.

Increase the synthesis of endogenous AMPs

The finding that compounds such as short-fatty acids, especially butyrate, and vitamin D$_3$ are able to increase the production of LL-37 by host cells has provided an alternative or complementary approach to promote the antimicrobial activities of endogenous AMPs. Both sodium butyrate and vitamin D$_3$ are being tested in clinical trials [72,73]. Remarkably, butyrate treatment was shown to induce LL-37 expression and reduce inflammation in the rectal epithelium of patients affected by Shigellosis [73].

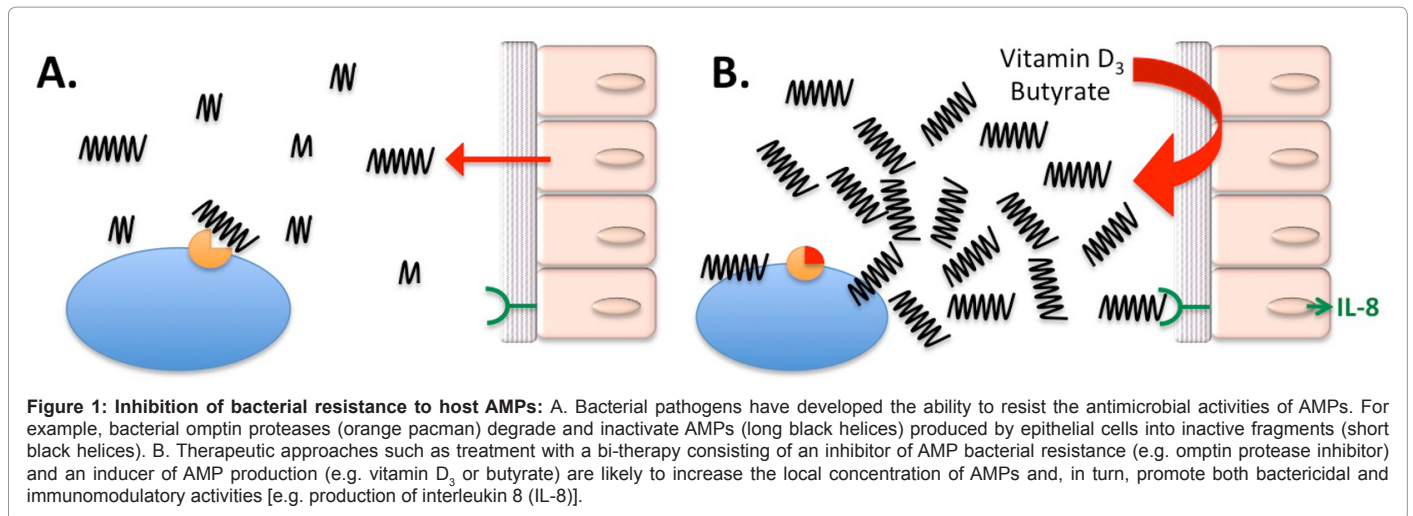### Synthetic bactericidal peptides

A number of synthetic peptides with potent bactericidal activity against both Gram-negative and positive bacteria were developed from natural AMPs. They were used as topical agents allowing the achievement of bactericidal concentrations and ensuring that peptides were minimally exposed to proteases. Several of these compounds went through clinical trials. Omiganan is a derivative of bovine indolicidin that was originally isolated from neutrophil granules. In phase III clinical trials, omiganan showed significant reduction of catheter-associated infections, but was dropped for further development [74]. Omiganan is currently in phase III trials for treatment of papulopustular rosacea. Another example is pexiganan, a synthetic analog of magainin isolated from frog skin that was used for the topical treatment of diabetic foot ulcer infections. Although Pexiganan demonstrated some efficacy, the Federal Drug Administration (FDA) did not approve it because of its lack of superiority over other therapies [75]. However, recent changes in the FDA regulations will bring pexiganan through new clinical trials [74].

Innate defense regulators (IDRs)

IDRs are synthetic peptides that were designed based on bovine bactenecin to have immunomodulatory properties and to be devoid of direct bactericidal activity [76]. Interestingly, these peptides selectively enhance innate immune mechanisms such as chemokine production and the recruitment of neutrophils and macrophages to the site of infection. Consequently, IDRs protect mice against various bacterial infections [77,78]. In addition, IDRs have been shown to favor wound healing [79] and promote increased survival of animals in a murine model of cerebral malaria [80]. These studies emphasize the importance of the immunomodulatory properties of AMPs.

Current Clinical Use of AMPs

The production of AMPs is not limited to multicellular organisms; bacteria can also synthesize AMPs that are active against other bacteria. These AMPs of bacterial origin that consist of non-ribosomally synthesized peptides such as polymyxins, bacitracin and gramicidins and ribosomally synthesized peptides such as bacteriocins, have been used for years [81,82]. Polymyxin E (also known as colistin) is a cyclic lipopetide produced by *Bacillus polymixa* [83]. Since 1959, polymyxin E has been used for the treatment of infections caused by Gram-negative bacteria. It was replaced by aminoglycosides in the 1980s, because of concern about toxicity. Polymyxin E has reemerged over the last 15 years and is currently one of the last-resort drugs for treatment of multi-drug resistant *Enterobacteriaceae*, *Pseudomonas* spp. and *Acinetobacter* spp. [84]. Gramicidin S, a cyclic decapeptide produced by *Bacillus brevis*, has been used as a topical antibiotic against Gram-positive bacteria

Figure 1: Inhibition of bacterial resistance to host AMPs: A. Bacterial pathogens have developed the ability to resist the antimicrobial activities of AMPs. For example, bacterial omptin proteases (orange pacman) degrade and inactivate AMPs (long black helices) produced by epithelial cells into inactive fragments (short black helices). B. Therapeutic approaches such as treatment with a bi-therapy consisting of an inhibitor of AMP bacterial resistance (e.g. omptin protease inhibitor) and an inducer of AMP production (e.g. vitamin D_3 or butyrate) are likely to increase the local concentration of AMPs and, in turn, promote both bactericidal and immunomodulatory activities [e.g. production of interleukin 8 (IL-8)].

since 1946 [85]. Nisin is a bacteriocin produced by *Lactococcus lactis* that acts primarily against Gram-positive bacteria and has been used safely as a food preservative for over fifty years [86].

Concluding Remarks

Although AMPs play a critical role in defending the host against potential infections, these defenses are sometimes overwhelmed by pathogens. It is possible to consider strategies aimed at boosting innate defenses and more specifically at promoting the various activities of AMPs. AMPs and their derivatives can be used in different ways to boost innate defenses and clear infections. They can be used alone as bactericidal and/or immunomodulatory agents, in combination with conventional antibiotics, or as endotoxin neutralizing compounds. In addition, bacterial pathogens may be sensitized to AMP killing by inhibiting their AMP-resistance mechanisms. This requires the characterization of the bacterial virulence factors involved in AMP resistance. These strategies are not mutually exclusive and can be combined to achieve resolution of infections. For example, inhibition of bacterial resistance to AMPs can be combined with the induction of AMP expression to enhance the local concentration of AMPs, this will promote both bactericidal and immunomodulatory effects of AMPs (Figure 1). The combination of AMPs with conventional antibiotics may also be considered. For example, bacteria living in biofilms may be sensitized to antibiotics by the biofilm-dispersing property of AMPs.

Nonetheless, there are a number of challenges or unknowns that currently impede the *in vivo* use of AMPs. Although it was suggested that AMPs are less likely than conventional antibiotics to induce resistance, several recent studies show that bacteria can rapidly develop AMP resistance mechanisms [87-89]. A major concern with synthetic peptides is their susceptibility to proteases. Several strategies including the use of D-amino acids, cyclisation and acylation were shown to increase AMP stability [90]. However, caution should be exerted when trying to stabilize AMPs, as this could have undesired consequences due to their multiple properties. Despite these challenges, the development of AMP-based therapies is a promising avenue that requires further exploration.

References

1. Otto M (2012) MRSA virulence and spread. Cell Microbiol 14: 1513-1521.

2. Pitout JD, Laupland KB (2008) Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 8: 159-166.

3. Nordmann P, Poirel L, Walsh TR, Livermore DM (2011) The emerging NDM carbapenemases. Trends Microbiol 19: 588-595.

4. Zasloff M (2002) Antimicrobial peptides of multicellular organisms. Nature 415: 389-395.

5. Hancock RE, Sahl HG (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 24: 1551-1557.

6. Rosenfeld Y, Papo N, Shai Y (2006) Endotoxin (lipopolysaccharide) neutralization by innate immunity host-defense peptides. Peptide properties and plausible modes of action. J Biol Chem 281: 1636-1643.

7. Kirikae T, Hirata M, Yamasu H, Kirikae F, Tamura H, et al. (1998) Protective effects of a human 18-kilodalton cationic antimicrobial protein (CAP18)-derived peptide against murine endotoxemia. Infect Immun 66: 1861-1868.

8. Overhage J, Campisano A, Bains M, Torfs EC, Rehm BH, et al. (2008) Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun 76: 4176-4182.

9. Tongaonkar P, Golji AE, Tran P, Ouellette AJ, Selsted ME (2012) High fidelity processing and activation of the human alpha-defensin HNP1 precursor by neutrophil elastase and proteinase 3. PLoS One 7: e32469.

10. Ghosh D, Porter E, Shen B, Lee SK, Wilk D, et al. (2002) Paneth cell trypsin is the processing enzyme for human defensin-5. Nat Immunol 3: 583-590.

11. Gudmundsson GH, Agerberth B, Odeberg J, Bergman T, Olsson B, et al. (1996) The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. Eur J Biochem 238: 325-332.

12. Gallo RL, Kim KJ, Bernfield M, Kozak CA, Zanetti M, et al. (1997) Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. J Biol Chem 272: 13088-13093.

13. Pestonjamasp VK, Huttner KH, Gallo RL (2001) Processing site and gene structure for the murine antimicrobial peptide CRAMP. Peptides 22: 1643-1650.

14. Sørensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, et al. (2001) Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood 97: 3951-3959.

15. Yamasaki K, Schauber J, Coda A, Lin H, Dorschner RA, et al. (2006) Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. FASEB J 20: 2068-2080.

16. Murakami M, Lopez-Garcia B, Braff M, Dorschner RA, Gallo RL (2004) Postsecretory processing generates multiple cathelicidins for enhanced topical antimicrobial defense. J Immunol 172: 3070-3077.

17. Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, et al. (2001) Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature 414: 454-457.

18. Chromek M, Slamová Z, Bergman P, Kovács L, Podracká L, et al. (2006) The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. Nat Med 12: 636-641.

19. Tai EK, Wu WK, Wang XJ, Wong HP, Yu L, et al. (2013) Intrarectal administration of mCRAMP-encoding plasmid reverses exacerbated colitis in Cnlp(-/-) mice. Gene Ther 20: 187-193.

20. Gombart AF, Borregaard N, Koeffler HP (2005) Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J 19: 1067-1077.

21. Hertting O, Holm Å, Lüthje P, Brauner H, Dyrdak R, et al. (2010) Vitamin D induction of the human antimicrobial Peptide cathelicidin in the urinary bladder. PLoS One 5: e15580.

22. Schrumpf JA, van Sterkenburg MA, Verhoosel RM, Zuyderduyn S, Hiemstra PS (2012) Interleukin 13 exposure enhances vitamin D-mediated expression of the human cathelicidin antimicrobial peptide 18/LL-37 in bronchial epithelial cells. Infect Immun 80: 4485-4494.

23. Steinmann J, Halldórsson S, Agerberth B, Gudmundsson GH (2009) Phenylbutyrate induces antimicrobial peptide expression. Antimicrob Agents Chemother 53: 5127-5133.

24. Sarker P, Ahmed S, Tiash S, Rekha RS, Stromberg R, et al. (2011) Phenylbutyrate counteracts Shigella mediated downregulation of cathelicidin in rabbit lung and intestinal epithelia: a potential therapeutic strategy. PLoS One 6: e20637.

25. Lehrer RI, Lu W (2012) α-Defensins in human innate immunity. Immunol Rev 245: 84-112.

26. Linzmeier RM, Ganz T (2005) Human defensin gene copy number polymorphisms: comprehensive analysis of independent variation in alpha- and beta-defensin regions at 8p22-p23. Genomics 86: 423-430.

27. O'Neil DA, Porter EM, Elewaut D, Anderson GM, Eckmann L, et al. (1999) Expression and regulation of the human beta-defensins hBD-1 and hBD-2 in intestinal epithelium. J Immunol 163: 6718-6724.

28. Zhao C, Wang I, Lehrer RI (1996) Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells. FEBS Lett 396: 319-322.

29. 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: 5707-5713.

30. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, et al. (2004) Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 173: 2909-2912.

31. Salzman NH, Ghosh D, Huttner KM, Paterson Y, Bevins CL (2003) Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin. Nature 422: 522-526.

32. Salzman NH, Hung K, Haribhai D, Chu H, Karlsson-Sjöberg J, et al. (2010) Enteric defensins are essential regulators of intestinal microbial ecology. Nat Immunol 11: 76-83.

33. Schneider T, Kruse T, Wimmer R, Wiedemann I, Sass V, et al. (2010) Plectasin, a fungal defensin, targets the bacterial cell wall precursor Lipid II. Science 328: 1168-1172.

34. Brogden KA (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol 3: 238-250.

35. Schroeder BO, Wu Z, Nuding S, Groscurth S, Marcinowski M, et al. (2011) Reduction of disulphide bonds unmasks potent antimicrobial activity of human β-defensin 1. Nature 469: 419-423.

36. Chu H, Pazgier M, Jung G, Nuccio SP, Castillo PA, et al. (2012) Human α-defensin 6 promotes mucosal innate immunity through self-assembled peptide nanonets. Science 337: 477-481.

37. Nijnik A, Hancock RE (2009) The roles of cathelicidin LL-37 in immune defences and novel clinical applications. Curr Opin Hematol 16: 41-47.

38. Lai Y, Gallo RL (2009) AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol 30: 131-141.

39. Bowdish DM, Davidson DJ, Hancock RE (2006) Immunomodulatory properties of defensins and cathelicidins. Curr Top Microbiol Immunol 306: 27-66.

40. Bowdish DM, Davidson DJ, Hancock RE (2005) A re-evaluation of the role of host defence peptides in mammalian immunity. Curr Protein Pept Sci 6: 35-51.

41. Davidson DJ, Currie AJ, Reid GS, Bowdish DM, MacDonald KL, et al. (2004) The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. J Immunol 172: 1146-1156.

42. Nijnik A, Pistolic J, Wyatt A, Tam S, Hancock RE (2009) Human cathelicidin peptide LL-37 modulates the effects of IFN-gamma on APCs. J Immunol 183: 5788-5798.

43. Mookherjee N, Brown KL, Bowdish DM, Doria S, Falsafi R, et al. (2006) Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37. J Immunol 176: 2455-2464.

44. Lillard JW Jr, Boyaka PN, Chertov O, Oppenheim JJ, McGhee JR (1999) Mechanisms for induction of acquired host immunity by neutrophil peptide defensins. Proc Natl Acad Sci U S A 96: 651-656.

45. Kin NW, Chen Y, Stefanov EK, Gallo RL, Kearney JF (2011) Cathelin-related antimicrobial peptide differentially regulates T- and B-cell function. Eur J Immunol 41: 3006-3016.

46. Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, et al. (1999) Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286: 525-528.

47. Heilborn JD, Nilsson MF, Kratz G, Weber G, Sørensen O, et al. (2003) The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. J Invest Dermatol 120: 379-389.

48. Koczulla R, von Degenfeld G, Kupatt C, Krötz F, Zahler S, et al. (2003) An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. J Clin Invest 111: 1665-1672.

49. Koprivnjak T, Peschel A (2011) Bacterial resistance mechanisms against host defense peptides. Cell Mol Life Sci 68: 2243-2254.

50. Nizet V (2006) Antimicrobial peptide resistance mechanisms of human bacterial pathogens. Curr Issues Mol Biol 8: 11-26.

51. Gruenheid S, Le Moual H (2012) Resistance to antimicrobial peptides in Gram-negative bacteria. FEMS Microbiol Lett 330: 81-89.

52. Bader MW, Sanowar S, Daley ME, Schneider AR, Cho U, et al. (2005) Recognition of antimicrobial peptides by a bacterial sensor kinase. Cell 122: 461-472.

53. Gryllos I, Tran-Winkler HJ, Cheng MF, Chung H, Bolcome R 3rd, et al. (2008) Induction of group A Streptococcus virulence by a human antimicrobial peptide. Proc Natl Acad Sci U S A 105: 16755-16760.

54. Campos MA, Vargas MA, Regueiro V, Llompart CM, Albertí S, et al. (2004) Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. Infect Immun 72: 7107-7114.

55. Llobet E, Tomás JM, Bengoechea JA (2008) Capsule polysaccharide is a bacterial decoy for antimicrobial peptides. Microbiology 154: 3877-3886.

56. Ilg K, Endt K, Misselwitz B, Stecher B, Aebi M, et al. (2009) O-antigen-negative Salmonella enterica serovar Typhimurium is attenuated in intestinal colonization but elicits colitis in streptomycin-treated mice. Infect Immun 77: 2568-2575.

57. Nagy G, Danino V, Dobrindt U, Pallen M, Chaudhuri R, et al. (2006) Down-regulation of key virulence factors makes the Salmonella enterica serovar Typhimurium rfaH mutant a promising live-attenuated vaccine candidate. Infect Immun 74: 5914-5925.

58. Kai-Larsen Y, Lüthje P, Chromek M, Peters V, Wang X, et al. (2010) Uropathogenic Escherichia coli modulates immune responses and its curli fimbriae interact with the antimicrobial peptide LL-37. PLoS Pathog 6: e1001010.

59. Lauth X, Von Kockritz-Blickwede M, McNamara CW, Myskowski S, Zinkernagel AS, et al. (2009) M1 protein allows group A streptococcal survival in phagocyte extracellular traps through cathelicidin inhibition. J Innate Immun 1: 202-214.

60. Thomassin JL, Brannon JR, Gibbs BF, Gruenheid S, Le Moual H (2012) OmpT outer membrane proteases of enterohemorrhagic and enteropathogenic Escherichia coli contribute differently to the degradation of human LL-37. Infect

Immun 80: 483-492.

61. Thomassin JL, Brannon JR, Kaiser J, Gruenheid S, Le Moual H (2012) Enterohemorrhagic and enteropathogenic Escherichia coli evolved different strategies to resist antimicrobial peptides. Gut Microbes 3: 556-561.

62. Belas R, Manos J, Suvanasuthi R (2004) Proteus mirabilis ZapA metalloprotease degrades a broad spectrum of substrates, including antimicrobial peptides. Infect Immun 72: 5159-5167.

63. Sieprawska-Lupa M, Mydel P, Krawczyk K, Wójcik K, Puklo M, et al. (2004) Degradation of human antimicrobial peptide LL-37 by Staphylococcus aureus-derived proteinases. Antimicrob Agents Chemother 48: 4673-4679.

64. Miller SI, Ernst RK, Bader MW (2005) LPS, TLR4 and infectious disease diversity. Nat Rev Microbiol 3: 36-46.

65. Gunn JS (2008) The Salmonella PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. Trends Microbiol 16: 284-290.

66. Gebhard S (2012) ABC transporters of antimicrobial peptides in Firmicutes bacteria - phylogeny, function and regulation. Mol Microbiol 86: 1295-1317.

67. Shelton CL, Raffel FK, Beatty WL, Johnson SM, Mason KM (2011) Sap transporter mediated import and subsequent degradation of antimicrobial peptides in Haemophilus. PLoS Pathog 7: e1002360.

68. Shafer WM, Qu X, Waring AJ, Lehrer RI (1998) Modulation of Neisseria gonorrhoeae susceptibility to vertebrate antibacterial peptides due to a member of the resistance/nodulation/division efflux pump family. Proc Natl Acad Sci U S A 95: 1829-1833.

69. Islam D, Bandholtz L, Nilsson J, Wigzell H, Christensson B, et al. (2001) Downregulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator. Nat Med 7: 180-185.

70. Sperandio B, Regnault B, Guo J, Zhang Z, Stanley SL Jr, et al. (2008) Virulent Shigella flexneri subverts the host innate immune response through manipulation of antimicrobial peptide gene expression. J Exp Med 205: 1121-1132.

71. Bauer B, Pang E, Holland C, Kessler M, Bartfeld S, et al. (2012) The Helicobacter pylori virulence effector CagA abrogates human Î²-defensin 3 expression via inactivation of EGFR signaling. Cell Host Microbe 11: 576-586.

72. Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, et al. (2011) High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 377: 242-250.

73. Raqib R, Sarker P, Mily A, Alam NH, Arifuzzaman AS, et al. (2012) Efficacy of sodium butyrate adjunct therapy in shigellosis: a randomized, double-blind, placebo-controlled clinical trial. BMC Infect Dis 12: 111.

74. Fox JL (2013) Antimicrobial peptides stage a comeback. Nat Biotechnol 31: 379-382.

75. Lipsky BA, Holroyd KJ, Zasloff M (2008) Topical versus systemic antimicrobial therapy for treating mildly infected diabetic foot ulcers: a randomized, controlled, double-blinded, multicenter trial of pexiganan cream. Clin Infect Dis 47: 1537-1545.

76. Easton DM, Nijnik A, Mayer ML, Hancock RE (2009) Potential of immunomodulatory host defense peptides as novel anti-infectives. Trends Biotechnol 27: 582-590.

77. Scott MG, Dullaghan E, Mookherjee N, Glavas N, Waldbrook M, et al. (2007) An anti-infective peptide that selectively modulates the innate immune response. Nat Biotechnol 25: 465-472.

78. Nijnik A, Madera L, Ma S, Waldbrook M, Elliott MR, et al. (2010) Synthetic cationic peptide IDR-1002 provides protection against bacterial infections through chemokine induction and enhanced leukocyte recruitment. J Immunol 184: 2539-2550.

79. Steinstraesser L, Hirsch T, Schulte M, Kueckelhaus M, Jacobsen F, et al. (2012) Innate defense regulator peptide 1018 in wound healing and wound infection. PLoS One 7: e39373.

80. Achtman AH, Pilat S, Law CW, Lynn DJ, Janot L, et al. (2012) Effective adjunctive therapy by an innate defense regulatory peptide in a preclinical model of severe malaria. Sci Transl Med 4: 135ra64.

81. Hancock RE, Chapple DS (1999) Peptide antibiotics. Antimicrob Agents Chemother 43: 1317-1323.

82. Cotter PD, Hill C, Ross RP (2005) Bacteriocins: developing innate immunity for food. Nat Rev Microbiol 3: 777-788.

83. Kumazawa J, Yagisawa M (2002) The history of antibiotics: the Japanese story. J Infect Chemother 8: 125-133.

84. Michalopoulos AS, Karatza DC (2010) Multidrug-resistant Gram-negative infections: the use of colistin. Expert Rev Anti Infect Ther 8: 1009-1017.

85. GAUSE GF (1946) Gramicidin S. Lancet 2: 46.

86. Cleveland J, Montville TJ, Nes IF, Chikindas ML (2001) Bacteriocins: safe, natural antimicrobials for food preservation. Int J Food Microbiol 71: 1-20.

87. Napier BA, Burd EM, Satola SW, Cagle SM, Ray SM, et al. (2013) Clinical use of colistin induces cross-resistance to host antimicrobials in Acinetobacter baumannii. MBio 4: e00021-00013.

88. Perron GG, Zasloff M, Bell G (2006) Experimental evolution of resistance to an antimicrobial peptide. Proc Biol Sci 273: 251-256.

89. Habets MG, Rozen DE, Brockhurst MA (2012) Variation in Streptococcus pneumoniae susceptibility to human antimicrobial peptides may mediate intraspecific competition. Proc Biol Sci 279: 3803-3811.

90. Fjell CD, Hiss JA, Hancock RE, Schneider G (2011) Designing antimicrobial peptides: form follows function. Nat Rev Drug Discov 11: 37-51.