Therapeutic Potential of Host Defense Peptides in Antibiotic-resistant Infections

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Abstract: The emergence of infections caused by multi-drug resistant (MDR) pathogens pose a major burden to modern healthcare. Exacerbating this issue is the substantial decline in development of new classes of antibiotics by pharmaceutical companies. This has led to renewed interest in the therapeutic potential of natural anti-infective agents such as host defense peptides (HDPs). The broad antimicrobial and immunomodulatory activities of HDPs and their synthetic derivatives, coupled with the fact that they do not readily induce microbial resistance, makes them extremely valuable leads in the development of new treatment strategies for MDR infections. This review examines our knowledge of the mechanisms behind multi-drug resistance as well as the properties of HDPs and their therapeutic potential, especially in the case of MDR infections. Challenges to their development as new therapeutics are also discussed.

Keywords: Host-defense peptide, multi-drug resistant bacteria, immunomodulatory, immunity, infection, therapeutic, chemoattractant, inflammation.

INTRODUCTION

Major medical advances in prevention and treatment of infectious diseases have vastly improved quality of life and significantly increased life expectancy. This is largely due to the discovery and development of antibiotics. The discovery of penicillin in 1928 ushered in the golden age of antibiotic innovation (1940s-1960s), during which the majority of antibiotic families in use today were identified [1]. Widespread and often inappropriate exploitation of these antibiotics has resulted in the emergence of multi-drug resistant (MDR) pathogens. Infections caused by MDR pathogens are a major burden to modern healthcare as a result of high morbidity/mortality rates, and the higher treatment cost of MDR infections [2]. Compounding this issue is the fact that the current rate of antibiotic development has declined substantially, with only six new antibiotics, none of which were truly innovative, approved since 2003 [3]. Pharmaceutical drug discovery and development is a lengthy (8-12 years) and expensive ($400–$800 million per approved agent) process, with many regulatory hurdles and low success rates [1, 4-7]. Anti-infective agents are generally used in short-course treatment of acute infections and there are significant market restrictions placed on newly developed anti-infectives. Thus, pharmaceutical companies looking for the greatest return on investment have shifted their focus to the development of drugs for chronic conditions such as hypertension, depression and arthritis [4].

Recent antibiotic research, especially target-based research, utilizing genomics, combinatorial chemistry and high-throughput screening, has not been as productive as expected. This led to a push to re-examine the therapeutic potential of natural compounds and their derivatives and to explore new concepts and approaches. Cationic host defense peptides (HDPs) and their synthetic derivatives constitute one such class of compounds, exhibiting broad-range anti-infective activity through both direct and adjunctive (immunomodulatory) action.

HDPs are produced by virtually all forms of life and are an evolutionarily ancient and apparently essential component of the host innate immune response to infectious agents. There have been more than 1200 HDPs identified or predicted, approximately 940 of which are found in eukaryotic organisms. A comprehensive list of HDPs can be found at http://aps.unmc.edu/AP/main.php [8, 9]. The broad range anti-infective activity of HDPs stems from the diversity of their functions including direct microbial killing; endotoxin neutralization; immune cell recruitment; modulation of pro-cytokine/chemokine production; suppression of potentially harmful inflammation; induction of cell differentiation; and enhancement of adaptive immune responses, cell survival, wound healing and angiogenesis. Interestingly, antimicrobial HDPs have retained their anti-infective activities over millions of years with few pathogens developing resistance mechanisms to them, while the broad immunomodulatory activities are not liable to resistance development, making them extremely attractive candidates for novel therapeutics targeting MDR pathogens. This review explores our current knowledge of HDPs and their synthetic derivatives as well as their potential use as novel therapeutics to combat MDR pathogens. Challenges facing their development are also discussed.

MULTI-DRUG RESISTANT BACTERIA

Until the 1960's, numerous classes of antibiotics (both natural and synthetic) were introduced into clinical practice, including sulfa drugs, β-lactams, phenyl propaniods, polyketides, aminoglycosides, macrolides, and quinolones. However, the rapid development of resistance to these drugs upon their introduction was under appreciated. Less than a year after the first-generation penicillin was used to treat *Staphylococcus* infections, penicillin-resistant *Staphylococcus aureus* strains were discovered. With few exceptions, the introduction of each new antibiotic has been followed, within a few years, by the first cases of resistance.

Over the last two decades, an alarming number of MDR pathogens have been identified [10]. For example, methicillin-resistant *S. aureus* (MRSA) is not only resistant to methicillin but often to aminoglycosides, macrolides, tetracycline, chloramphenicol and lincosamides [11]. MRSA is currently a major source of hospital-acquired infections associated with significant morbidity and mortality [12]. Although MRSA infections are treatable with vancomycin, vancomycin resistant-MRSA strains have been reported [13]. Strains of several other pathogens exhibit resistance to multiple or essentially all available antibacterial agents, notably *Enterococcus faecium, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.; known as the ESKAPE' pathogens [10]. Data published in 2004 by the US National Nosocomial Infection Surveillance (NNIS) System reported a substantial increase, from 1992to 2004, in the resistance rate and prevalence of multidrug resistant Gram-negative pathogens among healthcare-associated infections, especially ICUs. The NNIS also reported that 5.8% and nearly 21% of *Escherichia coli* and *K.*

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pneumoniae isolates, respectively, were resistant to third-generation cephalosporins [14].

Multidrug resistance in bacteria is generated by various mechanisms, including acquisition of resistance plasmids, transposons or integrons with genes encoding for resistance to specific antimicrobial agents, and/or induction due to mutation of regulatory loci controlling for example increased expression of multidrug efflux pumps that extrude a wide range of drugs [11]. Owing to the target specificity of each antibiotic, bacteria can become resistant through mutations that make the target less susceptible. For example, mutations in target enzymes, such as DNA topoisomerases, have been frequently identified in fluoroquinolone-resistant bacteria [15]. Resistance can also develop for non-proteinaceous targets, whereby an altered substrate structure is found in the resistant strain. For example, vancomycin binds to the lipid-linked disaccharide-pentapeptide, which is a precursor of cell wall peptidoglycan and consequently inhibits bacterial cell wall biosynthesis. In some vancomycin-resistant bacteria the substrate for vancomycin (the terminal two amino acids of the stem pentapeptide), D-Ala-D-Ala, is changed to D-Ala-D-lactic acid, reducing the binding affinity of vancomycin to its substrate [16]. In addition, bacteria may acquire, from other species, genes that encode less susceptible target proteins [17]. For example, MRSA expresses an alternative methicillin-resistant penicillin binding protein called PBP-2A. The gene for PBP-2A is located in the chromosome on a so-called genomic island that may have originated from *Staphylococcus sciuri* [18]. Bacteria may also acquire plasmids with genes encoding antibiotic inactivating enzymes; β-lactams such as penicillins and cephalosporins can be inactivated by plasmid-borne or derepressed chromosomal β-lactamases [19] Additional resistance mechanisms include reduced drug access to the target by acquiring genes encoding for drug-specific active efflux pumps [20]. As well, porin deficiency has been identified in some Gram-negative bacteria and appears to prevent influx of many antibiotics [21].

Multidrug resistance is not limited to bacteria, but can also be observed in other pathogens including parasites, viruses, and fungi. There are reports of widespread resistance to earlier generation antimalarial drugs, such as chloroquine and sulfadoxin-pyrimethamine in most malaria-endemic countries [22]. The emergence of resistance is also an increasing concern for treatment of HIV infections [23]; and due to acquired resistance to antifungal therapies, higher mortality and/or morbidity rates from invasive mycosis have been observed [24, 25].

The return to the pre-antibiotic era is rapidly becoming a reality in many parts of the world [26] and new strategies to prevent the spread of potentially lethal multi-drug resistant pathogens are urgently needed. One strategy garnering a lot of attention is the development of enhanced derivatives of the natural anti-infective molecules, HDPs, as novel therapeutics.

PHYSICAL PROPERTIES OF HOST DEFENSE PEPTIDES

Structure

Essentially all species of life produce HDPs, with individual organisms producing not only different classes of peptides but also a number of variants within each class. These peptides exhibit enormous diversity in sequence, size and secondary structures, however there are some common physical characteristics that define HDPs. They are generally between 12 and 50 amino acids in length with a predominance of basic over acidic amino acids, conferring a net positive charge of +2 to +9 [27]. In addition, approximately 50% of the amino acids are hydrophobic. The presence of basic and hydrophobic amino acids promotes folding of linear HDPs into amphipathic secondary structures upon contact with lipid bilayers [28].The secondary structures adopted by HDPs are used as the basis of their classification with four structural classes having been established. The two most common classes are β-sheet (e.g. defensins) and α-helical (eg. cathelicidins, magainin) peptides. Less common are looped peptides (e.g. bactenecin) and extended structures rich in arginine, glycine, histidine, proline, and/or tryptophan (e.g. indolicidin) [8, 29]. Structure-function studies suggest that certain biological properties of HDPs are dependent on specific structural characteristics.

The biological activities of HDPs are initiated through their interaction with cellular membranes. In their antibacterial action, first, they bind to the negatively charged surface of bacterial membranes then, through hydrophobic and electrostatic interactions, insert into the membrane. Thus the charge, hydrophobicity and amphipathicity are important to HDP function and explain why initial HDP structure-function studies focused on these characteristics in relation to *in vitro* antimicrobial activity [30-34]. In addition, the disulfide bonds found in the β-sheet and β-hairpin classes are essential for biological activity through stabilization of secondary structure [30, 35, 36]. For example the chemotactic and antimicrobial activity of the human β-defensin 3 (HBD-3) appears to be dependent on correct disulfide bond formation and peptide folding [30, 35]. It should be stated that the *in vitro* antimicrobial activity examined in these studies is likely to often have a limited physiological role due to the high inhibitory mono- and di- valent cation concentrations and polyanionic glycosaminoglycans and mucins found in biological fluids *in vivo* [37-39]. Nevertheless in circumstances in which these peptides are present in very high concentrations such as in phagocytic granules, in the immediate vicinity of degranulating phagocytes, and in the crypts of the intestine it seems likely that their direct antimicrobial activity could influence the outcome of infections. At lower concentrations the immunomodulatory activities might predominate as these are less affected by physiological salt concentrations.

To gain greater insight into the structure-function relationships behind HDP antimicrobial activity, Cherkasov *et al.* used the bovine cathelicidin bactenecin as the initial template to iteratively generate two large 9-amino acid peptide libraries with enhanced biological activity [40]. Experimental data generated from these two libraries was used in conjunction with machine learning quantitative structure activity (QSAR) approaches (discussed below), to relate atomic-resolution physical-chemical descriptors of HDPs to antimicrobial activity. Using this approach, the antimicrobial activities of 100,000 virtual peptides were effectively identified. Based on this study it is clear that the structure-function relationship is far more complex than once thought as the peptides predicted to be the most active were nearly identical in charge, hyrdophobicity and amphipathicity to the inactive peptides.

There have been far fewer *in vitro* studies into the structural requirements for immunomodulation, likely owing to the large number of immunomodulatory activities each HDP possesses. One *in vitro* study identified the N-terminal portion of the human cathelicidin LL-37 (residues 1-13) as associated with its chemotactic activity and ability to oligomerize [41, 42]. QSAR methodology has also been exploited to generate synthetic HDPs with enhanced immunomodulatory activities, known as Innate Defense Regulators (IDRs). For example, IDR-1, IDR-1002 and IDR-1018 were loose derivatives of Bac2A, a linear derivative of bactenecin that possesses both antimicrobial and immunomodulatory activities [43, 44]. Structure-function studies revealed that IDRs such as IDR-1018 adopt different conformations, depending the makeup of the membrane of target cells. In the presence of bacterial model membranes and mammalian model membranes, IDR-1018 adopts a β-turn and an α-helical conformation, respectively. In the presence of mammalian model membranes Bac2A also adopts an α-helical conformation however, its mean residue ellipticity differs from IDR-1018, suggesting that IDR-1018 is capable of stronger membrane interactions and is therefore likely to be more effective at translocating into cells [44, 45]. This may explain why IDRs are better immunomodulators, since many immunomodulatory activities depend on the ability of HDPs to traverse the plasma membrane and

interact with specific intracellular targets and receptors such as sequestosome-1, a key intracellular receptor of IDR-1 [46]. Further structural studies are required to determine if the ability to adopt α-helical conformations is a major factor in immunomodulatory activity and to identify other physical characteristics that regulate the immunomodulatory properties of peptides.

Expression

HDPs are encoded as individual genes, with HDP families often clustered together within the genome. For example the human α- and β-defensin genes co-localize to chromosome 8p21-23, while the sole cathelicidin LL-37 is found on chromosome 3p21.3 [9]. Regulation of gene expression varies by individual peptides, species, tissue, cell type, and differentiation state of a cell. Their expression is coordinately regulated at the transcriptional and post-translational levels and can be constitutive or up-regulated by inflammatory/immune stimuli such as immune mediators (e.g. cytokines) pathogens, or pathogen components (e.g. bacterial lipopolysaccharide) . HDPs are synthesized as inactive pro-forms, likely for intracellular storage purposes. During or after secretion they are proteolytically cleaved, releasing the biologically active peptide; thus the activity of HDPs is not only dependent on gene expression but also on the presence of specific proteases at the site of HDP secretion [9].

Despite substantial variation in HDP expression patterns, sequence, size, secondary structures, and anatomical site of production, all HDPs possess one or both of the two major functions discussed below, immunomodulation or direct microbicidal activity,.

Mechanisms of Action

I. Antimicrobial Activities

When investigating the direct antimicrobial activity of HDPs it is appropriate to refer to them as antimicrobial peptides (AMPs); which represent a promising lead for new, effective treatments for microbial diseases. AMPs initially attracted attention as alternative antibiotic candidates due to their ability to destabilize bacterial, fungal and possibly viral membranes [27], but the complexity of these molecules and their heterogeneity of action are now increasingly appreciated Fig. (**1**). Thus AMPs have been called 'dirty drugs' since they disrupt many biological processes in the target microbe with modest potency rather than acting on a specific target with high-affinity, reducing the selective pressure for *de novo* resistance development [47]. Virtually all AMPs are directly antimicrobial *in vitro* under appropriate conditions (e.g. in buffer or dilute medium). However, under physiological conditions, the direct bactericidal activity is often antagonized, as discussed above[48]. For example, LL-37 is protective against bacterial infections *in vivo* and exhibits direct bactericidal activity in phosphate buffer but does not reduce the bacterial load in physiologically relevant tissue culture medium [49].

Despite identification of an ever-growing number of AMPs (both natural and synthetic), their mode of action is only slowly being elucidated. The direct antimicrobial activity of HDPs is largely attributed to their ability to fold into structures that have both cationic and hydrophobic domains [47], although studies with synthetic peptides have revealed that the physical determinants of activity are very complex [40]. The overall net positive charge ensures initial attraction with and accumulation at the polyanionic microbial cell surface and insertion into anionic membranes of the Gram-negative and Gram-positive bacteria [47]. To reach the cytoplasmic membrane of bacterial cells, HDPs must cross the outer membrane and peptidoglycan of Gram-negative bacteria or the thicker peptidoglycan that contains associated anionic polymers such as lipoteichoic acid in Gram-positive bacteria. Since the interaction of cationic peptides with the outer membrane of Gram-negative bacteria is better understood, it will be described here. Following the initial binding of HDPs to the outer membrane, the peptides initiate their own passage across the outer membrane *via* the 'self-promoted uptake' mechanism [50]. The peptides possess greater affinity for the negatively charged surface LPS than native divalent cations, such as Ca^{2+} and Mg^{2+}, and as a result, the divalent cations are displaced, causing areas of disruption in the outer membrane. This enables the translocation across the outer membrane of the peptides, which can then associate with the phospholipids of the cytoplasmic membrane. Initially, the peptides insert at the interface between phospholipid head groups of the outer leaflet and the fatty acyl tails in parallel to the plane of the membrane [51]. Once the number of interacting peptides reaches a critical level, the peptides are proposed to aggregate or associate, and perturb the membrane bilayer. There is substantial disagreement as to what happens next; several hypotheses, including the barrel stave, torroidal pore, carpet and aggregate models (see refs [52-54] for an overview of these mechanisms), aim to explain peptide action on bacterial membranes in terms of lysis or membrane busting. However there is an increasing appreciation that although membrane busting does occur in model systems at high enough peptide concentrations, it is unlikely to be the actual mechanism in many cases [47].

Indeed we propose that membrane perturbation likely leads to one of (at least) three possible fates: (a) perturbation of membrane associated functions such as peptidoglycan biosynthesis, [55] energy generation, [56] cell division etc, (b) disruption of the physical integrity of the bilayer, through membrane thinning, transient poration and/or disruption of the barrier function [57] and/or (c) translocation across the cytoplasmic membrane to act on various cyto-

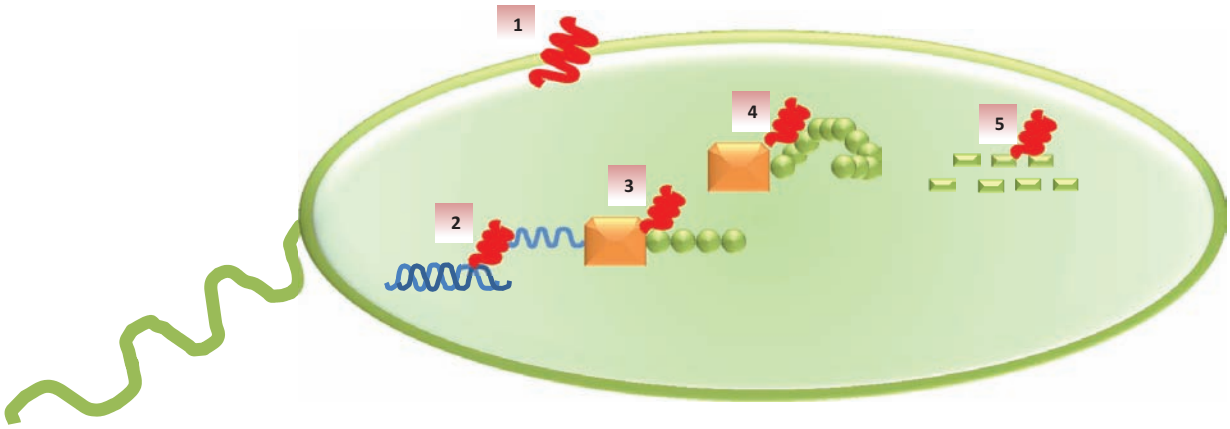

Fig. (1). Antimicrobial activity of host defence peptides (HDPs). The figure represents the different antimicrobial mechanisms of action exhibited by HDPs, such as disruption of the cytoplasmic membrane (1) or interaction with intracellular targets and disruption of cellular processes including DNA/RNA synthesis (2), interfering with protein synthesis (3), protein folding (4) and cell wall synthesis.

plasmic targets [47]. While the various models can explain the ability of cationic peptides to disrupt the cytoplasmic membrane, the aggregate model further describes how the peptides can cross the membrane to act on cytoplasmic targets [47]. It is worth mentioning that many peptides are also able to freely translocate across the membranes of eukaryotic cells and have properties that strongly resemble cell penetrating peptides [58].

An increasing number of HDPs have been suggested to act on internal targets, either as their major mechanism of action or in addition to membrane perturbation [47]. For example, HBD-3 and plectasin, a fungal defensin, act by binding to the bacterial cell-wall precursor Lipid II to inhibit cell wall biosynthesis [55, 59]. Other proposed cytoplasmic and/or membrane targets of peptides include DNA/RNA synthesis, condensation of intracellular DNA, protein synthesis/folding, cell wall synthesis/integrity and cell division [47, 60]. Pyrrhocoricin and its analogues interact with DnaK, a heat-shock protein involved in chaperone-assisted protein folding, inhibiting its ATPase activity, which results in accumulation of misfolded proteins and cell death [61].

Additionally, certain cationic HDPs at sub-inhibitory concentrations can also prevent bacterial biofilm formation, which is strongly implicated in the development of chronic infections. Established biofilms protect bacteria from host defense mechanisms and antibiotic therapy [62, 63]. Studies by Overhage *et al*. and Hell *et al*. demonstrated that at concentrations well below its minimum inhibitory concentration, LL-37 strongly inhibited biofilm formation and had an effect on existing *P. aeruginosa* and *S. epidermidis* biofilms [64, 65]. Overhage *et al*. tested a range of peptides and found this selective action against biofilms occurred only with certain peptides, e.g. with bovine indolicidin but not polymyxin B or bovine bactenecin.

HDPs have also been studied for their antiviral activity against a wide spectrum of viruses. Peptides can inhibit viruses through a wide variety of mechanisms involving almost every part of the viral life cycle [66]. For example, Dermaseptin directly interacts with the HIV virion, disrupting its membrane [67]. Antiviral peptides such as bovine and human lactoferricin can block viral entry by interacting with glycosaminoglycans on mammalian cell surfaces, specifically heparin sulfate, which are important for viral attachment [68, 69]. The spread of virions from one cell to a neighboring cell across tight junctions can also be inhibited by antiviral peptides such as the rabbit α-defensin NP-1, which inhibits cell-to-cell spread of HSV [70]. Antiviral peptides have also been demonstrated to block viral entry or attachment by interacting directly with specific viral receptors on the host cell and glycoproteins in the viral envelope. For example, the polyphemusin analogue T22 binds to the chemokine receptor, CXCR4, the T-cell surface protein CD4 and the viral envelope protein gp120 to inhibit binding and fusion of the HIV envelope with the host cell membrane [71, 72]. Antiviral peptides may also interact with and permeabilize the host cell membrane to interfere with viral entry. For instance, an eight-residue cyclic DL-α-peptide lowers the pH in endocytic vesicles, which inhibits entry of adenovirus particles through this pathway [73]. One major mechanism for the action of such peptides was revealed by studies that showed that antiviral peptides, such as LL-37 and human and bovine lactoferricin, can translocate across the plasma and nuclear membranes of host cells [74, 75]. Consequently, the internalized peptides could enhance host cell antiviral mechanisms [49]or block viral gene/protein expression.

Magainins and cecropins were among the first HDPs reported to display anti-parasitic activities [76], with magainin-2 causing swelling and eventual rupture of *Paramecium caudatum* [77]. Since then, anti-parasitic activities have been reported for numerous natural and synthetic HDPs [78, 79]. The underlying mechanism appears to be membrane disruption [80]. Due to differences in membrane composition, anti-parasitic peptides can exhibit a higher affinity for the membranes of infected erythrocytes than for normal erythrocytes [81, 82]. Hemolytic peptides induce hemoglobin leakage and parasite starvation while non-hemolytic peptides enter infected host cells and bind to the parasitic membrane [22]. Once bound, the peptides can disrupt the plasma membrane of the parasite modifying the parasite's membrane properties (e.g. charge and fluidity of lipid and protein components) to interfere with their normal functions and following internalization, disrupt essential biological processes [80, 82, 83].

An increasing number of antifungal peptides have been reported that induce cell lysis, interfere with cell wall synthesis, inhibit cellular energization and depolymerize the actin cytoskeleton [66]. Moerman *et al.* demonstrated that α-helical pore-forming peptides isolated from scorpion venom exhibited antifungal activities *via* permeabilization of fungal membranes [84]. Pn-AMP1, a small cysteine-rich peptide, caused depolymerization of the actin cytoskeleton in *Saccharomyces cerevisiae* and *C. albicans* [85]. Antifungal peptides can also enter the cytoplasm; the candidicidal activity of the glycine-rich peptide tenecin-3 is not a result of membrane permeabilization or depolarization but involves peptide entry into the cytoplasm [86].

II. Immunomodulatory Activities

The innate immune system is the first line of defense against microorganisms and is evolutionarily quite conserved among invertebrate and vertebrate animals. It is recognized as the most ancient arm of the immune system and its effectiveness is characterized by a rapid and effective response, intended to quickly eliminate threats. HDPs are an important component of the innate immune system. They are produced by many cells including epithelial and immune cells [87-89], acting at the site of infection in an autocrine and/or paracrine manner. HDPs can exhibit robust immunomodulatory activities and/or have direct antimicrobial activity Fig. (**2**). However as mentioned above, under physiological conditions their antimicrobial activities are considerably dampened which is not the case for their selective immunomodulatory activities, suggesting that these are the more likely explanation for their protective effects against microbes *in vivo* [37, 49]. These selective immunomodulatory capabilities make HDP the starting point for novel therapies against multi-drug resistant infections, acting on the host to boost protective immunity while modulating excessive inflammation [90]. Some of the most relevant immunomodulatory functions displayed by HDP are described below.

Chemotactic Activity

The presence of microorganisms, induces the local production and/or release of chemo-attractant (chemotactic) agents such as chemokines and HDPs, which are capable, with varying efficiency, of recruiting immune cells to the site of infection. HDPs also induce the expression of a broad range of chemokines such as CXCL8/IL8, CCL2/MCP1by neutrophils, monocytes and other immune cells [91, 92].These direct and indirect chemotactic activities are a conserved ability among HDPs, making this a strategic tool for screening and selecting for improved synthetic immunomodulatory HDPs (IDRs). This method was recently used to select three IDRs developed in our laboratory, IDR-1, IDR-1002 and IDR-1018 [93], which demonstrated and enhanced ability to induce chemokines in human peripheral blood mononuclear cells (PBMC). These activities also appear to underlie their protective effects in bacterial infections including those caused by MDR strains [94, 95].

Anti-endotoxin Activity

Lipopolysacharide (LPS), the outer membrane component of Gram-negative bacteria also known as endotoxin, is a classical inducer of systemic inflammation and deadly syndromes like sepsis [96]. Many antibiotics stimulate the release of endotoxin, enhancing the occurrence of systemic inflammation and consequent sepsis [97]. HDPs possess the capacity to dampen production of endotoxin-induced pro-inflammatory mediators such as tumor necrosis factor alpha (TNF-α) by blocking or modulating toll like receptor

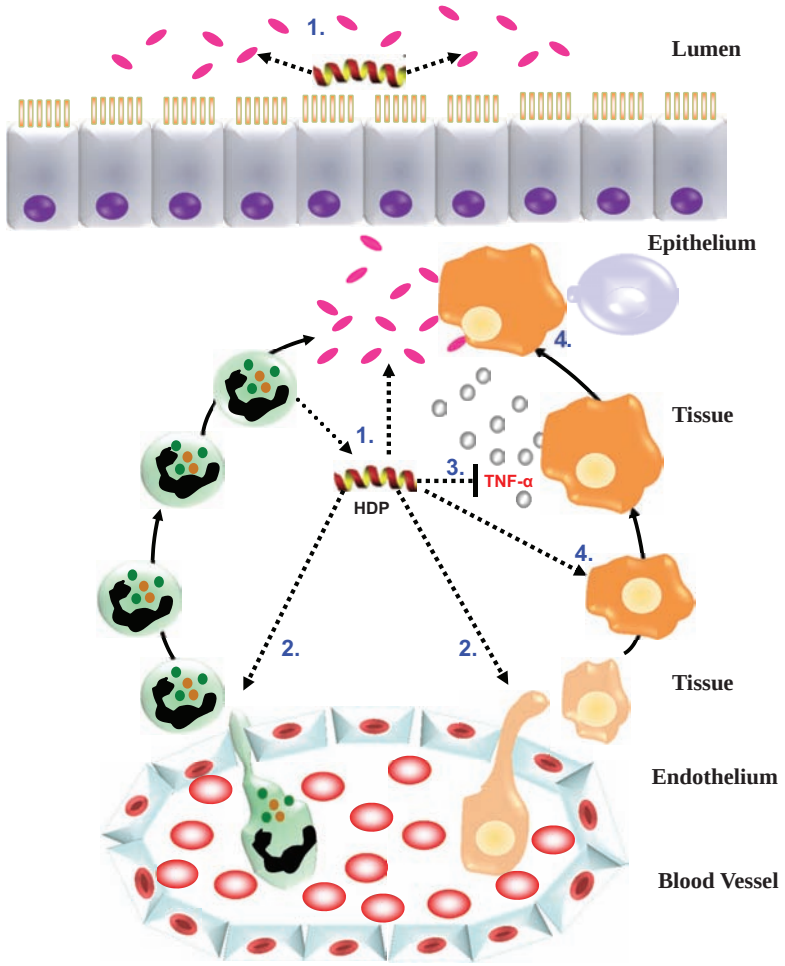

Fig. (2). Immunomodulatory activities of HDPs: In addition to their direct antimicrobial activity (1), HDPs possess a variety of immunomodulatory functions, acting at different locations within the affected micro-environment. These include the direct or indirect recruitment of immune cells to the site of infection (2) and inhibition of pro-inflammatory mediators such as TNF-a (3). HDPs also induce dendritic cell differentiation and activation thus connecting the innate and adaptive arms of the immune system (4).

(TLR) signaling pathways, and in some cases partly by direct LPS binding [98-100]. These activities may underlie the profound anti-inflammatory activity evident in infection models treated with IDR peptides [43, 94], as well as in sterile inflammation models.

Immune Cell Differentiation

Immune cell differentiation is essential to the proper development of immune responses. HDPs appear to have a direct link with this event as well. For example, LL-37 induces dendritic cell (DC) and bone forming-like cell differentiation [34, 101]. Similarly, hLF11, a lactoferrin-derived HDP well known for its *in vivo* protective effects on MDR infections, promotes the differentiation of a macrophage subset with pro and anti-inflammatory capabilities that are highly effective against bacterial pathogens [102]. Peptides also have very distinct activities when interacting with classical (M1) and alternatively activated (M2) macrophages [103].

Wound Healing and Angiogenesis

Wound healing involves the re-growth of epithelial layers and the formation of new blood vessels (angiogenesis), which is necessary for the wounded site to return to homeostasis. HDPs play an important role in this process by acting directly on epithelial and endothelial cells, inducing promoting re-epithelialization and angiogenesis, effects that have been demonstrated both *in vivo* and *in vitro* [104]. HDPs also induce wound healing indirectly through their chemotactic effects on epithelial cells and the induction of metalloproteinases [105, 106]. In fact, a lack of HDPs is linked to impaired re-epithelialization of chronic wounds [107, 108].

Other Functions

Enhancing cell survival and polarization of the adaptive immune system (adjuvant activity) are among other immunomodulatory activities attributed to HDPs [109, 110]. The molecular mechanisms by which these and other HDPs functions occur are complex and slowly being discovered. Mounting evidence suggests that HDPs target multiple processes within a given cell, with the responses depending on the nature of the peptide and the target cell type. In the case of LL-37, it appears to engage multiple receptors including the P2X7 receptor, formyl peptide receptor like-1 (FPRL-1) and other unknown Gi-protein coupled receptors, as well as GAPDH, an intracellular receptor [111-113].Some of the key signaling pathways that play a role in HDP immunomodulation include the mitogen-activated protein kinases (MAPK) p38, JNK, and extracellular signal-regulated kinase-1/2 (ERK1/2), as well as the Src-family kinases, NFκB (transiently) and PI3 kinase pathways [114].

The immunomodulatory activities displayed by HDPs have been demonstrated in animal models and appear to be important for pathogen clearance. Their role has been highlighted in models where the lack of HDPs leads to a reduced ability to clear infections, such as in transgenic mice lacking β-defensin 1 and cathelicidin-related antimicrobial peptide (CRAMP) or in human patients

lacking LL-37 and α-defensin 3. The reduced pathogen clearance is likely due to dysregulation of the immune response and/or decreased peptide mediated-killing leading to increased susceptibility to infections [115-118]. The immunomodulatory and antimicrobial properties of HDPs make them excellent candidates to treat infections; especially those caused by MDR pathogens, particularly in combination with other antimicrobial therapies.

THERAPEUTIC POTENTIAL OF HDPS

Infectious diseases, particularly those caused by MDR bacteria and parasites, are a leading cause of death, accounting for approximately one third of deaths worldwide [119]. HDPs and their synthetic derivatives possess several attributes that make them attractive candidates for novel anti-infectives targeted to MDR pathogens. Variants with enhanced antimicrobial activity can kill a broad range of pathogens acting on components of several essential pathways, which makes the development of effective resistance to them much less likely. As outlined above, most HDPs favorably modulate the innate immune response, which responds to and counteracts a broad range of infectious agents. Thus the anti-infective activity of immunomodulatory HDPs is very broad and not limited to a single class of pathogen [120, 121]. Furthermore, the ability to target the immune response rather than the pathogen also limits the selective pressure on the pathogen. The following sections explore HDPs in preclinical development or clinical trials, as well as peptides recently identified as potential lead compounds Table **1**.

HDPs in Clinical Trials

I. Directly Antimicrobial Peptides

Direct antimicrobial activity has been characterized for numerous synthetic peptides, several of which were introduced into clinical trials as topical anti-infectives. Migenix (www.migenix.com; now renamed BioWest Therapeutics) completed two separate Phase III clinical trials with Omiganan (MX-226/CSL-001), demonstrating safety in more than 3,000 patients and for the first time statistically significant efficacy in decreasing catheter colonization and reducing microbiologically confirmed tunnel infections ($p<0.02$). However, Omiganan is a first generation peptide of modest activity derived from bovine indolicidin, and likely for this reason missed its primary endpoint of physician-called infections ($p<0.08$). Nevertheless it was also statistically significantly effective in Phase II clinical trials against severe acne and Rosacea, but as an anti-inflammatory agent rather than a direct antimicrobial and should go into Phase III trials for Rosacea within the next 12 months.

Pexiganan acetate (MSI-78), a synthetic magainin derivative was tested in Phase III trials as an anti-infective for diabetic foot ulcers. Pexiganan completed Phase III clinical trials but in 1999 was denied approval by the food and drug administration because it did not prove more efficacious than standard fluoroquinolone therapy [122]. It was recently announced, by a new company Dipexium, that Pexiganan would re-enter Phase III trials to enable resubmission of a new drug application (http://www.dipexium pharmaceuticals.com/newsrel.html). In addition, it has been reported that second generation antimicrobial peptides based on Pexiganan have been developed that exhibit greater efficacy *in vitro* and hold the promise of greater efficacy *in vivo*.

Only a small number of HDPs with direct antimicrobial activity are in clinical trials for systemic use. Novozyme Inc.'s lead compound, currently in preclinical development, is a derivative of plectasin (NZ2114), a fungal defensin exhibits broad spectrum antimicrobial activity, especially against systemic, drug-resistant Gram positive infections including MRSA. Unlike many of the antimicrobial HDPs that cause pore formation, plectasin inhibits cell wall synthesis *via* interaction with Lipid II [59]. It has excellent activity both *in vitro* and *in vivo*, minimal toxicity, is recombinantly produced by fermentation thus reducing cost of goods, and as a disulphide bonded defensin molecule is quite protease resistant. PMX-30063 (Polymedix), a defensin mimetic made up of β-amino acids, was developed for the treatment of MRSA infections. PMX-30063 has shown potent broad-spectrum antibacterial activity against Gram positive and negative bacteria *in vitro* and *in vivo* and in Phase I trials demonstrated *ex vivo* antibacterial activity and that it was well tolerated systemically (www.polymedix.com/pdf/PMX-30063_fact_sheet.pdf).

II.Immunomodulatory Peptides

There have been a number of peptides developed that exhibit immunomodulatory activity. Some are solely immunomodulatory, like IMX942, while others possess a certain degree of antimicrobial activity, like hLF1-11 and talactoferrin. Inimex Pharmaceuticals has focused its research on the development of IDRs. Their lead peptide is IMX942, a 5-amino acid derivative of a bovine indolicidin, which was developed for the treatment of infections, especially in neutropenic patients, and displays efficacy in animal models against infections by a number of antibiotic resistant bacteria [94]. IMX942 has completed Phase IA clinical trials. The hormone peptide α-melanocyte-stimulating hormone (α-MSH) has become a lead for therapeutic development at Action Pharma owing to its extensive modulation of pro- and anti-inflammatory responses [123]. A derivative, AP214 is undergoing Phase IIB clinical trials for prevention of sepsis-induced kidney failure (http://www.actionpharma.com/index.dsp?page=73).

hLF1-11 developed by AM-Pharma is a human lactoferrin derivative intended to prevent bacteremia and fungal infections in immunocompromised individuals. It enhances macrophage-mediated phagocytosis and killing of *C. albicans* and S. *aureus* [102]. hLF1-11 has completed Phase I trials and the side effects were found to be much milder than many of the marketedantibiotics with no serious adverse events; however currently development is suspended. Talactoferrin, developed by Agennix (www. agennix.com/) is derivative recombinant form of human lactoferrin that possesses broad immunomodulatory activity including immune cell recruitment, activation of dendritic cells and enhancement of adaptive immune responses [124-126]. A double-blind, placebo-controlled Phase II trial evaluated talactoferrin versus placebo in 190 adult patients with severe sepsis enrolled at 24 leading centers across the U.S. The Phase II trial was reported at the American Thoracic Society International Conference to have achieved its primary endpoint of a reduction in 28-day all-cause mortality (12.5% absolute reduction, 46.5% relative reduction). Apparently exploratory analyses suggested that talactoferrin might be effective in reducing the levels of certain cytokines and chemokines that are important in the initiation and propagation of the inflammatory response in severe sepsis.

New Developments in Potential Lead HDPs and Alternative Design Methods

Many research groups continue to focus on identifying novel HDPs from natural sources that are already effective against MDR pathogens, several of which are listed in Table **I**. Many of these HDPs were discovered in phylogenetically old organisms such as the cnidarian, *Hydra magnipapillata*,which rely on rudimentary immune systems to survive infections [127, 128]. Although many of the novel HDPs are reported to have potent antimicrobial activity, these are often demonstrated under non-physiological conditions, therefore more *in vitro* and *in vivo* studies are required to assess their potential as leads compounds in the development of therapeutics.

New tools such as robotics and machine learning are being developed to facilitate rational design of peptides, minimizing the number and amount of peptides synthesized and tested. Natural and synthetic HDPs with desirable biological activities are used as templates to generate novel peptide libraries consisting of thousands of synthetic peptides through random amino-acid substitution, scrambling and truncation. These novel peptides are then tested *in vitro* to identify those with enhanced antimicrobial activity, which go on to

Table 1. Host Defense Peptides in Clinical Trials and Potential Lead Peptides with Described Activity Against MDR Pathogens

Drug/peptide	Description	Intended use	Activity against MDRs	Progress	References*
Antimicrobial/immunomodulatory					
Omiganan [MX-226/CSL-001] (Migenix)	Indolicidin derivative	Prevention of central veinous catheter infections, skin anti-sepsis, Rosacea	MRSA, VRE and ESBL *Escherichia coli* [146]	Phase IIIb/II	NCT 00231153 NCT 00608959
Pexiganan acetate [MSI-78] (Macrochem)	Maganin derivative	Topical antibiotic- diabetic ulcers	several resistant strains [147]	Phase III	NCT 00563394 NCT 00563433
PMX-30063 (Polymedix)	Defensin structural mimetic	Systemic antibiotic	MRSA	Phase Ib/II	NCT 01211470 www.polymedix.com/pipeline.php
NZ2114/SAR215500 (Novozyme)	Plectasin derivative	Systemic antibiotic	MRSA and *Pseudomonas aeuginosa* [59]	Preclinical	http://www.novozymes.com/en/news/news-archive/Pages/45873.aspx
Ceragenin [CSA-13] (Ceragenix)	Cholic acid based peptide mimetic	Topical antimicrobial- coat medical devices	MDR-*P. aeuruginosa* [148]	Phase III	http://www.ceragenix.com
LTX-109 (Lytix Biopharma)	peptidomimetic	Topical antibiotic	MRSA, VRE, MDR-*P. aeuruginosa*	Phase I/IIa	http://www.lytixbiopharma.com/?a=3&sub=48
PAC-113 (Pargen biopharmaceuticals)	Histatin derivative	antifungal	MDR-*Candida albicans*	Phase IIb	NCT 00659971
Iseganan [IB-367] (Ardea biociences)	Prointegrin-1 derivative	Prevention of oral mucositis	MRSA and *P. aeuginosa* [149]	Phase II	NCT 00022373
XOMA-629	BPI derivative	Impetigo	MRSA	Phase IIa	www.xoma.com
HB-1345 (Helix Biomedix)	lipohexapeptide	Acne	drug resistant *P. acnes*	Pre-phase I	www.helixbiomedix.com
Immunomodulatory/antimicrobial					
hLF1-11 (AM-Pharma)	Lactoferrin derivative	Prevention of bacteraemia and fungal infections	MRSA, MDR-*Acinetobacter baumannii*, fluconazole-resistant *C. Albicans* [150]	Phase I/II	NCT 00509938
OP-145 (Octoplus)	LL-37 derivative	Treatment of chronic middle ear infection		Phase I/II	http://www.octoplus.nl/index.cfm/octoplus/products/op-145/index.cfm
Talactoferrin (Agennix)	Lactoferrin derivative	Non-small cell lung cancer, diabetic ulcers and renal cancer, sepsis, cancer		Phase I/II/III	NCT 00923741 NCT 00630656 NCT 00095186
IMX942 [IDR-1] (Inimex)	Indolicidin derivative	Nosocomial infections, neutropenia	MRSA, VRE [94]	Phase Ia	www.inimexpharma.com/documents/pressrelease_firstclinicalstudy_apr2709.pdf
EA-230	β-hCG fragment	Sepsis		Phase II	www.expobio.com/clinical-trials/index.php
Opebacan [rBPI21] (XOMA)	BPI derivative	Endotoxemia in patients receiving stem cell transplants	MDR- *A. baumannii*[151]	Phase I/II	NCT 00462904
RDP58 (Genzyme)	HLA class-I derivative	Inflammatory bowel disease		Post phase II	www.genzyme.com/corp/licensing/genz_p_rdp58_login.asp
AP214 (Action Pharma)	α-MSH derivative	sepsis-induced kidney failure		Phase IIb	NCT 00903604 NCT 01256372

(Table 1) Contd.....

Drug/peptide	Description	Intended use	Activity against MDRs	Progress	References*
Prospective lead peptides					
Imcroporin (*I. maculates*)	α-helical	Antibacterial	MRSA		[152]
Vejovine (*Vaejovismexicanus*)	α-helical	Antibacterial	Clinical isolates of MDR-*P. aeuginosa, Klebsiella pneumoniae* and *A. baumannii*		[153]
Hyrdamacin-1 (*H. magnipapillata*)	α-helical/ β-sheet	Antibacterial	ESBL- *K. pneumoniae, K. oxytoca, E. Coli*		[127]
Arminin 1a (*H. magnipapillata*)		Antibacterial	MRSA, VRE		[128]
Oh-defensin (*Ornithoctonus hainana*)	defensin	Antibacterial/antifungal	*P. aeuruginosa,* *C. albicans*		[154]
Temporins [temporin-1Tb-1Tf] and esculentin [Esc 1-18 and 1-21] (*Pelophylax lessonae/ridibundus*)	α-helical polypeptides		MDR- *P. aeuruginosa* (*in vivo*) MDR-*S. maltophilia, E. Faecium,* *A. baumannii, S. aureus* (*in vitro*)		[155] [156]

*International clinical trial registration number as indexed on www.clinicaltrial.gov

Abbreviations: BPI, bactericidal/permeability-increasing protein; ESBL, extended spectrum beta-lactamase; HLA, human leukocyte antigen; MSH, melanocyte-stimulating hormone; MRSA, methicillin-resistant *Staphylococcus aureus*, MDR, multidrug resistant; VRE, vancomycin-resistant enterococci; β-hCG, beta-human chorionic gonadotropin

become templates for the synthesis of new libraries. Machine learning tools are used in conjunction with the experimental data generated from these libraries to generate predictive models of peptide activity. One example is QSAR analysis, which relates a series of quantifiable chemical and physical characteristics to a specific biological activity; thus the biological activity of a compound can be predicted based on its physical properties [129]. This approach facilitates rational large-scale design of novel therapeutic candidates because it allows for rapid and efficient virtual interrogation of large compound libraries. The pharmaceutical industry already utilizes QSAR during the small compound screening phase of drug discovery and in toxicological studies. QSAR analysis, relating the atomic-resolution physical-chemical descriptors to antimicrobial activity, combined with artificial intelligence/neural network approaches was recently employed, based on a large training dataset to predict the activity of 100 000 virtual 9-mer peptides [40]. *In vitro,* the peptides predicted to have the greatest antimicrobial activity were equally or more effective against a wide array of MDR bacteria than conventional antibiotics and were also protective in a murine *S. aureus* infection model. This demonstrates that unbiased computational approaches such as QSAR analysis coupled to artificial neural networks are viable, inexpensive and rapid methods for the discovery and development of novel HDPs with greater therapeutic potential.

Rational design of immunomodulatory peptides is more difficult largely because of their pleiotropic targets and effects and the relative novelty of the field. Thus rational design studies utilize specific immunomodulatory functions as markers of overall activity, such as suppression of TLR agonist mediated TNF-α production and induction of chemokines by PBMCs. Using this approach, innate defense regulator (IDR)-1, also called IMX-942, the first peptide designed to be purely immunomodulatory, was generated in our laboratory and proved protective in animal models of infection despite lacking direct antimicrobial activity [94]. The first generation synthetic immunomodulatory peptides are now being used as templates in an iterative design process, much like the one used for antimicrobial peptides, to generate new immunomodulatory peptides such as IDR-1002 with greater anti-infective activity [95].

In addition, a great deal of research now involves the development of alternative HDP design methods that improve efficacy while reducing cost, including ultrashort peptides, lipopeptides, peptides containing unnatural amino acids and self-assembling peptide nanoparticles [130-133]. Lipopeptides are potent antimicrobials (their mode of action is similar to directly antimicrobial HDPs) that are active against MDR bacteria. They are about 6 or 7 amino acids in length and can be linear or cyclic with a fatty acid moiety covalently linked to the N-terminus. N-terminal acylation of a number of natural and synthetic HDPs with C8-C18 long fatty acid moieties also enhances their antimicrobial activity, largely as a result of alterations to their hydrophobicity, oligomerization in solution and ability to interact with cellular membranes [134-136]. Makovitzki*et al.* have exploited these properties by engineering ultrashort cationic lipopeptides (USLiP), peptides of two to four amino acids conjugated to a fatty acid (12-16 carbon) chain [137]. They are potent antimicrobials, with MICs similar to that of native HDPs, and unlike many native lipopeptides and HDPs they exhibit low or no hemolytic activity. The structure of these USLiPs is unique in that the amino acid sequence seems to determine target cell specificity, while the fatty acid chain compensates for the lack of hydrophobic amino acids in the peptide and promotes oligomerization. The potent antimicrobial activity of USLiPs coupled with their simple composition make them economically viable leads for therapeutic development. Liu *et al* designed self-assembling nanoparticle micelles consisting of multiple HIV-1 TAT-derived peptides linked to cholesterol moieties. This design enhances the local density of cationic charge and peptide mass, thus improving the overall antimicrobial activity. The nanoparticles were effective against MRSA and VRE and in a rabbit model of *S. aureus*-induced

meningitis, crossed the blood-brain barrier, suppressed bacterial growth and limited the severity of infection [133].

Challenges Facing Therapeutic use of HDPs and Potential Solutions

Considering the numerous advantages of HDPs as novel therapeutics it may seem quite surprising that so few peptides have made it into clinical trials and none as yet into the clinic. To further develop HDPs into novel therapeutics several issues must first be addressed.

Cost of Manufacturing

Ideally therapeutic peptides should have a low production cost to ensure affordability. Unfortunately, the current method of solid-phase synthesis using fluorenylmethoxycarbonyl (FMOC) chemistry synthesis of HDPs is prohibitively expensive for large-scale production, although solution phase synthesis offers considerable promise. One approach to reducing cost is designing biologically active peptides with minimal length such as the 9-mer peptides of Cherkasov *et al.* or the USLiPs of Makovitzki *et al.*, thus reducing the amount of costly chemical precursors required [40, 137]. A new platform, designed specifically for commercial-scale peptide production is also required. A number of groups are attempting to address this through recombinant synthesis. Novozyme Inc. uses a high-yield fungal expression system in order to mass-produce a highly pure form of its lead peptide, NZ2114 [138]. Recently, a method involving recombinant synthesis and easily cleavable fusion protein partners was utilized for high yield production of several HDPs including LL-37, IDR-1 and MX-226 [139]. During the purification process, a naturally occurring protease, sumoase, which is cloned upstream of the target peptide, cleaves immediately downstream of the sumoase sequence freeing the pure peptide. Because this method utilizes an intrinsic cleavage system and a simple two-step purification process, it can easily be scaled up for industrial use while maintaining a low manufacturing cost [139].

Stability and Toxicity In vivo

Peptides are sensitive to proteolytic degradation, which substantially decreases their half-life. Our own, as-yet unpublished, pharmacokinetic studies have revealed half lives in the blood of as little as 2 minutes. Methods developed to enhance peptide resistance to proteases generally involve altering peptide structure to improve *in vivo* stability, although the down side to these approaches is that almost all of these substantially increase the cost of goods and are incompatible with recombinant synthesis. One approach is synthesis of peptide complexes such as tetrabranched structures (four peptides linked by a lysine core) or nanoparticles [133, 140]. Retro-inverso peptide isomers, in which the L-amino acids are replaced D-amino acids in the reverse sequence order, have also been developed. Because human proteases cannot metabolize the D-amino acid peptides, these isomers have significantly enhanced stability but maintain the spatial positioning and biological activity of the original peptide [141, 142]. This method has been successfully used to generate a biologically active Bac2A isomer (RI-Bac2A) that was more resistant to proteolytic degradation and exhibited reduced toxicity [142].

A number of HDPs exhibit some degree of systemic toxicity, which may explain why most peptides in clinical trials are used topically. The toxicity may be because HDPs interact with numerous cell types in different ways resulting in a complex mode of action however, little research has actually been done to address this issue. Although toxicity was initially thought to be a result of membrane disruption leading to cytolysis, many HDPs interact with and translocate across eukaryotic membranes [75, 94, 113] without disrupting them. This is probably because eukaryotic membranes lack negatively charged lipids and the strong membrane potential gradient found in prokaryotic membranes, and contain cholesterol [57]. Anti-infective HDPs can and have been designed that are protective in animal models of infection with little or no associated toxicity as is the case for IDR-1 [94]. Toxicity and pharmacokinetic studies must be carried out for HDPs in *ex vivo* human tissue models and relevant animal models to determine the mechanism behind the toxicity during systemic administration as well as identify other potential side effects, the appropriate formulations and alternate routes of administration.

Development of Resistance

There was concern as to whether widespread use of directly antimicrobial HDPs would promote the development of peptide-resistance by pathogens to both the therapeutic and endogenous HDPs. Several bacterial species already possess HDP-resistance mechanisms such as secretion of peptidases and proteases and alteration of surface charge [143].However, considering the broad target range exhibited by peptides it seems unlikely that the selective pressure placed on the pathogens would be great enough to incur resistance. Furthermore, there is no evidence that resistance mechanisms are easily acquired [143, 144]. Nisin a well recognized HDP has been used as food preservative for years with no effect on an individual's ability to fight infections [144, 145]. Finally, the use of immunomodulatory peptides could circumvent this issue completely as they target the immune response rather than the pathogen [94, 95].

CONCLUSION

The emergence of MDR pathogens has resulted in an urgent need to develop novel antimicrobial therapies. As anti-infective agents, HDPs can be directly antimicrobial and/or immunomodulatory. Thus, HDPs are unlikely to promote microbial resistance because they disrupt multiple biological processes in the pathogen or target the host immune system rather than the pathogen. Furthermore, HDPs can suppress the potentially harmful inflammation associated with infection. These properties make HDPs an attractive alternative in the treatment of MDR infections. Thousands of natural and synthetic HDPs have been identified however, only a handful of HDPs have made into clinical trials and none approved for clinical use in part because of issues regarding cost of manufacturing, peptide stability and toxicity. Although more work is needed, substantial strides have been made in recent years to overcome these setbacks, and the future looks promising for the development and widespread use of peptides as therapeutics targeted to MDR pathogens.

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REFERENCES

[1] Thomson CJ, Power E, Ruebsamen-Waigmann H, Labischinski H. Antibacterial research and development in the 21(st) Century--an industry perspective of the challenges. Curr Opin Microbiol 2004; 7: 445-50.

[2] Spellberg B. Antibiotic Resistance: Promoting Critically Needed Antibiotic Research and Development and Appropriate Use ("Stewardship") of these Precious Drugs. Infectious Diseases Society of America (IDSA) 2010.

[3] Donadio S, Maffioli S, Monciardini P, Sosio M, Jabes D. Antibiotic discovery in the twenty-first century: current trends and future perspectives. J Antibiot (Tokyo) 2010; 63: 423-30.

[4] Spellberg B, Powers JH, Brass EP, Miller LG, Edwards JE, Jr. Trends in antimicrobial drug development: implications for the future. Clin Infect Dis 2004; 38: 1279-86.
[5] Sellers LJ. Big pharma bails on anti-infectives research. Pharmaceutical Executive 2003.
[6] Projan SJ. Why is big Pharma getting out of antibacterial drug discovery? Curr Opin Microbiol 2003; 6: 427-30.
[7] Nathan C. Antibiotics at the crossroads. Nature 2004; 431: 899-902.
[8] Steinstraesser L, Kraneburg U, Jacobsen F, Al-Benna S. Host defense peptides and their antimicrobial-immunomodulatory duality. Immunobiology 2011; 216: 322-33.
[9] Lai Y, Gallo RL. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol 2009; 30: 131-41.
[10] Giamarellou H, Poulakou G. Multidrug-resistant Gram-negative infections: what are the treatment options? Drugs 2009; 69: 1879-901.
[11] Nikaido H. Multidrug resistance in bacteria. Annu Rev Biochem 2009; 78: 119-46.
[12] de Lencastre H, Oliveira D, Tomasz A. Antibiotic resistant Staphylococcus aureus: a paradigm of adaptive power. Curr Opin Microbiol 2007; 10: 428-35.
[13] Rossolini GM, Mantengoli E, Montagnani F, Pollini S. Epidemiology and clinical relevance of microbial resistance determinants versus anti-Gram-positive agents. Curr Opin Microbiol 2010; 13: 582-8.
[14] National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control 2004; 32: 470-85.
[15] Hooper DC. Mechanisms of action and resistance of older and newer fluoroquinolones. Clin Infect Dis 2000; 31 Suppl 2: S24-8.
[16] Courvalin P. Vancomycin resistance in gram-positive cocci. Clin Infect Dis 2006; 42 Suppl 1: S25-34.
[17] Spratt BG. Resistance to antibiotics mediated by tar*get al*terations. Science. 1994; 264: 388-93.
[18] Kuroda M, Ohta T, Uchiyama I, *et al.* Whole genome sequencing of meticillin-resistant Staphylococcus aureus. Lancet 2001; 357: 1225-40.
[19] Bush K. Alarming beta-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Curr Opin Microbiol 2010; 13: 558-64.
[20] Levy SB. Active efflux mechanisms for antimicrobial resistance. Antimicrob Agents Chemother. 1992; 36: 695-703.
[21] Harder KJ, Nikaido H, Matsuhashi M. Mutants of Escherichia coli that are resistant to certain beta-lactam compounds lack the ompF porin. Antimicrob Agents Chemother. 1981; 20: 549-52.
[22] Mor A. Multifunctional host defense peptides: antiparasitic activities. FEBS J 2009; 276: 6474-82.
[23] Perrin L, Telenti A. HIV treatment failure: testing for HIV resistance in clinical practice. Science. 1998; 280: 1871-3.
[24] Matejuk A, Leng Q, Begum MD, *et al.* Peptide-based Antifungal Therapies against Emerging Infections. Drugs Future 2010; 35: 197.
[25] Prasad R, Kapoor K. Multidrug resistance in yeast Candida. Int Rev Cytol 2005; 242: 215-48.
[26] French GL. The continuing crisis in antibiotic resistance. Int J Antimicrob Agents 2010; 36 Suppl 3: S3-7.
[27] Hancock REW, Diamond G. The role of cationic antimicrobial peptides in innate host defences. Trends in Microbiology 2000; 8: 402-10.
[28] Hancock RE, Rozek A. Role of membranes in the activities of antimicrobial cationic peptides. FEMS Microbiol Lett 2002; 206: 143-9.
[29] Guani-Guerra E, Santos-Mendoza T, Lugo-Reyes SO, Teran LM. Antimicrobial peptides: general overview and clinical implications in human health and disease. Clin Immunol 2010; 135: 1-11.
[30] Wu Z, Hoover DM, Yang D, *et al.* Engineering disulfide bridges to dissect antimicrobial and chemotactic activities of human β-defensin 3 Proc Natl Acad Sci USA 2003; 100: 8880-5.
[31] Circo R, Skerlavaj B, Gennaro R, Amoroso A, Zanetti M. Structural and functional characterization of hBD-1(Ser35), a peptide deduced from a DEFB1 polymorphism. Biochem Biophys Res Commun 2002; 293: 586-92.
[32] Kluver E, Schulz-Maronde S, Scheid S, *et al.* Structure-activity relation of human beta-defensin 3: influence of disulfide bonds and cysteine substitution on antimicrobial activity and cytotoxicity. Biochemistry 2005; 44: 9804-16.
[33] Taylor K, Barran PE, Dorin JR. Structure-activity relationships in beta-defensin peptides. Biopolymers 2008; 90: 1-7.
[34] Matsuzaki K, Sugishita K, Harada M, Fujii N, Miyajima K. Interactions of an antimicrobial peptide, magainin 2, with outer and inner membranes of Gram-negative bacteria. Biochim Biophys Acta. 1997; 1327: 119-30.
[35] Schroeder BO, Wu Z, Nuding S, *et al.* Reduction of disulphide bonds unmasks potent antimicrobial activity of human beta-defensin 1. Nature. 469: 419-23.
[36] Wang G. Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. J Biol Chem 2008; 283: 32637-43.
[37] Ciornei CD, Sigurdardottir T, Schmidtchen A, Bodelsson M. Antimicrobial and Chemoattractant Activity, Lipopolysaccharide Neutralization, Cytotoxicity, and Inhibition by Serum of Analogs of Human Cathelicidin LL-37. Antimicrob Agents Chemother 2005; 49: 2845-50.
[38] Scott MG, Davidson DJ, Gold MR, Bowdish D, Hancock REW. The Human Antimicrobial Peptide LL-37 Is a Multifunctional Modulator of Innate Immune Responses. The Journal of Immunology 2002; 169: 3883-91.
[39] BaraÅ„ska-Rybak W, Sonesson A, Nowicki R, Schmidtchen A. Glycosaminoglycans inhibit the antibacterial activity of LL-37 in biological fluids. Journal of Antimicrobial Chemotherapy 2006; 57: 260-5.
[40] Cherkasov A, Hilpert K, Jenssen H, *et al.* Use of artificial intelligence in the design of small peptide antibiotics effective against a broad spectrum of highly antibiotic-resistant superbugs. ACS Chem Biol 2009; 4: 65-74.
[41] Braff MH, Hawkins MA, Di Nardo A, *et al.* Structure-function relationships among human cathelicidin peptides: dissociation of antimicrobial properties from host immunostimulatory activities. J Immunol 2005; 174: 4271-8.
[42] Oren Z, Lerman JC, Gudmundsson GH, Agerberth B, Shai Y. Structure and organization of the human antimicrobial peptide LL-37 in phospholipid membranes: relevance to the molecular basis for its non-cell-selective activity. Biochem J. 1999; 341 (Pt 3): 501-13.
[43] Nijnik A, Madera L, Ma S, *et al.* Synthetic cationic peptide IDR-1002 provides protection against bacterial infections through chemokine induction and enhanced leukocyte recruitment. J Immunol. 184: 2539-50.
[44] Wieczorek M, Jenssen H, Kindrachuk J, *et al.* Structural studies of a peptide with immune modulating and direct antimicrobial activity. Chem Biol. 17: 970-80.
[45] Fischer PM, Zhelev NZ, Wang S, *et al.* Structure-activity relationship of truncated and substituted analogues of the intracellular delivery vector Penetratin. J Pept Res 2000; 55: 163-72.
[46] Yu HB, Kielczewska A, Rozek A, *et al.* Sequestosome-1/p62 Is the Key Intracellular Target of Innate Defense Regulator Peptide. Journal of Biological Chemistry 2009; 284: 36007-11.
[47] Hale JD, Hancock RE. Alternative mechanisms of action of cationic antimicrobial peptides on bacteria. Expert Rev Anti Infect Ther 2007; 5: 951-9.
[48] Mookherjee N, Wilson HL, Doria S, *et al.* Bovine and human cathelicidin cationic host defense peptides similarly suppress transcriptional responses to bacterial lipopolysaccharide. J Leukoc Biol 2006; 80: 1563-74.
[49] Bowdish DM, Davidson DJ, Lau YE, *et al.* Impact of LL-37 on anti-infective immunity. J Leukoc Biol 2005; 77: 451-9.
[50] Hancock RE. Peptide antibiotics. Lancet. 1997; 349: 418-22.
[51] Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol 2005; 3: 238-50.
[52] Wu M, Maier E, Benz R, Hancock RE. Mechanism of interaction of different classes of cationic antimicrobial peptides with planar bilayers and with the cytoplasmic membrane of Escherichia coli. Biochemistry. 1999; 38: 7235-42.
[53] Wu M, Hancock RE. Interaction of the cyclic antimicrobial cationic peptide bactenecin with the outer and cytoplasmic membrane. J Biol Chem. 1999; 274: 29-35.
[54] Matsuzaki K, Murase O, Fujii N, Miyajima K. An antimicrobial peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation. Biochemistry. 1996; 35: 11361-8.
[55] Sass V, Schneider T, Wilmes M, *et al.* Human beta-defensin 3 inhibits cell wall biosynthesis in Staphylococci. Infect Immun. 78: 2793-800.

[56] Spindler EC, Hale JD, Giddings TH, Jr., Hancock RE, Gill RT. Deciphering the mode of action of the synthetic antimicrobial peptide Bac8c. Antimicrob Agents Chemother. 55: 1706-16.

[57] Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 2006; 24: 1551-7.

[58] R.E.W. H. Introduction to the Theme: Seeking Clarity for Membrane-active Peptides. In: M.A.R C, editor. Membrane-active peptides: Methods and Results on Structure and Function: International University Line; 2010. p. 692.

[59] Schneider T, Kruse T, Wimmer R, *et al.* Plectasin, a fungal defensin, targets the bacterial cell wall precursor Lipid II. Science 2010; 328: 1168-72.

[60] Park CB, Kim HS, Kim SC. Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. Biochem Biophys Res Commun. 1998; 244: 253-7.

[61] Kragol G, Lovas S, Varadi G, *et al.* The antibacterial peptide pyrrhocoricin inhibits the ATPase actions of DnaK and prevents chaperone-assisted protein folding. Biochemistry 2001; 40: 3016-26.

[62] Hoiby N, Krogh Johansen H, Moser C, *et al.* Pseudomonas aeruginosa and the *in vitro* and *in vivo* biofilm mode of growth. Microbes Infect 2001; 3: 23-35.

[63] Drenkard E, Ausubel FM. Pseudomonas biofilm formation and antibiotic resistance are linked to phenotypic variation. Nature 2002; 416: 740-3.

[64] Overhage J, Campisano A, Bains M, *et al.* Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun 2008; 76: 4176-82.

[65] Hell E, Giske CG, Nelson A, Romling U, Marchini G. Human cathelicidin peptide LL37 inhibits both attachment capability and biofilm formation of Staphylococcus epidermidis. Lett Appl Microbiol 2010; 50: 211-5.

[66] Jenssen H, Hamill P, Hancock REW. Peptide Antimicrobial Agents. Clin Microbiol Rev 2006; 19: 491-511.

[67] Lorin C, Saidi H, Belaid A, *et al.* The antimicrobial peptide dermaseptin S4 inhibits HIV-1 infectivity *in vitro*. Virology 2005; 334: 264-75.

[68] Shieh MT, WuDunn D, Montgomery RI, Esko JD, Spear PG. Cell surface receptors for herpes simplex virus are heparan sulfate proteoglycans. J Cell Biol. 1992; 116: 1273-81.

[69] Spillmann D. Heparan sulfate: anchor for viral intruders? Biochimie 2001; 83: 811-7.

[70] Sinha S, Cheshenko N, Lehrer RI, Herold BC. NP-1, a rabbit alpha-defensin, prevents the entry and intercellular spread of herpes simplex virus type 2. Antimicrob Agents Chemother 2003; 47: 494-500.

[71] Tamamura H, Otaka A, Murakami T, *et al.* Interaction of an anti-HIV peptide, T22, with gp120 and CD4. Biochem Biophys Res Commun. 1996; 219: 555-9.

[72] Tamamura H, Xu Y, Hattori T, *et al.* A low-molecular-weight inhibitor against the chemokine receptor CXCR4: a strong anti-HIV peptide T140. Biochem Biophys Res Commun. 1998; 253: 877-82.

[73] Horne WS, Wiethoff CM, Cui C, *et al.* Antiviral cyclic D,L-alpha-peptides: targeting a general biochemical pathway in virus infections. Bioorg Med Chem 2005; 13: 5145-53.

[74] Haukland HH, Ulvatne H, Sandvik K, Vorland LH. The antimicrobial peptides lactoferricin B and magainin 2 cross over the bacterial cytoplasmic membrane and reside in the cytoplasm. FEBS Lett 2001; 508: 389-93.

[75] Lau YE, Rozek A, Scott MG, *et al.* Interaction and cellular localization of the human host defense peptide LL-37 with lung epithelial cells. Infect Immun 2005; 73: 583-91.

[76] Gwadz RW, Kaslow D, Lee JY, *et al.* Effects of magainins and cecropins on the sporogonic development of malaria parasites in mosquitoes. Infect Immun. 1989; 57: 2628-33.

[77] Zasloff M. Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc Natl Acad Sci USA. 1987; 84: 5449-53.

[78] Park Y, Jang SH, Lee DG, Hahm KS. Antinematodal effect of antimicrobial peptide, PMAP-23, isolated from porcine myeloid against Caenorhabditis elegans. J Pept Sci 2004; 10: 304-11.

[79] Alberola J, Rodriguez A, Francino O, *et al.* Safety and efficacy of antimicrobial peptides against naturally acquired leishmaniasis. Antimicrob Agents Chemother 2004; 48: 641-3.

[80] Efron L, Dagan A, Gaidukov L, Ginsburg H, Mor A. Direct interaction of dermaseptin S4 aminoheptanoyl derivative with intraerythrocytic malaria parasite leading to increased specific antiparasitic activity in culture. J Biol Chem 2002; 277: 24067-72.

[81] Rivas L, Luque-Ortega JR, Andreu D. Amphibian antimicrobial peptides and Protozoa: lessons from parasites. Biochim Biophys Acta 2009; 1788: 1570-81.

[82] Epand RM, Epand RF. Lipid domains in bacterial membranes and the action of antimicrobial agents. Biochim Biophys Acta 2009; 1788: 289-94.

[83] Rotem S, Mor A. Antimicrobial peptide mimics for improved therapeutic properties. Biochim Biophys Acta 2009; 1788: 1582-92.

[84] Moerman L, Bosteels S, Noppe W, *et al.* Antibacterial and antifungal properties of alpha-helical, cationic peptides in the venom of scorpions from southern Africa. Eur J Biochem 2002; 269: 4799-810.

[85] Koo JC, Lee B, Young ME, *et al.* Pn-AMP1, a plant defense protein, induces actin depolarization in yeasts. Plant Cell Physiol 2004; 45: 1669-80.

[86] Kim DH, Lee DG, Kim KL, Lee Y. Internalization of tenecin 3 by a fungal cellular process is essential for its fungicidal effect on Candida albicans. Eur J Biochem 2001; 268: 4449-58.

[87] Yang D, Chertov O, Oppenheim JJ. The role of mammalian antimicrobial peptides and proteins in awakening of innate host defenses and adaptive immunity. Cellular and Molecular Life Sciences 2001; 58: 978-89.

[88] Durr M, Peschel A. Chemokines Meet Defensins: the Merging Concepts of Chemoattractants and Antimicrobial Peptides in Host Defense. Infect Immun 2002; 70: 6515-7.

[89] Tjabringaa GS, Ninabera DK, Drijfhoutb JW, Rabea KF, Hiemstraa PS. Human Cathelicidin LL-37 Is a Chemoattractant for Eosinophils and Neutrophils That Acts *via* Formyl-Peptide Receptors. Int Arch Allergy Immunol 2006; 140: 103-12.

[90] Easton DM, Nijnik A, Mayer ML, Hancock REW. Potential of immunomodulatory host defense peptides as novel anti-infectives. Trends in Biotechnology 2009; 27: 582-90.

[91] Khine AA, Del Sorbo L, Vaschetto R, *et al.* Human neutrophil peptides induce interleukin-8 production through the P2Y6 signaling pathway. Blood 2006; 107: 2936-42.

[92] Mookherjee N, Hancock R. Cationic host defence peptides: Innate immune regulatory peptides as a novel approach for treating infections. Cellular and Molecular Life Sciences 2007; 64: 922-33.

[93] Wieczorek M, Jenssen H, Kindrachuk J, *et al.* Structural Studies of a Peptide with Immune Modulating and Direct Antimicrobial Activity. Chemistry & Biology 2010; 17: 970-80.

[94] Scott M, Dullaghan E, Mookherjee N, *et al.* An anti-infective peptide that selectively modulates the innate immune response. Nat Biotechnol 2007; 25: 465 - 72.

[95] Nijnik A, Madera L, Ma S, *et al.* Synthetic Cationic Peptide IDR-1002 Provides Protection against Bacterial Infections through Chemokine Induction and Enhanced Leukocyte Recruitment. The Journal of Immunology 2010; 184: 2539-50.

[96] Opal SM. New perspectives on immunomodulatory therapy for bacteraemia and sepsis. International Journal of Antimicrobial Agents 2010; 36: S70-S3.

[97] Lepper, Held, Schneider, *et al.* Clinical implications of antibiotic-induced endotoxin release in septic shock. Intensive Care Medicine 2002; 28: 824-33.

[98] Nagaoka I, Hirota S, Niyonsaba Fo, *et al.* Cathelicidin Family of Antibacterial Peptides CAP18 and CAP11 Inhibit the Expression of TNF-Î± by Blocking the Binding of LPS to CD14+ Cells. The Journal of Immunology 2001; 167: 3329-38.

[99] Scott MG, Vreugdenhil AC, Buurman WA, Hancock RE, Gold MR. Cutting edge: cationic antimicrobial peptides block the binding of lipopolysaccharide (LPS) to LPS binding protein. J Immunol 2000; 164: 549-53.

[100] Bowdish D, Hancock R. Anti-endotoxin properties of cationic host defence peptides and proteins. J endotoxin Res 2005; 11: 230-6.

[101] Davidson DJ, Currie AJ, Reid GSD, *et al.* The Cationic Antimicrobial Peptide LL-37 Modulates Dendritic Cell Differentiation and Dendritic Cell-Induced T Cell Polarization. J Immunol 2004; 172: 1146-56.

[102] van der Does AM, Bogaards SJP, Ravensbergen B, *et al.* Antimicrobial Peptide hLF1-11 Directs Granulocyte-Macrophage Colony-Stimulating Factor-Driven Monocyte Differentiation toward

Macrophages with Enhanced Recognition and Clearance of Pathogens. Antimicrob Agents Chemother 2010; 54: 811-6.
[103] Brown KL, Poon GFT, Birkenhead D, *et al.* Host Defense Peptide LL-37 Selectively Reduces Proinflammatory Macrophage Responses. The Journal of Immunology 2011; 186: 5497-505.
[104] Wu WKK, Wong CCM, Li ZJ, *et al.* Cathelicidins in inflammation and tissue repair: Potential therapeutic applications for gastrointestinal disorders. Acta Pharmacol Sin 2010; 31: 1118-22.
[105] Shaykhiev R, BeiÃŸwenger C, KÃ¤ndler K, *et al.* Human endogenous antibiotic LL-37 stimulates airway epithelial cell proliferation and wound closure. American Journal of Physiology - Lung Cellular and Molecular Physiology 2005; 289: L842-L8.
[106] Varoga D, Pufe T, Harder J, *et al.* Human β-defensin 3 mediates tissue remodeling processes in articular cartilage by increasing levels of metalloproteinases and reducing levels of their endogenous inhibitors. Arthritis & Rheumatism 2005; 52: 1736-45.
[107] Heilborn JD, Nilsson MF, Kratz G, *et al.* The Cathelicidin Anti-Microbial Peptide LL-37 is Involved in Re-Epithelialization of Human Skin Wounds and is Lacking in Chronic Ulcer Epithelium. J Investig Dermatol 2003; 120: 379-89.
[108] Koczulla R, von Degenfeld G, Kupatt C, *et al.* An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. The Journal of Clinical Investigation 2003; 111: 1665-72.
[109] Nicholls EF, Madera L, Hancock REW. Immunomodulators as adjuvants for vaccines and antimicrobial therapy. Annals of the New York Academy of Sciences. 1213: 46-61.
[110] Davidson DJ, Currie AJ, Reid GSD, *et al.* The Cationic Antimicrobial Peptide LL-37 Modulates Dendritic Cell Differentiation and Dendritic Cell-Induced T Cell Polarization. The Journal of Immunology 2004; 172: 1146-56.
[111] Tomasinsig L, Pizzirani C, Skerlavaj B, *et al.* The human cathelicidin LL-37 modulates the activities of the P2X7 receptor in a structure-dependent manner. J Bio Chem 2008; 283: 30471-81.
[112] Coffelt S, Tomchuck S, Zwezdaryk K, Danka E, Scandurro A. Leucine leucine-37 uses formyl peptide receptor-like 1 to activate signal transduction pathways, stimulate oncogenic gene expression, and enhance the invasiveness of ovarian cancer cells. Mol Cancer Res 2009; 7: 907-15.
[113] Mookherjee N, Lippert DND, Hamill P, *et al.* Intracellular Receptor for Human Host Defense Peptide LL-37 in Monocytes. J Immunol 2009; 183: 2688-96.
[114] Mookherjee N, Brown K, Bowdish D, *et al.* Modulation of the Toll-like receptor-mediated inflammatory response by the endogenous human host defence peptide LL-37. J Immunol 2006; 176: 2455 - 64.
[115] Moser C, Weiner DJ, Lysenko E, *et al.* {beta}-Defensin 1 Contributes to Pulmonary Innate Immunity in Mice. Infect Immun 2002; 70: 3068-72.
[116] Pütsep K, Carlsson G, Boman HG, Andersson M. Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study. The Lancet 2002; 360: 1144-9.
[117] Limura M, Gallo RL, Hase K, *et al.* Cathelicidin Mediates Innate Intestinal Defense against Colonization with Epithelial Adherent Bacterial Pathogens. J Immunol 2005; 174: 4901-7.
[118] Huang LC, Reins RY, Gallo RL, McDermott AM. Cathelicidin-deficient (Cnlp -/-) mice show increased susceptibility to Pseudomonas aeruginosa keratitis. Invest Ophthalmol Vis Sci 2007; 48: 4498-508.
[119] WHO. World Health Statistics on http://www.who.int/whosis/whostat/2009/en/index.html 2009.
[120] Scott MG, Rosenberger CM, Gold MR, Finlay BB, Hancock REW. An {alpha}-Helical Cationic Antimicrobial Peptide Selectively Modulates Macrophage Responses to Lipopolysaccharide and Directly Alters Macrophage Gene Expression. J Immunol 2000; 165: 3358-65.
[121] Scott MG, Davidson DJ, Gold MR, Bowdish D, Hancock RE. The human antimicrobial peptide LL-37 is a multifunctional modulator of innate immune responses. J Immunol 2002; 169: 3883-91.
[122] Lipsky BA, Holroyd KJ, Zasloff M. 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 2008; 47: 1537-45.
[123] Brzoska T, Luger TA, Maaser C, Abels C, Bohm M. Alpha-melanocyte-stimulating hormone and related tripeptides: biochemistry, antiinflammatory and protective effects *in vitro* and *in vivo*, and future perspectives for the treatment of immune-mediated inflammatory diseases. Endocr Rev 2008; 29: 581-602.
[124] Spadaro M, Caorsi C, Ceruti P, *et al.* Lactoferrin, a major defense protein of innate immunity, is a novel maturation factor for human dendritic cells. FASEB J 2008; 22: 2747-57.
[125] de la Rosa G, Yang D, Tewary P, Varadhachary A, Oppenheim JJ. Lactoferrin acts as an alarmin to promote the recruitment and activation of APCs and antigen-specific immune responses. J Immunol 2008; 180: 6868-76.
[126] Spadaro M, Curcio C, Varadhachary A, *et al.* Requirement for IFN-gamma, CD8+ T lymphocytes, and NKT cells in talactoferrin-induced inhibition of neu+ tumors. Cancer Res 2007; 67: 6425-32.
[127] Jung S, Dingley AJ, Augustin R, *et al.* Hydramacin-1, structure and antibacterial activity of a protein from the basal metazoan Hydra. J Biol Chem 2009; 284: 1896-905.
[128] Augustin R, Anton-Erxleben F, Jungnickel S, *et al.* Activity of the novel peptide arminin against multiresistant human pathogens shows the considerable potential of phylogenetically ancient organisms as drug sources. Antimicrob Agents Chemother 2009; 53: 5245-50.
[129] Fjell CD, Jenssen Hv, Hilpert K, *et al.* Identification of Novel Antibacterial Peptides by Chemoinformatics and Machine Learningâ€ Journal of Medicinal Chemistry 2009; 52: 2006-15.
[130] Jerala R. Synthetic lipopeptides: a novel class of anti-infectives. Expert Opin Investig Drugs 2007; 16: 1159-69.
[131] Hornef MW, Putsep K, Karlsson J, Refai E, Andersson M. Increased diversity of intestinal antimicrobial peptides by covalent dimer formation. Nat Immunol 2004; 5: 836-43.
[132] Giuliani A, Pirri G, Bozzi A, *et al.* Antimicrobial peptides: natural templates for synthetic membrane-active compounds. Cell Mol Life Sci 2008; 65: 2450-60.
[133] Liu L, Xu K, Wang H, *et al.* Self-assembled cationic peptide nanoparticles as an efficient antimicrobial agent. Nat Nanotechnol 2009; 4: 457-63.
[134] Avrahami D, Shai Y. Conjugation of a magainin analogue with lipophilic acids controls hydrophobicity, solution assembly, and cell selectivity. Biochemistry 2002; 41: 2254-63.
[135] Majerle A, Kidric J, Jerala R. Enhancement of antibacterial and lipopolysaccharide binding activities of a human lactoferrin peptide fragment by the addition of acyl chain. J Antimicrob Chemother 2003; 51: 1159-65.
[136] Lockwood NA, Haseman JR, Tirrell MV, Mayo KH. Acylation of SC4 dodecapeptide increases bactericidal potency against Gram-positive bacteria, including drug-resistant strains. Biochem J 2004; 378: 93-103.
[137] Makovitzki A, Avrahami D, Shai Y. Ultrashort antibacterial and antifungal lipopeptides. Proc Natl Acad Sci USA 2006; 103: 15997-6002.
[138] Mygind PH, Fischer RL, Schnorr KM, *et al.* Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. Nature 2005; 437: 975-80.
[139] Bommarius B, Jenssen H, Elliott M, *et al.* Cost-effective expression and purification of antimicrobial and host defense peptides in Escherichia coli. Peptides 2010; 31: 1957-65.
[140] Pini A, Falciani C, Mantengoli E, *et al.* A novel tetrabranched antimicrobial peptide that neutralizes bacterial lipopolysaccharide and prevents septic shock *in vivo*. FASEB J 2010; 24: 1015-22.
[141] Fischer PM. The design, synthesis and application of stereochemical and directional peptide isomers: a critical review. Curr Protein Pept Sci 2003; 4: 339-56.
[142] Kindrachuk J, Scruten E, Attah-Poku S, *et al.* Stability, toxicity and biological activity of host defense peptide BMAP28 and its inversed and retro-inversed isomers. Biopolymers 2010.
[143] Peschel A, Sahl HG. The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nat Rev Microbiol 2006; 4: 529-36.
[144] Hancock RE. Concerns regarding resistance to self-proteins. Microbiology 2003; 149: 3343-4; discussion 4-5.
[145] Bell G, Gouyon PH. Arming the enemy: the evolution of resistance to self-proteins. Microbiology 2003; 149: 1367-75.
[146] Fritsche TR, Rhomberg PR, Sader HS, Jones RN. Antimicrobial activity of omiganan pentahydrochloride tested against contemporary bacterial pathogens commonly responsible for catheter-associated infections. J Antimicrob Chemother 2008; 61: 1092-8.
[147] Maloy WL, Kari UP. Structure-activity studies on magainins and other host defense peptides. Biopolymers. 1995; 37: 105-22.

[148] Chin JN, Jones RN, Sader HS, Savage PB, Rybak MJ. Potential synergy activity of the novel ceragenin, CSA-13, against clinical isolates of Pseudomonas aeruginosa, including multidrug-resistant P. aeruginosa. J Antimicrob Chemother 2008; 61: 365-70.

[149] Mosca DA, Hurst MA, So W, *et al.* IB-367, a protegrin peptide with *in vitro* and *in vivo* activities against the microflora associated with oral mucositis. Antimicrob Agents Chemother 2000; 44: 1803-8.

[150] van der Does AM, Bogaards SJ, Ravensbergen B, *et al.* Antimicrobial peptide hLF1-11 directs granulocyte-macrophage colony-stimulating factor-driven monocyte differentiation toward macrophages with enhanced recognition and clearance of pathogens. Antimicrob Agents Chemother 2010; 54: 811-6.

[151] Urban C, Mariano N, Rahal JJ, *et al.* Polymyxin B-Resistant Acinetobacter baumannii Clinical Isolate Susceptible to Recombinant BPI and Cecropin P1. Antimicrob Agents Chemother 2001; 45: 994-5.

[152] Zhao Z, Ma Y, Dai C, *et al.* Imcroporin, a new cationic antimicrobial peptide from the venom of the scorpion Isometrus maculates. Antimicrob Agents Chemother 2009; 53: 3472-7.

[153] Hernandez-Aponte CA, Silva-Sanchez J, Quintero-Hernandez V, *et al.* Vejovine, a new antibiotic from the scorpion venom of Vaejovis mexicanus. Toxicon 2011; 57: 84-92.

[154] Zhao H, Kong Y, Wang H, *et al.* A defensin-like antimicrobial peptide from the venoms of spider, Ornithoctonus hainana. J Pept Sci 2011.

[155] Uccelletti D, Zanni E, Marcellini L, *et al.* Anti-Pseudomonas activity of frog skin antimicrobial peptides in a Caenorhabditis elegans infection model: a plausible mode of action *in vitro* and *in vivo*. Antimicrob Agents Chemother 2010; 54: 3853-60.

[156] Mangoni ML, Maisetta G, Di Luca M, *et al.* Comparative analysis of the bactericidal activities of amphibian peptide analogues against multidrug-resistant nosocomial bacterial strains. Antimicrob Agents Chemother 2008; 52: 85-91.

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