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Antimicrobial peptides: key components of the innate immune system.

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Abstract

Life-threatening infectious diseases are on their way to cause a worldwide crisis, as treating them effectively is becoming increasingly difficult due to the emergence of antibiotic resistant strains. Antimicrobial peptides (AMPs) form an ancient type of innate immunity found universally in all living organisms, providing a principal first-line of defense against the invading pathogens. The unique diverse function and architecture of AMPs has attracted considerable attention by scientists, both in terms of understanding the basic biology of the innate immune system, and as a tool in the design of molecular templates for new anti-infective drugs. AMPs are gene-encoded short (<100 amino acids), amphipathic molecules with hydrophobic and cationic amino acids arranged spatially, which exhibit broad spectrum antimicrobial activity. AMPs have been subject of natural evolution, as have the microbes, for hundreds of millions of years. Despite this long history of co-evolution, AMPs have not lost their ability to kill or inhibit the microbes totally, nor have the microbes learnt to avoid the lethal punch of AMPs. AMPs therefore have potential to provide an important break through and form the basis for a new class of antibiotics. In this review, we would like to give an overview of cationic antimicrobial peptides, origin, structure, functions, and mode of action of AMPs, which are highly expressed and found in humans, as well as a brief discussion about widely abundant, well characterized AMPs in mammals, in addition to pharmaceutical aspects and additional functions of AMPs.

1. Introduction

Throughout evolution, the ability of an organism to protect itself from microbial or other species invasion has been a key factor for survival. All species, from bacteria to humans, resist the invasion of microorganisms through a simple mechanism, but complex in functions, involving antimicrobial peptides (AMPs). AMPs are components of innate immunity, forming the first-line of defense used by many organisms against the invading pathogens (Jenssen et al., 2006). AMPs are gene-encoded short (<100 amino acids), amphipathic molecules with broad-spectrum antimicrobial activity, displaying multiple modes of action, including bacteriostatic, microbicidal, and cytolytic properties. Even though AMPs are the first-line of defense against the invading microbes, ironically they are a highly neglected aspect of immunology, and are normally only addressed passing in general immunology textbooks. It is predicted that each species could contain a wide variety of AMPs (Hancock and Rozek, 2002), hence describing all them is beyond the scope of any review. Hence a complete inventory of antimicrobial peptides is neither possible nor intended. Instead, illustrative examples are provided, demonstrating key functional aspects of such peptides, as well as considerations and issues of importance for their development to therapeutics.

2. History

A new area of research started in the 1960s, when Spitznagel and Zeya discovered that basic proteins and peptides in polymorphonuclear (PMN) leukocytes display antimicrobial properties (Zeya and Spitznagel, 1963; Zeya and Spitznagel, 1966), later named “AMPs” (Ganz et al., 1985; Selsted et al., 1985). However, full fledged

investigations on AMPs started in 1972, when Boman observed that *Drosophila melanogaster,* when injected with non-virulent strain of *Aerobacter cloacae* followed by a injection of virulent strain of the same organism after few days, survived even after subjection to high doses of these bacteria (Boman et al., 1972). In a following series of investigations in 1980, Boman and his group showed that a 35 amino acid molecule (cecropin) is responsible for the strong antibacterial activity (Hultmark et al., 1980; Steiner et al., 1981). Later, the significance of AMPs raised to higher level when Boman and his colleagues demonstrated that AMPs are present in almost all invertebrates investigated (Boman, 1981). A wide commercial interest in perusal of these molecules started with the discovery of magainins in frog skin by Zasloff in 1987 (Zasloff, 1987). This work for the first time showed that AMPs are not only molecules of lower invertebrates, but also components of higher vertebrates. Since then, more than 1400 AMPs have been isolated from bacteria, insects and other invertebrates, amphibians, birds, fishes, mammals, and plants (Wang and Wang, 2004; Wang et al., 2009a; Thomas et al., 2010). During last few years, an increasing number of researchers world-wide have contributed to the continued rapid expansion of the area.

3. Antimicrobial peptides

AMPs represent a universal feature of defense systems existing in all living forms, and their presence in all along the evolutionary scale demonstrates their effectiveness and significance in combating invading pathogens (Table 1). AMPs are promptly synthesized and readily available shortly after an infection to rapidly neutralize a broad range of microbes. The ability to produce AMPs is well preserved in almost all living organisms

and cell types. Although, AMPs have a certain degree of similarity among themselves regarding the biophysical properties, their sequence is rarely similar among closely related or distinct species/organisms. But, in the case of some AMPs, a certain degree of identity is found either in the pattern of amino acids or in the pro-region or a conserved regions (e.g cathelicidins). This phenomenon could probably reflect the species adaptation to the unique microbial environments that characterize the niche occupied by the species (Boman, 2000).

3.1 Defensins

Defensins are 18-45 amino acid long cationic peptides with six conserved cysteines and 3 disulfide bonds without glycosyl- or acyl- side-chain modifications. Defensins were first discovered in human neutrophiles as small cationic molecules (Ganz et al., 1985; Selsted et al., 1985). Until now, defensins have been discovered in mammals (Ganz et al., 1985; Selsted et al., 1985; Ganz and Lehrer, 1995), insects (Saito et al., 1995), Hymenopteran parasitic wasp *Nasonia vitripennis* (Tian et al., 2010) and plants (Thomma et al., 2002), and a related form also found in fungi (Galgóczy L et al., 2010). The evolution of defensins seems to have followed the "standard model" of gene evolution i.e., duplication of the ancestral gene, followed by mutation and natural selection based on the needs of the organism (Hughes, 1999). It is hypothesized that mammalian β-defensins originated prior to α-defensins, as the former resemble insect defensins more. In addition, mammalian α-defensins genes are present in variable non-determinable multiple copies in a very tight region of a single chromosome and expressed in a small range of tissues (Yang et al., 2004a). In humans, defensins have been identified in the granules of

neutrophils, paneth cells, monocytes, macrophages, keratinocytes or mucosal epithelial cells of the respiratory, digestive, urinary and reproductive systems (Duits et al., 2002; Auvynet and Rosenstein, 2009; Lai and Gallo, 2009). They are synthesized as 93-96 amino acids long pro-peptides consisting of a signal region, an anionic pre-segment, and C-terminal cationic region. In the case of α-defensin, release of the C-terminal region from the pro-segment by elastase, metallo-proteinase, or other proteolytic enzymes, activates the antimicrobial activity. The occurrence of disulphide-bridged defensins in a wide variety of organisms underscores that stabilized structure is important for activity. For example, replacement of cysteine residues by acidic amino acids (e.g aspartic acid) leads to a loss of activity, whereas replacement with hydrophobic amino acids (except alanine and leucine) retains the activity (Zhao, 2003). However, in case of Human neutrophil peptide-1 (HNP-1), Human β-defensins (HBD-3), and mouse defensins, neither structure nor disulphide bonds is necessary for antimicrobial activity and cytotoxicity (Kluver et al., 2005), but of utmost importance for antiviral activity (Daher et al., 1986), protease resistance (Peschel and Sahl, 2006), and chemotactic activity (Wu et al., 2003). Defensins have been shown to have a broad-spectrum antimicrobial activity against bacteria, fungi, and enveloped viruses, although most defensins lose much of their antimicrobial activity at physiological concentration of Na^{+}, Mg^{2+} or Ca^{2+} (Bals et al., 1998a; Bals et al., 1998b; Singh et al., 1998; Singh et al., 2000; Shafer et al., 2006). Having said that, it is also worth noting that electrolytes may have a more complex effect on peptide induced antimicrobial effects. For example, it has been shown that carbonate ion alters the gene expression pattern of key regulatory factors in the *S. aureus* and *E. coli,* thus making them more susceptible to antimicrobial peptides (Dorschner et al.,

2006).

Based on the site of expression, size, structure and pattern of disulphide bridges, defensins are classified into 3 types; α, β, and θ defensins.

3.1.1 α-Defensins

α-defensins are 29-35 residues long with a disulfide alignment pattern of 1-6, 2-4, and 3-5 (Figure 1). α-defensins are either stored as propeptides (in paneth cells) or as active processed matured peptides (in neutrophils). In humans, more than 30 α-defensin genes have been predicted using a bioinformatic approach (Schutte et al., 2002) , however at the protein level only 6 α-defensins have been reported. Of these, four are expressed in neutrophils and referred to as human neutrophil peptides (HNPs) and 2 human defensins (HD5 and HD6) are expressed in paneth cells (Jones and Bevins, 1993) and epithelial cells (Darmoul and Ouellette, 1996). In human, α-defensin genes are located on the chromosomes 8 (HNP-1, HNP-3) and 23 (HNP-4, HD-5, HD-6) (Sparkes et al., 1989; Liu et al., 1997). Interestingly, HNP-1, -2, -3 constitute 5% of the total protein, and constitute 99% of the total defensins content found in neutrophils. On the other hand, HNP-4 concentration is 100 fold lower than other HNPs (Shiomi et al., 1993; Ganz and Lehrer, 1994). Defensin genes are unequally inherited in multiple copies in different individuals due to which the concentration of defensins varies among individuals significantly (Lehrer et al., 1993; Yang et al., 2004a). After microbial stimulation, the concentration of the defensins within the crypt is estimated to reach 1-5 μM, which is sufficient for strong microbial action (Ayabe et al., 2000).

3.1.2 β-Defensins

β-defensins are 36-42 amino acids long peptides having a 1-5, 2-4, 3-6 disulfide alignment pattern and a longer N-terminal region, in comparison to α-defensins (Figure 1). Till now, only four types of human β-defensins (HBD) have been discovered in plasma, testis, gastric antrum (Schneider et al., 2005), epithelial cells and neutrophils (Namjoshi et al., 2008). Previously, it was thought that except HBD1, other HBDs are expressed mainly on inflammatory or infectious stimuli (Narayanan et al., 2003; De Smet and Contreras, 2005; Selsted and Ouellette, 2005). However, recent data suggest that HBD2 is expressed constitutively in prostate epithelial cells (Kim et al., 2011). It has also been demonstrated that defensins of this class can stimulate host adaptive immunity (Yang et al., 2004a; Meyer-Hoffert et al., 2010).

3.1.3 θ-Defensins

θ-Defensins are formed by post-translational ligation of two 9-residue sequences derived by heterodimeric splicing of α-defensin-related precursors. The mature θ-defensin peptide is a circular two-stranded β-sheet that is stabilized by three disulfides. However, the parallel orientation of the θ-defensin disulfide arrangement allows substantial flexibility around its short axis. θ-defensins have been isolated from neutrophils of rhesus monkey (*Rhesus macaque*) (Tran et al., 2008) and olive baboon (*Papio anubis*) leukocytes only (Garcia et al., 2008). Humans appear not to produce θ-defensins, due to a premature termination codon in the signal peptide (Cole et al., 2002), and no data exist about the presence of these molecules in other animals. θ-defensins have been shown to

possess potent antiviral properties, especially against human immunodeficiency virus (HIV) and herpes simplex virus (HSV) (Tang et al., 1999).

3.2 Cathelicidins

Cathelicidins form the second largest group of AMPs produced by mammals, and are characterized by far N-terminal end, a central conserved region, and a variable C-terminal region (Zanetti et al., 1995; Giuliani et al., 2007) (Figure 1). Like defensins, cathelicidins are synthesized as propeptides, which are cleaved in a two-step process to release the active peptides. To date, cathelicidins have been found in fish (Uzzell et al., 2003), birds (van Dijk et al., 2005), snakes (Wang et al., 2008; Zhao et al., 2008), and mammals (Zanetti et al., 1995). There is only one type of cathelicidin in humans (hCAP18), rat and mice (CRAMP), rhesus monkey (rhLL-37) (Bals et al., 2001), macaque and leaf-eating monkey (RL-37) (Zelezetsky et al., 2006). In contrast, cattle (Bactenecin, Indolicidin, Prophenins), and sheep (OaDode, SMAP-29) have different types of cathelicidins with varied C-terminal and conserved N-terminal (Zanetti, 2004; Tomasinsig and Zanetti, 2005; Durr et al., 2006).

In humans, the hCAP18 propeptide is processed by a serine proteinase 3 or aspartic protease gastricin in neutrophils to release the active fragment LL-37 or ALL-38 respectively (Sörensen et al., 2001; Sorensen et al., 2003; de Haar et al., 2006). LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) is a 37 amino acid long peptide with a highly hydrophobic N-terminal region and C-terminal region that adapt α-helical conformation in the presence of negatively charged lipids. Due to its amphipathic

nature, LL-37 binds to bacterial membranes and lipopolysaccharide (LPS), and also displays potent and broad spectrum antimicrobial properties (Larrick et al., 1995; Pasupuleti et al., 2009a). LL-37 is highly degradable by various enzymes (Sieprawska-Lupa et al., 2004; Pasupuleti et al., 2009a; Pasupuleti et al., 2009d; Stromstedt et al., 2009) and may lose its activity by binding to blood components such as plasma, albumin, Glycosaminoglycans (GAGs), etc (Pasupuleti et al., 2007; Schmidtchen et al., 2009; Bucki et al., 2010). Even in the presence of blood components, however, it can coordinate with other components of the innate immunity, such as recruiting neutrophils to the site of infections (Zanetti, 2004; Bowdish et al., 2005a). In humans, LL-37 is expressed in neutrophils, macrophages (Rivas-Santiago et al., 2008), monocytes, B-cells, T-cells (Agerberth et al., 2000) and in most types of epithelial cells such as skin, lung (Frohm et al., 1997), seminal plasma (Malm et al., 2000) and epididymis (Sorensen et al., 2008) etc. [For comprehensive review about LL-37, please refer see (Nijnik and Hancock, 2009; Bucki et al., 2010)

3.4 Histatins

Histatins are a group of histidine-rich peptides ranging in size from 7 to 38 amino acid residues. They are constitutively expressed by the parotid and submandibular/ sublingual salivary glands in humans (Rijnkels et al., 2003) and higher primates like macaques (Sabatini et al., 1989) with broad spectrum antimicrobial activity (De Smet and Contreras, 2005; Wiesner and Vilcinskas, 2010). Basically there are three types of gene-encoded histatins, which undergo cleavage by proteases to generate twleve different types of histatins of which histatin 1, 3 and 5 are prominent (Oppenheim et al., 1988; Sabatini

and Azen, 1989; Xu et al., 1992; Tsai and Bobek, 1998; Campese et al., 2009). The mode of action of histatins includes is not only by membrane permeabilization only, instead it also targets the mitochondria or mitochondrial F0F1 ATPase protein (Luque-Ortega et al., 2008) thereby causing efflux of ATP, resulting in depletion of intracellular ATP contents and ultimately death (Tsai and Bobek, 1998; Rothstein et al., 2001; Kavanagh and Dowd, 2004). Like most AMPs, histatins are not only antimicrobial, but also have other functions such as inhibition of hemagglutination (Murakami et al., 1990), co-aggregation and neutralisation of lipopolysaccharides (LPS) (Sugiyama, 1993; Cirioni et al., 2004), tannin binding (Yan and Bennick, 1995; Naurato et al., 1999) and wound healing (Oudhoff et al., 2008).

4. Antimicrobial proteins

In order to defend the host from infection, innate immunity is not only equipped with short cationic peptides that are synthesized prior to or after infection, but also with a large number of proteins which are on constant surveillance in the system. For example, bactericidal permeability increasing protein (BPI) is a 60 kDa protein with two distinct functional domains; the N-terminal 25 kDa fragment is antimicrobial, where as the C-terminal fragment is LPS-binding and antiangiogenic (van der Schaft et al., 2000). To date, BPI has been isolated from rabbit PMN cells, human epithelial cells and PMN cells (Canny et al., 2002). Strikingly, both rabbit and human BPI are very similar in structure and function. BPI inhibits Gram-negative bacteria, but is not active against Gram-positive bacteria or eukaryotic cells (Elsbach, 1990). In a sense, BPI is a unique molecule, as it acts in synergy with large number of diverse immune defence molecules e.g defensins, membrane attack complex of the complement system to acts at sites of inflammation.

Human heparin-binding protein (HBP) or CAP37 or azurocidin is 37 kDa basic, proteolytically inactive neutrophil elastase homologue, with heparin binding and antimicrobial activity (Heinzelmann et al., 1998). Histidine-rich glycoprotein (HRG) is a 67 kDa heparin-binding histidine-rich plasma protein, which is synthesized in liver and is present in human plasma at high concentration (1.5–2 μM) (Haupt and Heimburger, 1972; Heimburger et al., 1972; Jones et al., 2005a; Jones et al., 2005b). HRG contains two cystatin-like domains, a variable C-terminal region and a central histidine-rich region (HRR) with highly conserved GHHPH (Gly-His-His-Pro-His) tandem repeats flanked by proline-rich regions (Jones et al., 2005a; Jones et al., 2005b). HRG can acquire positive net charge either by incorporation of Zn^{2+}, or by protonation of histidine residues (~13%) in the HRR domain at acidic conditions (Jones et al., 2005a; Jones et al., 2005b; Kacprzyk et al., 2007), thereby obtaining antimicrobial activity (Kacprzyk et al., 2007; Rydengard et al., 2007; Rydengard et al., 2008). Recently, various novel roles have been discovered for HRG derived peptides, involving antiangiogenesis (Donate et al., 2004), antitumor activity (Olsson et al., 2004), chemotaxis, production of cytokine/chemokines in monocytes (Heinzelmann et al., 2001), as well as multiple interactions involving ligands such as heparin, plasminogen, fibrinogen, thrombospondin, heme, IgG, FcγR, and C1q (Rasmussen et al., 1996; Jones et al., 2005a).

Lactoferrin (80 kDa), is a major epididymal globular multifunctional secretory protein found abundantly at mucosa, secreted fluids, like semen, tears, and breast milk with a potent antimicrobial and immunomodulatory activity (Gonzalez-Chavez et al., 2009). Like cathelicidins, proteolysis of lactoferricin generates two different antimicrobial

peptides, the N-terminal derived lactoferricins (Gifford et al., 2005) and the kaliocins derived from an interior sequence (Viejo-Diaz et al., 2005). Lactoferrin can permeabilise membranes and disperse LPS through cation-mediated process especially chelating $Fe3^{+}$ (ferric state) ions (Valenti and Antonini, 2005). [For a comprehensive review about lactoferrin, please see (Gifford et al., 2005; Valenti and Antonini, 2005; Gonzalez-Chavez et al., 2009)]. Inaddition, lysozyme is a 14 kDa enzyme which is widely distributed in biological fluids, many cell types, and tissues. Lysozyme damages bacterial cell walls by catalysing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan. Lysozyme is active against Gram-positive bacteria by itself and can kill Gram-negative bacteria only in synergy with other AMPs e.g lactoferrin, HBDs, LL-37 (Chen et al., 2005). Similarly, major basic protein (MBP) is 13 kD small basic protein, rich in arginine and cystine-rich amino acids, which comprises almost half of the protein content in the large specific granules of mammalian eosinophils. Even though this protein is toxic to human cell, host tissue damage is negligible due to the delivery of the protein locally at the target site. At high concentrations, MBP has been shown to be antibacterial, antihelminthic, cytotoxic and also involved in immune hypersensitivity reactions, but the exact mode of antihelminthic action of this protein is remains unknown (Swaminathan et al., 2005). Finally**,** RNase 7 is a 14.5-kDa protein expressed in healthy skin as a part of host immune response (Harder and Schroder, 2002; Dyer and Rosenberg, 2006). It belongs to the ribonuclease A superfamily due to the presence of two histidines and one lysine, 6-8 cysteines with disulphide bonds, which form the catalytic site core. To date eight different types of RNase family members have been discovered. Of these, RNase 7 has been shown to

posses a broad spectrum antimicrobial activity (Harder and Schroder, 2002; Zhang et al., 2003).

5. AMPs generated by proteolysis

In addition to classical AMPs, the innate immune system seems to be based on a large number of antimicrobial polypeptides generated by proteolysis, by infection-associated enzymes of endogenous proteins such as prion, kininogen, growth factors, complement proteins, coagulation factors such as thrombin, tissue factor pathway inhibitor-derived peptides, superoxide dismutase, etc (Andersson et al., 2004; Nordahl et al., 2004; Nordahl et al., 2005; Frick et al., 2006; Malmsten et al., 2007; Pasupuleti et al., 2009b; Pasupuleti et al., 2009c; Papareddy et al., 2010a; Papareddy et al., 2010b). Of these, anaphylatoxins are small molecules, which are generated through complement activation (Zipfel and Reuter, 2009). These molecules play an important role in inflammation and are responsible for the activation of various components of the innate and adaptive immune system (Hugli, 1990). C3a, generated by activation of complement factor C3, is 77 amino acids long (MW of 9 kDa), contains α-helical cationic regions stabilized by three disufide bonds, and carries a net charge of +2 (pI 11.3). Similarly, C4a is derived from complement factor C4 by action of protease C1s, and is a cationic polypeptide with 77 residues and devoid of histidin, tryptophan, and carbohydrate (Moon et al., 1981). C5a, finally, is 74 amino acids long with four helices connected by loops and is released from C5 by the action of C5 convertase. C3a, C4a, but not C5a, have been shown to be antimicrobial and antifungal (Nordahl et al., 2004; Pasupuleti et al., 2007). It was concluded from the sequence comparison that C3a, C4a, and C5a are derived from

precursor molecules that share a common genetic origin. However, alignment and homology (dN/dS) studies between C3a, C4a, and C5a indicate only a 30% homology between C3a and C5a, and a 36% homology between C5a and C4a (Moon et al., 1981; Hugli, 1990; Pasupuleti et al., 2007). It is worth noting, however, that although the primary sequences of C3a, C4a and C5a differ significantly between species, the crucial elements required for the stability and integrity of molecules are conserved, especially cystein and arginine residues (Pasupuleti et al., 2007).

High molecular weight kininogen (HMWK) is a 120 kDa, multifunctional glycoprotein found in plasma and in mast cell α-granules. It consists of 5 different domains, each with different biological functions. It is a parent protein for bradykinin and serves as a cofactor for coagulation factor XI and prekallikrein assembly on biologic membranes. Unlike the complement system, cleavage of kininogen releases peptides with potent vasoactive, proinflammatory, and antiangiogenic properties (Colman et al., 2000). It has been shown that kininogen, when cleaved by neutrophil elastase enzymes releases a domain 5 fragment, which is antimicrobial in nature (Nordahl et al., 2005). Furthermore, cleavage by plasma kallikreins during contact system activation releases antibacterial peptides (Frick et al., 2006), as well as the bradykinin sequence.

6. AMP classification

Many AMPs share similar characteristics like positive net charge and amphipathicity, thus it not entirely straightforward to classify them based solely on physical characteristics. In addition, AMPs are present in all living forms and sequence diversity

among them is so large, that it is difficult to classify them based on sequence similarity or dissimilarity (Epand et al., 1999; van't Hof et al., 2001). As an alternative, therefore, the conformation adopted by a peptide on interaction with bacterial membranes may sometimes be a useful as a base for classification.

6.1 Helical peptides

Helical peptides are the most abundantly distributed and widely studied groups of AMPs, constituting about 27% of all AMPs with known secondary structure (Boman, 1995; Tossi et al., 2000). Many such peptides display distinct amphiphilic characteristics with about 50% hydrophobic residues, frequently appearing in repeated patterns. The reason for this is that when the peptide forms a α-helical structure the hydrophilic residues end up on the same side of the helix normal, thereby resulting in a conformation-dependent amphiphilicity. Frequently, these peptides are unstructured in an aqueous environment, but adopt an helical conformation upon encountering lipid membranes (Gesell et al., 1997). Capping at the N- and C-terminus stabilizes the helix further and results in AMPs less susceptible to electrolyte concentration (Park et al., 2004). Peptides belonging to this group usually kill microbes by creating membrane defects, leading to a loss of gradients in electrolytes, signal substances, and other factors. One of the best-studied peptide of this class is LL-37 (Durr et al., 2006). Furthermore, not only cationic peptides, but also hydrophobic anionic α-helical peptides e.g Lysenin, dermcidin (Schittek et al., 2001; Harris et al., 2009) belong to this group. However, due to their poor solubility (or solvency, rather) the later class exhibits a lower selectivity between microbes and mammalian cells (Kobayashi et al., 2004).

6.2 β-sheet peptides

In contrast to helical peptides, β-sheet peptides are frequently cyclic molecules constrained by intramolecular disulfide bridges. The best-studied peptides in this group are defensins (discussed above) and Protegrins (PG). Until now, dozens of PG mutants have been synthesized based upon the five naturally occurring forms (Ostberg and Kaznessis, 2005). Protegrins, originally isolated from the porcine leukocytes, are arginine-rich 16-18 amino acids long with two intramolecular cystine disulfide bonds, giving them an anti-parallel β-hairpin like structure (Kokryakov et al., 1993). Due to this highly constrained β-hairpin structure, PG possesses an amphipathic nature common to most AMPs. However, this rigid structure, in naturally occurring forms, prevents PG to undergo significant conformational changes upon association with membranes or upon oligomerization and also accounts for higher toxicity against eukaryotic cells (Ostberg and Kaznessis, 2005). In contrast to defensins, disulphide bond in PG is necessary for their antimicrobial activity, removing them decreases the activity significantly or abolishes it totally (Tamamura et al., 1995; Qu et al., 1997; Cho et al., 1998b). Recently Wang et al., showed that disulphide in Ib-AMP1, a 20-residue β-sheet AMP found in the seeds of *Impatiens balsamina*, is responsible for the intracellular activity. Interestingly, disulfide bonds in this system were found not to be needed for activity, although target specificity depends on the disulfide bond, as linear Ib-AMP1 were found to be more membrane active than their disulfide analogues, which were oriented to intracellular targets (Wang et al., 2009b). It has furthermore been found that for antimicrobial activity, maintenance of a suitable hydrophobic and hydrophilic balance, as well as cyclization is

important for this group of peptides (Matsuzaki et al., 1997; Rao, 1999; Powers and Hancock, 2003).

6.3 Over representation of one or more amino acids

Not all AMPs belong to the above-mentioned classes, and instead display largely disordered random coil structures. As indicated above, AMPs have a high frequency of cationic lysine and arginine (in a few cases also histidine) residues, while anionic aspartic and glutamic acid are relatively rare. Most AMPs therefore carry a net positive charge, the average net charge being +4. Another amino acid that stands out is tryptophan, which is 50% more common in AMPs than its general occurrence (The Antimicrobial Peptide Database. http://aps.unmc.edu/AP/). The preference for these amino acids in AMPs is motivated by the specific characteristics they confer to peptide performance. For example, the cationic charge of the vast majority of AMPs is a result of the anionic nature of most microorganisms. By charge attraction, the peptides reach lytic concentration in anionic bacteria membranes. In addition, amphiphilicity is important for many AMPs in order to adsorb to and disrupt membrane bilayers, requiring hydrophobic and/or surface active amino acids.

7. Biophysical parameters influence the antimicrobial activity of AMPs

As selective toxicity is crucial for AMPs, they must have a set of biophysical themes or constrains which contribute to AMPs selectivity.

7.1 Sequence

The most characteristic feature found in AMPs is a high degree of functional conservation, but little conservation in peptide sequence. For example, most helical AMPs, irrespective of sequence, contain 30 - 60% of hydrophobic amino acids (Giangaspero et al., 2001) arranged in the pattern of i+3 or i+4 (Pasupuleti et al., 2008). The reason being, when the peptide assumes helical structures all the hydrophobic and hydrophilic amino acids are on two different planes forming a perfect amphipathic structure. Although there is limited sequence homology among AMPs even those belonging to similar families or isolated from the same animals, there is some degree of conservation of specific amino acids at significant positions. In most AMPs, aspartic acid and glutamic acid are rarely seen, whereas cationic amino acids like lysine or arganine are highly overrepresented. In addition, cysteines and tryptophan residues are involved in disulfide bonds and hydrophobic interactions, respectively. Moreover, glycine is frequently found at the C- or N-terminal position as it is a good capping agent, helix stabilizer, provides protection from amino or carboxypeptidases (Tossi et al., 2000; Pasupuleti et al., 2008), and forms a substrate for C-terminal amidation. Based on the low degree of specific sequence conservation in AMPs, it has been suggested that thermodynamics plays a major role in specificity and mechanism of peptide action, rather than the sequence itself. The fundamental reason for the divergence in sequence and retained antimicrobial function is due to the necessity of the host immune system to adapt successfully to different environments by retaining its efficiency against specific microbial pathogens (Tossi et al., 2000). Also contributing to this, AMPs have evolved to act, under different physiological conditions, and against distinct microbial targets that

differ in their membrane characteristics.

7.2 Net charge (Q)

Most AMPs carry a net charge of +2 - +11 due to the overrepresentation of lysine and/or arginine (Yeaman and Yount, 2003; Niyonsaba et al., 2006) and the absence or sparsity of aspartic or glutamic acid (Yount and Yeaman, 2005). It is widely accepted that cationicity is primarily responsible for the initial interaction of the AMP with the negatively charged membrane surface of the bacteria (Yount and Yeaman, 2005; Yount and Yeaman, 2006). For example, in the membrane of red blood cells, phosphatidylcholine (PC) and sphingomyelin (SM) are abundant in the outer layer, while the inner leaflet mainly contains amino lipids (phosphatidylethanolamine (PE) and phosphatidylserine (PS)). These lipids are zwitterionic (except for PS), rendering the outer part of the membrane uncharged. On the contrary, the outer membrane of bacteria is rich in anionic lipids. For example, *E. coli* membranes are typically composed of about 91% PE, 3% phosphatidylglycerol (PG), and 6% cardiolipin, while the cytoplasmic membrane contains 82% PE, 6% PG, and 12% cardiolipin (Lugtenberg and Peters, 1976). *S. aureus* membranes, on the other hand, contain PG lipids only, typically of the order 36% PG, 7% cardiolipin, and 57% Lysyl Phosphatidylglycerol (Bonsen et al., 1967; Haest et al., 1972). In addition to peptidoglycan, Gram-negative bacteria contain negatively charged LPS (up to 50% of the outer membrane), and Gram-positive bacteria teichoic acid, which causes additional negative charge on the surface (Yeaman and Yount, 2003). Studies with magainin (Dathe et al., 2001) and other helical peptides (Giangaspero et al., 2001; Pasupuleti et al., 2008) have demonstrated a direct correlation

between peptide charge and potency. However, increasing the charge beyond +7 does not increase the activity further, due to strong interactions between the peptide and phospholipid head groups, which prevents structuring (Tossi et al., 1994) and translocation into the deeper layers of membranes. At the same time, however, studies with acetylated and non-acetylated defensin peptides reveal some complexity in the relationship between antimicrobial property and charge (Papanastasiou et al., 2009).

7.3 Hydrophobicity (H)

Mean hydrophobicity of a peptide is defined as the proportion of hydrophobic residues within a peptide and is typically around 50% for most AMPs. Hydrophobicity is an important physicochemical characteristic of AMPs, which is considered to be independent of other structural parameters (Yount and Yeaman, 2005; Pasupuleti et al., 2009a; Pasupuleti et al., 2009d; Schmidtchen et al., 2009). Biophysical studies have shown that hydrophobicity can modulate the antimicrobial efficiency and specificity of individual α-helical AMPs, as hydrophobicity govern the extent to which a peptide can partition into the lipid bilayers (Giangaspero et al., 2001; Dennison et al., 2005a; Ringstad et al., 2007; Ringstad et al., 2008). It is noteworthy that different strains and types of microbes respond differently to increasing hydrophobicity (Jiang et al., 2008). Although hydrophobicity is required for membrane permeabilization, above optimum levels leads to a loss of antimicrobial activity (Yount and Yeaman, 2005), and the increase in mammalian toxicity (Chen et al., 2007; Khandelia et al., 2008; Pasupuleti et al., 2008; Schmidtchen et al., 2009). The reason for increased toxicity, with highly hydrophobic peptides, in eukaryotic cells is that peptides with higher hydrophobicity

experience poor solubility conditions in aqueous solution, therefore binding to (and disrupting) also eukaryotic cell membranes (Dennison et al., 2005b). Thus, a strong correlation is observed between cytotoxicity and hydrophobicity (Blondelle and Houghten, 1992; Bessalle et al., 1993; Javadpour et al., 1996; Skerlavaj et al., 1996; Pasupuleti et al., 2008). Since hydrophobic interactions are needed for antimicrobial activity at physiological electrolyte concentrations, a balance is therefore needed between electrostatic and hydrophobic interactions.

Related to hydrophobicity and amphiphilicity (the latter frequently expressed as a hydrophobic moment), polar angle (θ) is a measure of the relative proportion of polar versus non-polar facets of a peptide conformed to an amphipathic helix (Yount and Yeaman, 2005). For hypothetical helical peptides, composed solely of hydrophobic residues on one face and hydrophilic residues on the other side, the polar angle will be 180°. Most naturally occurring helical AMPs have a polar angle of 140° - 180° (Tossi et al., 2000). The polar angle has been shown to influence the overall stability and halflife of the peptide-induced membrane pores (Uematsu and Matsuzaki, 2000). In studies with natural and synthetic AMPs, peptides with a smaller polar angle, i.e., greater hydrophobic surface, have been shown to induce more extensive membrane permeabilization, translocation, and pore formation rates than peptides with higher polar angle (Dathe et al., 1997; Wieprecht et al., 1997).

In conclusion, the activity of AMPs is not determined by a single factor but by a subtle combination of factors such as sequence, net charge, hydrophobicity and position of

cationic residues. Amphipathicity, hydrophobicity, and conformation of a peptide also play a role in the antimicrobial activity, however there is no strict rule regarding the optimal number of charged and hydrophobic residues for maximum antimicrobial activity and minimum cytotoxicity as it varies widely among different peptides and even within a given structural group (Khandelia et al., 2008).

8. Mode of action

Despite the great success in identifying novel AMPs from various sources, there are still some areas where there is a great dearth of information, especially with regard to the mode of action. Enhanced understanding of this will be of great use in peptide-based drug designing (Zasloff, 1992; Zasloff, 2002; Hale and Hancock, 2007). Although AMPs belong to innate immunity, the mechanism by which they kill the microbes is quite different from that of cytokines and phagocytes. Before we look at the mode of action, however, we need to understand the membrane biology of bacteria, fungi and eukaryotic membranes, which are the primary target for most AMPs. Universally, all cell membranes are fluid mosaics of proteins and phospholipids, which are arranged as bilayers with hydrophobic and hydrophilic domains. However, there exists a significant lipid compositional difference between the prokaryotic and eukaryotic membranes as well as among cell types. Bacterial membranes are made up of negatively charged phospholipids such as PG, cardiolipin (CL), or PS (Cronan, 2003), which are stabilized by the divalent cations such as Mg^{+2} or/and Ca^{+2}. Even though there is not much difference in lipid composition, Gram-negative bacteria differ from Gram-positive bacteria as the former have a smaller peptidoglycan layer and an outer membrane, in

addition to a cytoplasmic membrane containing LPS, which acts as permeability barrier (Hancock, 2001). Interestingly some peptide binding to Gram-negative bacterial membranes induce Mg^{2+} ion displacement between the LPS, thus destabilizing the membranes arrangement (Takahashi et al., 2010). In contrast to bacteria, fungal membranes are rich in phosphomannans and other related constituents such as negatively charged phosphatidylinositol (PI), PS, and diphosphatidylglycerol (DPG), which give a higher negative charge surface for the membranes (Prasad and Ghannoum, 1996; Yount and Yeaman, 2006; Jiang et al., 2008).

On the other hand, mammalian membranes are rich in zwitterionic phospholipids with neutral net charge, including PE, phosphatidylcholine (PC), or sphingomyelin (SM). Moreover, cholesterol is present in significant amounts in mammalian membranes and can reduce the activity of AMPs by affecting the fluidity and dipole potential of phospholipids, in addition to stabilizing the lipid bilayers and delaying the binding of peptides to the membranes (Tytler et al., 1995; Matsuzaki, 1999). Therefore, sterol in the mammalian membranes is thought to be involved in differentiating mammalian and fungal cells from prokaryotes (Tytler et al., 1995). However, cholesterol in the membrane is not the sole molecule that influences the specificity because *Fusiarum moliniforme*, a fungus containing cholesterol, is as sensitive to cecropin as ergosterol-containing *Fusiarum oxysporium* and *Aspergillus sp* (De Lucca et al., 1998a; De Lucca et al., 1998b; van't Hof et al., 2001). These studies point out that in addition to cholesterol, membrane potential and asymmetric distribution of phospholipids in eukaryotic membranes contribute to prevention of AMPs binding (Matsuzaki, 1999; Lai and Gallo, 2009).

A widely accepted notion is that electrostatic interaction between the positively charged amino acids and negatively charged LPS /phospholipid head group of the target cell is involved in the binding and accumulation of the peptides on the surface of the membrane (Epand et al., 2010). Although the specific orientation of a peptide may vary between systems, it will normally reside at the membrane interface until a threshold in peptide interfacial concentration is reached (Yount and Yeaman, 2005). Parameters influencing the threshold concentration include the propensity of peptide self-assembly, peptide charge, amphipathicity, and hydrophobicity, as well as membrane fluidity and composition (Yang et al., 2000; Yount et al., 2006). Through peptide binding and incorporation into the lipid membrane, packing and defects are introduced through one or several mechanisms (Figure 2).

Barrel-stave model, also known as helical bundle model, was first proposed by Ehrenstein and Lecar in 1977 (Ehrenstein and Lecar, 1977). According to this model, a variable number of individual peptide molecules are arranged to form a barrel-like pore or channel (Figure 2). In this mechanism, peptide hydrophobic surfaces interact with the acyl chains of lipid in the membrane, generating an aqueous pore consisting of at least four peptides (Ehrenstein and Lecar, 1977; Reddy et al., 2004). A crucial step in this model is that peptides have to recognize each other in the membrane bound state. It is highly energetically unfavorable for a single peptide to traverse the membrane, hence the peptides aggregate on the surface until the threshold concentration is reached, and then insert into the hydrophobic core of the membrane by undergoing a conformational phase

transition, forcing polar–phospholipids head groups aside to induce localized membrane thinning (Yeaman and Yount, 2003). This event is followed, by additional recruitment of peptides around/in the channel, leading to an increase in pore size and stabilization, thus killing the microbe by leakage of intracellular components (Figure 2). Aggregation may be required simply because the amount of stabilization that one peptide provides is not sufficient for pore formation and stronger binding of peptides to pores could be due to packing reasons as lipid head groups are less tightly packed in pores (Mihajlovic and Lazaridis, 2010b; Mihajlovic and Lazaridis, 2010a). Interestingly, a large number of membrane conductance studies with Alamethcin (Sansom, 1993), Pardaxin (Rapaport and Shai, 1991) and α-5 segment of the Cry delta-endotoxin family (Gazit et al., 1994) have suggested that transmembrane pore formation is not a single step process but involving multiple steps, however this needs to be further investigated (Sansom, 1993; Yeaman and Yount, 2003). On the other hand, this type of pores demands quite specific peptide properties in terms of size, helicity, and amphiphilicity, rendering this mechanism less frequent. Indeed, barrel-stave pores have only been experimentally demonstrated to occur for a few peptides, alamethicin being the most well-known example (Qian et al., 2008; Mihajlovic and Lazaridis, 2010b). Although, barrel stave model is frequently viewed as prototypic for peptide induced transmembrane pores, it can only applied to very small group of AMPs.

Contrary to pores of the “barrel- stave” type, toroidal pores can be formed by a much greater variety of peptides (Figure 2). Prior to formation of both barrel-stave and toroidal pores, the peptide adsorbs parallel to the membrane surface (Huang, 2006; Huang, 2009;

Melo et al., 2009). When a certain (local) concentration is reached, the peptide either inserts into the membrane, or induces a positive curvature strain in the membrane, resulting in an opening, the so-called toroidal pore. Upon further increasing the peptide concentration, or simultaneously with toroidal pore formation, two additional scenarios may take place. In one of these, higher peptide amounts on the membrane surface may eventually cause micellization in a detergent-like manner (Shai, 2002), although initial pore formation is not a prerequisite for this action. In the second one, the chemical potential imbalance across the bilayer due to peptide adsorption on the outer leaflet results in peptide translocation across the membrane to the inner membrane leaflet, which can take place through transient toroidal pores or without pore formation (Ludtke et al., 1995; Pokorny and Almeida, 2004). In addition, peptide adsorption in the polar head group region causes lateral expansion of the lipid membrane, which allows relaxation of the alkyl chains and results in membrane thinning, further facilitating membrane rupture (Ludtke et al., 1995; Wu et al., 1995; Mecke et al., 2005). Depending on the composition of the membrane, also peptide-induced phase transitions or lipid segregation may cause membrane rupture (Lohner et al., 2008). Membrane lipids such as PE, an abundant component of bacterial membranes, are also sensitive to phase transitions, and experimental data have shown that peptides may induce transitions from lamellar to cubic (Angelova et al., 2000) or reversed hexagonal phases (El Jastimi and Lafleur, 1999) in PE-containing membranes. Segregation of membrane lipids, e.g., due to favorable interactions between cationic peptides and anionic membrane lipids, may also cause membrane rupture (Epand and Epand, 2009).

Although it has been extensively demonstrated that the AMPs permeabilize lipid membranes and bacterial walls, and that this correlates to the antimicrobial effects, AMPs may have also other targets. Thus, an increasing number of studies have showed that peptides translocate the membrane, block essential cellular processes, and kill the bacteria without damaging the membrane extensively (Zhang et al., 2001; Patrzykat et al., 2002). In such studies, multiple cell targets or alternative mode of action have been demonstrated, including inhibition of nucleic acid synthesis (Pleurocidin, Buforin–II) (Subbalakshmi and Sitaram, 1998; Patrzykat et al., 2002), RNA synthesis (Bac5, Bac 7) (Park et al., 1998), protein synthesis (Indolicidin , PR-39, Attacins) (Carlsson et al., 1991; Boman et al., 1993; Subbalakshmi and Sitaram, 1998), enzymatic activity (Pyrrhocidin, Apidaecin and Drosocin) (Otvos et al., 2000; Kragol et al., 2001), ATP efflux (histatins) (Tsai and Bobek, 1998; Rothstein et al., 2001; Kavanagh and Dowd, 2004; De Smet and Contreras, 2005), and cell wall synthesis (Nisin) (Brumfitt et al., 2002). Importantly, whatever target for such metabolic effects, AMPs have to interact with the membrane(s) prior to entering the pathogen interior (Hancock, 2005). It is likely that the mode of action of individual peptides may vary in accordance with the microbe targeted, concentrations at which they are assayed, and the physical properties of the interacting membranes (Jenssen et al., 2006).

9. Basis for antimicrobial activity vs. toxicity

A key factor for AMP action is their target specificity by which they kill microbes but not mammalian cells. Although a lot of information has been obtained about structure-activity relationships in AMPs, basic rules governing the differences in selectivity and

toxicity among peptides remain to be fully understood (Glukhov et al., 2005). It has been proposed that the negatively charged outer surface of bacteria and the higher negative transmembrane potential ($\Delta\Psi$) accounts for the preferential binding of AMPs, which have a net positive charge (Matsuzaki, 1999). For example, pathogenic bacteria in the mid logphase have a $\Delta\Psi$ ranging from -130 to -150 mV (Yount et al., 2006). On the contrary, eukaryotic cells have a $\Delta\Psi$ ranging from -90 to -110 mV. In addition, fluidity of bilayers, dipole moment, curvature, and content of acidic phospholipids such as PG or PS in bilayers also play a minor role in the activity. The significance of these factors is more prominently seen for the activity difference among the sub-strains of bacteria (Matsuzaki et al., 1991; Oren et al., 1997).

Under physiological conditions, human cells are resistant to AMPs, but some AMPs such as LL-37 and DP1, show cytotoxicity at high concentrations (Johansson et al., 1998; Shaykhiev et al., 2005). Therefore, AMP cytotoxicity should be assessed carefully with an understanding of the limitation of the experiment. Generally, it is believed that differences in the distribution of hydrophilic and hydrophobic residues in the peptides are responsible for different modes of action on the membrane, even the hemolytic ability (Rodziewicz-Motowidlo et al., 2010). The hemolytic assays with human erythrocytes are used as a simple way to demonstrate toxic ability of peptide against human cells, with good noise to signal ratio. However, data derived from *in vitro* and *ex vivo* erythrocyte assays or hemolysis have limited utility, as such assays are carried out in austere buffer rather than in complex biomatrices and *in vivo* (Maisetta et al., 2008). As many AMPs display limited eukaryotic membrane binding at physiologic ionic strength and presence

of serum, the predictive capacity may be compromised (Makovitzki and Shai, 2005; Maisetta et al., 2008; Pasupuleti et al., 2008; Pasupuleti et al., 2009d; Schmidtchen et al., 2009). Taken together, the degree to which AMPs permeabilise or lyse human erythrocytes may therefore not reflect their relevant potential cytotoxicy *in vivo*.

Adding to the selectivity of AMPs for bacteria over mammalian cells is the presence of cholesterol in the latter (Tytler et al., 1995; Silvestro et al., 1997). Cholesterol has a condensing effect on the membrane by increasing lipid order while only marginally reducing fluidity of the alkyl chains. The membrane-condensing effect reduces the lateral density fluctuations and increases the membrane expansion modulus, thus precluding peptide binding and incorporation in the membrane. In this context, it is also interesting to note that while normal eukaryotic cells can resist AMPs action, tumor cells are more susceptible to AMP action due to an elevated expression of anionic molecules such as PS, O-glycosylated mucins, sialilated gangliosides and heparan sulfates (Dobrzynska et al., 2005; Lee et al., 2008; Schweizer, 2009). This alteration in composition leads to partial loss of lipid symmetry and architecture which in turn lead to an increase of negative membrane potential, thus facilitating in efficient binding of AMPs to membranes (Utsugi et al., 1991). Apart from the difference in cell membrane composition, fluidity, higher electro potential and glycosylation pattern of membrane-associated proteins have also been proposed as reasons for AMPs antitumor activity (Hoskin and Ramamoorthy, 2008). Until now, it is not very clear what factors are responsible for antitumor activity and whether the molecular mechanism(s) underlying the antibacterial and anticancer activities of AMPs are the same or not (Hoskin and Ramamoorthy, 2008; Schweizer, 2009).

10. Optimization strategies to enhance the antimicrobial activity

Many naturally occurring AMPs are not optimized for efficient activity and need to be improved before they can be used as therapeutics. Until now, modifications in charge and hydrophobicity have been favored tools for increasing peptide activity, although frequently at the cost of increased toxicity and/or other detrimental effects (Pasupuleti et al., 2008; Pasupuleti et al., 2009d; Schmidtchen et al., 2009). Therefore, various other methods have recently been used for peptide optimization, including Quantitative structure-activity relationship (QSAR) (Frecer, 2006; Jenssen et al., 2008; Pasupuleti et al., 2008), altering structure by cyclisation (Dathe et al., 2004; Wessolowski et al., 2004), introducing fluorine atoms or trifluromethyl groups (Gimenez et al., 2006), increasing positive charge or hydrophobicity by tagging (Dathe et al., 2002; Schmidtchen et al., 2009).

10.1 Random mutagenesis

Random mutagenesis includes methods that modify natural peptide by addition/deletion/replacement of single or more residues, or truncations at the N- or C-terminal or generation of chimeric peptides using a combination of both methods. Unfortunately, random mutagenesis is informative only in few circumstances, and often raises more questions than the answers it provides (Tossi et al., 2000). Therefore this method is rarely used unless to answer a specific question or for generation of ultra short peptides (Pasupuleti et al., 2009a; Pasupuleti et al., 2009d; Schmidtchen et al., 2009).

10.2 Quantitative structure-activity relationships (QSAR)

Much like other chemical molecules, AMPs can also be easily improved with appropriate amino acid substitutions, provided the structure-function relationship (SAR) and factors that govern the activity/specificity and toxicity are known. QSAR is a mathematical relationship between biological activity of a molecular system and its geometric and chemical characteristics. Simplistically, QSAR studies attempt to find a consistent relationship between biological activity and molecular properties, so that these "rules" can be used to evaluate the activity of new compounds. AMP-based QSAR studies involve limited sets of systemic modifications of residues in naturally occurring molecules to form optimum or amphipathic structures, which are then subjected to determination of biological activity range via a number of methods. In these studies, instead of using a pool of amino acids for analysis, only a few amino acids with specific characteristics, such as basic (lysine or arginine) or hydrophobic (alanine, leucine, phenylalanine or tryptophan) amino acids are used in order to obtain a peptide with maximum activity and minimum toxicity towards the host.

Most AMP based QSAR studies of have focused on using peptides differing from one another at several sequence positions with defined and easily alignable properties or descriptors (e.g. amphipathicity, hydrophobicity and helical content) to determine a statistically significant correlations between the activity and structure (Frecer et al., 2004; Lejon et al., 2004; Taboureau et al., 2006; Hilpert et al., 2008; Langham et al., 2008). In QSAR studies, the choice of descriptors/parameters plays a crucial step in understanding the structure-activity relationship (Bhonsle et al., 2007). Various SAR studies indicate

that at least seven parameters/descriptors (size, sequence, charge, amphipathicity, hydrophobicity, helical content, distance between the hydrophobic and hydrophilic faces of the helix) can influence the spectrum and activity range of helical peptides. From structure-function based studies with various AMPs, it has been learned that antimicrobial activity is dependent upon hydrophobicity, charge, and amphipathicity, and the extent of role played by these factors depends upon the bacteria. The major advantage of QSAR studies is that it reduces the number of peptides to be analysed and the experiments to be done in order to obtain meaningful data and a molecule with desired characteristics (Tossi et al., 2000; Jenssen et al., 2008). On the other hand, selective toxicity depends more upon the amphipathicity and environmental conditions (Frecer, 2006; Jenssen et al., 2008; Pasupuleti et al., 2008). Due to the technical difficulties and experimentation limitations, mostly *in vitro* studies are carried out, including radial diffusion assay (RDA), minimum inhibitory concentrations (MIC) assay, bacterial membrane/model membrane lysis, kinetics studies, time killing experiments etc, whereas extensive *in vivo* studies are very seldomly performed (Chen et al., 2000), thereby limiting the predictive value of QSAR *in vivo*.

QSAR analysis uses mean of peptide properties or descriptors for drawing conclusions but not the positional information of amino acids or molecules. Theoretically, modifying amino acid(s) at the N or C terminal has no profound effect on the mean of peptide properties, but may have dramatic effects on peptide activity and selectivity. Unfortunately, owing to the above factor, QSAR results obtained using a set of peptides belonging to a family (e.g. helical peptides) can't be transferred to other set or family

(e.g. beta sheet peptides) of peptide. Nevertheless, QSAR studies help is a powerful tool for generating molecules with required activity in robust manner, essential in an industrial drug development setting (Juretic et al., 2011; Torrent et al., 2011).

10.3 Increasing proteolysis resistance

Another widely used method for peptide optimization is to reduce the protease sensitivity and increase the half-life of the peptide under *in vivo* conditions. In order to increase the protease resistance, various strategies have been proposed, including cyclisation, use of nonnatural amino acids (Goodman et al., 2001; Adessi and Soto, 2002; Giuliani et al., 2007), amidation at the N-terminus (Stromstedt et al., 2009), introduction of disulphide bonds (Peschel and Sahl, 2006), preventing protease binding by amino acid substitutions (Banerjee et al., 1996), modifying the peptide bonds by nitrogen alkylation (Ostresh et al., 1996), and polyethylene glycol(PEG) mixing or modification (Ganz and Lehrer, 1995). The half-life of a peptide is highly enhanced by conjugation of the free amino groups of the peptides with PEG as this prevents the attack of proteases by steric hindrance as well as serum protein binding and macrophage up take (Ganz and Lehrer, 1995). Interestingly, reduced protease susceptibility and enhanced activities were obtained by introducing disulphide bonds or lactam bonds in indolicidin and cecropin-melittin hybrid peptide, respectively (Houston et al., 1998; Rozek et al., 2003). It is also noteworthy that in addition to increased antibacterial activity, introduction of disulphide bonds in the sakacin peptide leads to broadened spectrum of the peptides (Uteng et al., 2003).

10.4 Enhancers

Recently Ueno et al. reported that AMP activity against some types of bacteria can be increased by adding enhancer peptides along with native AMPs (Ueno et al., 2010). In this work, they used a non-antimicrobial peptide fragment, NP4P, obtained by substitution of all acidic amino acid residues with amides (i.e., Glu →Gln, and Asp → Asn) in pro-region of nematode cecropin P4. NP4P is not antimicrobial nor does it interfere with bacterial growth by itself. However, its presence reduced the minimum Bactericidal Concentration (MBC) values by 10 times for peptides (polymyxin B and ASABF-a) against *S. aureus*. Interestingly, this enhanced activity was not observed with the peptides such as indolicidin or antibiotics such as ampicillin, kanamycin and enrofloxacin, which do not act on membranes. Furthermore while enhanced effects were observed against *E. coli* and *S. aureus*, less synergistic effects were observed for other microbes, such as *M. luteus* IFO12708, *B. subtilis* IFO3134, *P. aeruginosa* IFO3899 and *S. marcescens* IFO3736. Although this appears to be a strain-specific behavior, the origin and implications of this observation needs to be pursued further. Of course, synergistic studies are not new in the area of AMPs, instead various researchers have investigated the effects of using two different AMPs or antibiotics together (Rosenfeld et al., 2006; Gueguen et al., 2009; Jeong et al., 2010; Lin et al., 2010). However, Ueno's work differs from the previous work in the sense that others have used two peptides, which are individually antimicrobial, where as these researchers have shown that a non-antimicrobial peptide can also increase the efficiency of an antibiotic or an antimicrobial peptide.

11. Significance and other functions of AMPs

The terms “antimicrobial peptide” or “natural antibiotic” have biased the interpretation of the function of AMPs, and most likely delayed the discovery of other functions (Lai and Gallo, 2009). Increasing new evidence, however, is indicating that the role of AMPs in host defense goes well beyond direct killing of microorganisms (Figure 3). AMPs, in addition to their microbicidal activities in host tissues, thus exhibit a plethora of activities such as stimulating cell proliferation, activating the immune system, and exhibiting cytotoxic effect on tumor cells (McDermott, 2009). Furthermore, some AMPs possess antiviral, antitumor (Bhutia and Maiti, 2008; Rodrigues et al., 2009), antiobesity (Dodd et al., 2010), angiogenesis, and vasculogenesis properties (Koczulla and Bals, 2003; Koczulla et al., 2003), as well as immuno-modulatory activity such as promotion of wound healing (Hancock, 2001; Bowdish et al., 2005c; Tokumaru et al., 2005), inhibition of pro-inflammatory responses induced by LPS (Sawa et al., 1998; Bals et al., 1999a; Bals et al., 1999b), recruitment of leukocyte (Welling et al., 1998), chemokine production (Territo et al., 1989; Chertov et al., 1996; Scott et al., 2002; Grutkoski et al., 2003), and anti-inflammatory properties (Turner et al., 1998; Scott and Hancock, 2000; Scott et al., 2000a; Scott et al., 2000b; Nagaoka et al., 2001).

Till now, primarily beneficial aspects of AMPs have been reported in literature. However, increasing recent evidences show that AMPs are “double edged sword”, as they have both pro and antitumor activity. Interestingly, HBD-3 and LL-37 can activate epidermal growth factor receptor (EGFR) and phosphorylate STAT1 and STAT3 (Niyonsaba et al., 2007). It is known that activation of EGFR induces the phosphorylation

of STAT proteins which aids in keratinocyte migration and tumor proliferation (Chan et al., 2004). Thus, a fresh look at the role of AMPs in the tumor scenario is needed. The primary reason for multiple functions of AMPs is their amphipathic and cationic charge, which give them an ability to interact with a wide variety of receptors instead of a specific target. Based on these multiple roles of AMPs, much current interest is directed to unravel the coupling between the innate and adaptive immunity, which influences the quantity of the immune response and damage/pathogen clearance effect (Brogden et al., 2003a; Brogden et al., 2003b; Fritz et al., 2004; Bowdish et al., 2005b). [For comprehensive review about immunomodulatory see (Bowdish et al., 2006; Niyonsaba et al., 2006)]

12. Problems and bottlenecks of AMP therapeutics

From their discovery, microbiologists have been tempted by the idea to develop AMPs into 21th century novel antibiotics. The recent rise in antibiotic resistance has drawn the attention of researchers and companies alike towards these molecules (Table 2). Peptides are therefore being developed as promising therapeutics due to their small size, wide-spread occurrence in living organisms, long evolutionary history, potential ability to overcome bacterial resistance and the added benefit of delivery routes other than intravenous injection (Lien and Lowman, 2003). However, the biggest hurdle to develop these evolutionary conserved and interesting molecules into peptide therapeutics is to overcome the limitations imposed by nature from time immemorial.

In all living forms, the various biological processes are controlled by regulatory processes comprising of initiation or inhibition via specific protein-protein interactions. Thus, AMPs are well suited to control complicated processes occurring in biological systems. This unique biocompatibility feature should put peptides on the top of a "probable future therapeutic molecule" list. As in all drug developments, despite the promising results in animal disease models, there are obstacles that remain to be solved before the wide-spread development of peptides as therapeutics kicks-off is achieved. The major issues that need attention are susceptibility of peptides to proteolytic enzymes, lack of information regarding antigenicity, immunogenicity, potential toxicities of relatively large and highly charged peptides, ability to achieve high microbicidal activity under physiological conditions and comparatively, various effects on normal cells, unclear mode of action, interactions with immune/inflammatory cells, high costs associated with peptide synthesis (Sewald and Jakubke, 2002; Jenssen et al., 2006).

12.1 Synthesis

Currently, there are three methods for the production of AMPs, i.e. isolation from natural sources, chemical synthesis, and expression using biological systems. Isolation from natural sources is important for novel peptide discovery, but may not always be practical for clinical trials and use. Considering this and other issues, peptide synthesis has instead taken a rapidly expanding role in this context. The typical standard peptide synthesis approach, based on Fmoc chemistry, allows synthesis of peptides up to about 50 amino acids in length with greater success rate. Although peptides as long as about 100 amino acids are possible to generate by this approach, this is rarely attempted on lab scale due to the inefficiency of coupling of longer chains.

The alternative cost effective way of producing ultra short AMPs is by genetic engineering techniques in bacteria or yeast, the latter being organisms of choice since the cost involved in insects, plants and cell-free systems is typically higher. Interestingly, most researchers have focused on production of AMPs in bacteria due to the availability of well-defined plasmid vectors, screening techniques, practical importance of large-scale peptide production by fermentation, where a large amount of information about the dynamics of bacterial growth in fermentors is available. However, direct expression of AMPs is usually difficult since these peptides are toxic to the expression host (Kim et al., 2008). Consequently, these peptides are usually expressed as fusion proteins in protease-deficient bacterial strains, e.g. *E. coli* BL21. The fusion technique improves solubility and avoids degradation of, and toxicity to, expressing cells (Panavas et al., 2009). Nevertheless, problem in expressing fusion proteins is waste of energy/protein synthesis machinery, in addition to problems related to cleavage and purification of the AMP from the fusion molecules. Hence there is a need for new methods of synthesis of high quality and quantity of AMP in bacterial systems. The major problem in this scenario is conditional toxicity, i.e. the AMP should not be toxic to the expression host during the formation time, but active when used against the infectious bacteria. As a solution to this problem, Qing et al. developed pCold vectors, which allow a high expression of the target gene by cold shock, i.e. bacterial growth at 37°C and protein expression at 15°C (Qing et al., 2004). So far, however, the wider utility of this method in large-scale production remains uncertain from fermentation economics point of view.

A literature review identifies no more than 30 papers aiming to address this problem (Yang et al., 2004b; Moon et al., 2006; Mehrnejad et al., 2008; Renye and Somkuti, 2008; Panavas et al., 2009; Bommarius et al., 2010; Cao et al., 2010; Li et al., 2010; Shen et al., 2010). In addition, AMPs synthesized by humans and other vertebrates are amidated at the C-terminal, due to which these peptides are able to withstand bacterial enzymes for long times (Mueller and Driscoll, 2008; Stromstedt et al., 2009). Since bacterial systems effectively lack post-translational modification machinery, recombinantly expressed peptides lack this modification and are susceptible to enzymatic digestion or have a lower antimicrobial activity. In order to solve this problem, Cao et al., expressed a Cecropin- melittin hybrid peptide in the yeast *Pichia pastoris* X-33Strain (Cao et al., 2010) with antibacterial activity, however no information regarding antifungal activity was presented. In spite of technical difficulties, plectasin was isolated from the fungus *Pseudoplectania nigrella* and expressed in *Aspergillus sp* by using recombinant technology (Mygind et al., 2005). Although various researchers have shown lab scale production of AMPs in bacteria, no one has so far reported the chemical, protease and *in vivo* stability of recombinantly produced AMPs.

12.2 Purification and cost aspects

Despite low yields, maximum ~500-1000 mg per batch, the only current method of synthesizing AMPs is through chemical synthesis, which is quite expensive. The high cost in such peptide synthesis is due to the use of quite long, cumbersome, and expensive synthesis procedures and the requirement of a purification step after each cycle. The other bottleneck is the purification step, which follows the synthesis of each of the products

used in the peptide synthesis (Ayoub and Scheidegger, 2006). The most widely used step in this purification is the use of HPLC, either alone or in combination with other methods. The main drawback of any single HPLC method is that it only provides negative identification, i.e., it shows impurities that resolve from the main peak and not the impurities that co-elute. Thus, a single product has to pass through round of separate purification systems, which adds to the cost of the final product (Lax and Michael, 2006) and a loss of peptides in significant amounts.

Another issue, precluding the eventual use of AMPs in standard antibacterial therapeutics, is the cost involved in AMP production. For treating a single patient with an infection, one may need up to mg amounts per kg body weight per day, and production alone of such a quantity through chemical synthesis will cost around 5-300 US $, far exceeding current costs for antibiotics (Marr et al., 2006). Hence, reducing cost of goods is an important issue, needed to be addressed. Alternatively, cost of goods may be reduced by identifying ultrashort and highly efficient peptides which can be produced by using genetic engineering techniques in yeast and/or enhancing bioavailability of AMPs by using efficient drug delivery systems. Apart from the issues mentioned above, another major problem in producing AMPs in bacterial systems is LPS contamination. Purification from LPS in large scale production therefore requires further downstream steps, which add to the cost of production.

12.3 Lack of specificity

The single most important property of any therapeutic drug is its target specificity and

non-toxicity to other components of the host. The major problem with AMPs in this context is their lack of a unique specific target site. Out of hundreds of AMPs discovered at crucial sites of infection in various cell types and in concentrations well above the MIC values, only very few have been found to be potent in physiologically relevant conditions (Mookherjee and Hancock, 2007). Despite this, infection model studies in animals have convincingly shown that cationic peptides are able to limit or clear the infection, despite the loss of direct antimicrobial activity under physiological conditions illustrating the complexity of AMP pharmacokinetics and pharmacodynamics (Mookherjee and Hancock, 2007).

By logic, peptides derived from human sources should not be toxic to humans, unfortunately this is not necessarily the case. Since AMPs share features with eukaryotic nuclear localization signal peptides, they are able to translocate into cells and cause mast cell degranulation (Dos Santos Cabrera et al., 2009), thus leading to cytotoxicity. Due to the drug regulations in many countries, much attention to the development of AMP therapeutics has been shifted to topically applied agents, as opposed to internal drug agents (Giuliani et al., 2007). However, even this approach poses some inherent problems, since skin, intestine and mouth are colonized by normal flora, and AMPs therefore risk eliminating of total microbial flora. In order to narrow down the spectrum, recently He et al. have shown generation of a versatile platform called specifically-targeted antimicrobial peptide (STAMP). A completed STAMP consists of conjoined but functionally independent targeting and killing regions, separated by a small flexible linker, all within a linear peptide sequence (He et al., 2010). By using STAMP, these

researchers showed that previously inactive peptides against *S. mutans* can become active by adding a targeting sequence. Interestingly, it was observed that the location of target peptide (i.e, N or C terminal region) in STAMP decides the efficiency of the peptide. For unknown reasons, STAMP's with targeting peptide in C-terminal region have significantly higher antimicrobial than those having at N-terminal (He et al., 2010). This observation is analogous to findings showing that attaching a hydrophobic tag at C-terminal, instead of N-terminal region, enhances the AMP activity (Pasupuleti et al., 2009a; Schmidtchen et al., 2009).

12.4 Protease susceptibility

Sensitivity to proteases, unfavorable pharmokinetics, and rapid clearance of AMPs severely restrict their application as therapeutics. Interestingly, most pathogenic bacteria express specific and non-specific virulence factors such as endotoxins, proteases and peptidases in order to evade the host immune response. To circumvent these problems, amidation at the N-terminus has been applied (Stromstedt et al., 2009), as has the introduction of D-amino acids at L-form at cleavage sites (Stromstedt et al., 2009). This approach works very well on a lab scale, but the synthesis of entirely D-form peptides or in combination with L-form adds extra cost as well as technical problems. Also, peptides consisting entirely of D-form are potential immunogens and could cause side effects, including hypersensitivity reactions. In addition, there is little information about the general immunogenicity of a peptide with a combination of D and L amino acids (Guichard et al., 1994). Another unresolved question which needs to be answered is whether a peptide should be susceptible to degradation in order to avoid the toxic side

effects, or should be stable in order to give a long term effect.

12.5 Pharmacological drawbacks

Unfortunately, AMPs do not exhibit advantageous pharmacokinetic properties and deny the "golden lipinski rule of five for drug likeliness" (Lipinski et al., 2001; Zhang and Wilkinson, 2007). Despite this obvious drawback, many peptides have entered into clinical trails, with varying degree of success (Table 2). Although considerable progress has been made regarding the other areas discussed here, three major problems which need urgent attention are poor distribution, frequent toxicity, and fast decomposition or half life (Cassone and Otvos, 2010). Notably, a direct relationship is observed between the half-life and prolonged effect of peptide (Kjeldsen et al., 1998; Kim et al., 2002). In this context, a potential approach is to fuse the peptides to albumin (Koehler et al., 2002) or anti-tissue factor Fab (Lien and Lowman, 2003) by using a linker (Koehler et al., 2002) or through peptide addition (Dennis et al., 2002), with the intention to increase half-life. Using this approach, it was found that the half-life of albumin–peptide conjugates did not differ significantly from that of albumin itself, which has a half-life of 19 days (Peters, 1985).

At present, there is still some ambiguity regarding peptides being antigenic by themselves, i.e., in the absence of any adjuvant. Until recently, owing to short life span in plasma, it was presumed that the peptides are unable to cause any kind of antibody response. However, various reports have started to appear, showing that peptides can act as adjuvants and induce a high level of humoral response and antibody production

(Garlapati et al., 2009; Kindrachuk et al., 2009; Kovacs-Nolan et al., 2009). Although the origin of this adjuvant effect of the peptides remains to be elucidated, the peptide presumably acts through affecting the ability of the antigen recipient to develop either humoral or cytotoxic immune response by changing the number and activity of immune cells (Kieber-Emmons et al., 1997). Unfortunately, this unexpected novel function has raised a new question on whether AMPs act as adjuvants in combination with pharmaceutical formulations, thus raising antibodies against the components in the formulation, or alternatively, those components in the formulation might act as adjuvants and induce antibody production against AMPs. This is an important question to answer, especially when AMPs are to be used as immunomodulatory molecules. Taken the above into account, it would not be entirely surprising if antibodies are raised on intravenous injection. Based upon the data obtained from clinical trials, however, where AMPs were used as tropical agents, no data exists regarding the development of immune response for the peptides when applied topically (Hancock, 2000). At present, there is little or no complete information regarding the pharmacology and pharmacokinetics of AMPs, as very few studies have been done. Thus, there is urgent need to carry out additional studies on this issue (van't Hof et al., 2001; Jenssen et al., 2006).

13. **Probability of development of resistance by microbes to AMPs**

Co-habitation, in symbiosis or open warfare, over millions of years has resulted in host-pathogen interactions, where the key issues in pathogen survival is its ability to evade host immune attack. Successful pathogens have developed an array of inducible or constitutive counter measures to avoid multiple host defence mechanisms (Yount and

Yeaman, 2005). Due to the rarity of reports regarding emergence of AMPs resistance several researchers have speculated that microbes are unable to develop resistance to AMPs based on two points. Firstly, the membrane has to be modified and this is so finely tuned that extensive modifications are difficult and have more adverse functional consequences. Secondly, due to the presence of large numbers of AMPs in the host, it is difficult for microbes develop resistance to all AMPs at the same time. More recently, however, a number of resistance mechanisms in bacteria against AMPs have been discovered and investigated, including release of GAGs, polysaccharides and other polyanionic species able to scavenge cationic AMPs, upregulation of proteolytic enzymes able to degrade AMPs, membrane modifications resulting in a decreased negative surface potential of bacterial membranes, just to mention a few; [for comprehensive review, see Nizet (Nizet, 2007)]. In addition, individual cellular resistance mechanisms may drive the emergence and propagation of the antimicrobial resistance, as occurred with antibiotics (Peschel and Sahl, 2006). In a laboratory setting, it has recently been shown that *E. coli* and *P. fluorescens* can independently evolve heritable resistance mechanism in the presence of increasing concentration of pexiganan over a period of 600-700 generations (Perron et al., 2006). Thus, the presence of increasing concentrations of pexiganan alone, induces a selection pressure on the bacteria leading to specific adaptation (Nizet, 2006).

It has been reported that *S. aureus* can resist various AMPs just by reducing the net charge of the membrane by introduction of cationic residues in the membranes (Peschel et al., 2001). Interestingly, not all microbes are following the same strategy. For example, *Serratia sp* and *Morganella sp* both express an outer membrane that lacking the acidic

lipids for appropriate peptide binding (Giuliani et al., 2007), while *Shigella* sp releases plasmid DNA, therby inactivating AMPs by scavenging. On the other hand, *S. pyogenes* (Nyberg et al., 2004), *Pseudomonas sp* (Schmidtchen et al., 2001b), *S. aureus* (Sieprawska-Lupa et al., 2004), *S. epidermidis* (Lai et al., 2007) and *P. gingivalis* (Zasloff, 2002) all produce proteases, which degrade AMPs. Interestingly, some bacteria such as *Pseudomonas sp* and *S. epidermidis,* in addition to protease production use other methods such as forming biofilms, changing hydrophobicity and permeability of outer membranes (Guo et al., 1998; Peschel, 2002; Vuong et al., 2004a; Vuong et al., 2004b; Otto, 2006) to protect themselves from the AMPs. It is noteworthy that not all bacteria use a simple mechanism. For example, *S. enterica* (Gunn et al., 2000), *S. aureus* (Peschel et al., 2001; Li et al., 2007a; Li et al., 2007b), and *S. epidermidis* (Li et al., 2007b) use PhoP/PhoQ and/or analogous AMP sensing system consisting of several complex components to sense and block AMP activity. Furthermore, not all factors produced by microbes attack the host-derived AMPs directly, but rather the control mechanism encrypted in the host to control a pathway. For example, *S. aureus* produces clumping factor A, which binds to C3a and enhances its degradation by factor I, in turn regulating the complement factor (Hair et al., 2008; Potempa and Pike, 2009). In addition, some pathogens such as *Pseudomonas sp, E. faecalis, S. pyogenes* degrade host macromolecules, e.g., GAG and collagen, so that that the degradation products can interfere with AMP function (Schmidtchen et al., 2001a; Schmidtchen et al., 2002; Schmidtchen et al., 2003).

Despite of conclusive evidence regarding resistance mechanisms shown by various

pathogens *in vitro*, potential resistance development to AMPs under *in vivo* conditions remains to be clarified (Yount and Yeaman, 2005). Recently, in *Salmonella enterica*, it has been shown that mutations in the putative transport protein Sbm A is responsible for resistance against PR-39 peptides (proline rich peptides). Somewhat unexpectedly, Sbm A mutants were found to be as fit as the wild type in terms of growth and long term survivability in artificial medium (Pranting and Andersson, 2010). On the other hand, investigations with similar strain of *Salmonella enterica* with protamine peptide (cationic and basic) showed opposite results (Pranting et al., 2008). In addition, protamine resistance isolates showed characteristics of small colony variants, a phenotype often associated with persistent and recurrent infections that are difficult to treat. Both these studies show that stable mutants arise at a very high rate and the mutants, in comparison to the wild type, are similarly susceptible to different types of antibiotics and AMPs (Pranting et al., 2008; Pranting and Andersson, 2010). Here, it should be noted that the conditions experienced by bacteria in a laboratory setting are quite different from those in nature. In nature, the microbes are exposed to kaleidoscopic array of AMPs, which reduces or disturbs the selection pressure that causes resistance development. Furthermore, by default generation after generation, microbes are not continuously exposed to same specific adaptation inducing molecules or stress, as they encounter different AMPs within the host tissue and species from time to time (Perron et al., 2006). A further point is that, in addition to acting as direct antimicrobial agent, some AMPs have immunomodulatory functions which create a second wave of attack on the pathogen with different type of molecules, thereby precluding resistance development further. It is noteworthy that microbes which show resistance to naturally derived AMPs such as

defensins, have been found to be susceptible to other synthetic AMPs (Peschel, 2002). Based upon the above observations, it is tempting to speculate that cross resistance against a panel of AMPs is not possible under natural conditions, even if occurs, each mutant has a resistance against single AMPs with different type of characteristic behaviour. Nonetheless, advanced understanding of the mechanism of AMP action and resistance will reveal novel potentially vulnerable targets for novel anti-infective agents.

14. Why research on AMPs?

Without doubt, antibiotics are the most successful anti-infective agents used to control infectious diseases and until the last decade or so, pharamaceutical industry has continuously upgraded/modified the existing antibiotics and developed newer antibiotics (Gordon et al., 2005; Hancock, 2007). Despite the unquestionable success of antibiotics, a major concern in the health sector is increasing problem of antibiotic resistance, which has rendered most current antibiotics useless against an increasing number of pathogenic bacteria. Experimental studies and theoretical models show that, if preventive measures such as antibiotic cycling are enforced, antibiotic resistance might fall back (Ohlsen et al., 2008; Andersson and Hughes, 2010), but will never reach zero. Moreover it leaves a mark in the microbe with every probability to rebound rapidly to become dominant on renewed exposure to the antibiotic. Resistance is a natural consequence of adaptation, an inherent factor in the evolution of organisms, hence it is impossible to stop antibiotic resistance altogether (Salyers and Amabile-Cuevas, 1997). The short life-cycles of bacteria facilitate rapid genetic changes and adaptation to a constantly changing ecosystem for better survival. Studies on fitness and dynamics of resistance development

in various pathogens have led to the conclusion that antibiotic resistance does not occur in some cases, or occurs only at a very slow rate, as this is too costly in term of fitness (Andersson and Hughes, 2010). Therefore, newer types of molecules are needed, which can replace the antibiotics in the long run. Nevertheless, AMPs are less prone to induce resistance than conventional antibiotics, as they act on multiple targets simultaneously.

Due to their broad-spectrum antimicrobial activity and multiple functions, AMPs are promising therapeutic agents against infectious disease. AMPs are versatile molecules with multiple functions in bridging the innate and adaptive immune response (Figure 4). Although a substantial amount of work has been done, there are some areas, where concerted research efforts are needed, such as the role of AMPs in chronic diseases. A major challenge in the work to unravel the true biological functions of AMPs is the lack of true representative models. For example, mice and humans have a different set up of AMPs, and thus do not serve adequately to represent the human condition. Furthermore, the condition is not so different with knockout mice due to the inherent problem of synergistic activity of AMPs, complicating interpretation of *in vivo* studies. Despite these hurdles, innovative strategies and drugs to combat bacterial infection will hopefully emerge from the research in innate immunity, especially AMPs (Bochud et al., 2007; Sambhara and Lehrer, 2007), as they represent natural mechanisms of combating pathogenic challenge by rapid microbicidal activity and there is no little doubt about the efficiency of AMPs as novel therapeutics (Hancock and Lehrer, 1998; Lien and Lowman, 2003; Marshall, 2003; Yount and Yeaman, 2005; Hancock and Sahl, 2006; Giuliani et al., 2007). It is also noteworthy in this context that conventional antibiotics have failed

dramatically to reverse the disastrous trend of antibiotic resistance development in human pathogens, which is rising at an alarming rate. In comparison to this, AMPs may offer at least potential advantages over currently available and widely used antibiotics.

Conclusion

Antimicrobial or host defense peptides are widely distributed in animals and plants, as part of a strategy of innate immunity to control bacteria and prevent diseases. With some exceptions, AMPs described to date are small with a marked cationic and amphipathic character. Cationicity helps in initial binding of the peptides preferably to the bacterial surface and the amphipathic nature facilitates AMP binding to bacterial membranes, and their subsequent disintegration. Although there exist an ambiguity regarding the exact role, most agree that AMPs primary role is to control and limit the microbial infections either by acting directly or/and modulating the immune system. Unlike antibiotics, which kill every microbe, natural AMPs may spare the normal flora and kill only the pathogenic bacteria. Furthermore they are optimized to function only in a given fluid composition and environment, thus avoiding cross-reactions. Combined, the primary task of AMPs is thus to prevent the colonization and invasion of the host epithelia by potential pathogens. Although AMPs have been primarily thought to be natural antibiotics, the interest in AMPs has risen to a higher level during the last few years, both in academic research and in industrial development, with the discovery of immunomodulatory function of some AMPs. Despite technical hurdles, such as cost of goods, chemical and biological degradation, and unfavourable pharmacokinetics, several peptides have advanced to clinical trials (Table 2). There is no doubt that in an age where many pathogens have

developed extensive resistance to commonly used antibiotics, AMPs represent attractive worthwhile candidates to pursue as therapeutic molecules owing to their small size, wide spread occurrence among animals and plants, and potent antimicrobial and immunomodulation functions.

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Conflict of interest

No declarations of interest

Legends

Figure 1: Schematic representation of α-defensins, β-defensins and cathelicidin (hCAP18). The conserved cysteines are highlighted in black box. The disulfide linkages for the α-defensins (1-6, 2-4, 3-5) and β-defensins (1-5, 2-4, 3-6) are represented with the lines. Cathelicidins are synthesized as inactive precursor proteins with a signal sequence at N-terminal, central conserved cathelin-like region and an inactive AMP at the C-terminal region. Serine proteinase 3 and or aspartic protease gastricin cleave between the conserved region and variable region to generate an active AMP (e.g., LL-37).

Figure 2: Models depicting the mode of action by membrane active AMPs. Peptides are usually unstructured in solutions and on binding to the membranes, they undergo a rapid restructuring to adopt favorable structure for translocations into the membrane. Later based upon the biophysical property of the peptides they destabilize the membranes either by barrel stove model (left), toroidal pore model (center) or carpet model (right).

Figure 3: Different functions of AMPs in the host immune protection. AMPs can induce plethora of responses such as incitation of wound repair mechanism, genesis of vascular system, preventing obesity development, promote leukocyte recruitment at the site of infections, induction of cell differentiation process, binding to LPS and preventing pro-inflammatory responses. The overall

advantage of all these functions is protection from infections as well as host-induced pro-inflammatory responses.

Figure 4: AMPs activate innate immune system, which provides shorter, non-specific wide spectrum protection to the host without any memory. On the contrary, Vaccines activates adaptive immune system that provides long-term single pathogen protection with memory.

Table 1: List of the major classes of AMPs produced in various organisms.

Table 2: List of peptides that went into clinical trials and their current status/outcome.

References

Adessi C, Soto C. (2002). Converting a peptide into a drug: strategies to improve stability and bioavailability. Curr Med Chem, 9, 963-78.

Agerberth B, Charo J, Werr J, Olsson B, Idali F, Lindbom L, Kiessling R, Jörnvall H, Wigzell H, Gudmundsson GH. (2000). The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. Blood, 96, 3086-93.

Andersson DI, Hughes D. (2010). Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol, 8, 260-71.

Andersson E, Rydengård V, Sonesson A, Mörgelin M, Björck L, Schmidtchen A. (2004). Antimicrobial activities of heparin-binding peptides. Eur J Biochem, 271, 1219-26.

Angelova A, Ionov R, Koch MH, Rapp G. (2000). Interaction of the peptide antibiotic alamethicin with bilayer- and non-bilayer-forming lipids: influence of increasing alamethicin concentration on the lipids supramolecular structures. Arch Biochem Biophys, 378, 93-106.

Auvynet C, Rosenstein Y. (2009). Multifunctional host defense peptides: antimicrobial peptides, the small yet big players in innate and adaptive immunity. FEBS J, 276, 6497-508.

Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME, Ouellette AJ. (2000). Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. Nat Immunol, 1, 113-8.

Ayoub M, Scheidegger D. (2006). Peptide drugs, overcoming the challenges, a growing business. Chimica Oggi-Chemistry Today, 24, 46-48.

Badria FA, Guirguis AN, Perovic S, Steffen R, Muller WE, Schroder HC. (1998). Sarcophytolide: a new neuroprotective compound from the soft coral *Sarcophyton glaucum*. Toxicology, 131, 133-43.

Bals R, Goldman MJ, Wilson JM. (1998a). Mouse beta-defensin 1 is a salt-sensitive antimicrobial peptide present in epithelia of the lung and urogenital tract. Infect Immun, 66, 1225-32.

Bals R, Wang X, Wu Z, Freeman T, Bafna V, Zasloff M, Wilson JM. (1998b). Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. J Clin Invest, 102, 874-80.

Bals R, Weiner DJ, Moscioni AD, Meegalla RL, Wilson JM. (1999a). Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide. Infect Immun, 67, 6084-9.

Bals R, Weiner DJ, Wilson JM. (1999b). The innate immune system in cystic fibrosis lung disease. J Clin Invest, 103, 303-7.

Bals R, Lang C, Weiner DJ, Vogelmeier C, Welsch U, Wilson JM. (2001). Rhesus monkey (*Macaca mulatta*) mucosal antimicrobial peptides are close homologues of human molecules. Clin Diagn Lab Immunol, 8, 370-5.

Banerjee A, Pramanik A, Bhattacharjya S, Balaram P. (1996). Omega amino acids in peptide design: incorporation into helices. Biopolymers, 39, 769-77.

Bessalle R, Gorea A, Shalit I, Metzger JW, Dass C, Desiderio DM, Fridkin M. (1993). Structure-function studies of amphiphilic antibacterial peptides. J Med Chem, 36, 1203-9.

Bhonsle JB, Venugopal D, Huddler DP, Magill AJ, Hicks RP. (2007). Application of 3D-QSAR for identification of descriptors defining bioactivity of antimicrobial peptides. J Med Chem, 50, 6545-53.

Bhutia SK, Maiti TK. (2008). Targeting tumors with peptides from natural sources. Trends Biotechnol, 26, 210-7.

Biggs JS, Rosenfeld Y, Shai Y, Olivera BM. (2007). Conolysin-Mt: a conus peptide that disrupts cellular membranes. Biochemistry, 46, 12586-93.

Blondelle SE, Houghten RA. (1992). Design of model amphipathic peptides having potent antimicrobial activities. Biochemistry, 31, 12688-94.

Bochud PY, Bochud M, Telenti A, Calandra T. (2007). Innate immunogenetics: a tool for exploring new frontiers of host defence. Lancet Infect Dis, 7, 531-42.

Boman HG, Nilsson I, Rasmuson B. (1972). Inducible antibacterial defence system in Drosophila. Nature, 237, 232-5.

Boman HG. (1981). *in Microbial Control of Insects, Mites and Plant Diseases*. New York: Academic.769−784

Boman HG, Agerberth B, Boman A. (1993). Mechanisms of action on *Escherichia coli* of cecropin P1 and PR-39, two antibacterial peptides from pig intestine. Infect Immun, 61, 2978-84.

Boman HG. (1995). Peptide antibiotics and their role in innate immunity. Annu Rev Immunol, 13, 61-92.

Boman HG. (2000). Innate immunity and the normal microflora. Immunol Rev, 173, 5-16.

Bommarius B, Jenssen H, Elliott M, Kindrachuk J, Pasupuleti M, Gieren H, Jaeger KE, Hancock RE, Kalman D. (2010). Cost-effective expression and purification of antimicrobial and host defense peptides in Escherichia coli. Peptides, 31, 1957-65.

Bonsen PP, de Haas GH, van Deenen LL. (1967). Synthetic and structural investigations on 3-phosphatidyl-1'-(3'-O-L-lysyl)glycerol. Biochemistry, 6, 1114-20.

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

Bowdish DM, Davidson DJ, Lau YE, Lee K, Scott MG, Hancock RE. (2005b). Impact of LL-37 on anti-infective immunity. J Leukoc Biol, 77, 451-9.

Bowdish DM, Davidson DJ, Scott MG, Hancock RE. (2005c). Immunomodulatory activities of small host defense peptides. Antimicrob Agents Chemother, 49, 1727-32.

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

Brogden KA, Ackermann M, McCray PB, Jr., Tack BF. (2003a). Antimicrobial peptides in animals and their role in host defences. Int J Antimicrob Agents, 22, 465-78.

Brogden KA, Heidari M, Sacco RE, Palmquist D, Guthmiller JM, Johnson GK, Jia HP, Tack BF, McCray PB. (2003b). Defensin-induced adaptive immunity in mice and its potential in preventing periodontal disease. Oral Microbiol Immunol, 18, 95-9.

Brumfitt W, Salton MR, Hamilton-Miller JM. (2002). Nisin, alone and combined with peptidoglycan-modulating antibiotics: activity against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *enterococci*. J Antimicrob Chemother, 50, 731-4.

Bucki R, Leszczynska K, Namiot A, Sokolowski W. (2010). Cathelicidin LL-37: a multitask antimicrobial peptide. Arch Immunol Ther Exp (Warsz), 58, 15-25.

Campese M, Sun X, Bosch JA, Oppenheim FG, Helmerhorst EJ. (2009). Concentration and fate of histatins and acidic proline-rich proteins in the oral environment. Arch Oral Biol, 54, 345-53.

Canny G, Levy O, Furuta GT, Narravula-Alipati S, Sisson RB, Serhan CN, Colgan SP. (2002). Lipid mediator-induced expression of bactericidal/ permeability-increasing protein (BPI) in human mucosal epithelia. Proc Natl Acad Sci U S A, 99, 3902-7.

Cao Y, Yu RQ, Liu Y, Zhou HX, Song LL, Qiao DR. (2010). Design, Recombinant Expression, and Antibacterial Activity of the Cecropins-Melittin Hybrid Antimicrobial Peptides. Curr Microbiol, 61, 169-75.

Carlsson A, Engstrom P, Palva ET, Bennich H. (1991). Attacin, an antibacterial protein from *Hyalophora cecropia*, inhibits synthesis of outer membrane proteins in *Escherichia coli* by interfering with omp gene transcription. Infect Immun, 59, 3040-5.

Cassone M, Otvos L, Jr. (2010). Synergy among antibacterial peptides and between peptides and small-molecule antibiotics. Expert Rev Anti Infect Ther, 8, 703-16.

Chan KS, Carbajal S, Kiguchi K, Clifford J, Sano S, DiGiovanni J. (2004). Epidermal growth factor receptor-mediated activation of Stat3 during multistage skin carcinogenesis. Cancer Res, 64, 2382-9.

Charlet M, Chernysh S, Philippe H, Hetru C, Hoffmann JA, Bulet P. (1996). Innate immunity. Isolation of several cysteine-rich antimicrobial peptides from the blood of a mollusc, *Mytilus edulis*. J Biol Chem, 271, 21808-13.

Chen J, Falla TJ, Liu H, Hurst MA, Fujii CA, Mosca DA, Embree JR, Loury DJ, Radel PA, Cheng Chang C, Gu L, Fiddes JC. (2000). Development of protegrins for the treatment and prevention of oral mucositis: structure-activity relationships of synthetic protegrin analogues. Biopolymers, 55, 88-98.

Chen X, Niyonsaba F, Ushio H, Okuda D, Nagaoka I, Ikeda S, Okumura K, Ogawa H. (2005). Synergistic effect of antibacterial agents human beta-defensins, cathelicidin LL-37 and lysozyme against *Staphylococcus aureus* and *Escherichia coli*. J Dermatol Sci, 40, 123-32.

Chen X, Niyonsaba F, Ushio H, Hara M, Yokoi H, Matsumoto K, Saito H, Nagaoka I, Ikeda S, Okumura K, Ogawa H. (2007). Antimicrobial peptides human beta-defensin

(hBD)-3 and hBD-4 activate mast cells and increase skin vascular permeability. Eur J Immunol, 37, 434-44.

Chertov O, Michiel DF, Xu L, Wang JM, Tani K, Murphy WJ, Longo DL, Taub DD, Oppenheim JJ. (1996). Identification of defensin-1, defensin-2, and CAP37/azurocidin as T-cell chemoattractant proteins released from interleukin-8-stimulated neutrophils. J Biol Chem, 271, 2935-40.

Cho JH, Park CB, Yoon YG, Kim SC. (1998a). Lumbricin I, a novel proline-rich antimicrobial peptide from the earthworm: purification, cDNA cloning and molecular characterization. Biochim Biophys Acta, 1408, 67-76.

Cho Y, Turner JS, Dinh NN, Lehrer RI. (1998b). Activity of protegrins against yeast-phase *Candida albicans*. Infect Immun, 66, 2486-93.

Chu KT, Ng TB. (2003). Isolation of a large thaumatin-like antifungal protein from seeds of the Kweilin chestnut *Castanopsis chinensis*. Biochem Biophys Res Commun, 301, 364-70.

Cirioni O, Giacometti A, Ghiselli R, Orlando F, Kamysz W, D'Amato G, Mocchegiani F, Lukasiak J, Silvestri C, Saba V, Scalise G. (2004). Potential therapeutic role of histatin derivative P-113d in experimental rat models of *Pseudomonas aeruginosa* sepsis. J Infect Dis, 190, 356-64.

Cole AM, Weis P, Diamond G. (1997). Isolation and characterization of pleurocidin, an antimicrobial peptide in the skin secretions of winter flounder. J Biol Chem, 272, 12008-13.

Cole AM, Hong T, Boo LM, Nguyen T, Zhao C, Bristol G, Zack JA, Waring AJ, Yang OO, Lehrer RI. (2002). Retrocyclin: a primate peptide that protects cells from infection by T- and M-tropic strains of HIV-1. Proc Natl Acad Sci U S A, 99, 1813-8.

Colman RW, Jameson BA, Lin Y, Johnson D, Mousa SA. (2000). Domain 5 of high molecular weight kininogen (kininostatin) down-regulates endothelial cell proliferation and migration and inhibits angiogenesis. Blood, 95, 543-50.

Costa MM, Dios S, Alonso-Gutierrez J, Romero A, Novoa B, Figueras A. (2009). Evidence of high individual diversity on myticin C in mussel (*Mytilus galloprovincialis*). Dev Comp Immunol, 33, 162-70.

Cronan JE. (2003). Bacterial membrane lipids: where do we stand? Annu Rev Microbiol, 57, 203-24.

Daher KA, Selsted ME, Lehrer RI. (1986). Direct inactivation of viruses by human granulocyte defensins. J Virol, 60, 1068-74.

Darmoul D, Ouellette AJ. (1996). Positional specificity of defensin gene expression reveals Paneth cell heterogeneity in mouse small intestine. Am J Physiol, 271, G68-74.

Dathe M, Wieprecht T, Nikolenko H, Handel L, Maloy WL, MacDonald DL, Beyermann M, Bienert M. (1997). Hydrophobicity, hydrophobic moment and angle subtended by charged residues modulate antibacterial and haemolytic activity of amphipathic helical peptides. FEBS Lett, 403, 208-12.

Dathe M, Nikolenko H, Meyer J, Beyermann M, Bienert M. (2001). Optimization of the antimicrobial activity of magainin peptides by modification of charge. FEBS Lett, 501, 146-50.

Dathe M, Meyer J, Beyermann M, Maul B, Hoischen C, Bienert M. (2002). General aspects of peptide selectivity towards lipid bilayers and cell membranes studied by variation of the structural parameters of amphipathic helical model peptides. Biochim Biophys Acta, 1558, 171-86.

Dathe M, Nikolenko H, Klose J, Bienert M. (2004). Cyclization increases the antimicrobial activity and selectivity of arginine- and tryptophan-containing hexapeptides. Biochemistry, 43, 9140-50.

de Haar SF, Hiemstra PS, van Steenbergen MT, Everts V, Beertsen W. (2006). Role of polymorphonuclear leukocyte-derived serine proteinases in defense against *Actinobacillus actinomycetemcomitans*. Infect Immun, 74, 5284-91.

De Lucca AJ, Bland JM, Grimm C, Jacks TJ, Cary JW, Jaynes JM, Cleveland TE, Walsh TJ. (1998a). Fungicidal properties, sterol binding, and proteolytic resistance of the synthetic peptide D4E1. Can J Microbiol, 44, 514-20.

De Lucca AJ, Bland JM, Jacks TJ, Grimm C, Walsh TJ. (1998b). Fungicidal and binding properties of the natural peptides cecropin B and dermaseptin. Med Mycol, 36, 291-8.

De Lucca AJ, Cleveland TE, Wedge DE. (2005). Plant-derived antifungal proteins and peptides. Can J Microbiol, 51, 1001-14.

De Smet K, Contreras R. (2005). Human antimicrobial peptides: defensins, cathelicidins and histatins. Biotechnol Lett, 27, 1337-47.

Dennis MS, Zhang M, Meng YG, Kadkhodayan M, Kirchhofer D, Combs D, Damico LA. (2002). Albumin binding as a general strategy for improving the pharmacokinetics of proteins. J Biol Chem, 277, 35035-43.

Dennison SR, Harris F, Phoenix DA. (2005a). Are oblique orientated alpha-helices used by antimicrobial peptides for membrane invasion? Protein Pept Lett, 12, 27-9.

Dennison SR, Wallace J, Harris F, Phoenix DA. (2005b). Amphiphilic alpha-helical antimicrobial peptides and their structure/function relationships. Protein Pept Lett, 12, 31-9.

Destoumieux D, Munoz M, Bulet P, Bachere E. (2000). Penaeidins, a family of antimicrobial peptides from penaeid shrimp (Crustacea, Decapoda). Cell Mol Life Sci, 57, 1260-71.

Dobrzynska I, Szachowicz-Petelska B, Sulkowski S, Figaszewski Z. (2005). Changes in electric charge and phospholipids composition in human colorectal cancer cells. Mol Cell Biochem, 276, 113-9.

Dodd GT, Mancini G, Lutz B, Luckman SM. (2010). The peptide hemopressin acts through CB1 cannabinoid receptors to reduce food intake in rats and mice. J Neurosci, 30, 7369-76.

Donate F, Juarez JC, Guan X, Shipulina NV, Plunkett ML, Tel-Tsur Z, Shaw DE,

Morgan WT, Mazar AP. (2004). Peptides derived from the histidine-proline domain of the histidine-proline-rich glycoprotein bind to tropomyosin and have antiangiogenic and antitumor activities. Cancer Res, 64, 5812-7.

Dorschner RA, Lopez-Garcia B, Peschel A, Kraus D, Morikawa K, Nizet V, Gallo RL. (2006). The mammalian ionic environment dictates microbial susceptibility to antimicrobial defense peptides. Faseb J, 20, 35-42.

Dos Santos Cabrera MP, Arcisio-Miranda M, da Costa LC, de Souza BM, Broggio Costa ST, Palma MS, Ruggiero Neto J, Procopio J. (2009). Interactions of mast cell degranulating peptides with model membranes: a comparative biophysical study. Arch Biochem Biophys, 486, 1-11.

Duits LA, Ravensbergen B, Rademaker M, Hiemstra PS, Nibbering PH. (2002). Expression of beta-defensin 1 and 2 mRNA by human monocytes, macrophages and dendritic cells. Immunology, 106, 517-25.

Durr UH, Sudheendra US, Ramamoorthy A. (2006). LL-37, the only human member of the cathelicidin family of antimicrobial peptides. Biochim Biophys Acta, 1758, 1408-25.

Dyer KD, Rosenberg HF. (2006). The RNase a superfamily: generation of diversity and innate host defense. Mol Divers, 10, 585-97.

Ehrenstein G, Lecar H. (1977). Electrically gated ionic channels in lipid bilayers. Q Rev Biophys, 10, 1-34.

Ehret-Sabatier L, Loew D, Goyffon M, Fehlbaum P, Hoffmann JA, van Dorsselaer A, Bulet P. (1996). Characterization of novel cysteine-rich antimicrobial peptides from scorpion blood. J Biol Chem, 271, 29537-44.

El Jastimi R, Lafleur M. (1999). Nisin promotes the formation of non-lamellar inverted phases in unsaturated phosphatidylethanolamines. Biochim Biophys Acta, 1418, 97-105.

Elsbach P. (1990). Antibiotics from within: antibacterials from human and animal sources. Trends Biotechnol, 8, 26-30.

Epand RF, Epand RM, Monaco V, Stoia S, Formaggio F, Crisma M, Toniolo C. (1999). The antimicrobial peptide trichogin and its interaction with phospholipid membranes. Eur J Biochem, 266, 1021-8.

Epand RF, Maloy L, Ramamoorthy A, Epand RM. (2010). Amphipathic helical cationic antimicrobial peptides promote rapid formation of crystalline States in the presence of phosphatidylglycerol: lipid clustering in anionic membranes. Biophys J, 98, 2564-73.

Epand RM, Epand RF. (2009). Lipid domains in bacterial membranes and the action of antimicrobial agents. Biochim Biophys Acta, 1788, 289-94.

Fernandes JM, Kemp GD, Molle MG, Smith VJ. (2002). Anti-microbial properties of histone H2A from skin secretions of rainbow trout, *Oncorhynchus mykiss*. Biochem J, 368, 611-20.

Frecer V, Ho B, Ding JL. (2004). De novo design of potent antimicrobial peptides. Antimicrob Agents Chemother, 48, 3349-57.

Frecer V. (2006). QSAR analysis of antimicrobial and haemolytic effects of cyclic

cationic antimicrobial peptides derived from protegrin-1. Bioorg Med Chem, 14, 6065-74.

Frick IM, Akesson P, Herwald H, Morgelin M, Malmsten M, Nagler DK, Bjorck L. (2006). The contact system--a novel branch of innate immunity generating antibacterial peptides. Embo J, 25, 5569-78.

Fritz JH, Brunner S, Birnstiel ML, Buschle M, Gabain A, Mattner F, Zauner W. (2004). The artificial antimicrobial peptide KLKLLLLLKLK induces predominantly a TH2-type immune response to co-injected antigens. Vaccine, 22, 3274-84.

Frohm M, Agerberth B, Ahangari G, Stahle-Backdahl M, Liden S, Wigzell H, Gudmundsson GH. (1997). The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. J Biol Chem, 272, 15258-63.

Galgóczy L, Kovács L, CS V. (2010). Defensin-like antifungal proteins secreted by filamentous fungi.Current Research, Technology and Education.Topics in Applied Microbiology and. Microbial Biotechnology. "Available at" http://www.formatex.info/microbiology2/550-559.pdf

Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, Lehrer RI. (1985). Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest, 76, 1427-35.

Ganz T, Lehrer RI. (1994). Defensins. Curr Opin Immunol, 6, 584-9.

Ganz T, Lehrer RI. (1995). Defensins. Pharmacol Ther, 66, 191-205.

Garcia AE, Osapay G, Tran PA, Yuan J, Selsted ME. (2008). Isolation, synthesis, and antimicrobial activities of naturally occurring theta-defensin isoforms from baboon leukocytes. Infect Immun, 76, 5883-91.

Garlapati S, Facci M, Polewicz M, Strom S, Babiuk LA, Mutwiri G, Hancock RE, Elliott MR, Gerdts V. (2009). Strategies to link innate and adaptive immunity when designing vaccine adjuvants. Vet Immunol Immunopathol, 128, 184-91.

Gazit E, Bach D, Kerr ID, Sansom MS, Chejanovsky N, Shai Y. (1994). The alpha-5 segment of Bacillus thuringiensis delta-endotoxin: in vitro activity, ion channel formation and molecular modelling. Biochem J, 304, 895-902.

Gesell J, Zasloff M, Opella SJ. (1997). Two-dimensional 1H NMR experiments show that the 23-residue magainin antibiotic peptide is an alpha-helix in dodecylphosphocholine micelles, sodium dodecylsulfate micelles, and trifluoroethanol/water solution. J Biomol NMR, 9, 127-35.

Giangaspero A, Sandri L, Tossi A. (2001). Amphipathic alpha helical antimicrobial peptides. Eur J Biochem, 268, 5589-600.

Gifford JL, Hunter HN, Vogel HJ. (2005). Lactoferricin: a lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. Cell Mol Life Sci, 62, 2588-98.

Gimenez D, Andreu C, del Olmo M, Varea T, Diaz D, Asensio G. (2006). The introduction of fluorine atoms or trifluoromethyl groups in short cationic peptides

enhances their antimicrobial activity. Bioorg Med Chem, 14, 6971-8.

Giuliani A, Pirri G, Nicoletto SF. (2007). Antimicrobial peptides: an overview of a promising class of therapeutics. Central European Journal of Biology 2, 1-33.

Glukhov E, Stark M, Burrows LL, Deber CM. (2005). Basis for selectivity of cationic antimicrobial peptides for bacterial versus mammalian membranes. J Biol Chem, 280, 33960-7.

Gonzalez-Chavez SA, Arevalo-Gallegos S, Rascon-Cruz Q. (2009). Lactoferrin: structure, function and applications. Int J Antimicrob Agents, 33, 301 e1-8.

Goodman M, Zapf C, Rew Y. (2001). New reagents, reactions, and peptidomimetics for drug design. Biopolymers, 60, 229-45.

Gordon YJ, Romanowski EG, McDermott AM. (2005). A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. Curr Eye Res, 30, 505-15.

Grutkoski PS, Graeber CT, Lim YP, Ayala A, Simms HH. (2003). Alpha-defensin 1 (human neutrophil protein 1) as an antichemotactic agent for human polymorphonuclear leukocytes. Antimicrob Agents Chemother, 47, 2666-8.

Gueguen Y, Bernard R, Julie F, Paulina S, Delphine DG, Franck V, Philippe B, Evelyne B. (2009). Oyster hemocytes express a proline-rich peptide displaying synergistic antimicrobial activity with a defensin. Mol Immunol, 46, 516-22.

Guichard G, Benkirane N, Zeder-Lutz G, van Regenmortel MH, Briand JP, Muller S. (1994). Antigenic mimicry of natural L-peptides with retro-inverso-peptidomimetics. Proc Natl Acad Sci U S A, 91, 9765-9.

Gunn JS, Ryan SS, Van Velkinburgh JC, Ernst RK, Miller SI. (2000). Genetic and functional analysis of a PmrA-PmrB-regulated locus necessary for lipopolysaccharide modification, antimicrobial peptide resistance, and oral virulence of *Salmonella enterica serovar typhimurium*. Infect Immun, 68, 6139-46.

Guo L, Lim KB, Poduje CM, Daniel M, Gunn JS, Hackett M, Miller SI. (1998). Lipid A acylation and bacterial resistance against vertebrate antimicrobial peptides. Cell, 95, 189-98.

Haest CW, de Gier J, den Kamp JO, Bartels P, van Deenen LL. (1972). Chages in permeability of Staphylococcus aureus and derived liposomes with varying lipid composition. Biochim Biophys Acta, 255, 720-33.

Hair PS, Ward MD, Semmes OJ, Foster TJ, Cunnion KM. (2008). *Staphylococcus aureus* clumping factor A binds to complement regulator factor I and increases factor I cleavage of C3b. J Infect Dis, 198, 125-33.

Hale JD, Hancock RE. (2007). Alternative mechanisms of action of cationic antimicrobial peptides on bacteria. Expert Rev Anti Infect Ther, 5, 951-9.

Hancock RE, Lehrer R. (1998). Cationic peptides: a new source of antibiotics. Trends Biotechnol, 16, 82-8.

Hancock RE. (2000). Cationic antimicrobial peptides: towards clinical applications. Expert Opin Investig Drugs, 9, 1723-9.

Hancock RE. (2001). Cationic peptides: effectors in innate immunity and novel antimicrobials. Lancet Infect Dis, 1, 156-64.

Hancock RE, Rozek A. (2002). Role of membranes in the activities of antimicrobial cationic peptides. FEMS Microbiol Lett, 206, 143-9.

Hancock RE. (2005). Mechanisms of action of newer antibiotics for Gram-positive pathogens. Lancet Infect Dis, 5, 209-18.

Hancock RE, Sahl HG. (2006). Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol, 24, 1551-7.

Hancock RE. (2007). The complexities of antibiotic action. Mol Syst Biol, 3, 142.

Harder J, Schroder JM. (2002). RNase 7, a novel innate immune defense antimicrobial protein of healthy human skin. J Biol Chem, 277, 46779-84.

Harris F, Dennison SR, Phoenix DA. (2009). Anionic antimicrobial peptides from eukaryotic organisms. Curr Protein Pept Sci, 10, 585-606.

Haupt H, Heimburger N. (1972). Human serum proteins with high affinity for carboxymethylcellulose. I. Isolation of lysozyme, C1q and 2 hitherto unknown -globulins. Hoppe Seylers Z Physiol Chem, 353, 1125-32.

Hazlett L, Wu M. (2011). Defensins in innate immunity. Cell Tissue Res, 343, 175-88.

He J, Yarbrough DK, Kreth J, Anderson MH, Shi W, Eckert R. (2010). Systematic approach to optimizing specifically targeted antimicrobial peptides against Streptococcus mutans. Antimicrob Agents Chemother, 54, 2143-51.

Heimburger N, Haupt H, Kranz T, Baudner S. (1972). Human serum proteins with high affinity to carboxymethylcellulose. II. Physico-chemical and immunological characterization of a histidine-rich 3,8S- 2 -glycoportein (CM-protein I). Hoppe Seylers Z Physiol Chem, 353, 1133-40.

Heinzelmann M, Mercer-Jones MA, Flodgaard H, Miller FN. (1998). Heparin-binding protein (CAP37) is internalized in monocytes and increases LPS-induced monocyte activation. J Immunol, 160, 5530-6.

Heinzelmann M, Kim E, Hofmeister A, Gordon LE, Platz A, Cheadle WG. (2001). Heparin binding protein (CAP37) differentially modulates endotoxin-induced cytokine production. Int J Surg Investig, 2, 457-66.

Hilpert K, Fjell CD, Cherkasov A. (2008). Short linear cationic antimicrobial peptides: screening, optimizing, and prediction. Methods Mol Biol, 494, 127-59.

Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA. (1999). Phylogenetic perspectives in innate immunity. Science, 284, 1313-8.

Hoskin DW, Ramamoorthy A. (2008). Studies on anticancer activities of antimicrobial peptides. Biochim Biophys Acta, 1778, 357-75.

Houston ME, Jr., Kondejewski LH, Karunaratne DN, Gough M, Fidai S, Hodges RS, Hancock RE. (1998). Influence of preformed alpha-helix and alpha-helix induction on the activity of cationic antimicrobial peptides. J Pept Res, 52, 81-8.

Huang HW. (2006). Molecular mechanism of antimicrobial peptides: the origin of cooperativity. Biochim Biophys Acta, 1758, 1292-302.

Huang HW. (2009). Free energies of molecular bound states in lipid bilayers: lethal concentrations of antimicrobial peptides. Biophys J, 96, 3263-72.

Hughes AL. (1999). Evolutionary diversification of the mammalian defensins. Cell Mol Life Sci, 56, 94-103.

Hugli TE. (1990). Structure and function of C3a anaphylatoxin. Curr Top Microbiol Immunol, 153, 181-208.

Hultmark D, Steiner H, Rasmuson T, Boman HG. (1980). Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of *Hyalophora cecropia*. Eur J Biochem, 106, 7-16.

Imamura M, Wada S, Koizumi N, Kadotani T, Yaoi K, Sato R, Iwahana H. (1999). Acaloleptins A: inducible antibacterial peptides from larvae of the beetle, *Acalolepta luxuriosa*. Arch Insect Biochem Physiol, 40, 88-98.

Javadpour MM, Juban MM, Lo WC, Bishop SM, Alberty JB, Cowell SM, Becker CL, McLaughlin ML. (1996). De novo antimicrobial peptides with low mammalian cell toxicity. J Med Chem, 39, 3107-13.

Jenssen H, Hamill P, Hancock RE. (2006). Peptide antimicrobial agents. Clin Microbiol Rev, 19, 491-511.

Jenssen H, Fjell CD, Cherkasov A, Hancock RE. (2008). QSAR modeling and computer-aided design of antimicrobial peptides. J Pept Sci, 14, 110-4.

Jeong N, Kim JY, Park SC, Lee JK, Gopal R, Yoo S, Son BK, Hahm JS, Park Y, Hahm KS. (2010). Antibiotic and synergistic effect of Leu-Lys rich peptide against antibiotic resistant microorganisms isolated from patients with cholelithiasis. Biochem Biophys Res Commun, 399, 581-6.

Jiang Z, Kullberg BJ, van der Lee H, Vasil AI, Hale JD, Mant CT, Hancock RE, Vasil ML, Netea MG, Hodges RS. (2008). Effects of hydrophobicity on the antifungal activity of alpha-helical antimicrobial peptides. Chem Biol Drug Des, 72, 483-95.

Johansson J, Gudmundsson GH, Rottenberg ME, Berndt KD, Agerberth B. (1998). Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37. J Biol Chem, 273, 3718-24.

Jones AL, Hulett MD, Parish CR. (2005a). Histidine-rich glycoprotein: A novel adaptor protein in plasma that modulates the immune, vascular and coagulation systems. Immunol Cell Biol, 83, 106-18.

Jones AL, Poon IK, Hulett MD, Parish CR. (2005b). Histidine-rich glycoprotein specifically binds to necrotic cells via its amino-terminal domain and facilitates necrotic cell phagocytosis. J Biol Chem, 280, 35733-41.

Jones DE, Bevins CL. (1993). Defensin-6 mRNA in human Paneth cells: implications for antimicrobial peptides in host defense of the human bowel. FEBS Lett, 315, 187-92.

Jung S, Dingley AJ, Augustin R, Anton-Erxleben F, Stanisak M, Gelhaus C, Gutsmann

T, Hammer MU, Podschun R, Bonvin AM, Leippe M, Bosch TC, Grotzinger J. (2009). Hydramacin-1, structure and antibacterial activity of a protein from the basal metazoan Hydra. J Biol Chem, 284, 1896-905.

Juretic D, Vukicevic D, Petrov D, Novkovic M, Bojovic V, Lucic B, Ilic N, Tossi A. (2011). Knowledge-based computational methods for identifying or designing novel, non-homologous antimicrobial peptides. Eur Biophys J, 40, 371-85.

Kacprzyk L, Rydengard V, Morgelin M, Davoudi M, Pasupuleti M, Malmsten M, Schmidtchen A. (2007). Antimicrobial activity of histidine-rich peptides is dependent on acidic conditions. Biochim Biophys Acta, 1768, 2667-80.

Kavanagh K, Dowd S. (2004). Histatins: antimicrobial peptides with therapeutic potential. J Pharm Pharmacol, 56, 285-9.

Khandelia H, Ipsen JH, Mouritsen OG. (2008). The impact of peptides on lipid membranes. Biochim Biophys Acta, 1778, 1528-36.

Kieber-Emmons T, Murali R, Greene MI. (1997). Therapeutic peptides and peptidomimetics. Curr Opin Biotechnol, 8, 435-41.

Kim HJ, Jung JR, Lee SY, Chang IH, Lee TJ, Kim W, Myung SC. (2011). Expression of human beta-defensin-2 in the prostate. BJU Int, 107, 144-9.

Kim JM, Jang SA, Yu BJ, Sung BH, Cho JH, Kim SC. (2008). High-level expression of an antimicrobial peptide histonin as a natural form by multimerization and furin-mediated cleavage. Appl Microbiol Biotechnol, 78, 123-30.

Kim TH, Lee H, Park TG. (2002). Pegylated recombinant human epidermal growth factor (rhEGF) for sustained release from biodegradable PLGA microspheres. Biomaterials, 23, 2311-7.

Kindrachuk J, Jenssen H, Elliott M, Townsend R, Nijnik A, Lee SF, Gerdts V, Babiuk LA, Halperin SA, Hancock RE. (2009). A novel vaccine adjuvant comprised of a synthetic innate defence regulator peptide and CpG oligonucleotide links innate and adaptive immunity. Vaccine, 27, 4662-71.

Kjeldsen T, Pettersson AF, Drube L, Kurtzhals P, Jonassen I, Havelund S, Hansen PH, Markussen J. (1998). Secretory expression of human albumin domains in *Saccharomyces cerevisiae* and their binding of myristic acid and an acylated insulin analogue. Protein Expr Purif, 13, 163-9.

Kluver E, Schulz-Maronde S, Scheid S, Meyer B, Forssmann WG, Adermann K. (2005). Structure-activity relation of human beta-defensin 3: influence of disulfide bonds and cysteine substitution on antimicrobial activity and cytotoxicity. Biochemistry, 44, 9804-16.

Kobayashi H, Ohta N, Umeda M. (2004). Biology of lysenin, a protein in the coelomic fluid of the earthworm Eisenia foetida. Int Rev Cytol, 236, 45-99.

Koczulla AR, Bals R. (2003). Antimicrobial peptides: current status and therapeutic potential. Drugs, 63, 389-406.

Koczulla R, von Degenfeld G, Kupatt C, Krotz F, Zahler S, Gloe T, Issbrucker K,

Unterberger P, Zaiou M, Lebherz C, Karl A, Raake P, Pfosser A, Boekstegers P, Welsch U, Hiemstra PS, Vogelmeier C, Gallo RL, Clauss M, Bals R. (2003). An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. J Clin Invest, 111, 1665-72.

Koehler MF, Zobel K, Beresini MH, Caris LD, Combs D, Paasch BD, Lazarus RA. (2002). Albumin affinity tags increase peptide half-life in vivo. Bioorg Med Chem Lett, 12, 2883-6.

Kokryakov VN, Harwig SS, Panyutich EA, Shevchenko AA, Aleshina GM, Shamova OV, Korneva HA, Lehrer RI. (1993). Protegrins: leukocyte antimicrobial peptides that combine features of corticostatic defensins and tachyplesins. FEBS Lett, 327, 231-6.

Kovacs-Nolan J, Latimer L, Landi A, Jenssen H, Hancock RE, Babiuk LA, van Drunen Littel-van den Hurk S. (2009). The novel adjuvant combination of CpG ODN, indolicidin and polyphosphazene induces potent antibody- and cell-mediated immune responses in mice. Vaccine, 27, 2055-64.

Kragol G, Lovas S, Varadi G, Condie BA, Hoffmann R, Otvos L, Jr. (2001). The antibacterial peptide pyrrhocoricin inhibits the ATPase actions of DnaK and prevents chaperone-assisted protein folding. Biochemistry, 40, 3016-26.

Kuhn-Nentwig L, Muller J, Schaller J, Walz A, Dathe M, Nentwig W. (2002). Cupiennin 1, a new family of highly basic antimicrobial peptides in the venom of the spider Cupiennius salei (Ctenidae). J Biol Chem, 277, 11208-16.

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

Lai Y, Gallo RL. (2009). AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol, 30, 131-41.

Langham AA, Khandelia H, Schuster B, Waring AJ, Lehrer RI, Kaznessis YN. (2008). Correlation between simulated physicochemical properties and hemolycity of protegrin-like antimicrobial peptides: predicting experimental toxicity. Peptides, 29, 1085-93.

Larrick JW, Hirata M, Balint RF, Lee J, Zhong J, Wright SC. (1995). Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. Infect Immun, 63, 1291-7.

Lax R, Michael V. (2006). Are low-priced peptides affordable? Chimica oggi 24, 38-40.

Lee HS, Park CB, Kim JM, Jang SA, Park IY, Kim MS, Cho JH, Kim SC. (2008). Mechanism of anticancer activity of buforin IIb, a histone H2A-derived peptide. Cancer Lett, 271, 47-55.

Lehrer RI, Lichtenstein AK, Ganz T. (1993). Defensins: antimicrobial and cytotoxic peptides of mammalian cells. Annu Rev Immunol, 11, 105-28.

Lehrer RI, Ganz T. (2002). Cathelicidins: a family of endogenous antimicrobial peptides. Curr Opin Hematol, 9, 18-22.

Lejon T, Stiberg T, Strom MB, Svendsen JS. (2004). Prediction of antibiotic activity and synthesis of new pentadecapeptides based on lactoferricins. J Pept Sci, 10, 329-35.

Li C, Haug T, Styrvold OB, Jorgensen TO, Stensvag K. (2008). Strongylocins, novel

antimicrobial peptides from the green sea urchin, *Strongylocentrotus droebachiensis*. Dev Comp Immunol, 32, 1430-40.

Li JF, Zhang J, Zhang Z, Ma HW, Zhang JX, Zhang SQ. (2010). Production of Bioactive Human Beta-Defensin-4 in Escherichia coli Using SUMO Fusion Partner. Protein J, 29, 314-9.

Li M, Cha DJ, Lai Y, Villaruz AE, Sturdevant DE, Otto M. (2007a). The antimicrobial peptide-sensing system aps of *Staphylococcus aureus*. Mol Microbiol, 66, 1136-47.

Li M, Lai Y, Villaruz AE, Cha DJ, Sturdevant DE, Otto M. (2007b). Gram-positive three-component antimicrobial peptide-sensing system. Proc Natl Acad Sci U S A, 104, 9469-74.

Lien S, Lowman HB. (2003). Therapeutic peptides. Trends Biotechnol, 21, 556-62.

Lin KH, Chuang YC, Lee SH, Yu WL. (2010). In vitro synergistic antimicrobial effect of imipenem and colistin against an isolate of multidrug-resistant *Enterobacter cloacae*. J Microbiol Immunol Infect, 43, 317-22.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings Adv Drug Deliv Rev 46, 3-26.

Liu L, Zhao C, Heng HH, Ganz T. (1997). The human beta-defensin-1 and alpha-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry. Genomics, 43, 316-20.

Liu YQ, Sun ZJ, Wang C, Li SJ, Liu YZ. (2004). Purification of a novel antibacterial short peptide in earthworm Eisenia foetida. Acta Biochim Biophys Sin (Shanghai), 36, 297-302.

Lohner K, Sevcsik E, Pabst G. (2008). *Liposome-based biomembrane mimetic systems: Implications for lipid-peptide interactions,* . Edited by A L-L. Amsterdam: Elsevier.

Ludtke S, He K, Huang H. (1995). Membrane thinning caused by magainin 2. Biochemistry, 34, 16764-9.

Lugtenberg EJ, Peters R. (1976). Distribution of lipids in cytoplasmic and outer membranes of *Escherichia coli* K12. Biochim Biophys Acta, 441, 38-47.

Luque-Ortega JR, van't Hof W, Veerman EC, Saugar JM, Rivas L. (2008). Human antimicrobial peptide histatin 5 is a cell-penetrating peptide targeting mitochondrial ATP synthesis in Leishmania. Faseb J, 22, 1817-28.

Ma DY, Liu SW, Han ZX, Li YJ, Shan AS. (2008). Expression and characterization of recombinant gallinacin-9 and gallinacin-8 in *Escherichia coli*. Protein Expr Purif, 58, 284-91.

Maisetta G, Di Luca M, Esin S, Florio W, Brancatisano FL, Bottai D, Campa M, Batoni G. (2008). Evaluation of the inhibitory effects of human serum components on bactericidal activity of human beta defensin 3. Peptides, 29, 1-6.

Makovitzki A, Shai Y. (2005). pH-dependent antifungal lipopeptides and their plausible mode of action. Biochemistry, 44, 9775-84.

Malm J, Sorensen O, Persson T, Frohm-Nilsson M, Johansson B, Bjartell A, Lilja H, Stahle-Backdahl M, Borregaard N, Egesten A. (2000). The human cationic antimicrobial protein (hCAP-18) is expressed in the epithelium of human epididymis, is present in seminal plasma at high concentrations, and is attached to spermatozoa. Infect Immun, 68, 4297-302.

Malmsten M, Davoudi M, Walse B, Rydengard V, Pasupuleti M, Morgelin M, Schmidtchen A. (2007). Antimicrobial peptides derived from growth factors. Growth Factors, 25, 60-70.

Marr AK, Gooderham WJ, Hancock RE. (2006). Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol, 6, 468-72.

Marshall SH. (2003). Antimicrobial peptides: A natural alternative to chemical antibiotics and a potential for applied biotechnology. Electronic Journal of Biotechnology, 6 http://www.ejbiotechnology.info/content/vol6/issue3/full/1.

Matsunaga S, Fusetani N, Konosu S. (1985). Bioactive marine metabolites, IV. Isolation and the amino acid composition of discodermin A, an antimicrobial peptide, from the marine sponge *Discodermia kiiensis*. J Nat Prod, 48, 236-41.

Matsuzaki K, Harada M, Funakoshi S, Fujii N, Miyajima K. (1991). Physicochemical determinants for the interactions of magainins 1 and 2 with acidic lipid bilayers. Biochim Biophys Acta, 1063, 162-70.

Matsuzaki K, Yoneyama S, Fujii N, Miyajima K, Yamada K, Kirino Y, Anzai K. (1997). Membrane permeabilization mechanisms of a cyclic antimicrobial peptide, tachyplesin I, and its linear analog. Biochemistry, 36, 9799-806.

Matsuzaki K. (1999). Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochim Biophys Acta, 1462, 1-10.

McDermott AM. (2009). The role of antimicrobial peptides at the ocular surface. Ophthalmic Res, 41, 60-75.

Mecke A, Lee DK, Ramamoorthy A, Orr BG, Banaszak Holl MM. (2005). Membrane thinning due to antimicrobial peptide binding: an atomic force microscopy study of MSI-78 in lipid bilayers. Biophys J, 89, 4043-50.

Mehrnejad F, Naderi-Manesh H, Ranjbar B, Maroufi B, Asoodeh A, Doustdar F. (2008). PCR-based gene synthesis, molecular cloning, high level expression, purification, and characterization of novel antimicrobial peptide, brevinin-2R, in *Escherichia coli*. Appl Biochem Biotechnol, 149, 109-18.

Melo MN, Ferre R, Castanho MA. (2009). Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. Nat Rev Microbiol, 7, 245-50.

Meyer-Hoffert U, Schwarz T, Schroder JM, Glaser R. (2010). Increased expression of human beta-defensin 3 in mollusca contagiosum. Clin Exp Dermatol, 35, 190-2.

Mihajlovic M, Lazaridis T. (2010a). Antimicrobial peptides bind more strongly to membrane pores. Biochim Biophys Acta, 1798, 1494-502.

Mihajlovic M, Lazaridis T. (2010b). Antimicrobial peptides in toroidal and cylindrical

pores. Biochim Biophys Acta, 1798, 1485-93.

Miyashita M, Sakai A, Matsushita N, Hanai Y, Nakagawa Y, Miyagawa H. (2010). A novel amphipathic linear peptide with both insect toxicity and antimicrobial activity from the venom of the scorpion *Isometrus maculatus*. Biosci Biotechnol Biochem, 74, 364-9.

Mohammed R, Peng J, Kelly M, Hamann MT. (2006). Cyclic heptapeptides from the Jamaican sponge *Stylissa caribica*. J Nat Prod, 69, 1739-44.

Mookherjee N, Hancock RE. (2007). Cationic host defence peptides: innate immune regulatory peptides as a novel approach for treating infections. Cell Mol Life Sci, 64, 922-33.

Moon JY, Henzler-Wildman KA, Ramamoorthy A. (2006). Expression and purification of a recombinant LL-37 from *Escherichia coli*. Biochim Biophys Acta, 1758, 1351-8.

Moon KE, Gorski JP, Hugli TE. (1981). Complete primary structure of human C4a anaphylatoxin. J Biol Chem, 256, 8685-92.

Mueller GP, Driscoll WJ. (2008). alpha-Amidated peptides: approaches for analysis. Methods Mol Biol, 446, 67-84.

Murakami Y, Takeshita T, Shizukuishi S, Tsunemitsu A, Aimoto S. (1990). Inhibitory effects of synthetic histidine-rich peptides on haemagglutination by *Bacteroides gingivalis* 381. Arch Oral Biol, 35, 775-7.

Mygind PH, Fischer RL, Schnorr KM, Hansen MT, Sonksen CP, Ludvigsen S, Raventos D, Buskov S, Christensen B, De Maria L, Taboureau O, Yaver D, Elvig-Jorgensen SG, Sorensen MV, Christensen BE, Kjaerulff S, Frimodt-Moller N, Lehrer RI, Zasloff M, Kristensen HH. (2005). Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. Nature, 437, 975-80.

Nagaoka I, Hirota S, Niyonsaba F, Hirata M, Adachi Y, Tamura H, Heumann D. (2001). Cathelicidin family of antibacterial peptides CAP18 and CAP11 inhibit the expression of TNF-alpha by blocking the binding of LPS to CD14(+) cells. J Immunol, 167, 3329-38.

Nair DG, Fry BG, Alewood P, Kumar PP, Kini RM. (2007). Antimicrobial activity of omwaprin, a new member of the waprin family of snake venom proteins. Biochem J, 402, 93-104.

Namjoshi S, Caccetta R, Benson HA. (2008). Skin peptides: biological activity and therapeutic opportunities. J Pharm Sci, 97, 2524-42.

Narayanan S, Miller WL, McDermott AM. (2003). Expression of human beta-defensins in conjunctival epithelium: relevance to dry eye disease. Invest Ophthalmol Vis Sci, 44, 3795-801.

Naurato N, Wong P, Lu Y, Wroblewski K, Bennick A. (1999). Interaction of tannin with human salivary histatins. J Agric Food Chem, 47, 2229-34.

Nguyen LT, Schibli DJ, Vogel HJ. (2005). Structural studies and model membrane interactions of two peptides derived from bovine lactoferricin. J Pept Sci, 11, 379-89.

Nijnik A, Hancock RE. (2009). The roles of cathelicidin LL-37 in immune defences and novel clinical applications. Curr Opin Hematol, 16, 41-7.

Niyonsaba F, Nagaoka I, Ogawa H. (2006). Human defensins and cathelicidins in the skin: beyond direct antimicrobial properties. Crit Rev Immunol, 26, 545-76.

Niyonsaba F, Ushio H, Nakano N, Ng W, Sayama K, Hashimoto K, Nagaoka I, Okumura K, Ogawa H. (2007). Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. J Invest Dermatol, 127, 594-604.

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

Nizet V. (2007). Understanding how leading bacterial pathogens subvert innate immunity to reveal novel therapeutic targets. J Allergy Clin Immunol, 120, 13-22.

Noga EJ, Silphaduang U. (2003). Piscidins: a novel family of peptide antibiotics from fish. Drug News Perspect, 16, 87-92.

Nordahl EA, Rydengård V, Nyberg P, Nitsche DP, Mörgelin M, Malmsten M, Björck L, Schmidtchen A. (2004). Activation of the complement system generates antibacterial peptides. Proc Natl Acad Sci U S A, 101, 16879-84.

Nordahl EA, Rydengård V, Mörgelin M, Schmidtchen A. (2005). Domain 5 of high molecular weight kininogen is antibacterial. J Biol Chem, 280, 34832-9.

Nyberg P, Rasmussen M, Bjorck L. (2004). alpha2-Macroglobulin-proteinase complexes protect *Streptococcus pyogenes* from killing by the antimicrobial peptide LL-37. J Biol Chem, 279, 52820-3.

Ohlsen K, Dandekar G, Schwarz R, Dandekar T. (2008). New trends in pharmacogenomic strategies against resistance development in microbial infections. Pharmacogenomics, 9, 1711-23.

Olsson AK, Larsson H, Dixelius J, Johansson I, Lee C, Oellig C, Björk I, Claesson-Welsh L. (2004). A fragment of histidine-rich glycoprotein is a potent inhibitor of tumor vascularization. Cancer Res, 64, 599-605.

Oppenheim FG, Xu T, McMillian FM, Levitz SM, Diamond RD, Offner GD, Troxler RF. (1988). Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on Candida albicans. J Biol Chem, 263, 7472-7.

Oren Z, Hong J, Shai Y. (1997). A repertoire of novel antibacterial diastereomeric peptides with selective cytolytic activity. J Biol Chem, 272, 14643-9.

Orivel J, Redeker V, Le Caer JP, Krier F, Revol-Junelles AM, Longeon A, Chaffotte A, Dejean A, Rossier J. (2001). Ponericins, new antibacterial and insecticidal peptides from the venom of the ant *Pachycondyla goeldii*. J Biol Chem, 276, 17823-9.

Ostberg N, Kaznessis Y. (2005). Protegrin structure-activity relationships: using homology models of synthetic sequences to determine structural characteristics important for activity. Peptides, 26, 197-206.

Ostresh JM, Blondelle SE, Dorner B, Houghten RA. (1996). Generation and use of nonsupport-bound peptide and peptidomimetic combinatorial libraries. Methods

Enzymol, 267, 220-34.

Otto M. (2006). Bacterial evasion of antimicrobial peptides by biofilm formation. Curr Top Microbiol Immunol, 306, 251-8.

Otvos L, Jr., O I, Rogers ME, Consolvo PJ, Condie BA, Lovas S, Bulet P, Blaszczyk-Thurin M. (2000). Interaction between heat shock proteins and antimicrobial peptides. Biochemistry, 39, 14150-9.

Oudhoff MJ, Bolscher JG, Nazmi K, Kalay H, van 't Hof W, Amerongen AV, Veerman EC. (2008). Histatins are the major wound-closure stimulating factors in human saliva as identified in a cell culture assay. Faseb J, 22, 3805-12.

Ovchinnikova TV, Balandin SV, Aleshina GM, Tagaev AA, Leonova YF, Krasnodembsky ED, Men'shenin AV, Kokryakov VN. (2006). Aurelin, a novel antimicrobial peptide from jellyfish *Aurelia aurita* with structural features of defensins and channel-blocking toxins. Biochem Biophys Res Commun, 348, 514-23.

Pan W, Liu X, Ge F, Han J, Zheng T. (2004). Perinerin, a novel antimicrobial peptide purified from the clamworm *Perinereis aibuhitensis* grube and its partial characterization. J Biochem, 135, 297-304.

Panavas T, Sanders C, Butt TR. (2009). SUMO fusion technology for enhanced protein production in prokaryotic and eukaryotic expression systems. Methods Mol Biol, 497, 303-17.

Papanastasiou EA, Hua Q, Sandouk A, Son UH, Christenson AJ, Van Hoek ML, Bishop BM. (2009). Role of acetylation and charge in antimicrobial peptides based on human beta-defensin-3. APMIS, 117, 492-9.

Papareddy P, Kalle M, Kasetty G, Morgelin M, Rydengard V, Albiger B, Lundqvist K, Malmsten M, Schmidtchen A. (2010a). C-terminal peptides of tissue factor pathway inhibitor are novel host defense molecules. J Biol Chem, 285, 28387-98.

Papareddy P, Rydengard V, Pasupuleti M, Walse B, Morgelin M, Chalupka A, Malmsten M, Schmidtchen A. (2010b). Proteolysis of human thrombin generates novel host defense peptides. PLoS Pathog, 6, e1000857.

Park CB, Kim HS, Kim SC. (1998). 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, 244, 253-7.

Park IY, Cho JH, Kim KS, Kim YB, Kim MS, Kim SC. (2004). Helix stability confers salt resistance upon helical antimicrobial peptides. J Biol Chem, 279, 13896-901.

Pasupuleti M, Walse B, Nordahl EA, Morgelin M, Malmsten M, Schmidtchen A. (2007). Preservation of antimicrobial properties of complement peptide C3a, from invertebrates to humans. J Biol Chem, 282, 2520-8.

Pasupuleti M, Walse B, Svensson B, Malmsten M, Schmidtchen A. (2008). Rational design of antimicrobial C3a analogues with enhanced effects against Staphylococci using an integrated structure and function-based approach. Biochemistry, 47, 9057-70.

Pasupuleti M, Chalupka A, Morgelin M, Schmidtchen A, Malmsten M. (2009a).

Tryptophan end-tagging of antimicrobial peptides for increased potency against *Pseudomonas aeruginosa*. Biochim Biophys Acta, 1790, 800-08

Pasupuleti M, Davoudi M, Malmsten M, Schmidtchen A. (2009b). Antimicrobial activity of a C-terminal peptide from human extracellular superoxide dismutase. BMC Res Notes, 2, 136.

Pasupuleti M, Roupe M, Rydengard V, Surewicz K, Surewicz WK, Chalupka A, Malmsten M, Sorensen OE, Schmidtchen A. (2009c). Antimicrobial activity of human prion protein is mediated by its N-terminal region. PLoS ONE, 4, e7358.

Pasupuleti M, Schmidtchen A, Chalupka A, Ringstad L, Malmsten M. (2009d). End-tagging of ultra-short antimicrobial peptides by W/F stretches to facilitate bacterial killing. PLoS One, 4, e5285.

Patrzykat A, Friedrich CL, Zhang L, Mendoza V, Hancock RE. (2002). Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli*. Antimicrob Agents Chemother, 46, 605-14.

Perron GG, Zasloff M, Bell G. (2006). Experimental evolution of resistance to an antimicrobial peptide. Proc Biol Sci, 273, 251-6.

Peschel A, Jack RW, Otto M, Collins LV, Staubitz P, Nicholson G, Kalbacher H, Nieuwenhuizen WF, Jung G, Tarkowski A, van Kessel KP, van Strijp JA. (2001). *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with l-lysine. J Exp Med, 193, 1067-76.

Peschel A. (2002). How do bacteria resist human antimicrobial peptides? Trends Microbiol, 10, 179-86.

Peschel A, Sahl HG. (2006). The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nat Rev Microbiol, 4, 529-36.

Peters T, Jr. (1985). Serum albumin. Adv Protein Chem, 37, 161-245.

Pokorny A, Almeida PF. (2004). Kinetics of dye efflux and lipid flip-flop induced by delta-lysin in phosphatidylcholine vesicles and the mechanism of graded release by amphipathic, alpha-helical peptides. Biochemistry, 43, 8846-57.

Potempa J, Pike RN. (2009). Corruption of innate immunity by bacterial proteases. J Innate Immun, 1, 70-87.

Powers JP, Hancock RE. (2003). The relationship between peptide structure and antibacterial activity. Peptides, 24, 1681-91.

Pranting M, Negrea A, Rhen M, Andersson DI. (2008). Mechanism and fitness costs of PR-39 resistance in *Salmonella enterica serovar Typhimurium* LT2. Antimicrob Agents Chemother, 52, 2734-41.

Pranting M, Andersson DI. (2010). Mechanisms and physiological effects of protamine resistance in *Salmonella enterica serovar Typhimurium* LT2. J Antimicrob Chemother, 65, 876-87.

Prasad R, Ghannoum AM. (1996). *Lipids of Pathogenic Fungi* CRC press

Qian S, Wang W, Yang L, Huang HW. (2008). Structure of the alamethicin pore reconstructed by x-ray diffraction analysis. Biophys J, 94, 3512-22.

Qing G, Ma LC, Khorchid A, Swapna GV, Mal TK, Takayama MM, Xia B, Phadtare S, Ke H, Acton T, Montelione GT, Ikura M, Inouye M. (2004). Cold-shock induced high-yield protein production in *Escherichia coli*. Nat Biotechnol, 22, 877-82.

Qu XD, Harwig SS, Shafer WM, Lehrer RI. (1997). Protegrin structure and activity against *Neisseria gonorrhoeae*. Infect Immun, 65, 636-9.

Rao AG. (1999). Conformation and antimicrobial activity of linear derivatives of tachyplesin lacking disulfide bonds. Arch Biochem Biophys, 361, 127-34.

Rapaport D, Shai Y. (1991). Interaction of fluorescently labeled pardaxin and its analogues with lipid bilayers. J Biol Chem, 266, 23769-75.

Rasmussen PB, Bjorn S, Hastrup S, Nielsen PF, Norris K, Thim L, Wiberg FC, Flodgaard H. (1996). Characterization of recombinant human HBP/CAP37/azurocidin, a pleiotropic mediator of inflammation-enhancing LPS-induced cytokine release from monocytes. FEBS Lett, 390, 109-12.

Reddy KV, Yedery RD, Aranha C. (2004). Antimicrobial peptides: premises and promises. Int J Antimicrob Agents, 24, 536-47.

Renye JA, Jr., Somkuti GA. (2008). Cloning of milk-derived bioactive peptides in *Streptococcus thermophilus*. Biotechnol Lett, 30, 723-30.

Rijnkels M, Elnitski L, Miller W, Rosen JM. (2003). Multispecies comparative analysis of a mammalian-specific genomic domain encoding secretory proteins. Genomics, 82, 417-32.

Ringstad L, Andersson Nordahl E, Schmidtchen A, Malmsten M. (2007). Composition Effect on Peptide Interaction with Lipids and Bacteria: Variants of C3a Peptide CNY21. Biophys J, 92, 87-98.

Ringstad L, Protopapa E, Lindholm-Sethson B, Schmidtchen A, Nelson A, Malmsten M. (2008). An electrochemical study into the interaction between complement-derived peptides and DOPC mono- and bilayers. Langmuir, 24, 208-16.

Rivas-Santiago B, Hernandez-Pando R, Carranza C, Juarez E, Contreras JL, Aguilar-Leon D, Torres M, Sada E. (2008). Expression of cathelicidin LL-37 during *Mycobacterium tuberculosis* infection in human alveolar macrophages, monocytes, neutrophils, and epithelial cells. Infect Immun, 76, 935-41.

Rodrigues EG, Dobroff AS, Taborda CP, Travassos LR. (2009). Antifungal and antitumor models of bioactive protective peptides. An Acad Bras Cienc, 81, 503-20.

Rodziewicz-Motowidlo S, Mickiewicz B, Greber K, Sikorska E, Szultka L, Kamysz E, Kamysz W. (2010). Antimicrobial and conformational studies of the active and inactive analogues of the protegrin-1 peptide. Febs J, 277, 1010-22.

Rosenfeld Y, Barra D, Simmaco M, Shai Y, Mangoni ML. (2006). A synergism between temporins toward Gram-negative bacteria overcomes resistance imposed by the

lipopolysaccharide protective layer. J Biol Chem, 281, 28565-74.

Rothstein DM, Spacciapoli P, Tran LT, Xu T, Roberts FD, Dalla Serra M, Buxton DK, Oppenheim FG, Friden P. (2001). Anticandida activity is retained in P-113, a 12-amino-acid fragment of histatin 5. Antimicrob Agents Chemother, 45, 1367-73.

Rozek A, Powers JP, Friedrich CL, Hancock RE. (2003). Structure-based design of an indolicidin peptide analogue with increased protease stability. Biochemistry, 42, 14130-8.

Rydengard V, Olsson AK, Morgelin M, Schmidtchen A. (2007). Histidine-rich glycoprotein exerts antibacterial activity. Febs J, 274, 377-89.

Rydengard V, Shannon O, Lundqvist K, Kacprzyk L, Chalupka A, Olsson AK, Morgelin M, Jahnen-Dechent W, Malmsten M, Schmidtchen A. (2008). Histidine-rich glycoprotein protects from systemic Candida infection. PLoS Pathog, 4, e1000116.

Sabatini LM, Azen EA. (1989). Histatins, a family of salivary histidine-rich proteins, are encoded by at least two loci (HIS1 and HIS2). Biochem Biophys Res Commun, 160, 495-502.

Sabatini LM, Warner TF, Saitoh E, Azen EA. (1989). Tissue distribution of RNAs for cystatins, histatins, statherin, and proline-rich salivary proteins in humans and macaques. J Dent Res, 68, 1138-45.

Saito T, Kawabata S, Shigenaga T, Takayenoki Y, Cho J, Nakajima H, Hirata M, Iwanaga S. (1995). A novel big defensin identified in horseshoe crab hemocytes: isolation, amino acid sequence, and antibacterial activity. J Biochem, 117, 1131-7.

Salyers AA, Amabile-Cuevas CF. (1997). Why are antibiotic resistance genes so resistant to elimination? Antimicrob Agents Chemother, 41, 2321-5.

Sambhara S, Lehrer RI. (2007). The innate immune system: a repository for future drugs? Expert Rev Anti Infect Ther, 5, 1-5.

Sansom MS. (1993). Alamethicin and related peptaibols--model ion channels. Eur Biophys J, 22, 105-24.

Sawa T, Kurahashi K, Ohara M, Gropper MA, Doshi V, Larrick JW, Wiener-Kronish JP. (1998). Evaluation of antimicrobial and lipopolysaccharide-neutralizing effects of a synthetic CAP18 fragment against *Pseudomonas aeruginosa* in a mouse model. Antimicrob Agents Chemother, 42, 3269-75.

Schittek B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, Schirle M, Schroeder K, Blin N, Meier F, Rassner G, Garbe C. (2001). Dermcidin: a novel human antibiotic peptide secreted by sweat glands. Nat Immunol, 2, 1133-7.

Schmidtchen A, Frick IM, Björck L. (2001a). Dermatan sulphate is released by proteinases of common pathogenic bacteria and inactivates antibacterial alpha-defensin. Mol Microbiol, 39, 708-13.

Schmidtchen A, Wolff H, Hansson C. (2001b). Differential proteinase expression by *Pseudomonas aeruginosa* derived from chronic leg ulcers. Acta Derm Venereol, 81, 406-9.

Schmidtchen A, Frick IM, Andersson E, Tapper H, Bjorck L. (2002). Proteinases of

common pathogenic bacteria degrade and inactivate the antibacterial peptide LL-37. Mol Microbiol, 46, 157-68.

Schmidtchen A, Holst E, Tapper H, Bjorck L. (2003). Elastase-producing *Pseudomonas aeruginosa* degrade plasma proteins and extracellular products of human skin and fibroblasts, and inhibit fibroblast growth. Microb Pathog, 34, 47-55.

Schmidtchen A, Pasupuleti M, Morgelin M, Davoudi M, Alenfall J, Chalupka A, Malmsten M. (2009). Boosting antimicrobial peptides by hydrophobic oligopeptide end tags. J Biol Chem, 284, 17584-94.

Schneider JJ, Unholzer A, Schaller M, Schafer-Korting M, Korting HC. (2005). Human defensins. J Mol Med, 83, 587-95.

Schutte BC, Mitros JP, Bartlett JA, Walters JD, Jia HP, Welsh MJ, Casavant TL, McCray PB, Jr. (2002). Discovery of five conserved beta -defensin gene clusters using a computational search strategy. Proc Natl Acad Sci U S A, 99, 2129-33.

Schweizer F. (2009). Cationic amphiphilic peptides with cancer-selective toxicity. Eur J Pharmacol, 625, 190-4.

Scott MG, Hancock RE. (2000). Cationic antimicrobial peptides and their multifunctional role in the immune system. Crit Rev Immunol, 20, 407-31.

Scott MG, Rosenberger CM, Gold MR, Finlay BB, Hancock RE. (2000a). An alpha-helical cationic antimicrobial peptide selectively modulates macrophage responses to lipopolysaccharide and directly alters macrophage gene expression. J Immunol, 165, 3358-65.

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

Scott MG, Davidson DJ, Gold MR, Bowdish D, Hancock RE. (2002). The human antimicrobial peptide LL-37 is a multifunctional modulator of innate immune responses. J Immunol, 169, 3883-91.

Selsted ME, Harwig SS, Ganz T, Schilling JW, Lehrer RI. (1985). Primary structures of three human neutrophil defensins. J Clin Invest, 76, 1436-9.

Selsted ME, Ouellette AJ. (2005). Mammalian defensins in the antimicrobial immune response. Nat Immunol, 6, 551-7.

Sewald N, Jakubke HD. (2002). *Peptides: Chemistry and Biology* edn First Edition: Wiley-VCH.

Shafer WM, Bowdish D, Davidson D, Hancock R: Immunomodulatory Properties of Defensins and Cathelicidins. In *Antimicrobial Peptides and Human Disease*. Edited by: Springer Berlin Heidelberg; 2006:27-66. Current Topics in Microbiology and Immunology, vol 306.]

Shai Y. (2002). Mode of action of membrane active antimicrobial peptides. Biopolymers, 66, 236-48.

Shaykhiev R, Beisswenger C, Kandler K, Senske J, Puchner A, Damm T, Behr J, Bals R.

(2005). Human endogenous antibiotic LL-37 stimulates airway epithelial cell proliferation and wound closure. Am J Physiol Lung Cell Mol Physiol, 289, L842-8.

Shen Y, Ai HX, Song R, Liang ZN, Li JF, Zhang SQ. (2010). Expression and purification of moricin CM4 and human beta-defensins 4 in Escherichia coli using a new technology. Microbiol Res, 165, 713-8.

Shiomi K, Nakazato M, Ihi T, Kangawa K, Matsuo H, Matsukura S. (1993). Establishment of radioimmunoassay for human neutrophil peptides and their increases in plasma and neutrophil in infection. Biochem Biophys Res Commun, 195, 1336-44.

Sieprawska-Lupa M, Mydel P, Krawczyk K, Wojcik K, Puklo M, Lupa B, Suder P, Silberring J, Reed M, Pohl J, Shafer W, McAleese F, Foster T, Travis J, Potempa J. (2004). Degradation of human antimicrobial peptide LL-37 by *Staphylococcus aureus*-derived proteinases. Antimicrob Agents Chemother, 48, 4673-9.

Silvestro L, Gupta K, Weiser JN, Axelsen PH. (1997). The concentration-dependent membrane activity of cecropin A. Biochemistry, 36, 11452-60.

Singh PK, Jia HP, Wiles K, Hesselberth J, Liu L, Conway BA, Greenberg EP, Valore EV, Welsh MJ, Ganz T, Tack BF, McCray PB, Jr. (1998). Production of beta-defensins by human airway epithelia. Proc Natl Acad Sci U S A, 95, 14961-6.

Singh PK, Tack BF, McCray PB, Jr., Welsh MJ. (2000). Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. Am J Physiol Lung Cell Mol Physiol, 279, L799-805.

Sitaram N, Nagaraj R. (1999). Interaction of antimicrobial peptides with biological and model membranes: structural and charge requirements for activity. Biochim Biophys Acta, 1462, 29-54.

Skerlavaj B, Gennaro R, Bagella L, Merluzzi L, Risso A, Zanetti M. (1996). Biological characterization of two novel cathelicidin-derived peptides and identification of structural requirements for their antimicrobial and cell lytic activities. J Biol Chem, 271, 28375-81.

Solstad T, Larsen AN, Seppola M, Jorgensen TO. (2008). Identification, cloning and expression analysis of a hepcidin cDNA of the Atlantic cod (*Gadus morhua L.*). Fish Shellfish Immunol, 25, 298-310.

Sorensen OE, Gram L, Johnsen AH, Andersson E, Bangsboll S, Tjabringa GS, Hiemstra PS, Malm J, Egesten A, Borregaard N. (2003). Processing of seminal plasma hCAP-18 to ALL-38 by gastricsin: a novel mechanism of generating antimicrobial peptides in vagina. J Biol Chem, 278, 28540-6.

Sorensen OE, Borregaard N, Cole AM. (2008). Antimicrobial peptides in innate immune responses. Contrib Microbiol, 15, 61-77.

Sörensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, Borregaard N. (2001). Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood, 97, 3951-9.

Sparkes RS, Kronenberg M, Heinzmann C, Daher KA, Klisak I, Ganz T, Mohandas T. (1989). Assignment of defensin gene(s) to human chromosome 8p23. Genomics, 5, 240-4.

Steiner H, Hultmark D, Engström A, Bennich H, Boman HG. (1981). Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature, 292, 246-8.

Stromstedt AA, Pasupuleti M, Schmidtchen A, Malmsten M. (2009). Evaluation of strategies for improving proteolytic resistance of antimicrobial peptides by using variants of EFK17, an internal segment of LL-37. Antimicrob Agents Chemother, 53, 593-602.

Subbalakshmi C, Sitaram N. (1998). Mechanism of antimicrobial action of indolicidin. FEMS Microbiol Lett, 160, 91-6.

Sugiarto H, Yu PL. (2006). Identification of three novel ostricacins: an update on the phylogenetic perspective of beta-defensins. Int J Antimicrob Agents, 27, 229-35.

Sugiyama K. (1993). Anti-lipopolysaccharide activity of histatins, peptides from human saliva. Experientia, 49, 1095-7.

Swaminathan GJ, Myszka DG, Katsamba PS, Ohnuki LE, Gleich GJ, Acharya KR. (2005). Eosinophil-granule major basic protein, a C-type lectin, binds heparin. Biochemistry, 44, 14152-8.

Taboureau O, Olsen OH, Nielsen JD, Raventos D, Mygind PH, Kristensen HH. (2006). Design of novispirin antimicrobial peptides by quantitative structure-activity relationship. Chem Biol Drug Des, 68, 48-57.

Takahashi D, Shukla SK, Prakash O, Zhang G. (2010). Structural determinants of host defense peptides for antimicrobial activity and target cell selectivity. Biochimie, 92, 1236-41.

Tamamura H, Murakami T, Horiuchi S, Sugihara K, Otaka A, Takada W, Ibuka T, Waki M, Yamamoto N, Fujii N. (1995). Synthesis of protegrin-related peptides and their antibacterial and anti-human immunodeficiency virus activity. Chem Pharm Bull (Tokyo), 43, 853-8.

Tang YQ, Yuan J, Miller CJ, Selsted ME. (1999). Isolation, characterization, cDNA cloning, and antimicrobial properties of two distinct subfamilies of alpha-defensins from rhesus macaque leukocytes. Infect Immun, 67, 6139-44.

Tasiemski A, Schikorski D, Le Marrec-Croq F, Pontoire-Van Camp C, Boidin-Wichlacz C, Sautiere PE. (2007). Hedistin: A novel antimicrobial peptide containing bromotryptophan constitutively expressed in the NK cells-like of the marine annelid, *Nereis diversicolor*. Dev Comp Immunol, 31, 749-62.

Territo MC, Ganz T, Selsted ME, Lehrer R. (1989). Monocyte-chemotactic activity of defensins from human neutrophils. J Clin Invest, 84, 2017-20.

Thomas S, Karnik S, Barai RS, Jayaraman VK, Idicula-Thomas S. (2010). CAMP: a useful resource for research on antimicrobial peptides. Nucleic Acids Res, 38, D774-80.

Thomma BP, Cammue BP, Thevissen K. (2002). Plant defensins. Planta, 216, 193-202.

Tian C, Gao B, Fang Q, Ye G, Zhu S. (2010). Antimicrobial peptide-like genes in Nasonia vitripennis: a genomic perspective. BMC Genomics, 11, 187.

Tokumaru S, Sayama K, Shirakata Y, Komatsuzawa H, Ouhara K, Hanakawa Y, Yahata Y, Dai X, Tohyama M, Nagai H, Yang L, Higashiyama S, Yoshimura A, Sugai M,

Hashimoto K. (2005). Induction of keratinocyte migration via transactivation of the epidermal growth factor receptor by the antimicrobial peptide LL-37. J Immunol, 175, 4662-8.

Tomasinsig L, Zanetti M. (2005). The cathelicidins--structure, function and evolution. Curr Protein Pept Sci, 6, 23-34.

Torrent M, Andreu D, Nogues VM, Boix E. (2011). Connecting peptide physicochemical and antimicrobial properties by a rational prediction model. PLoS ONE, 6, e16968.

Tossi A, Scocchi M, Skerlavaj B, Gennaro R. (1994). Identification and characterization of a primary antibacterial domain in CAP18, a lipopolysaccharide binding protein from rabbit leukocytes. FEBS Lett, 339, 108-12.

Tossi A, Sandri L, Giangaspero A. (2000). Amphipathic, alpha-helical antimicrobial peptides. Biopolymers, 55, 4-30.

Tran D, Tran P, Roberts K, Osapay G, Schaal J, Ouellette A, Selsted ME. (2008). Microbicidal properties and cytocidal selectivity of *Rhesus macaque* theta defensins. Antimicrob Agents Chemother, 52, 944-53.

Tsai H, Bobek LA. (1998). Human salivary histatins: promising anti-fungal therapeutic agents. Crit Rev Oral Biol Med, 9, 480-97.

Turner J, Cho Y, Dinh NN, Waring AJ, Lehrer RI. (1998). Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. Antimicrob Agents Chemother, 42, 2206-14.

Tytler EM, Anantharamaiah GM, Walker DE, Mishra VK, Palgunachari MN, Segrest JP. (1995). Molecular basis for prokaryotic specificity of magainin-induced lysis. Biochemistry, 34, 4393-401.

Uematsu N, Matsuzaki K. (2000). Polar angle as a determinant of amphipathic alpha-helix-lipid interactions: a model peptide study. Biophys J, 79, 2075-83.

Ueno S, Kusaka K, Tamada Y, Zhang H, Minaba M, Kato Y. (2010). An enhancer peptide for membrane-disrupting antimicrobial peptides. BMC Microbiol, 10, 46.

Ullal AJ, Litaker RW, Noga EJ. (2008). Antimicrobial peptides derived from hemoglobin are expressed in epithelium of channel catfish (*Ictalurus punctatus, Rafinesque*). Dev Comp Immunol, 32, 1301-12.

Uteng M, Hauge HH, Markwick PR, Fimland G, Mantzilas D, Nissen-Meyer J, Muhle-Goll C. (2003). Three-dimensional structure in lipid micelles of the pediocin-like antimicrobial peptide sakacin P and a sakacin P variant that is structurally stabilized by an inserted C-terminal disulfide bridge. Biochemistry, 42, 11417-26.

Utsugi T, Schroit AJ, Connor J, Bucana CD, Fidler IJ. (1991). Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes. Cancer Res, 51, 3062-6.

Uzzell T, Stolzenberg ED, Shinnar AE, Zasloff M. (2003). Hagfish intestinal antimicrobial peptides are ancient cathelicidins. Peptides, 24, 1655-67.

Valenti P, Antonini G. (2005). Lactoferrin: an important host defence against microbial

and viral attack. Cell Mol Life Sci, 62, 2576-87.

van't Hof W, Veerman EC, Helmerhorst EJ, Amerongen AV. (2001). Antimicrobial peptides: properties and applicability. Biol Chem, 382, 597-619.

van der Schaft DW, Toebes EA, Haseman JR, Mayo KH, Griffioen AW. (2000). Bactericidal/permeability-increasing protein (BPI) inhibits angiogenesis via induction of apoptosis in vascular endothelial cells. Blood, 96, 176-81.

van Dijk A, Veldhuizen EJ, van Asten AJ, Haagsman HP. (2005). CMAP27, a novel chicken cathelicidin-like antimicrobial protein. Vet Immunol Immunopathol, 106, 321-7.

Viejo-Diaz M, Andres MT, Fierro JF. (2005). Different anti-Candida activities of two human lactoferrin-derived peptides, Lfpep and kaliocin-1. Antimicrob Agents Chemother, 49, 2583-8.

Vuong C, Kocianova S, Voyich JM, Yao Y, Fischer ER, DeLeo FR, Otto M. (2004a). A crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence. J Biol Chem, 279, 54881-6.

Vuong C, Voyich JM, Fischer ER, Braughton KR, Whitney AR, DeLeo FR, Otto M. (2004b). Polysaccharide intercellular adhesin (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system. Cell Microbiol, 6, 269-75.

Wang G, Li X, Wang Z. (2009a). APD2: the updated antimicrobial peptide database and its application in peptide design. Nucleic Acids Res, 37, D933-7.

Wang P, Bang JK, Kim HJ, Kim JK, Kim Y, Shin SY. (2009b). Antimicrobial specificity and mechanism of action of disulfide-removed linear analogs of the plant-derived Cys-rich antimicrobial peptide Ib-AMP1. Peptides, 30, 2144-9.

Wang Y, Hong J, Liu X, Yang H, Liu R, Wu J, Wang A, Lin D, Lai R. (2008). Snake cathelicidin from Bungarus fasciatus is a potent peptide antibiotics. PLoS ONE, 3, e3217.

Wang Z, Wang G. (2004). APD: the Antimicrobial Peptide Database. Nucleic Acids Res, 32, D590-2.

Welling MM, Hiemstra PS, van den Barselaar MT, Paulusma-Annema A, Nibbering PH, Pauwels EK, Calame W. (1998). Antibacterial activity of human neutrophil defensins in experimental infections in mice is accompanied by increased leukocyte accumulation. J Clin Invest, 102, 1583-90.

Wessolowski A, Bienert M, Dathe M. (2004). Antimicrobial activity of arginine- and tryptophan-rich hexapeptides: the effects of aromatic clusters, D-amino acid substitution and cyclization. J Pept Res, 64, 159-69.

Wieprecht T, Dathe M, Epand RM, Beyermann M, Krause E, Maloy WL, MacDonald DL, Bienert M. (1997). Influence of the angle subtended by the positively charged helix face on the membrane activity of amphipathic, antibacterial peptides. Biochemistry, 36, 12869-80.

Wiesner J, Vilcinskas A. (2010). Antimicrobial peptides: the ancient arm of the human immune system. Virulence, 1, 440-64.

Willey JM, van der Donk WA. (2007). Lantibiotics: peptides of diverse structure and function. Annu Rev Microbiol, 61, 477-501.

Wu Y, He K, Ludtke SJ, Huang HW. (1995). X-ray diffraction study of lipid bilayer membranes interacting with amphiphilic helical peptides: diphytanoyl phosphatidylcholine with alamethicin at low concentrations. Biophys J, 68, 2361-9.

Wu Z, Hoover DM, Yang D, Boulegue C, Santamaria F, Oppenheim JJ, Lubkowski J, Lu W. (2003). Engineering disulfide bridges to dissect antimicrobial and chemotactic activities of human beta-defensin 3. Proc Natl Acad Sci U S A, 100, 8880-5.

Xiao Y, Cai Y, Bommineni YR, Fernando SC, Prakash O, Gilliland SE, Zhang G. (2006). Identification and functional characterization of three chicken cathelicidins with potent antimicrobial activity. J Biol Chem, 281, 2858-67.

Xu L, Lal K, Pollock JJ. (1992). Histatins 2 and 4 are autoproteolytic degradation products of human parotid saliva. Oral Microbiol Immunol, 7, 127-8.

Yan Q, Bennick A. (1995). Identification of histatins as tannin-binding proteins in human saliva. Biochem J, 311 (Pt 1), 341-7.

Yang D, Biragyn A, Hoover DM, Lubkowski J, Oppenheim JJ. (2004a). Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense. Annu Rev Immunol, 22, 181-215.

Yang L, Weiss TM, Lehrer RI, Huang HW. (2000). Crystallization of antimicrobial pores in membranes: magainin and protegrin. Biophys J, 79, 2002-9.

Yang WH, Zhang WC, Lu XM, Jiang GS, Gao PJ. (2009). Characterization of a novel antibacterial glycopeptide produced by *Penicillium sp. M03*. Lett Appl Microbiol, 48, 393-7.

Yang YH, Zheng GG, Li G, Zhang XJ, Cao ZY, Rao Q, Wu KF. (2004b). Expression of bioactive recombinant GSLL-39, a variant of human antimicrobial peptide LL-37, in *Escherichia coli*. Protein Expr Purif, 37, 229-35.

Yeaman MR, Yount NY. (2003). Mechanisms of antimicrobial peptide action and resistance. Pharmacol Rev, 55, 27-55.

Yount NY, Yeaman MR. (2005). Immunocontinuum: perspectives in antimicrobial peptide mechanisms of action and resistance. Protein Pept Lett, 12, 49-67.

Yount NY, Bayer AS, Xiong YQ, Yeaman MR. (2006). Advances in antimicrobial peptide immunobiology. Biopolymers, 84, 435-58.

Yount NY, Yeaman MR. (2006). Structural congruence among membrane-active host defense polypeptides of diverse phylogeny. Biochim Biophys Acta, 1758, 1373-86.

Zanetti M, Gennaro R, Romeo D. (1995). Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. FEBS Lett, 374, 1-5.

Zanetti M. (2004). Cathelicidins, multifunctional peptides of the innate immunity. J Leukoc Biol, 75, 39-48.

Zasloff M. (1987). 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 U S A, 84, 5449-53.

Zasloff M. (1992). Antibiotic peptides as mediators of innate immunity. Curr Opin Immunol, 4, 3-7.

Zasloff M. (2002). Antimicrobial peptides of multicellular organisms. Nature, 415, 389-95.

Zelezetsky I, Pontillo A, Puzzi L, Antcheva N, Segat L, Pacor S, Crovella S, Tossi A. (2006). Evolution of the primate cathelicidin. Correlation between structural variations and antimicrobial activity. J Biol Chem, 281, 19861-71.

Zeya HI, Spitznagel JK. (1963). Antibacterial and Enzymic Basic Proteins from Leukocyte Lysosomes: Separation and Identification. Science, 142, 1085-7.

Zeya HI, Spitznagel JK. (1966). Cationic proteins of polymorphonuclear leukocyte lysosomes. I. Resolution of antibacterial and enzymatic activities. J Bacteriol, 91, 750-4.

Zhang J, Dyer KD, Rosenberg HF. (2003). Human RNase 7: a new cationic ribonuclease of the RNase A superfamily. Nucleic Acids Res, 31, 602-7.

Zhang L, Scott MG, Yan H, Mayer LD, Hancock RE. (2000). Interaction of polyphemusin I and structural analogs with bacterial membranes, lipopolysaccharide, and lipid monolayers. Biochemistry, 39, 14504-14.

Zhang L, Rozek A, Hancock RE. (2001). Interaction of cationic antimicrobial peptides with model membranes. J Biol Chem, 276, 35714-22.

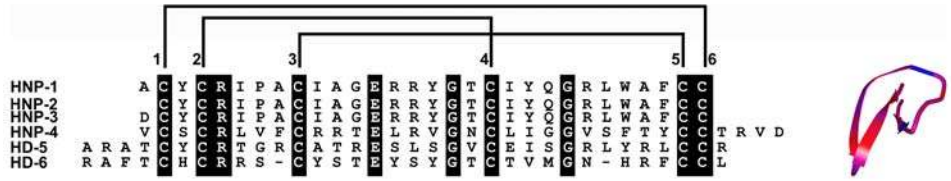
Zhang MQ, Wilkinson B. (2007). Drug discovery beyond the 'rule-of-five'. Curr Opin Biotechnol, 18, 478-88.

Zhao H: Mode of action of antimicrobial peptides [Academic dissertation]. Helsinki: University of Helsinki: 2003.

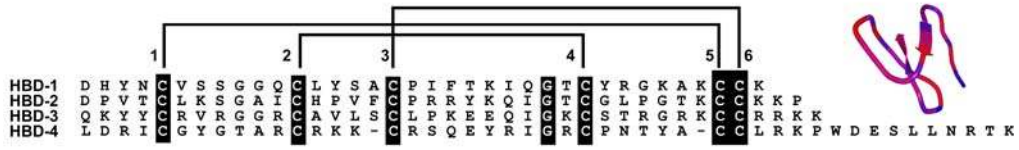
Zhao H, Gan TX, Liu XD, Jin Y, Lee WH, Shen JH, Zhang Y. (2008). Identification and characterization of novel reptile cathelicidins from elapid snakes. Peptides, 29, 1685-91.

Zipfel PF, Reuter M. (2009). Complement Activation Products C3a and C4a as Endogenous Antimicrobial Peptides. International Journal of Peptide Research and Therapeutics, 15, 87-95.

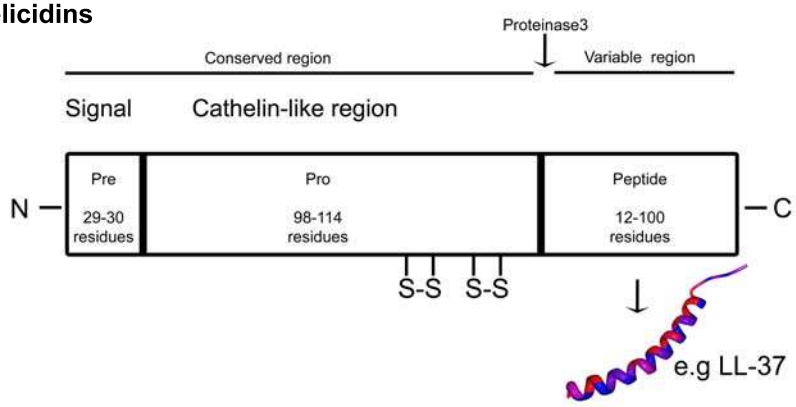
α-Defensins

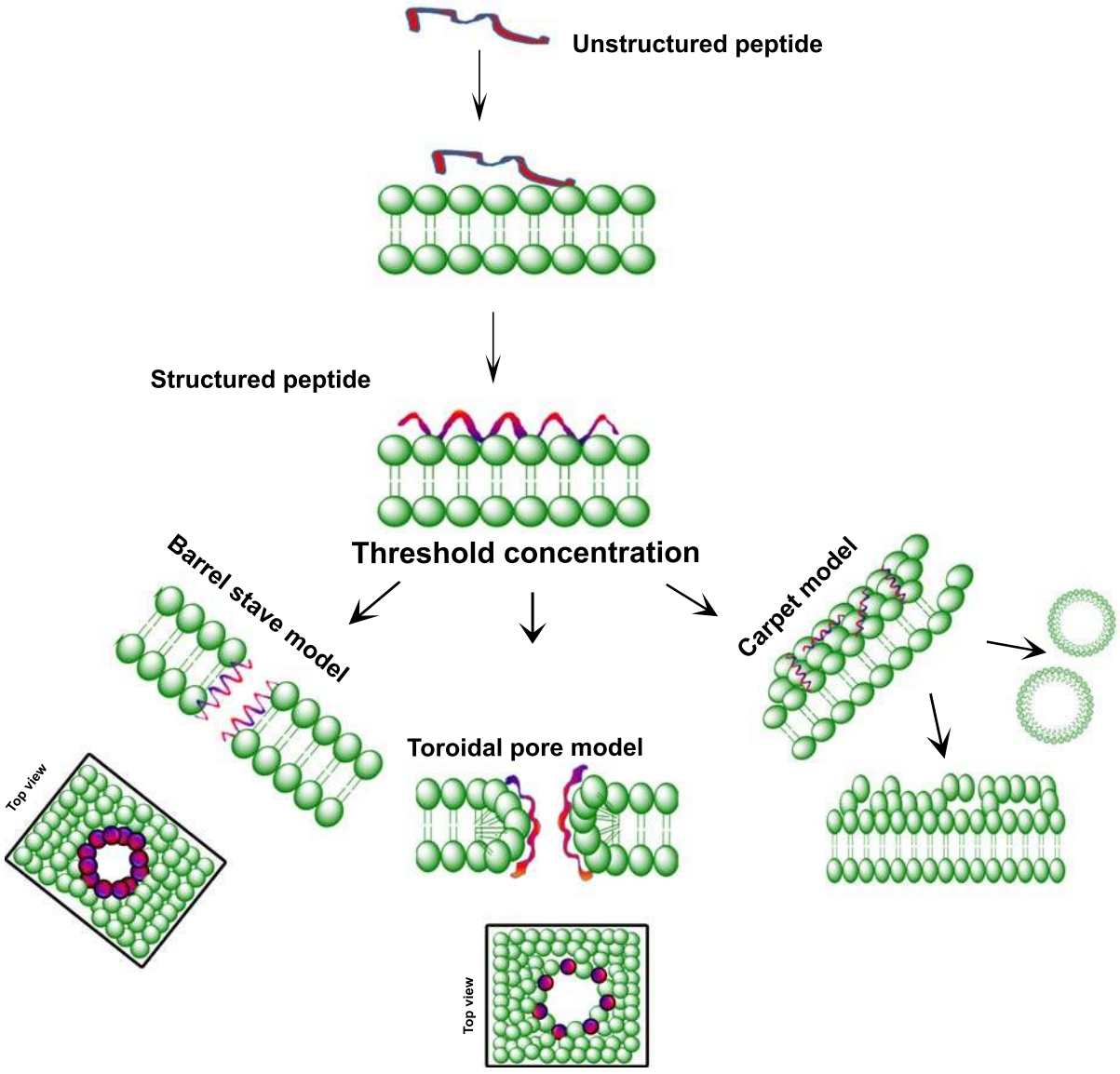


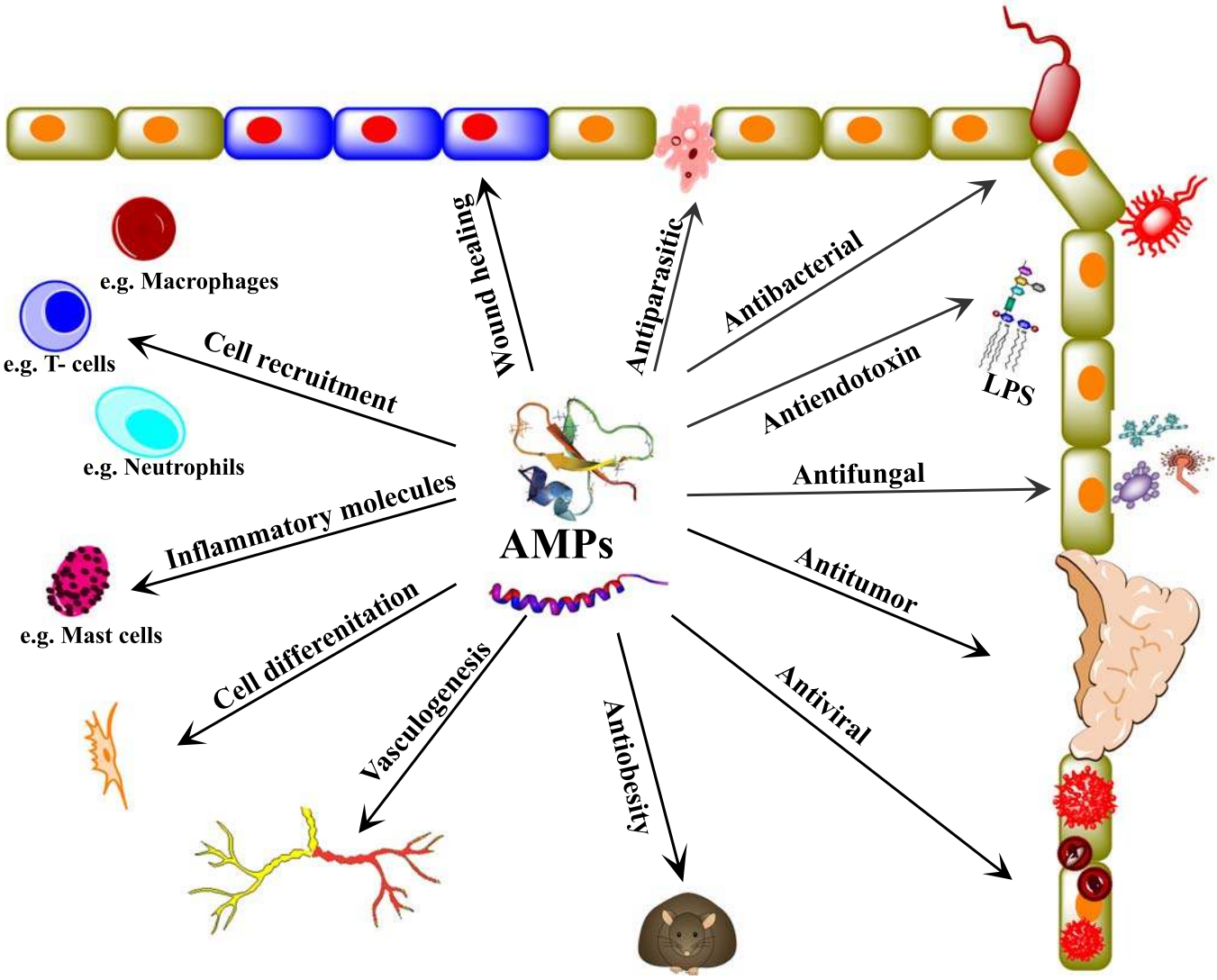
β-Defensins



Cathelicidins




Unstructured peptide
Structured peptide
Threshold concentration
Barrel stave model
Carpet model
Toroidal pore model
Top view
Top view


e.g. Macrophages
e.g. T- cells
e.g. Neutrophils
e.g. Mast cells
Cell recruitment
Wound healing
Antiparasitic
Antibacterial
Antiendotoxin
LPS
Antifungal
Antitumor
Antiviral
Antiobesity
Vasculogenesis
Cell differenitation
Inflammatory molecules
AMPs

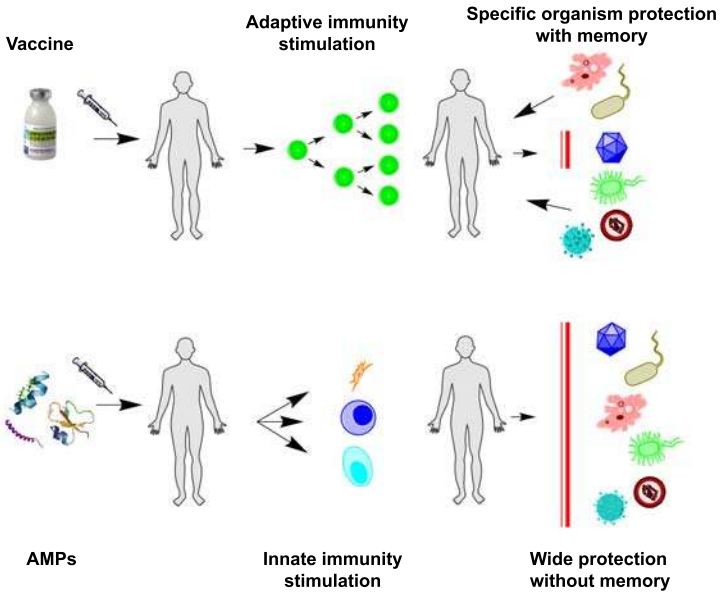

Vaccine
Adaptive immunity stimulation
Specific organism protection with memory
AMPs
Innate immunity stimulation
Wide protection without memory

Table 1: List of the major classes of AMPs produced in various organisms.

Tree	Phylla/class	*Species*	AMP produced
Bacteria	Gram negative bacteria		Bacteriocins (Jenssen et al., 2006) Lantibiotics (Willey and van der Donk, 2007)
Fungi	Ascomycota saprophytic ascomycete	*Penicillium sp* *Pseudoplectania nigrella*	AF (Yang et al., 2009) Plectasin (Mygind et al., 2005)
Plants		*Castanopsis chinensis*	TLPs (Chu and Ng, 2003; De Lucca et al., 2005)
Animal kingdom	Porifera	*Stylissa caribica* *Discodermia kiiensis*	Stylisin (Mohammed et al., 2006) Discodermin A (Matsunaga et al., 1985)
	Cnidaria	*Hydra sp* *Aurelia aurita* *Sarcophyton glaucum*	Hydramacin-1 (Jung et al., 2009) Aurelin (Ovchinnikova et al., 2006) Sarcophytolide (Badria et al., 1998)
	Mollusk	*Mytilus galloprovincialis* *Conus mustelinus*	Myticin C (Costa et al., 2009) Conolysin-Mt (Biggs

			et al., 2007)
	Annelida	*Nereis diversicolor*	Hedistin (Tasiemski et al., 2007)
		Eisenia foetida	OEP3121 (Liu et al., 2004)
		Perinereis aibuhitensis	Perinerin (Pan et al., 2004)
		Lumbricus rubellus	Lumbricin (Cho et al., 1998a)
	Arthopoda	*Carcinoscorpius rotundicauda*	Tachyplesins, Polyphemusin, and big defensin (Zhang et al., 2000)
		Drosophila melanogaster,	Drosomycin, Cecropins, Diptericin, Drosocin, Attacin and Metchnikowin (Hoffmann et al., 1999)
		Pachycondyla goeldii	Ponericidins (Orivel et al., 2001)
		Acalolepta luxuriosa	Acaloleptin A1, A2 and A3 (Imamura et al., 1999),
		Cupiennius salei	Lycotoxins and Cupiennin-1 (Kuhn-Nentwig et al., 2002),
		Apis mellifera	Melittin (Sitaram and Nagaraj, 1999),
		Androctonus australis	Androctonin (Ehret-Sabatier et al., 1996),
		Litopenaeus vannamei,	Penaeidins

			(Destoumieux et al., 2000)
		Mytilus galloprovincialis	Mytilin, Mytimycin (Charlet et al., 1996)
		Insects belonging to lepidoptera and diptera, Marine protochordate and porcine intestine	Cecropins (Steiner et al., 1981)
		Isometrus maculatus	(Miyashita et al., 2010)
	Echinodermata	*Strongylocentrotus droebachiensis*	Strongylocins (Li et al., 2008)
	Fishes	Gadus morhua L	Hepcidin (Solstad et al., 2008)
		Ictalurus punctatus Rafinesque.	HbbetaP-1 (Ullal et al., 2008)
		Morone chrysops	Piscidins (Noga and Silphaduang, 2003)
		Oncorhynchus mykiss	Histone H2A (Fernandes et al., 2002)
		Pleuronectes americanus	Pleurocidin (Cole et al., 1997)
	Reptiles	*Bungarus fasciatus*	Cathelicidin-BF (Wang et al., 2008)
		Oxyuranus microlepidotus	Omwaprin (Nair et al., 2007)
	Amphibian	*Xenopus Sp*	Magainin (Zasloff, 1987)
	Birds	*Gallus gallus*	Gallinacins (Ma et al.,

			2008)
		Gallus gallus	Fowlicidin (Xiao et al., 2006)
		Struthio camelus	Ostricacins (Sugiarto and Yu, 2006)
	Mammals	*Bos taurus*	LfcinB (Nguyen et al., 2005)
		Homo sapiens	Defensins (De Smet and Contreras, 2005; Hazlett and Wu, 2011) Cathelidicins (Lehrer and Ganz, 2002; Zanetti, 2004; De Smet and Contreras, 2005; Bucki et al., 2010)

Table 2: List of peptides that went into clinical trials and their current status/outcome

Peptide name	Company	Phase of study	Development for treatment of
CLS001	Cutanea Life Science	Phase II	Rosacea.
DPK-060	DermaGen	Phase III	Atopic dermatitis
EA-230	Exponential Biotherapies	Phase II	sepsis
HB1345	Helix BioMedix	Phase I	skin infections
IMX942	Inimex Pharmaceuticals	Phase I	Hospital related infections
LTX-109	Lytix Biopharma AS	Phase I/IIa clinical trial	MRSA
NZ2114	Novozymes A/S and *Sanofi-Aventis*	Phase I	Hospital related infections
Pexiganan	Access Pharmaceuticals	Study completed	Hospital related infections
Plectasin	Novozymes AS	Preclinical	Pneumococcal and streptococcal infections
PMX-30063	PolyMedix	currently recruiting participants	Broad spectrum antibiotics
POL-7080	Polyphor	Phase I	Broad spectrum antibiotics
RDP58	Genzyme	Phase II	Anti inflammatory properties
SCV-07	SciClone	Recruiting participants	Chronic hepatitis C virus infection
Talactoferrin	Agennix AG	Recruiting participants for Phase III	Non Small Cell Lung Cancer

Zadaxin	SciClone	Phase II	Antiviral properties
Omiganan	Migenix	FDA approval not granted	Anti inflammatory properties
Iseganan (IB-367)	Intrabiotics Pharmaceuticals, Inc	Failed Phase II	None.
Neuprex (rBPI21)	Xoma (US) Berkeley	Failed Phase III	None
XMP.629	Xoma	Failed Phase III trial	None
CZEN-002	Zengen/Zensano	Study terminated / Failed	Vaginal yeast infection
hLF1-11	AM-Pharma Holding B.V.	Study terminated / Failed	Hospital related infections
MX-2401	Microbiologix Biotech	Study terminated / Failed	Sepsis
Oglufanide disodium	Implicit Bioscience	Study terminated / Failed	Immune suppression
Omigard™ (MBI-226)	Biowest Therapeutics Inc	Study terminated / Failed	Catheter related infections
PAC-113	Pacgen Biopharmaceuticals	Study terminated / Failed	oral Candidiasis infections