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REVIEW ARTICLE

PEPTIDE-BASED ANTIFUNGAL THERAPIES AGAINST EMERGING INFECTIONS

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SUMMARY

Acquired drug resistance to mycotic infections is rapidly emerging as a major medical problem. Opportunistic fungal infections create therapeutic challenges, particularly in high-risk immunocompromised patients with AIDS, cancer, and those undergoing transplantation. Higher mortality and/or morbidity rates due to invasive mycosis have been increasing over the last 20 years, and in light of growing resistance to commonly used antibiotics, novel antifungal drugs and approaches are required. Currently, there is considerable interest in antifungal peptides that are ubiquitous in plant and animal kingdoms. These small cationic peptides may have specific targets or may be multifunctional in their mechanism of action. On the basis of recent advances in protein engineering and solid-phase syntheses, the utility and potential of selected peptides as efficient antifungal drugs with acceptable toxicity profiles are being realized. This review will discuss recent advances in peptide therapy for opportunistic fungal infections.

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INTRODUCTION

In an era of increased incidence of fungal infections in immunocompromised patients (1, 2) and greater resistance to "frontline" antifungal therapies (3), there is a growing need to discover new antifungal therapies. Although newer azole derivatives, such as voriconazole, are more effective and show fungicidal activity against filamentous fungi such as *Aspergillus fumigatus* (4), these derivatives are fungistatic and not fungicidal against pathogenic yeasts. The inability to kill yeasts leads to azole resistance in prolonged infections and increases the likelihood that these agents will lack efficacy in severe *Candida* infections in immunosuppressed patients. Amphotericin B has also been commonly used to treat serious fungal infections, and in contrast to azoles, it is fungicidal against yeasts. Nevertheless, resistance to amphotericin B is slowly developing in selected *Candida* species (5) and its use is associated with significant side effects, including nephrotoxicity. Although recently developed antifungals (e.g., the peptide-based agents micafungin sodium and caspofungin acetate) have been developed and are very promising, resistance to these therapies has already been reported (6-8) and will no doubt become more widespread. The development of resistance to current antifungal agents, as well as the limited efficacy and the side effects associated with several of these agents, increase the importance of continued development of new alternative approaches. This review will examine both synthetic and natural peptides as antifungal therapies, divided into peptides that have a primarily antifungal mechanism of action and peptides that broadly inhibit microbes, including bacteria, fungi and enveloped viruses (9). Because a large number of diverse antifungal peptides exists in nature, we will mainly focus on those that show promise in treating agricultural and human diseases.

OVERVIEW OF ANTIMICROBIAL AND ANTIFUNGAL PEPTIDES

Antimicrobial and antimycotic peptides are small cationic and amphipathic molecules, generally made up of fewer than 50 amino acids. These peptides are omnipresent and have been isolated from prokaryotes and eukaryotes in the plant, bacterial, fungal and animal kingdoms (10-13). Nature has strategically placed antimicrobial and antifungal peptides at the first line of defense between the host organism and its surrounding environment because these peptides

are able to quickly inhibit a wide spectrum of infectious microbes without significant toxicity to the host organism. When insects are infected within a short period of time, they secrete an array of cationic peptides to combat the invading organism (14). Although antimicrobial peptides (AMPs) are the primary means of combating pathogens in lower forms of life, these peptides have an adjunct role to the immune system in phylogenetically more advanced organisms. Indeed, in humans cationic peptides have an important role and they are produced and secreted by several different tissues, including salivary glands, skin, eyes and liver, as well as epithelial and platelet cells and neutrophils (15). Several antifungal peptides display selective toxicity for the microbial target by identifying conserved molecular determinants of pathogens (16, 17). A classic example is the echinocandin family, which targets 1,3-β-glucan synthase, an enzyme essential for cell wall integrity of fungi (18). In most instances, however, AMPs are less specific in their targeting, resulting in exhibition of a broad spectrum of inhibitory/cidal activity, not only against fungi but also against bacteria and envelope-containing viruses (19). Broad-spectrum AMPs often target and lyse the membrane of the microbe, yet these peptides frequently have less proclivity to lyse mammalian cell membranes, such as those of red blood cells. The interaction between AMPs and target microbes is complex, but the positive charge of the peptide is essential to its binding with negatively charged membrane/wall elements, such as the mannoproteins in yeasts. Moreover, despite targeting and lysing microbial membranes, the potencies and spectra of activities of these broad-spectrum AMPs against different classes of microbes vary and depend on the membrane composition of the pathogen and the structure of the peptide. Much remains to be learned about the subtle differences in microbial membranes that may affect efficacy of the AMPs.

Although the focus of this review is on the direct action of peptides against disease-causing fungi, accumulating evidence suggests that the antifungal activity of AMPs is multifactorial. For example, AMPs stimulate the immune system in mammals by several mechanisms: 1) activation of T cells; 2) stimulation of Toll-like receptors; 3) amplification of phagocyte action; 4) activation of dendritic cells; and 5) chemoattraction of neutrophils (20-25). Moreover, these activated cells and receptors may reduce the growth of fungi in vivo by modifying levels of various cytokines, chemokines and integrins (26, 27). Thus, similar to other antifungal agents (28, 29), the interplay between antifungal peptides, their modulation of the immune system, and the host's immune status will likely determine the efficacy of the peptide. In addition to their role in providing immunosurveillance against pathogens and maintaining a healthy floral milieu, studies have shown potential for antimicrobial cationic peptides in cancer and gene therapy (30-33). As peptide-engineering methods develop, the potential for producing sufficient amounts of naturally occurring peptides increases significantly. As a result, the antifungal peptides offer promise for the future treatment of infectious diseases in a diverse range of organisms, including humans. Figure 1 shows representatives of specific and broad-spectrum antifungal peptides, as well as their mechanisms of action.

Because of the disparate structure of antifungal peptides and the incomplete knowledge of their mechanisms of action, classifying the various antifungal peptides is a daunting task. Whereas some lipopeptides (e.g., echinocandins) or histidine-rich peptides (e.g., the linear histatins or branched HK peptides) have primarily antifungal activity, membrane-disrupting peptides (e.g., magainins, protegrins) inhibit a diverse group of microorganisms, including fungi, bacteria and viruses. Structurally, linear cationic antifungal peptides (e.g., LL-37, magainins) form α-helical structures in a hydrophobic milieu, while cysteine-containing peptides containing from one to multiple disulfide bonds (e.g., protegrins and defensins) form β-sheet-enriched structures. The formation of these α-helical and/or β-sheet secondary structures may increase the amphipathicity of the peptides and enable them to act specifically with their targets in the fungal membrane. In addition to histidine-rich peptides such as histatins, other peptides (e.g., apidaecins, indolicidin) have a high percentage of certain amino acids such as proline and tryptophan. Interestingly, potent antifungal linear peptide fragments from larger proteins (e.g., lactoferrin, casein and lysozyme) are able to inhibit fungi because of multiple direct and indirect effects (34). Notably, for optimal antimicrobial activity, it may be necessary for the peptide structures to undergo post-translational modifications, including glycosylation, formation of D-amino acid enantiomers, amidation, halogenation or phosphorylation (20, 35-39).

Nearly 1,200 AMPs have now been identified. In this review, we have selected examples of peptides that have antifungal properties with varied mechanisms of action (Fig. 1). For supplementary reviews on AMPs, see those by Jensen and Hancock (40), Yeaman (41), De Lucca (42) and Bulet (13).

PEPTIDES WITH PRIMARILY ANTIFUNGAL PROPERTIES

Naturally occurring peptides with primarily or exclusively antifungal properties are less ubiquitous than those with broad antimicrobial activity. From an evolutionary perspective, it was important for nature to form peptides that were functionally omnipotent and could protect all life forms from a great variety of infectious pathogens. Thus, the vast majority of small peptides are actually antimicrobial, affecting bacteria, fungi and enveloped viruses (43). Nevertheless, there are some peptides, both natural and synthetic, which exhibit primarily antifungal activity. The potential advantage of these is that the therapeutic window for peptides specific for fungi is greater than that of peptides with broad antimicrobial activity. In contrast to broad-spectrum AMPs, which induce membrane lysis, most of these antifungal peptides have specific targets that are intracellular, on the cell membrane or on the cell wall. Because of the diverse targets, the structure of these AMPs can vary significantly and includes linear, open-ended cysteine-rich cyclic peptides and cyclic lipopeptides (see Table I).

1,3-β-Glucan synthesis inhibitors

These specific fungal inhibitors are cyclic lipoproteins that noncompetitively inhibit the multiunit membrane-integrated enzyme β-glucan synthase, critical for cell wall integrity. Inhibition of β-glucan synthase results in destabilization of the cell wall, leading to susceptibility to osmotic stress and cell lysis. In addition to the cell wall, 1,3-β-glucans have a role in the division septum and assembly of the acropore wall and are therefore also sensitive to the synthase inhibitors. β-Glucan synthase has a widespread distribution in fungi, including *Candida*, *Aspergillus*, *Cryptococcus* and *Pneumocystis* spp. Mycelious fungi such as *Aspergillus* spp., however, are less sensitive

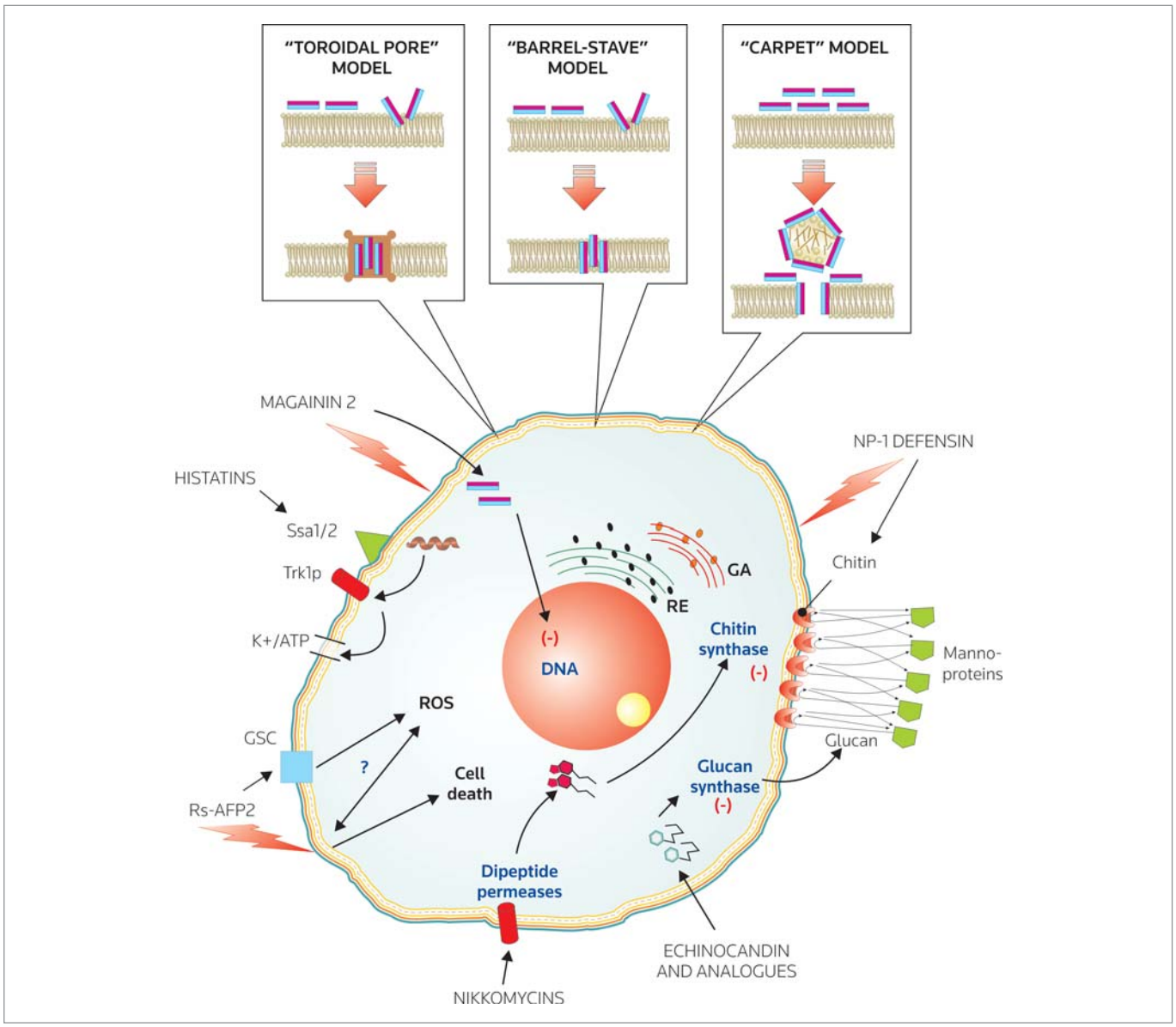

Figure 1. Modes of action proposed for antifungal peptides. The majority of antimicrobial peptides (AMPs) inhibit filamentous fungi and yeasts by membrane lysis. Three mechanisms have been proposed by which AMPs induce membrane lysis: the toroidal pore, barrel-stave and carpet. In addition, there are other AMPs that are more selective in their antifungal activity. Several antifungal peptides disrupt the function and/or structure of the cell wall. Defensin NP-1 binds tightly to chitin in the cell wall and this may be important in mediating selective membrane lysis. In addition, chitin biosynthesis is blocked by nikkomycins, which enter fungal cells via dipeptide permeases. The lipopeptide echinocandin and its synthetic analogues inhibit the synthesis of 1,3-β-glucans, a key component of the cell wall. For histatins, one mechanistic concept is based on specific binding with extracellular receptor-like heat shock protein SSA1/2, internalization and binding to the K^+ channel transporter Trk1p, and as a consequence, efflux of adenosine triphosphate (ATP) and potassium ions from the cell. MAG-2 magainin kills fungi via cell membrane disruption and DNA damage. The plant defensin Rs-AFP2 causes membrane permeabilization via interaction with glucosylceramide (GSC) and formation of reactive oxygen species (ROS). M, mitochondria; N, nucleus; E, endosomes; L, lysosomes; RE, endoplasmic reticulum; GA, Golgi apparatus.

to these inhibitors because the synthase is found primarily in tips of the growing hyphae. Inhibition of β-glucan synthase results in negative feedback, causing cell cycle arrest. The family of echinocandins consists of several lipopeptides that differ slightly in their peptide core. This family includes the echinocandins, pneumocandins, mulundocandins, aculeacins and WF-11899 (44-46). Notably, members of the echinocandin family are the only peptides thus far approved for severe localized (e.g., pneumonitis) or systemic fungal infections.

Echinocandins and pneumocandins

Several analogues from these two classes of β-glucan synthase inhibitors have shown promise in preclinical or clinical studies for the

Table I. *Antimicrobial peptides that specifically target fungi.*

Name of the group	Representative peptides	Origin	Structure	Mechanism	MW[1]	Susceptible strain	Ref.
β-Glucan synthase inhibitors	Echinocandins Pneumocandins Aculeacins	Fungi	Cyclic lipopeptides	Inhibition of glucan synthesis	1,000	*Candida* spp., *Pneumocystis carinii, Aspergillus* spp.	47 50 58
	Mulundocandins	Fungi	Threonine is substituted by serine and lineoyl by 12-methylmyristoyl	Inhibition of glucan synthesis	1,000	*Candida* spp. *Pneumocystis carinii, Aspergillus* spp.	60 61
Cell wall chitin inhibitors	Nikkomycins Polyoxins	Bacteria	Nucleoside peptide antibiotic	Inhibition of chitin synthesis	Nikkomycin Z: 495	*Candida* spp., *Coccidioides immitis Blastomyces dermatitidis, Histoplasma capsulatum*	66 67
	Aureobasidins	Fungi	Cyclic lipophilic depsipeptide with 8 amino acids and an α-hydroxy acid	Inhibition of actin and chitin assembly, synthesis of sphingolipids	1,100	*Candida* spp., *Cryptococcus neoformans*	69 70 71
Membrane-targeting inhibitors	Rs-AFP2	Plants	α-Helical 3-stranded β-sheets, 4 disulfide bridges	Targets membrane glucosylceramide, induces reactive oxygen species, membrane lysis (?)	5,730	*Candida albicans*, *Candida krusei, Aspergillus flavus*, *Fusarium solani*	76 73
	Drosomycin	Insects	α-Helical 3-stranded β-sheets, 4 disulfide bridges	Targets voltage-gated sodium channel (?), membrane lysis (?)	5,250	*Fusarium oxysporum, Neurospora crassa, Saccharomyces cerevisiae*	74 276 277 77
	Bacillomycin F Iturin A	Bacteria	Cyclic with lipid-soluble β-amino acid linked to the D/L-amino acid	Lysis by pore formation and leakage of key ions	1,000	*Aspergillus niger, Candida albicans*, *Fusarium oxysporum, Aspergillus flavus, Fusarium moniliforme*	81 82
Histatins	Histatin 1-12	Mammals	α-Helical in hydrophobic environment; increased histidine content	Mechanism uncertain (targets mitochondria vs. high-affinity potassium transport protein vs. lysis of energized membrane)	Histatin 1: 4,880 Histatin 3: 4,060 Histatin 5: 3,040	*Candida* spp., *Trichosporon pollulans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*	88 90 91 108 105 278

[1]Approximate molecular weight.

treatment of invasive and systemic *Candida* and *Aspergillus* infections. Of the three subfamilies of echinocandins (B, C and D), analogues of group B have been most useful in the development of antifungal drugs. Echinocandin B, produced by *Aspergillus nidulans* and *Aspergillus rugulosus*, was found to have potent antifungal activity, with an MIC between 0.20 and 0.35 μg/mL[1] for *Candida* spp. (47). Nevertheless, it was an unsuitable antifungal candidate because it induced lysis of red blood cells. Compared with echinocandins, pneumocandins have a broader spectrum of fungicidal activities (48, 49). This group of cyclic lipopeptides, including the most studied, pneumocandin A_0 (L-671329), is produced by *Zalerion asboricola* and has shown potent activity against *Candida* spp. and *Pneumocystis carinii* (50). Similar to echinocandin B, pneumocandin A_0 also caused hemolysis. After significant research and screening of a number of analogues with varied *N*-acyl side-chain substitutions, the unwanted side effect of hemolysis from these lipopeptides was

[1] Because investigators use different MIC units (molar vs. weight/vol), the approximate molecular weights are listed in Tables I and II to enable conversion and comparison of the activities of AMPs.

mitigated with the echinocandin analogue cilofungin (LY-121019). While there was no effect on its antifungal activities against *A. fumigatus* and *Candida* spp. (51), cilofungin had a 10-fold lower hemolysis than echinocandin B. Nevertheless, clinical trials on cilofungin were stopped because of side effects (52).

Analogues with an improved efficacy and safety profile and greater aqueous solubility have now been developed and have received FDA approval for antifungal treatment (53). Currently, there are three approved echinocandins/pneumocandins: caspofungin (second-generation pneumocandin, MK-0991) was approved in 2001 and micafungin sodium (an echinocandin, FK-463) in 2004, while anidulafungin (an echinocandin, LY-30366) was most recently approved in 2006. All three drugs have fungicidal activity against the majority of *Candida* spp. (*C. albicans, C. glabrata, C. tropicalis, C. dubliniensis* and *C. krusei*), including those that are resistant to fluconazole or amphotericin. Moreover, these antifungal lipopeptides are fungistatic toward *Aspergillus* spp. As a representative of these lipopeptides, the MIC of micafungin ranged from 0.0156 to 2 μg/mL for *Candida* spp., while for *Aspergillus* spp. the MIC ranged from 0.0078 to 0.0156 μg/mL (54). There are a number of fungi against which these echinocandins/pneumocandins are less effective (e.g., *Candida parapsilosis*, *Candida guilliermondii*, *Cryptococcus neoformans*, mucormycoses [*Rhizopus oryzae*] and yeast forms of *Histoplasma capsulatum*, *Blastomyces dermatitidis* and *Coccidioides immitis*). Currently, these three lipopeptides have been approved for various *Candida* infections and caspofungin has been approved to treat invasive aspergillosis when patients are unable to tolerate or are resistant to first-line therapies. Because there are a number of fungi against which the echinocandin family shows poor activity, care must be taken to select the appropriate clinical setting to administer these drugs.

Other β-glucan synthase inhibitors

In addition to echinocandins/pneumocandins, there are a number of other related echinocandin family members that will be mentioned briefly. To date, these cyclic lipoprotein analogues have not been clinically approved, and in general, the following classes have reduced antifungal activity and/or greater toxicity compared with the pneumocandins/echinocandins discussed previously.

Aculeacins (A-D, F), isolated from *Aspergillus aculeatus*, have potent antifungal activity (55-57). In general, aculeacins have an MIC < 0.31 μg/mL for most *Candida* spp., but they are not active against *C. tropicalis* or most filamentous fungi (58). When aculeacin A was compared with pneumocandin A_0, the aculeacin induced significantly more hemolysis at a lower concentration and was slightly less effective in vivo against systemic *C. albicans* infection in a mouse model (50).

Mulundocandins differ structurally from echinocandins by one amino acid; threonine is substituted by serine and the lipophilic side chain consists of 12-methylmyristoyl and not lineoyl (59). Mulundocandins are produced by *Aspergillus syndowi* var. *mulundenis* (59, 60) and are effective against *C. albican*s (MIC = 0.5-4.0 μg/mL), *C. glabrata* (MIC = 2.0-4.0 μg/mL) and *C. tropicalis* (MIC = 1.0-8.0 μg/mL). Against other *Candida* spp., mulundocandins are less active and show little or no activity against *C. neoformans* or filamentous fungi (61).

WF-11899A, B and C possess potent anti-*Candida* activities that are superior to cilofungin and equivalent to those of fluconazole. Unfortunately, they lyse murine red blood cells at low concentrations (62).

Inhibitors of chitin in the cell wall

Nikkomycins and polyoxins

Nikkomycins, produced by *Streptomyces tendae* and *Streptomyces ansochromogenes,* and polyoxins, produced by *Streptomyces cacaoi,* are the most widely studied peptidyl nucleoside inhibitors of chitin synthase (63-65). Synthase inhibitors such as nikkomycin are structural analogues of uridine diphosphate-*N*-acetylglucosamine, a major constituent of chitin. Absent in vertebrates, chitin is a cell wall component that is essential to maintain the structural integrity of the fungus. Nikkomycins were able to inhibit chitin synthesis in *C. albicans* in both in vitro and in vivo studies (66) and they are not toxic to human cells. They also show significant activity against *C. immitis* and *B. dermatitidis*, and moderate activity against *H. capsulatum* (67, 68), but these agents are not active against filamentous fungi. Despite their advantages, the use of these inhibitors has been limited because of their unfavorable pharmacokinetics.

Aureobasidins

There are 18 members of the aureobasidin family produced by *Aureobasidium pullulans*. They are cyclic depsipeptide lipophilic antibiotics made up of eight amino acids and an α-hydroxy acid (69). Two modes of action have been proposed for aureobasidins: one is based on disruption of the cell wall/membranes by altering the assembly of actin and chitin (70) and the other is based on interrupted synthesis of sphingolipids (71). Several members of the aureobasidin family are active against *Candida* spp. (e.g., aureobasidin A MIC < 0.05-0.2 μg/mL) and *C. neoformans* (e.g., aureobasidin A MIC = 0.78 μg/mL) (72).

Membrane-active selective antimicrobial peptides

Rs-AFP2, Hs-AFP1, Dm-AMP1 and heliomicin

Isolated from radish seeds, Rs-AFP2 is a 50-amino-acid residue plant defensin that has an α-helix and three-stranded β-sheets stabilized by four disulfide bridges. Rs-AFP2 shares structural and functional homology with other plant defensins, Hs-AFP1 and Dm-AMP1, and the insect defensin heliomicin (73-75). Notably, these defensins have no significant effect on bacteria but have marked antifungal effects. Rs-AFP2 causes rapid K^+ efflux, Ca^{2+} uptake and alkalinization of the medium by targeting the fungus-specific membrane glucosylceramide and by inducing membrane permeability. After targeting the ceramide in the membrane, this defensin also induces intracellular reactive oxygen species that are toxic to the fungi. Following 7.5 h of incubation, Rs-AFP2 (10 μM) inhibited *C. albicans* and *C. krusei* by 99.8% and 91.1%, respectively (73). *C. glabrata*, which does not contain this fungus-specific ceramide, was not inhibited. Furthermore, the MICs of Rs-AFP2 toward *Aspergillus flavus* and *Fusarium solani* were 0.7 and 0.04 μM, respectively. Analogues of Rs-AFP2, in which arginines replaced neutral amino acids, were more effective against *F. solani*. Moreover, replacement of cysteines with α-aminobutyric acid improved the antifungal

potency of Rs-AFP2 (76). Unlike iturins discussed later in this section, Rs-AFP2 and its close analogues show little cytotoxicity for mammalian cells at doses that are inhibitory to fungal pathogens.

Drosomycin

An insect defensin structurally related to Rs-AFP2, drosomycin is an inducible 44-residue cysteine-rich peptide produced by *Drosophila melanogaster* (74, 75). Despite its sequence homology with Rs-AFP2, the underlying antifungal mechanism of drosomycin remains unclear. Based on the interaction of drosomycin with the voltage-gated sodium channel in *D. melanogaster*, Cohen and colleagues have suggested that this interaction may have a role in the inhibition of microbial pathogens (77). More is understood about the signal transduction pathways that upregulate drosomycin levels in the hemolymph of *D. melanogaster*. After insects are challenged by bacteria, drosomycin, under the control of activated Toll receptors, reaches its peak concentration in the hemolymph at 16 h (74, 78). Despite its induction by bacteria, this defensin has no significant antibacterial effect (74). Drosomycin has significant fungicidal activity against filamentous fungi (e.g., MIC < 5 μM against *Fusarium oxysporum* and *Neurospora crassa*) (79) and recent studies have revealed its antiyeast (MIC = 12 μM against *Saccharomyces cerevisiae*) activities (80).

Iturins

Produced by *Bacillus subtilis*, iturins are cyclic peptides with a lipid-soluble β-amino acid linked to an array of D- and L-amino acids. Iturins act on microbial membranes, causing pore formation and leakage of key ions from fungi (81). Their antimicrobial activity is limited primarily to fungi, with little effect on bacteria, but they are toxic to mammalian cell membranes. In contrast to most other AMPs, which are cationic, iturins may be anionic (bafilomycin L) or neutral (iturin A). One member of the family, bafilomycin F, effectively inhibits *Aspergillus niger (*MIC = 40 μg/mL), *C. albicans* (MIC = 40 μg/mL), *C. tropicalis* (MIC = 40 μg/mL) and several phytopathogens such as *Mycosphaerella pinodes* (MIC = 10 μg/mL) (82). In contrast to its potent antifungal activity, bafilomycin F modestly inhibits the bacterium *Micrococcus luteus* (MIC = 200 μg/mL) but has no inhibitory effect on other bacteria tested (e.g., MIC > 400 μg/mL against *Escherichia coli* K12, *Streptomyces albus* G, *Staphylococcus aureus*). In addition, using a radial diffusion assay, Klich et al. found that iturin A had marked antifungal activity against phytopathogens and human pathogens such as *A. flavus* (83); most fungi were inhibited at 7.7 μg/mL, with no change in the zone of inhibition for several weeks. Although this group of peptides is effective against dermatomycoses in humans and animals, they induce unacceptable levels of red blood cell lysis (84). Nevertheless, there is a growing interest in substituting toxic pesticides with these peptides for the treatment of fungal infections in plants.

Histidine-rich peptides: histatins

Histatins are a group of linear cationic peptides that are isolated from human saliva and have potent and specific biological activity against fungi. Their weak amphipathic character, lack of disulfide bonds and high histidine content distinguish them from other cationic peptides (85). Whereas histatins form random polymers in aqueous environments, they adopt α-helical structures in hydrophobic environments and this has been posited to have a role in their antifungal activity (86, 87). A key feature of histatins is their strong candidacidal activity (e.g., against *C. albicans, C. glabrata, C. guilliermondii, C. krusei, C. lambica, C. parapsilosis, C. pseudotropicalis, C. stellatoidea* and *C. tropicalis* [88, 89], *C. neoformans* and *A. fumigatus* [90, 91]). This family of 12 small (3-4 kD) histidine-rich peptides is created by proteolytic cleavage of histatin 1 and histatin 3 (92) and is exclusively found in saliva of primates (93-95). Of these peptides, histatin 5 has the strongest fungicidal activity, with an MIC of 100 μM against *C. albicans* (96). P-113, a 12-amino-acid fragment of histatin 5, is the smallest peptide that retains full anticandidal activity compared to its parent peptide (97). In addition to inhibiting yeast cells, histatin 5 and two synthetic variants (Dhar4 and Dhar5) markedly inhibited formation of the drug-resistant *C. albicans* biofilm (98-100). Besides mammals, arthropods also produce histidine-rich peptides that are active against certain fungi such as *C. albicans* but are not active against bacteria (101-103). Compared to histatin 5, synthetic branched histidine-rich peptides are significantly more inhibitory (about 15-fold on a molar basis) against *C. albicans* (104). Moreover, these branched antifungal histidine-rich peptides are highly selective and show little toxicity toward mammalian cells.

Of interest is the rationale for the use of these histidine-rich peptides, which provide selective fungicidal activity. Although several mechanisms have been proposed, including one put forth in a study suggesting that histatins interact with an energized membrane (105), most data indicate that histidine-rich peptides must traverse the membrane and interact with an intracellular target (106-108) (Fig. 1). Interestingly, in our laboratory, histidine-rich peptides have been developed as nucleic acid carriers because of their ability to traverse membranes and disrupt acidic endosomes (32, 33, 109). Although we think that the fungicidal mechanisms of histatins and branched HK polymers are closely linked, this supposition has not yet been proven.

ANTIFUNGAL PEPTIDES WITH BROAD-SPECTRUM ANTIMICROBIAL ACTIVITY

In contrast to the peptides that specifically target fungi, most AMPs affect a number of organisms, including bacteria, fungi and envelope-containing viruses. A common theme among most of these broad-spectrum AMPs is that they lyse the membranes of the pathogen. Despite this nonspecific mechanism, many of these peptides do not lyse mammalian membranes at pathogen-inhibiting concentrations. Because the number of AMPs that can affect bacteria, fungi and viruses is extensive, we will discuss selected examples of linear and cyclic antifungal peptides produced by several different species (Table II).

Linear peptides

Small, linear, primarily α-helical peptides are the most common and well-studied group of AMPs and include families such as cecropins (110), magainins (111) and dermaseptins (112). Because α-helical amphipathic peptides differ in amino acid composition, as well as in length and positive charge, their antimicrobial activity is likely determined by their global structural components rather than by their specific amino acid sequence (113). Because the final common path-

Table II. *Antimicrobial peptides that specifically target fungi.*

Group name	Representative peptides	Origin	Structure	Mechanism	MW[1]	Susceptible strain	Ref.
Cecropins	60 members; cecropin A	Insects	α-Helical, tryptophan in the first or second position, *C*-amidated end	Membrane lysis	2,800-4,000	*Aspergillus* spp., *Fusarium moniliforme, Fusarium oxysporum*	121 124
Magainins	Magainin 2	Frogs	α-Helical, glycine- and serine-rich	Membrane lysis, DNA damage	2,500-3,000	*Candida albicans, Cryptococcus neoformans, Saccharomyces cerevisiae*	111 114
Bombinin-like and bombinin-H	BLP-1, BLP-3, bombinins H1-7	Frogs	α-Helical, glycine-rich, *C*-amidated end	Membrane lysis	2,300-4,000	*Candida albicans, Candida guilliermondii, Candida tropicalis*	137
Dermaseptins	Dermaseptins S1-5	Frogs	α-Helical, lysine-rich	Membrane lysis, apoptosis induction	2,500-3,500	*Aspergillus fumigatus*	112 114
Cathelicidins (without cysteines)	LL-37, mCRAMP, BMAP-27, BMAP-28	Humans, cattle, pigs, goats, mice	α-Helical	Membrane lysis	3,000-4,000	*Candida albicans*, *Cryptococcus neoformans*	279 160
	Indolicin, tritrpticin	Humans, cattle, pigs, goats, mice	Extended wedge, tryptophan-rich	Membrane lysis	1,900-2,000	*Cryptoccocus neoformans, Candida* spp., *Aspergillus fumigatus*	166 167
PAF-26	PAF-26	Combinatorial library	Penatratin-like hexapeptide	Unknown	950	*Penicillum digitatum*, *Saccharomyces cerevisiae*	280
Kaxins	dF21-10K	Synthetic design	Nonamphipathic hydrophobic core, *N*-terminal lysine-rich	Membrane lysis	1,830-2,350	*Candida* spp.	175
Thanatin	Thanatin	Insects	β-Sheet, *C*-terminal single disulfide bond-forming	Membrane lysis	2,400	*Aspergillus fumigatus, Neurospora crassa, Trichophyton mentagrophytes*	176 281
Cathelicidins (with cysteines)	Protegrins 1-5	Pigs	β-Sheet, 2 disulfide bridges	Membrane lysis	1,900-2,200	*Candida albicans*	282
α-Defensins	HNPs 1-4, HDs 5-6, NP-1	Mammals	Cyclic, 3 disulfide bridges (C1-6, C2-4, C3-5)	Membrane lysis	3,000-4,000	*Candida* spp., *Aspergillus fumigatus*, *Cryptococcus neoformans*	30 283 202
β-Defensins	HBDs 1-4, bovine tracheal antimicrobial peptide	Mammals	Cyclic, disulfide bridges (C1-5, C2-4, C3-6)	Membrane lysis	3,500-5,000	*Candida* spp., *Aspergillus fumigatus*	224 222
θ-Defensins	rTD1-3	Rhesus monkeys	Circular octadecapeptides, 2 antiparallel β-sheets and 3 disulfide bonds	Membrane lysis	2,100	*Candida albicans*, *Cryptococcus neoformans*	20 225

Continuation

Table II (Cont.) *Antimicrobial peptides that specifically target fungi.*

Group name	Representative peptides	Origin	Structure	Mechanism	MW[1]	Susceptible strain	Ref.
Gallinacins	Gallinacin-1 and -1α, gallinacin-2 and gallinacin-6	Chickens	β-Sheet, lysine- and arginine-rich, 3 disulfide bonds	Membrane lysis, effect on DNA replication, RNA and protein synthesis	2,000-5,000	*Candida albicans, Saccharomyces cerevisiae*	227
Macrocycles	Kalata, circulin A, circulin B, cyclopsychotride	Plants	Cyclic knot	Membrane lysis	2,800-3,400	*Candida kefyr, Candida tropicalis*	228
Syringomycins Pseudomycins	Syringomycin E, syringotoxin B, syringostatin A	Bacteria	Lipodepsinona-peptides	Membrane lysis	2,300-3,500	*Candida* spp., *Aspergillus*	229 231
Lactoferrin[1]-derived peptides	Peptide 2, lactoferricin	Mammals	Mixture of α-helix and β-sheet peptides	Membrane lysis	2,600-4,660	*Candida albicans, Cryptococcus albidus, Dekkera bruxellensis, Pichia membranifaciens, Saccharomyces cerevisiae, Zygosaccharomyces bisporus*	284 242 241
BPI[1] domain III analogues	XMP-284, XMP-366, XMP-391	Mammals	*N*-Terminal lysine-rich	Membrane lysis	1,550	*Candida* spp., *Cryptococcus neoformans*, *Aspergillus fumigatus, Histoplasma capsulatum*	245

[1]The molecular weights of lactoferrin and BPI are 55,000 and 80,000, respectively. MW, approximate molecular weight; BMAP, bovine myeloid antimicrobial peptide; HNPs 1-4, human neutrophil peptides 1-4.

way for these α-helical amphipathic peptides is primarily disruption of the cell membrane, these peptides have widespread activity against bacteria, fungi and membrane-enveloped viruses. Nevertheless, accumulating data suggest that at least some of these peptides also have unique intracellular targets (114-116). In addition, many are able to lyse cancer cells at concentrations up to 10-fold lower than those required to lyse normal human cells (117).

Cecropins

Cecropins and cecropin-like peptides have a broad spectrum of antimicrobial activity (bacteria and fungi) and have primarily been isolated from the hemolymph of silkworm moths (118, 119). These peptides are 29-42 amino acids in length and form α-helices in hydrophobic environments, such as the plasma membrane. With few exceptions among the insect cecropins, two distinguishing characteristics are a tryptophan at the first or second positions and an amidated *C*-terminal amino acid (13). Although the primary target of cecropins is the plasma membrane, cecropin A at its microbicidal dose does not affect mammalian cells and numerous studies have shown that this peptide can be administered safely to animals (120-122). At concentrations between 25 and 100 μg/mL, cecropin A effectively killed *Aspergillus* spp. and at 12.5 μg/mL it effectively killed *Fusarium moniliforme* and *F. oxysporum* (123, 124). Notably, genetically modified rice expressing the cecropin A gene was completely protected from the blast fungus *Magnaporthe grisea* (125). In addition, no toxicity was reported in transgenic mice expressing a cecropin-like peptide (e.g., Shiva-1) (122). Thus, cecropins are a promising class of antifungal peptides because of their safe therapeutic profile.

Magainins

Isolated from the skin of *Xenopus laevis* (the African frog), magainins are a family of cationic amphipathic peptides that range between 21 and 26 amino acids in length and are enriched in glycine and serine

residues (111, 126, 127). The magainin family of peptides includes magainin 1, 2, II, PGLa peptides, xenopsin and the caerulein precursor fragment (128-130). Some of these family members (including magainin 2 and PGLa) may form heterodimers, which increases their ability to permeabilize the membranes of pathogens (131). In addition to cidal activity of magainins against Gram-negative and Gram-positive bacteria and protozoa, magainins have antifungal activity against *Candida* spp., *C. neoformans* and *S. cerevisiae*. Magainin 2 is particularly active against *C. neoformans* (MIC = 6.25 μg/mL), with greater activity than three other cationic peptides (132). Although magainin 2 has moderate activity against *C. albicans* (MIC > 80 μg/mL), it potently inhibits *C. glabrata* (MIC = 25.0 μg/mL), *C. tropicalis* (MIC = 12.5 μg/mL) and *C. krusei* (MIC = 12.5-25.0 μg/mL) (111, 132). Similar to many other AMPs, magainins show selectivity in their cidal activity toward pathogenic fungi and bacteria. Nevertheless, the therapeutic window for natural magainins is not great enough to allow treatment of systemic infections in humans. As a result, magainin analogues or hybrid peptides with greater activity are being developed (133-135). For example, Avrahami and colleagues showed that conjugating magainins with a lipophile greatly increased their activity against *C. neoformans* (136). In addition to its membrane-lysing mechanism, magainin 2 has an alternative antifungal action by interfering with the DNA integrity of fungi (114). This study raises the question as to whether many cationic peptides kill fungi by mechanisms other than targeting the membrane.

Bombinin-H

Bombinin-H and bombinin-like peptides isolated from the skin of toads of the *Bombina* genus (137, 138) are glycine-rich, weakly cationic peptides with an amidated *C*-terminal amino acid (139). In addition to inhibiting bacteria, bombinin-like peptides (BLP-1, BLP-3) are active against fungi, especially *C. albicans* (MIC BLP-1, BLP-3 = 0.4 μM). Importantly, these peptides have little hemolytic activity (< 10% at 15 μM). Bombinin-H peptides have varied antimicrobial and hemolytic activity (139, 140). Bombinins H2 and H4, which damage cell membranes of microbes, were found to be active against *C. albicans* (MIC H2 = 3.1 μM; MIC H4 = 1.6 μM), *C. guilliermondii* (MIC H2 = 1.3 μM; MIC H4 = 0.7 μM) and *C. tropicalis* (MIC H2 = 1.1 μM; MIC H4 = 0.6 μM) (141, 142). While bombinin H2 induces 11% hemolysis, bombinin H4 induces 28% hemolysis at 15 μM. Other bombinin-H peptides with greater hydrophobicity, bombinin H6 and bombinin H7, have lower antimicrobial activity and induce greater hemolysis than do bombinin H2 and bombinin H4. Notably, bombinin H2 and bombinin H4 peptides are active against the spores of the fungus *Phytophthora nicotianae*, with a minimal fungistatic concentration of 10 and 18 μM, respectively (143). Although some bombinin-H peptides differ in their conformations as a result of stereochemistry modifications at the second position, these bombinins have similar antimicrobial activity (144).

Dermaseptins S and B

Dermaseptins S and B have between 28 and 34 amino acids and are lysine-rich peptides that characteristically have a tryptophan at position 3. They were identified in the skin of tree frogs of the genus *Phyllomedusa* (112). By interfering with lipid layers, which leads to osmotic imbalance, dermaseptins lyse a wide spectrum of microorganisms. Remarkably, dermaseptins often exhibit synergy with one another, resulting in a 100-fold increase in antimicrobial activity compared with dermaseptins separately (145). In addition to their antibacterial, antiviral and antiprotozoal activity, they are cidal to pathogenic fungi (146, 147), including yeasts and some filamentous fungi (*A. fumigatus*) (148). For example, a synthetic dermaseptin S1 analogue, a 16-mer peptide, shows marked activity against *C. albicans* (MIC = 5.8 μM) and notably this analogue has little hemolytic activity (149). Interestingly, the presence of a phenyl group at position 3 selectively inhibits *C. albicans* more effectively than bacteria. In addition to pathogenic fungi, a synthetic derivative of dermaseptin (i.e., MsrA2 [*N*-Met-dermaseptin B1]) elicits strong antimicrobial activity against virulent phytopathogenic fungi and protects transgenic potatoes from a broad spectrum of fungal infections (150).

Metchnikowin

Metchnikowin is a 26-residue proline-rich inducible peptide isolated from *Drosophila*. This peptide exhibits activity against Gram-negative bacteria and filamentous fungi, but does not inhibit Gram-positive bacteria. Studies with metchnikowin against fungi have been quite limited, but it has shown potent activity against *N. crassa* (0.5-1 μM).

LL-37

LL-37 (hCAP-18[104-140]) is the only member of the cathelicidin family of host defense peptides expressed in humans (151). Members of the cathelicidin family are characterized by a conserved region and a highly variable domain that contains the mature antimicrobial peptide. In some species (cattle, pigs, goats), there are multiple antimicrobial cathelicidin peptides that show marked diversity in size, charge and structure, including α-helical or β-sheet formations. LL-37, however, is an α-helical antimicrobial peptide named after the two consecutive *N*-terminal leucines and its length of 37 amino acids. Widely distributed in human skin, gastrointestinal tract, urinary and respiratory tract (152, 153), it is expressed by a number of cells, including monocytes, neutrophils, natural killer cells, B and T cells (154-156), and is upregulated by immune stimuli (153) and by vitamin D (157, 158). In mice, both LL-37 and a close analogue, mCRAMP, have a similar MIC range for *C. albicans* of 15-20 μM (159). In one study, mCRAMP was induced by *C. albicans* at the skin surface in a mouse model, demonstrating that these peptides provide a natural barrier to fungal infections. Other cathelicidin α-helical peptides have shown activity (bovine myeloid antimicrobial peptide 27 [BMAP-27], BMAP-28 from cows) against *Candida* spp. and *C. neoformans* but were less active against filamentous fungi (160). Besides its antifungal activity, LL-37 has potent antimicrobial activity against all isolates of *Burkholderia pseudomallei* independent of their lipopolysaccharide phenotype (161). LL-37 also has an essential role in promoting angiogenesis and as a wound-healing agent (162). At higher concentrations it induces the production of cytokines and chemokines and at physiological concentrations it alters interleukin-8 (IL-8) production by keratinocytes and bronchial epithelial cells in response to inflammatory conditions (163). Thus, LL-37 plays an important role in epithelial innate immunity (152). In addition to its antimicrobial activity, reduction or excess production of LL-37 has been associated with the skin conditions atopic dermatitis and rosacea, respectively (164, 165).

Indolicidin and tritrpticin

Indolicidin and tritrpticin are tryptophan-rich AMPs that also belong to the cathelicidin family (166, 167). They are expressed in neutrophils (168) of cows and pigs, respectively. Unlike the α-helical peptides previously discussed, these tryptophan-rich peptides have an extended wedge shape conformation in hydrophobic environments such as the plasma membrane (166, 167, 169). Indolicidin is a 13-amino-acid peptide (ILPWKWPWWPWRR) that contains 5 tryptophans (39%) and 3 prolines (25%) and an amidated *C*-terminal amino acid. In addition to its high activity against *S. aureus* and *E. coli* (167), indolicidin has potent antifungal activity against *C. neoformans* (MIC = 2-4 μg/mL) and good to moderate activity against *Candida* spp. (MIC = 8-32 μg/mL), with the exception of *C. guilliermondii* (MIC > 32 μg/mL) (160). In addition, Ahmad and colleagues reported that incorporation of indolicidin within liposomes minimized its toxicity, enabling higher doses to be administered to cure mice with systemic *A. fumigatus* infections. In contrast, maximum tolerated doses of free indolicidin did not cure mice with such infections (170). Although an important mechanism for killing microbes may be indolicidin's action as a membrane ionophore, two studies have suggested that the DNA of the microbe is also an important target of the peptide (115, 116).

Tritrpticin is also a 13-amino-acid peptide (VRRFPWWWPFLRR) containing 3 tryptophans (23%), 4 arginines (30%) and 2 prolines (15%). Although no in vivo studies have been performed to test its efficacy, Lawyer et al. determined that this peptide had weak activity toward *C. albicans* (MIC = 1000 μg/mL) and *A. fumigatus* (MIC = 250 μg/mL) (166). Unlike indolicidin's *C*-terminal amino acid, which is amidated, tritrpticin's *C*-terminal amino acid is not. When the *C*-terminal amino acid of tritrpticin is amidated, antibacterial activity increases two- to eightfold, while hemolytic activity decreases significantly (171). It is not known whether this increased antimicrobial activity extends to killing fungi. Interestingly, Yang et al. found that prolines in tritrpticin were essential for microbial selectivity, in that replacing them with alanine markedly increased hemolysis (169).

PAF-26

PAF-26, a penetratin-like hexapeptide with the sequence Ac-RKKWFW-NH_2, was identified as a lead candidate against phytopathogenic fungi after screening a combinatorial library of hexapeptides. The MIC of PAF-26 against *Penicillium digitatum*, a fungus that causes postharvest decay in fruits, is 4 μM. Munoz and colleagues recently found that addition of tryptophan to the *N*-terminal amino acid increased its activity against yeast (MIC against *S. cerevisiae* = 16 μM) and Gram-negative bacteria (MIC against *E. coli* = 4 μM) (172). PAF-26 and its analogues showed little to no hemolysis at 100 μM. In addition, PAF-26 has been fused with magainin to augment the agents' antimicrobial activity (173).

Kaxins

Kaxins are synthetic cationic AMPs that have a nonamphipathic hydrophobic core segment (174, 175). By inserting lysines at the *N*-terminal end and creating D-enantiomer peptides, Burrows and colleagues developed kaxins with potent candidacidal activity with little lysis. One kaxin, dF21-10K (KKKKKKKKKKAAFAAWAAFAA-NH_2), showed MIC values of 16-64 μg/mL against all fluconazole-sensitive and -resistant *Candida* spp. (*C. albicans, C. dubliniensis, C. glabrata, C. guilliermondii, C. krusei, C. lusitaniae, C. parapsilosis* and *C. tropicalis*) (175). Notably, dF21-10K showed marked activity with complete killing against biofilms formed by *C. albicans* or *C. tropicalis.* Although elimination of biofilms by antifungal molecules usually requires concentrations 30-2,000 times higher than their MIC (100), dF21-10K eradicated biofilms at only 10 times its MIC (175).

Cyclic peptides

The vast majority of broad-spectrum cyclic AMPs contain between one and four disulfide bonds and adopt a β-sheet-enriched structure including β-hairpin, β-sheet or α-helix/β-sheet mixed structures. Most of these AMPs contain open-ended cyclic structures formed by internal disulfide bonds, but θ-defensins, in addition to internal bonds, form closed-ended cyclic structures. With peptides such as defensins that contain multiple disulfide bonds, formation of the correct bond remains a challenge for developing peptide technologies.

Thanatin

Thanatin is an inducible and nonhemolytic 21-amino-acid peptide isolated from the insect *Podisus maculiventris.* Compared with other arthropod AMPs, it has broad-spectrum antimicrobial activity. Thanatin has a single disulfide bond, forming a *C*-terminal loop that possesses a strong positive charge. Although it has little homology with other arthropod AMPs, thanatin does have homology in its amino acid sequence, structure and biological function with brevenins found in frog secretions. Thanatins are fungicidal against several phytomycotic organisms (e.g., MIC against *N. crassa*, *Botrytis cinerea*, *Nectria haematococca*, *Trichoderma viride*, *Alternaria brassicola* and *Fusarium culmorum* < 5 μM) and two pathogenic fungi in humans (MIC against *A. fumigatus* = 10-20 μM; MIC against *Trichophyton mentagrophytes* = 20-40 μM) (176). Although these peptides show no activity against yeasts such as *S. cerevisiae* or *C. albicans*, interestingly, close homologues of thanatin isolated from skin secretions of frogs do (MIC brevinin-1E = 4.7 μM) (177).

Protegrins

Protegrins are approximately 2-kD cysteine-rich β-sheet peptides found in neutrophils of pigs. Similar to LL-37 and indolicidin, they belong to the cathelicidin peptide family, but unlike these two linear peptides, protegrins are cyclic AMPs. They contain 16-18 amino acids and have 2 disulfide bridges, which are essential for their antimicrobial activities, especially at physiological salt concentrations (178, 179). In contrast to defensins, protegrins display full antimicrobial activity in the presence of physiological saline (180). The ability to maintain activity in the presence of salt is critical for AMPs to exhibit their antimicrobial effects systemically or perhaps via aerosolized therapy. Although protegrins have limited homology with defensins, they share significant homology with tachyplesins found in hemocytes of horseshoe crabs (181, 182). Protegrins display broad-spectrum activity against bacteria, fungi, protozoa and viruses (20, 179, 183-186) and their primary mechanism of microbial action is lysis of the microbial membrane (187). Cho and colleagues examined the anticandidal activity of protegrins 1-5 (188) and found that protegrins 1-3 and 5 had greater anticandidal activity (MIC = 2.50-2.85

μM) than protegrin 4 (MIC = 4.78 μM). In addition, a D-enantiomer of protegrin 1 had slightly higher activity compared to protegrin 1. Other analogues of protegrin have been prepared with either greater antimicrobial activity and/or decreased hemolysis, but they have not been tested against fungi.

Mammalian defensins

Mammalian defensins are a family of cationic peptides containing six highly conserved cysteine residues with three disulfide bridges (189, 190) that are divided into three subfamilies: α- and β-defensins, found in many mammalian species, and θ-defensins, found in rhesus macaques. The α- and β-defensins differ in amino acid sequence and in the location of disulfide bonds, but their 3-D structures are virtually identical; they both contain three antiparallel β-sheets and one α-helix (191). Whereas mammalian defensins usually have antimycotic properties against *C. albicans* (192, 193), there are distinct variations in their antifungal activities.

α-Defensins

The mature α-defensin peptide contains 29-36 amino acids, with 3 disulfide bridges between cysteines 1-6, 2-4 and 3-5. Four α-defensins (human neutrophil peptides 1-4 [HNPs 1-4]) are constitutively expressed in human neutrophils (30, 194-196) and two other α-defensins, HD-5 and HD-6, are expressed in specialized epithelial cells (e.g., paneth cells) of the intestine and female urogenital tract (197-199). In addition to creating membrane "pores" as a mechanism for microbial inhibition, HNP-1 and HNP-2 are able to inhibit protein, RNA and DNA synthesis (200, 201). Lehrer et al. compared defensins HNPs 1-3 for their anticandidal activity and found that HNP-1 was most effective. Although HNP-3 (at 50 μg/mL) showed little to no inhibition of *C. albicans*, HNP-1 greatly reduced the number of colony-forming units and was about 10-fold more effective than HNP-2. Furthermore, α-defensins from rabbits (i.e., NP-1, NP-2 and NP-3) are highly effective against *C. albicans* (193). Indeed, NP-1 had 10-fold greater candidacidal activity than HNP-1. In addition to *Candida* spp., the rabbit defensins killed *A. fumigatus* (minimal fungicidal concentration NP-1 = 25 μg/mL, NP-2 = 50 μg/mL and NP-3 = 100 μg/mL), as determined by an MTT assay (202). Moreover, both rabbit NP (MIC NP-1 = 3.75-15 μg/mL) and human HNP inhibited the growth of *C. neoformans* (203, 204). Because NP-1 and NP-2 defensins bind tightly to chitin and its fragments, this interaction with the fungal cell wall may be important in these peptides' cidal activity (202).

β-Defensins

β-Defensins are generally larger than their α-counterparts and contain 38-47 amino acids, with disulfide bonds between cysteines 1-5, 2-4 and 3-6. The human β-defensin (HBD) family consists of at least 6 members. HBD-1, HBD-2 and HBD-3 are mainly produced by epithelial cells and play an important role in immunity of mucosal and body surfaces (205-210). In contrast to HBDs 1-3, which are present in many tissues, HBD-4 is found primarily in the gastric antrum and testes (211) and HBD-5 and HBD-6 are specifically expressed in the epididymis (212). β-Defensins such as HBD-1 may be expressed constitutively (209, 213), while others (e.g., HBD-2 or HBD-3) may be induced by various proinflammatory mediators (e.g., TNF-α, IL-1β) (214-221). Whereas the antibacterial activity of HBDs 1-4 has been studied by several groups, antifungal studies of β-defensins have been limited to the inducible peptides HBD-2 and HBD-3. Although there was a strong correlation between HBD-2 and HBD-3 in their inhibition of *Candida* spp., HBD-3 was generally more effective. HBD-2 and HBD-3 were active against several *Candida* spp.: *C. albicans* (MIC HBD-2 = 4.6-59.2 μg/mL; MIC HBD-3 = 2.8-7.1 μg/mL), *C. tropicalis* (MIC HBD-2 = 3.9-13.1 μg/mL; MIC HBD-3 = 3.3-14.4 μg/mL), *C. parapsilosis* (MIC HBD-2 = 9.3-17.8 μg/mL; MIC HBD-3 = 1.4-12.4 μg/mL), *C. krusei* (MIC HBD-2 = 12.2 to > 250 μg/mL; MIC HBD-3 = 2.0-13.7 μg/mL) and *C. glabrata* (MIC HBD-2 = 22.7 to > 250 μg/mL; MIC HBD-3 = 33.8 to > 250 μg/mL) (222). Notably, HBD-3 inhibited the growth of the three isolates of *C. krusei* while one of the three isolates was resistant to HBD-2. Bovine tracheal antimicrobial peptide (TAP), an analogue of HBD-2 that is secreted by respiratory epithelial cells, inhibits *C. albicans* (MIC = 400 μg/mL [223] vs. MIC = 6-12 μg/mL [224]) and the hyphal form of *A. fumigatus* (400 μg/mL) (223). This discrepancy of MICs against *C. albicans* may be due to differences in the strains or growth media. In addition to their direct antimicrobial properties, β-defensins can modulate the function of immunocompetent cells (23).

θ-Defensins

Three θ-defensins (rTD-1, rTD-2 and rTD-3) were isolated from extracts of granulocytes from rhesus monkeys. These defensins are circular octadecapeptides that contain two antiparallel β-sheets and three disulfide bonds that are formed by post-translational splicing of two nonapeptides, which are derived from α-related defensin precursors (20). θ-Defensins possess a broad spectrum of salt-independent antimicrobial activity against bacteria, viruses (both enveloped and nonenveloped) and fungi. Although they share similar sequence and structural homology with protegrins, they induce significantly less hemolysis. Interestingly, θ-defensins differ from other AMPs in their interaction with membranes, which perhaps explains their reduced proclivity to induce hemolysis. The defensins rTD1-3 show significant activity against *C. albicans* (MIC rTD-1 and rTD-12 = 1 μg/mL; MIC rTD-3 = 3 μg/mL) (20, 225) and are also active against *C. neoformans* (20). Their activity against other fungi has not been reported.

Poultry gallinacins

Poultry gallinacins, produced by leukocytes, are rich in arginines and lysines, have three disulfide bonds and are functionally and structurally equivalent to mammalian β-defensins. While gallinacin-1 and gallinacin-1α inhibit *C. albicans,* gallinacin-2 does not (226). Strong bactericidal and fungicidal activities (MIC = 8 μg/mL against *C. albicans* and *S. cereviseae*) were observed for gallinacin-6, which is expressed in the digestive tract of chickens (227). Because of potential resistance developing in microbes exposed to low levels of antibiotics, there has been increasing interest in stimulating endogenous AMPs such as gallinacin-6 to suppress microbial growth in chickens.

Macrocyclic peptides

Isolated from coffee plants, macrocyclic peptides contain four end-to-end large cyclic peptides with six disulfide bonds. Four macrocyclic cysteine-knot peptides of 29-31 residues, sharing 45%

homology with one another, belong to this family (kalata, circulin A [CirA] and circulin B [CirB] and cyclopsychotride [Cpt]). In contrast to kalata and CirA, which are relatively ineffective against Gram-negative bacteria such as *E. coli* and *Pseudomonas aeruginosa*, CirB and Cpt kill both Gram-positive and Gram-negative bacteria, in particular *E. coli*. Although the cyclic peptides were inactive against *C. albicans* in a low-salt assay, all peptides were moderately active against *Candida kefyr* (MIC = 14.0-29.0 μM) and two of the four peptides were active against *C. tropicalis* (MIC CirA = 21.4 μM; MIC Cpt = 56.5 μM) (228). The antifungal and antibacterial activities of these peptides were significantly reduced when 100 mM sodium chloride was added to the assay medium. Unless improved analogues are discovered, macrocyclic peptides will likely play a limited role as antifungal agents.

Syringomycins and related peptides

Syringomycins and related peptides are a group of cyclic lipopeptides, called lipodepsinonapeptides, produced by *Pseudomonas syringae*. This group of peptides induces ion channels that affect membrane function, including membrane potential, protein phosphorylation and H^+-ATPase activity (229, 230). The family members (syringomycin E, syringotoxin B and syringostatin A) were effective in vitro against a number of isolates of *Candida* spp. (MIC = 2.5-25 μg/mL), *C. neoformans* (MIC = 0.8-10 μg/mL) and *A. fumigatus* (MIC = 5-40 μg/mL) (231). Generally, the three cyclic peptides were more effective against yeasts than against filamentous fungi. Moreover, syringotoxin B was the least effective antifungal cyclic peptide. In a subsequent study, syringomycin E was also found to be effective in treating vaginal candidiasis in mice (232).

ANTIMICROBIAL PEPTIDES THAT ARE PROTEOLYTIC FRAGMENTS OF PROTEINS

The majority of peptides derived from proteins such as lactoferrin, bactericidal permeability-increasing protein (BPI) and pepsinogen A have broad-spectrum antimicrobial activity. For example, hydrolysates of lactoferrin have marked activity against Gram-negative and Gram-positive bacteria (233). Depending on which site of the protein the peptides are derived from, the peptides may have an α-helical or β-sheet structure. Moreover, these proteolytic peptide fragments frequently have greater antifungal activity than the parent proteins. Although we discuss mammalian peptides from lactoferrin and BPI in this review, amphibian AMPs derived from the *N*-terminal domains of pepsinogen A and C have also been identified (234).

Lactoferrin

Lactoferrin is an iron-binding antimicrobial glycoprotein (78 kDa) that is present in neutrophil granules, in breast milk and on many mucosal surfaces (235, 236). It is multifunctional and can influence a wide range of biological processes, including immune responses. There are two mechanisms by which lactoferrin is thought to inhibit fungal and bacterial growth: 1) by depriving the fungi of iron, which is essential for their growth; and 2) by generating antifungal peptides from proteolytic enzymatic digestion of lactoferrin. Kirkpatrick et al. initially proposed that the iron-binding properties of lactoferrin were responsible for its inhibition of *C. albicans* (237). Later, Zarember and colleagues showed that iron sequestration had an important role in the inhibition of *A. fumigatus* by lactoferrin (238). A recombinant lactoferrin (talactoferrin; Agennix) is now in clinical trials for the treatment of cancer, diabetic skin ulcers and sepsis. Enzymatic degradation of lactoferrin results in several antimicrobial/antifungal peptides (i.e., lactoferricin H, lactoferricin B, peptide 2, kaliocin-1) (239-242). Except for kaliocin, the AMPs are derived from the *N*-terminal region of lactoferrin. Many of these proteolytic peptides possess stronger antimicrobial activity than the lactoferrin protein (233). The *N*-terminal lactoferrin-derived peptides that lack the iron-binding domain of the parent protein exert their antifungal activity by affecting the permeability of the membrane (243). After the *N*-terminal lactoferrin H peptide was first identified, several smaller peptides were identified with antifungal activity. Viejo-Diaz et al. discovered Lfpep (residues 18-40 of human lactoferrin), which was candidicidal against fluconazole- and amphotericin-resistant *Candida* spp. (MIC = 9.3-18.7 μM) (241). Another well-known antifungal peptide derived from lactoferrin is peptide 2. Ueta and colleagues compared six *N*-terminal peptides derived from lactoferrin and found that peptide 2 (FKCRRWQWRM) had the greatest antifungal activity. Against two strains of *Candida* its MIC was approximately 17 μM, which was close to the MIC of miconazole. Interestingly, replacing the single cysteine of peptide 2 with alanine markedly mitigated its anticandidal activity. Although peptide 2 cannot form a cyclic structure, perhaps the single cysteine enables the formation of dimers, which may be more potent antifungal agents. Moreover, peptide 2 augments the candidacidal activity of polymorphonuclear leukocytes by inducing superoxides (242).

Bactericidal permeability-increasing protein (BPI) domain III analogues

BPI is a 55-kDa cationic protein that is stored in azurophilic granules of neutrophils. BPI inhibits Gram-negative bacteria by binding lipopolysaccharide with high affinity, and it also shows antifungal activity against *H. capsulatum* (MIC_{50} = 1 μg/mL; MIC = 80-90 μg/mL). Moreover, BPI together with defensins and cathepsin G are additive in their inhibitory activity against *H. capsulatum* (244). Compared with the parent protein, proteolytic fragments of BPI have significantly more antifungal activity towards *Candida* spp., *C. neoformans* and *A. fumigatus* (245). On the basis of the BPI domain III structure, three synthetic peptides (XMP-284, XMP-366, XMP-391) have been reported to be effective against several *Candida* spp. and showed synergistic activity with fluconazole (246). Additionally, XMP-391 was found to increase the antifungal activity of amphotericin B against systemic aspergillosis in a murine model (247).

SYNTHETIC STRATEGIES FOR PRODUCING PEPTIDES FOR PRECLINICAL AND CLINICAL APPLICATIONS

Naturally occurring broad-spectrum AMPs and their analogues have been examined for their utility as therapeutic antibiotics by many groups and the ability to produce AMPs economically is a key factor that will determine which AMPs will be widely used to treat fungal infections. To date, the only approved antifungal peptides belong to the echinocandin family (micafungin sodium, caspofungin acetate and anidulafungin); precursors of these FDA-approved peptides are isolated from various molds and further modified chemically.

Nonetheless, depending on the original source of the peptide, isolation of natural compounds can often be prohibitively expensive and peptide isolates may be sensitive to protease digestion. In many cases, peptide technologies, including solid-phase peptide synthesis and recombinant engineering methods, provide mechanistic insight about the peptide and offer the potential of creating more effective antifungal therapies in a cost-saving manner.

Several groups have synthesized peptides by a solid-phase peptide method for their antimicrobial and antifungal studies. The synthesized peptides have included linear (170, 223, 225, 248, 249) and cyclic peptides with one to multiple disulfide bonds (76, 228, 250, 251) and closed-ended cyclic θ-defensin peptides (225). Synthesized peptides have ranged in size from 6 to 31 residues. Solid-phase peptide syntheses are ideally suited for structure–function studies because the amino acid sequences can rapidly be altered to compare their antimicrobial activities with the parent peptide. For example, Chen and colleagues synthesized 20 analogues of protegrins to gain understanding of the relationship between the primary and secondary structure and the antimicrobial activity (252). From these structure–activity relationship (SAR) studies, the IB-367 analogue was identified for further clinical investigation. Several such SAR studies have been done to identify analogues with increased efficacy and reduced toxicity (176). In addition to the SAR function studies, several hybrid peptides (e.g., cecropin A–magainin, HP ribosomal peptide–magainin, cecropin A–mellitin) have been created by peptide synthesis with increased biological activity toward microbes compared with either peptide alone (134, 135, 253). For clinical studies, it is likely that peptide synthesis will prove cost-effective in AMP production for smaller peptides (i.e., those composed of approximately 25 amino acids or less). Of note, the length of a peptide that can be synthesized without significant truncation depends on its amino acid sequence and content. Thus far, analogues of protegrins, histatins and indolicidin have been made for clinical trials by peptide synthesis (254). Therapeutic peptides with increased length and complexity continue to be made. Improvements in synthesis and development of higher-quality resins for solid-phase synthesis, price reductions for commonly used reagents and increased competition are enabling efficient and cost-effective production of these peptides. Peptide synthesis is an evolving methodology in which further advances are anticipated.

Recombinant engineering is an alternative technology that has been used to synthesize various AMPs (255-260). There is significant overlap in the antimicrobial studies that have been conducted with recombinant engineering and solid-phase synthesis technologies. For example, SAR studies and hybrid AMPs have also used recombinant engineering methods (133, 173, 261). Recombinant engineering is more appropriate and cost-effective for larger AMPs, such as defensins, which are composed of 30-50 amino acids and 3 or more disulfide bonds. Several organisms have been used to synthesize recombinant AMPs, including *E. coli*, baculovirus insect cells, fungi (*Pichia pastoris, S. cerevisiae*, *Aspergillus* spp.) and chloroplast expression systems (262). Thus far, expression in *E. coli* and fungi has yielded promising results. Fusion proteins produced in *E. coli* are the most widely used in recombinant engineering because of their low cost and the large amount of information on the strains and available plasmids (255). Many AMPs are toxic to bacteria, so these peptides are often fused with proteins such as thioredoxin and glutathione *S*-transferase (255, 263), which, in addition to preventing cell lysis, aid in disulfide bond formation of the antimicrobial peptide (264). Although most expression plasmids produced low levels of AMPs in *E. coli*, the isopropyl-β-D-1-thiogalactopyranoside (IPTG)-inducible pET-32a(+) expression vector produced levels of HBD-2 reaching 1.3 g/L (265). To express antifungal peptides in yeast and filamentous fungi, resistant strains of these organisms lacking the target to these peptides have been developed. In *P. pastoris*, the plant defensin Psd1, fused with MFa1 signal peptide and a pro-region, produced fusion protein levels of 60 mg/L. Expression systems in filamentous fungi have yielded even higher levels of proteins (≥ 10 g/L). Talactoferrin alfa, which has entered phase III clinical trials for the treatment of foot ulcers, sepsis and cancer, is expressed by *Aspergillus* spp.

Another synthetic strategy to develop cost-effective antibiotics is to utilize antimicrobial peptide-mimetic agents. For example, Beckloff and colleagues developed *meta*-phenylene ethynylene (mPE) compounds that have an amphipathic structure similar to that of magainins. mPE exhibited antimicrobial activity at nanomolar concentrations against a variety of bacteria and *Candida* spp. found in oral infections (266). Other magainin-mimetic compounds, such as MSI-751, have also been developed (267). Using electron microscopy, these mimetics were shown to form ion channels in pathogenic organisms, similar to magainins. Moreover, experimental studies to identify the determinants underlying the configuration of these peptides will likely provide adequate information for computer simulation methods to explore their fungicidal mechanism at the atomic level. Interactions between peptides and lipid bilayers are being investigated by molecular dynamics methods (268). By understanding structural and dynamic properties of peptides and their target, molecular dynamics-based methodology increases the likelihood for rational design of antimicrobial peptidomimetics (269, 270).

CONCLUSIONS

Both plants and animals are constantly exposed to harmful pathogens present in all environmental niches. Moreover, some organisms lack components related to specialized immunity, such as lymphocytes or antibodies. Nonetheless, living organisms have found a way to live and thrive. During the evolutionary process, nature was able not only to create but also strategically localize small AMPs with relatively simple modes of action, resulting in survival of life forms on this planet. The pharmaceutical use of these AMPs and analogues may also have an important role in treating human infectious diseases because of increased resistance to the widespread use of currently used antibiotics. Whether resistance to these AMPs will occur has been debated, but it is likely that this will be at a reduced rate and that it will depend on how the antimicrobial peptide is administered. Combination therapy of the antimicrobial peptide with antibiotics or with other peptides will likely reduce the development of resistance markedly. One area that merits further attention and research is the use of cationic peptides to prevent the formation of polymicrobial biofilms. Although there are promising peptide candidates that have demonstrated marked reduction of biofilms caused by bacteria or *Candida* spp. (98, 175, 266, 271-273), we are not aware of studies demonstrating the efficacy of these peptides against biofilms caused by multiple orgainisms (274). Regardless of the effectiveness of naturally occurring peptides as

antifungal agents, new peptide strategies offer the potential of developing more potent, specific and nontoxic therapies. Several examples given in this review have shown that modified peptides are more effective than their parent peptides and most AMPs in clinical trials are analogues of the parent peptide.

Current antimycotic therapies face many difficulties, including fast-growing drug resistance, limited choice of antifungal drugs and increased fungal infections in immunosuppressed AIDS, organ transplant or cancer patients. Candidemia in these patients leads to an overall mortality of 40% in the United States (275). Although AMPs and their analogues bring the hope of treating these serious antifungal infections more effectively, interactions of new therapies with biological pathways will need to be addressed in terms of safety concerns. To date, the only antifungal peptides to receive FDA approval are the echinocandin family of β-glucan inhibitors. These inhibitors have been approved for the treatment of candidemia, the empiric treatment of neutropenic febrile patients and the treatment of invasive aspergillosis. In addition, there are a number of other antifungal AMPs in various stages of preclinical and clinical trials, primarily involving topical and aerosolized approaches to infections (see reviews by Gordon [254] and Jenssen and Hancock [40]). With the many promising antifungal peptides that show selective toxicity toward pathogenic fungi, we anticipate that peptide analogues will continue to be developed with increased therapeutic efficacy and have an important role in treating patients with fungal infections.

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REFERENCES

1. Ostrosky-Zeichner, L., Rex, J.H., Bennett, J., Kullberg, B.J. *Deeply invasive candidiasis.* Infect Dis Clin North Am 2002, 16(4): 821-35.
2. Venkatesan, P., Perfect, J.R., Myers, S.A. *Evaluation and management of fungal infections in immunocompromised patients.* Dermatol Ther 2005, 18(1): 44-57.
3. Prasad, R., Kapoor, K. *Multidrug resistance in yeast Candida.* Int Rev Cytol 2005, 242: 215-48.
4. Chandrasekar, P.H., Cutright, J., Manavathu, E. *Efficacy of voriconazole against invasive pulmonary aspergillosis in a guinea-pig model.* J Antimicrob Chemother 2000, 45(5): 673-6.
5. Perea, S., Patterson, T.F. *Antifungal resistance in pathogenic fungi.* Clin Infect Dis 2002, 35(9): 1073-80.
6. Hernandez, S., López-Ribot, J.L., Najvar, L.K., McCarthy, D.I., Bocanegra, R., Graybill, J.R. *Caspofungin resistance in Candida albicans: Correlating clinical outcome with laboratory susceptibility testing of three isogenic isolates serially obtained from a patient with progressive Candida esophagitis.* Antimicrob Agents Chemother 2004, 48(4): 1382-3.
7. Hakki, M., Staab, J.F., Marr, K.A. *Emergence of a Candida krusei isolate with reduced susceptibility to caspofungin during therapy.* Antimicrob Agents Chemother 2006, 50(7): 2522-4.
8. Thompson, G.R., 3rd, Wiederhold, N.P., Vallor, A.C., Villareal, N.C., Lewis, J.S., 2nd, Patterson, T.F. *Development of caspofungin resistance following prolonged therapy for invasive candidiasis secondary to Candida glabrata infection.* Antimicrob Agents Chemother 2008, 52(10): 3783-5.
9. Reddy, K.V., Yedery, R.D., Aranha, C. *Antimicrobial peptides: Premises and promises.* Int J Antimicrob Agents 2004, 24(6): 536-47.
10. Hoffmann, J.A., Kafatos, F.C., Janeway, C.A., Ezekowitz, R.A. *Phylogenetic perspectives in innate immunity.* Science 1999, 284(5418): 1313-8.
11. Zasloff, M. *Antimicrobial peptides of multicellular organisms.* Nature 2002, 415(6870): 389-95.
12. Broekaert, W.F., Terras, F.R., Cammue, B.P., Osborn, R.W. *Plant defensins: Novel antimicrobial peptides as components of the host defense system.* Plant Physiol 1995, 108(4): 1353-8.
13. Bulet, P., Stocklin, R., Menin, L. *Anti-microbial peptides: From invertebrates to vertebrates.* Immunol Rev 2004, 198: 169-84.
14. Otvos, L., Jr., Bokonyi, K., Varga, I. et al. *Insect peptides with improved protease-resistance protect mice against bacterial infection.* Protein Sci 2000, 9(4): 742-9.
15. Ganz, T., Lehrer, R.I. *Antimicrobial peptides of leukocytes.* Curr Opin Hematol 1997, 4(1): 53-8.
16. Thevissen, K., Terras, F.R., Broekaert, W.F. *Permeabilization of fungal membranes by plant defensins inhibits fungal growth.* Appl Environ Microbiol 1999, 65(12): 5451-8.
17. Thevissen, K., Osborn, R.W., Acland, D.P., Broekaert, W.F. *Specific binding sites for an antifungal plant defensin from Dahlia (Dahlia merckii) on fungal cells are required for antifungal activity.* Mol Plant Microbe Interact 2000, 13(1): 54-61.
18. Schmatz, D.M., Abruzzo, G., Powles, M.A. et al. *Pneumocandins from Zalerion arboricola. IV. Biological evaluation of natural and semisynthetic pneumocandins for activity against Pneumocystis carinii and Candida species.* J Antibiot (Tokyo) 1992, 45(12): 1886-91.
19. Jigami, Y., Odani, T. *Mannosylphosphate transfer to yeast mannan.* Biochim Biophys Acta 1999, 1426(2): 335-45.
20. Tang, Y.Q., Yuan, J., Osapay, G. et al. *A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated alpha-defensins.* Science 1999, 286(5439): 498-502.
21. Biragyn, A., Ruffini, P.A., Leifer, C.A. et al. *Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2.* Science 2002, 298(5595): 1025-9.
22. Chong-Cerrillo, C., Selsted, M.E., Peterson, E.M., de la Maza, L.M. *Susceptibility of human and murine Chlamydia trachomatis serovars to granulocyte- and epithelium-derived antimicrobial peptides.* J Pept Res 2003, 61(5): 237-42.
23. Yang, D., Chertov, O., Bykovskaia, S.N. et al. *Beta-defensins: Linking innate and adaptive immunity through dendritic and T cell CCR6.* Science 1999, 286(5439): 525-8.
24. Yang, D., Chen, Q., Chertov, O., Oppenheim, J.J. et al. *Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells.* J Leukoc Biol 2000, 68(1): 9-14.
25. Yang, D., Biragyn, A., Kwak, L.W., Oppenheim, J.J. et al. *Mammalian defensins in immunity: More than just microbicidal.* Trends Immunol 2002, 23(6): 291-6.
26. Durr, M., Peschel, A. *Chemokines meet defensins: The merging concepts of chemoattractants and antimicrobial peptides in host defense.* Infect Immun 2002, 70(12): 6515-7.
27. Hancock, R.E., Rozek, A. *Role of membranes in the activities of antimicrobial cationic peptides.* FEMS Microbiol Lett 2002, 206(2): 143-9.
28. Ben-Ami, R., Lewis, R.E., Kontoyiannis, D.P. *Immunocompromised hosts: Immunopharmacology of modern antifungals.* Clin Infect Dis 2008, 47(2): 226-35.

29. Balloy, V., Huerre, M., Latgé, J.P., Chignard, M. *Differences in patterns of infection and inflammation for corticosteroid treatment and chemotherapy in experimental invasive pulmonary aspergillosis.* Infect Immun 2005, 73(1): 494-503.
30. Ganz, T. *Immunology. Versatile defensins.* Science 2002, 298(5595): 977-9.
31. Okumura, K., Itoh, A., Isogai, E. et al. *C-terminal domain of human CAP18 antimicrobial peptide induces apoptosis in oral squamous cell carcinoma SAS-H1 cells.* Cancer Lett 2004, 212(2): 185-94.
32. Chen, Q.R., Zhang, L., Stass, S.A., Mixson, A.J. *Branched co-polymers of histidine and lysine are efficient carriers of plasmids.* Nucleic Acids Res 2001, 29(6): 1334-40.
33. Leng, Q., Mixson, A.J. *Modified branched peptides with a histidine-rich tail enhance in vitro gene transfection.* Nucleic Acids Res 2005, 33(4): e40.
34. Lopez-Exposito, I., Recio, I. *Protective effect of milk peptides: Antibacterial and antitumor properties.* Adv Exp Med Biol 2008, 606: 271-93.
35. Kuipers, O.P., Bierbaum, G., Ottenwälder B. et al. *Protein engineering of lantibiotics.* Antonie Van Leeuwenhoek 1996, 69(2): 161-69.
36. Andreu, D., Rivas, L. *Animal antimicrobial peptides: An overview.* Biopolymers 1998, 47(6): 415-33.
37. Cheigh, C.I., Pyun, Y.R. *Nisin biosynthesis and its properties.* Biotechnol Lett 2005, 27(21): 1641-8.
38. Jack, R.W., Jung, G. *Lantibiotics and microcins: Polypeptides with unusual chemical diversity.* Curr Opin Chem Biol 2000, 4(3): 310-7.
39. Xie, L., van der Donk, W.A. *Post-translational modifications during lantibiotic biosynthesis.* Curr Opin Chem Biol 2004, 8(5): 498-507.
40. Jenssen, H., P. Hamill, Hancock, R.E. *Peptide antimicrobial agents.* Clin Microbiol Rev 2006, 19(3): 491-511.
41. Yeaman, M.R., Yount, N.Y. *Mechanisms of antimicrobial peptide action and resistance.* Pharmacol Rev 2003, 55(1): 27-55.
42. De Lucca, A.J. *Antifungal peptides: Potential candidates for the treatment of fungal infections.* Expert Opin Investig Drugs 2000, 9(2): 273-99.
43. Cole, A.M., Ganz, T. *Human antimicrobial peptides: Analysis and application.* Biotechniques 2000, 29(4): 822-6, 828, 830-1.
44. Georgopapadakou, N.H., Walsh, T.J. *Antifungal agents: Chemotherapeutic targets and immunologic strategies.* Antimicrob Agents Chemother 1996, 40(2): 279-91.
45. Kurtz, M.B., Douglas, C.M. *Lipopeptide inhibitors of fungal glucan synthase.* J Med Vet Mycol 1997, 35(2): 79-86.
46. Turner, W.W., Current, W.L. *Echinocandin antifungal agents.* In: Biotechnology of Antibiotics, 2nd Ed. W.R. Strohl (Ed.). Marcel Dekker, Inc.: New York, 1997, 315-34.
47. Benz, F., Knuesel, F., Nuesch, J., Treicher, H., Voser, W., Nyfeler, R., Keller-Schlerein, W. *Echinocandin B, ein neuartiges Polypetid Antibioticum aus Aspergillus nidulans var echinlatus: Isolierung and Baudsteine.* Helv Chim Acta 1974, 57: 2459-77.
48. Schmatz, D.M., Romancheck, M.A., Pittarelli, L.A. et al. *Treatment of Pneumocystis carinii pneumonia with 1,3-beta-glucan synthesis inhibitors.* Proc Natl Acad Sci U S A 1990, 87(15): 5950-4.
49. Schmatz, D.M., Powles, M.A., McFadden, D.C. et al. *Antipneumocystis activity of water-soluble lipopeptide L-693,989 in rats.* Antimicrob Agents Chemother 1992, 36(9): 1964-70.
50. Fromtling, R.A., Abruzzo, G.K. *L-671,329, a new antifungal agent. III. In vitro activity, toxicity and efficacy in comparison to aculeacin.* J Antibiot (Tokyo) 1989, 42(2): 174-8.
51. Zambias, R.A., Hammond, M.L., Heck, J.V., Bartizal, K., Trainor, C., Abruzzo, G., Schmatz, D.M., Nollstadt, K.M. *Preparation and structure-activity relationships of simplified analogues of the antifungal agent cilofungin: A total synthesis approach.* J Med Chem 1992, 35(15): 2843-55.
52. Gordee, R.S., Zeckner, D.J., Howard, L.C., Alborn, W.E., Jr., Debono, M. *Anti-Candida activity and toxicology of LY121019, a novel semisynthetic polypeptide antifungal antibiotic.* Ann N Y Acad Sci 1988, 544: 294-309.
53. Sucher, A.J., E.B. Chahine, H.E. Balcer, *Echinocandins: The newest class of antifungals.* Ann Pharmacother 2009. 43(10): 1647-57.
54. Tawara, S., Ikeda, F., Maki, K. et al. *In vitro activities of a new lipopeptide antifungal agent, FK463, against a variety of clinically important fungi.* Antimicrob Agents Chemother 2000, 44(1): 57-62.
55. Mizuno, K., Yagi, A., Satoi, S., Takada, M., Hayashi, M. *Studies on aculeacin. I. Isolation and characterization of aculeacin A.* J Antibiot (Tokyo) 1977, 30(4): 297-302.
56. Satoi, S., Yagi, A., Asano, K., Mizuno, K., Watanabe, T. *Studies on aculeacin. II. Isolation and characterization of aculeacins B, C, D, E, F and G.* J Antibiot (Tokyo) 1977, 30(4): 303-7.
57. Mizoguchi, J., Saito, T., Mizuno, K., Hayano, K. *On the mode of action of a new antifungal antibiotic, aculeacin A: Inhibition of cell wall synthesis in Saccharomyces cerevisiae.* J Antibiot (Tokyo) 1977, 30(4): 308-13.
58. Iwata, K., Yamamoto, Y., Yamaguchi, H., Hiratani, T. *In vitro studies of aculeacin A, a new antifungal antibiotic.* J Antibiot (Tokyo) 1982, 35(2): 203-9.
59. Mukhopadhyay, T., Ganguli, B.N., Fehlhaber, H.W., Kogler, H., Vertesy, L. *Mulundocandin, a new lipopeptide antibiotic. II. Structure elucidation.* J Antibiot (Tokyo) 1987, 40(3): 281-9.
60. Roy, K., Mukhopadhyay, T., Reddy, G.C., Desikan, K.R., Ganguli, B.N. *Mulundocandin, a new lipopeptide antibiotic. I. Taxonomy, fermentation, isolation and characterization.* J Antibiot (Tokyo) 1987, 40(3): 275-80.
61. Hawser, S., Borgonovi, M., Markus, A., Isert, D. *Mulundocandin, an echinocandin-like lipopeptide antifungal agent: Biological activities in vitro.* J Antibiot (Tokyo) 1999, 52(3): 305-10.
62. Iwamoto, T., Fujie, A., Nitta, K., Hashimoto, S., Okuhara, M., Kohsaka, M. *WF11899A, B and C, novel antifungal lipopeptides. II. Biological properties.* J Antibiot (Tokyo) 1994, 47(10): 1092-7.
63. Hori, M., Eguchi, J., Kakiki, K., Misato, T. *Studies on the mode of action of polyoxins. VI. Effect of polyoxin B on chitin synthesis in polyoxin-sensitive and resistant strains of Alternaria kikuchiana.* J Antibiot (Tokyo) 1974, 27(4): 260-6.
64. Brillinger, G.U. *Metabolic products of microorganisms. 181. Chitin synthase from fungi, a test model for substances with insecticidal properties.* Arch Microbiol 1979, 121(1): 71-4.
65. Chen, W., H. Zeng, Tan, H. *Cloning, sequencing, and function of sanF: A gene involved in nikkomycin biosynthesis of Streptomyces ansochromogenes.* Curr Microbiol 2000, 41(5): 312-6.
66. McCarthy, P.J., Troke, P.F., Gull, K. *Mechanism of action of nikkomycin and the peptide transport system of Candida albicans.* J Gen Microbiol 1985, 131(4): 775-80.
67. Hector, R.F., Zimmer, B.L., Pappagianis, D. *Evaluation of nikkomycins X and Z in murine models of coccidioidomycosis, histoplasmosis, and blastomycosis.* Antimicrob Agents Chemother 1990, 34(4): 587-93.
68. Clemons, K.V., Stevens, D.A. *Efficacy of nikkomycin Z against experimental pulmonary blastomycosis.* Antimicrob Agents Chemother 1997, 41(9): 2026-8.
69. Ikai, K., Takesako, K., Shiomi, K., Moriguchi, M., Umeda, Y., Yamamoto, J., Kato, I., Naganawa, H. *Structure of aureobasidin A.* J Antibiot (Tokyo) 1991, 44(9): 925-33.
70. Endo, M., Takesako, K., Kato, I., Yamaguchi, H. *Fungicidal action of aureobasidin A, a cyclic depsipeptide antifungal antibiotic, against*

Saccharomyces cerevisiae. Antimicrob Agents Chemother 1997, 41(3): 672-6.

71. Nagiec, M.M., Nagiec, E.E., Baltisberger, J.A., Wells, G.B., Lester, R.L., Dickson, R.C. *Sphingolipid synthesis as a target for antifungal drugs. Complementation of the inositol phosphorylceramide synthase defect in a mutant strain of Saccharomyces cerevisiae by the AUR1 gene.* J Biol Chem 1997, 272(15): 9809-17.
72. Takesako, K., Kuroda, H., Inoue, T. et al. *Biological properties of aureobasidin A, a cyclic depsipeptide antifungal antibiotic.* J Antibiot (Tokyo) 1993, 46(9): 1414-20.
73. Thevissen, K., Kristensen, H.H., Thomma, B.P., Cammue, B.P., François, I.E. *Therapeutic potential of antifungal plant and insect defensins.* Drug Discov Today 2007, 12(21-22): 966-71.
74. Fehlbaum, P., Bulet, P., Michaut, L., Lagueux, M., Broekaert, W.F., Hetru, C., Hoffmann, J.A. *Insect immunity. Septic injury of Drosophila induces the synthesis of a potent antifungal peptide with sequence homology to plant antifungal peptides.* J Biol Chem 1994, 269(52): 33159-63.
75. Gao, B., Zhu, S.Y. *Differential potency of drosomycin to Neurospora crassa and its mutant: Implications for evolutionary relationship between defensins from insects and plants.* Insect Mol Biol 2008, 17(4): 405-11.
76. Schaaper, W.M., Posthuma, G.A., Plasman, H.H. et al. *Synthetic peptides derived from the beta2-beta3 loop of Raphanus sativus antifungal protein 2 that mimic the active site.* J Pept Res 2001, 57(5): 409-18.
77. Cohen, L., Moran, Y., Sharon, A., Segal, D., Gordon, D., Gurevitz, M. *Drosomycin, an innate immunity peptide of Drosophila melanogaster, interacts with the fly voltage-gated sodium channel.* J Biol Chem 2009, 284(35): 23558-63.
78. Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.M., Hoffmann, J.A. *The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults.* Cell 1996, 86(6): 973-83.
79. Michaut, L., Fehlbaum, P., Moniatte, M., Van Dorsselaer, A., Reichhart, J.M., Bulet, P. *Determination of the disulfide array of the first inducible antifungal peptide from insects: Drosomycin from Drosophila melanogaster.* FEBS Lett 1996, 395(1): 6-10.
80. Zhang, Z.T., Zhu, S.Y. *Drosomycin, an essential component of antifungal defence in Drosophila.* Insect Mol Biol 2009, 18(5): 549-56.
81. Besson, F., Michel, G. *Action of the antibiotics of the iturin group on artificial membranes.* J Antibiot (Tokyo) 1984, 37(6): 646-51.
82. Mhammedi, A., Peypoux, F., Besson, F., Michel, G. *Bacillomycin F, a new antibiotic of iturin group: Isolation and characterization.* J Antibiot (Tokyo) 1982, 35(3): 306-11.
83. Klich, M.A., Lax, A.R., Bland, J.M. *Inhibition of some mycotoxigenic fungi by iturin A, a peptidolipid produced by Bacillus subtilis.* Mycopathologia 1991, 116(2): 77-80.
84. Latoud, C., Peypoux, F., Michel, G., Genet, R., Morgat, J.L. *Interactions of antibiotics of the iturin group with human erythrocytes.* Biochim Biophys Acta 1986, 856(3): 526-35.
85. Brewer, D., Hunter, H., Lajoie, G. *NMR studies of the antimicrobial salivary peptides histatin 3 and histatin 5 in aqueous and nonaqueous solutions.* Biochem Cell Biol 1998, 76(2-3): 247-56.
86. Raj, P.A., Soni, S.D., Levine, M.J. *Membrane-induced helical conformation of an active candidacidal fragment of salivary histatins.* J Biol Chem 1994, 269(13): 9610-9.
87. Raj, P.A., Marcus, E., Sukumaran, D.K. *Structure of human salivary histatin 5 in aqueous and nonaqueous solutions.* Biopolymers 1998, 45(1): 51-67.
88. Pollock, J.J., Denepitiya, L., MacKay, B.J., Iacono, V.J. *Fungistatic and fungicidal activity of human parotid salivary histidine-rich polypeptides on Candida albicans.* Infect Immun 1984, 44(3): 702-7.
89. Rayhan, R. Xu, L., Santarpia, R.P. 3rd, Tylenda, C.A., Pollock, J.J. *Antifungal activities of salivary histidine-rich polypeptides against Candida albicans and other oral yeast isolates.* Oral Microbiol Immunol 1992, 7(1): 51-2.
90. Helmerhorst, E.J., Reijnders, I.M., van't Hof, W., Simoons-Smit, I., Veerman, E.C., Amerongen, A.V. *Amphotericin B- and fluconazole-resistant Candida spp., Aspergillus fumigatus, and other newly emerging pathogenic fungi are susceptible to basic antifungal peptides.* Antimicrob Agents Chemother 1999, 43(3): 702-4.
91. Tsai, H., Bobek, L.A. *Human salivary histatin-5 exerts potent fungicidal activity against Cryptococcus neoformans.* Biochim Biophys Acta 1997, 1336(3): 367-9.
92. Castagnola, M., Inzitari, R., Rossetti, D.V. et al. *A cascade of 24 histatins (histatin 3 fragments) in human saliva. Suggestions for a pre-secretory sequential cleavage pathway.* J Biol Chem 2004, 279(40): 41436-43.
93. MacKay, B.J., Pollock, J.J., Iacono, V.J., Baum, B.J. *Isolation of milligram quantities of a group of histidine-rich polypeptides from human parotid saliva.* Infect Immun 1984, 44(3): 688-94.
94. vanderSpek, J.C., Wyandt, H.E., Skare, J.C., Milunsky, A., Oppenheim, F.G., Troxler, R.F. *Localization of the genes for histatins to human chromosome 4q13 and tissue distribution of the mRNAs.* Am J Hum Genet 1989, 45(3): 381-7.
95. Ahmad, M., Piludu, M., Oppenheim, F.G., Helmerhorst, E.J., Hand, A.R. *Immunocytochemical localization of histatins in human salivary glands.* J Histochem Cytochem 2004, 52(3): 361-70.
96. Raj, P.A., Edgerton, M., Levine, M.J. *Salivary histatin 5: Dependence of sequence, chain length, and helical conformation for candidacidal activity.* J Biol Chem 1990, 265(7): 3898-905.
97. Rothstein, D.M., Spacciapoli, P., Tran, L.T. et al. *Anticandida activity is retained in P-113, a 12-amino-acid fragment of histatin 5.* Antimicrob Agents Chemother 2001, 45(5): 1367-73.
98. De Prijck, K., De Smet, N., Rymarczyk-Machal, M. et al. *Candida albicans biofilm formation on peptide functionalized polydimethylsiloxane.* Biofouling 2010, 26(3): 269-75.
99. Pusateri, C.R., Monaco, E.A., Edgerton, M. *Sensitivity of Candida albicans biofilm cells grown on denture acrylic to antifungal proteins and chlorhexidine.* Arch Oral Biol 2009, 54(6): 588-94.
100. Douglas, L.J. *Candida biofilms and their role in infection.* Trends Microbiol 2003, 11(1): 30-6.
101. Lee, S.Y., Moon, H.J., Kawabata, S., Kurata, S., Natori, S., Lee, B.L. *A sapecin homologue of Holotrichia diomphalia: Purification, sequencing and determination of disulfide pairs.* Biol Pharm Bull 1995, 18(3): 457-9.
102. Lee, S.Y., Moon, H.J., Kurata, S., Natori, S., Lee, B.L. *Purification and cDNA cloning of an antifungal protein from the hemolymph of Holotrichia diomphalia larvae.* Biol Pharm Bull 1995, 18(8): 1049-52.
103. Iijima, R., Kurata, S., Natori, S. *Purification, characterization, and cDNA cloning of an antifungal protein from the hemolymph of Sarcophaga peregrina (flesh fly) larvae.* J Biol Chem 1993, 268(16): 12055-61.
104. Zhu, J., Luther, P.W., Leng, Q., Mixson, A.J. *Synthetic histidine-rich peptides inhibit Candida species and other fungi in vitro: Role of endocytosis and treatment implications.* Antimicrob Agents Chemother 2006, 50(8): 2797-805.
105. Mochon, A.B., Liu, H. *The antimicrobial peptide histatin-5 causes a spatially restricted disruption on the Candida albicans surface, allowing rapid entry of the peptide into the cytoplasm.* PLoS Pathog 2008, 4(10): e1000190.
106. Helmerhorst, E.J., van't Hof, W., Breeuwer, P. et al. *Characterization of histatin 5 with respect to amphipathicity, hydrophobicity, and effects on cell and mitochondrial membrane integrity excludes a candidacidal mechanism of pore formation.* J Biol Chem 2001, 276(8): 5643-9.

107. Helmerhorst, E.J., Breeuwer, P., van't Hof, W. et al. *The cellular target of histatin 5 on Candida albicans is the energized mitochondrion.* J Biol Chem 1999, 274(11): 7286-91.

108. Baev, D., Rivetta, A., Vylkova, S., Sun, J.N., Zeng, G.F., Slayman, C.L., Edgerton, M. *The TRK1 potassium transporter is the critical effector for killing of Candida albicans by the cationic protein, Histatin 5.* J Biol Chem 2004, 279(53): 55060-72.

109. Leng, Q., Scaria, P., Zhu, J., Ambulos, N., Campbell, P., Mixson, A.J. *Highly branched HK peptides are effective carriers of siRNA.* J Gene Med 2005, 7(7): 977-86.

110. Steiner, H., Hultmark, D., Engström, A., Bennich, H., Boman, H.G. *Sequence and specificity of two antibacterial proteins involved in insect immunity.* Nature 1981, 292(5820): 246-8.

111. Zasloff, M. *Magainins, a class of antimicrobial peptides from Xenopus skin: Isolation, characterization of two active forms, and partial cDNA sequence of a precursor.* Proc Natl Acad Sci U S A 1987, 84(15): 5449-53.

112. Mor, A., Nguyen, V.H., Delfour, A., Migliore-Samour, D., Nicolas, P. *Isolation, amino acid sequence, and synthesis of dermaseptin, a novel antimicrobial peptide of amphibian skin.* Biochemistry 1991, 30(36): 8824-30.

113. Dathe, M., Wieprecht, T. *Structural features of helical antimicrobial peptides: Their potential to modulate activity on model membranes and biological cells.* Biochim Biophys Acta 1999, 1462(1-2): 71-87.

114. Morton, C.O., Hayes, A., Wilson, M., Rash, B.M., Oliver, S.G., Coote, P. *Global phenotype screening and transcript analysis outlines the inhibitory mode(s) of action of two amphibian-derived, alpha-helical, cationic peptides on Saccharomyces cerevisiae.* Antimicrob Agents Chemother 2007, 51(11): 3948-59.

115. Hsu, C.H., Chen, C., Jou, M.L. et al. *Structural and DNA-binding studies on the bovine antimicrobial peptide, indolicidin: Evidence for multiple conformations involved in binding to membranes and DNA.* Nucleic Acids Res 2005, 33(13): 4053-64.

116. Marchand, C., Krajewski, K., Lee, H.F. et al. *Covalent binding of the natural antimicrobial peptide indolicidin to DNA abasic sites.* Nucleic Acids Res 2006, 34(18): 5157-65.

117. Cruciani, R.A., Barker, J.L., Zasloff, M., Chen, H.C., Colamonici, O. *Antibiotic magainins exert cytolytic activity against transformed cell lines through channel formation.* Proc Natl Acad Sci U S A 1991, 88(9): 3792-6.

118. Merrifield, R.B., Merrifield, E.L., Juvvadi, P., Andreu, D., Boman, H.G. *Design and synthesis of antimicrobial peptides.* Ciba Found Symp 1994, 186: 5-20.

119. Hultmark, D. *Immune reactions in Drosophila and other insects: A model for innate immunity.* Trends Genet 1993, 9(5): 178-83.

120. Moore, A.J., Devine, D.A., Bibby, M.C. *Preliminary experimental anticancer activity of cecropins.* Pept Res 1994, 7(5): 265-9.

121. Giacometti, A., Cirioni, O., Ghiselli, R. et al. *Effect of mono-dose intraperitoneal cecropins in experimental septic shock.* Crit Care Med 2001, 29(9): 1666-9.

122. Reed, W.A., Elzer, P.H., Enright, F.M., Jaynes, J.M., Morrey, J.D., White, K.L. *Interleukin 2 promoter/enhancer controlled expression of a synthetic cecropin-class lytic peptide in transgenic mice and subsequent resistance to Brucella abortus.* Transgenic Res 1997, 6(5): 337-47.

123. De Lucca, A.J., Bland, J.M., Jacks, T.J., Grimm, C., Walsh, T.J. *Fungicidal and binding properties of the natural peptides cecropin B and dermaseptin.* Med Mycol 1998, 36(5): 291-8.

124. De Lucca, A.J., Bland, J.M., Grimm, C. et al. *Fungicidal properties, sterol binding, and proteolytic resistance of the synthetic peptide D4E1.* Can J Microbiol 1998, 44(6): 514-20.

125. Coca, M., Peñas, G., Gómez, J., Campo, S., Bortolotti, C., Messeguer, J., Segundo, B.S. *Enhanced resistance to the rice blast fungus Magnaporthe grisea conferred by expression of a cecropin A gene in transgenic rice.* Planta 2006, 223(3): 392-406.

126. Giovannini, M.G., Poulter, L., Gibson, B.W., Williams, D.H. *Biosynthesis and degradation of peptides derived from Xenopus laevis prohormones.* Biochem J 1987, 243(1): 113-20.

127. Terry, A.S., Poulter, L., Williams, D.H., Nutkins, J.C., Giovannini, M.G., Moore, C.H., Gibson, B.W. *The cDNA sequence coding for prepro-PGS (prepro-magainins) and aspects of the processing of this prepro-polypeptide.* J Biol Chem 1988, 263(12): 5745-51.

128. Bevins, C.L., Zasloff, M. *Peptides from frog skin.* Annu Rev Biochem 1990, 59: 395-414.

129. Sures, I., Crippa, M. *Xenopsin: The neurotensin-like octapeptide from Xenopus skin at the carboxyl terminus of its precursor.* Proc Natl Acad Sci U S A 1984, 81(2): 380-4.

130. Gibson, B.W., Poulter, L., Williams, D.H., Maggio, J.E. *Novel peptide fragments originating from PGLa and the caerulein and xenopsin precursors from Xenopus laevis.* J Biol Chem 1986. 261(12): 5341-9.

131. Hara, T., Mitani, Y., Tanaka, K. et al. *Heterodimer formation between the antimicrobial peptides magainin 2 and PGLa in lipid bilayers: A cross-linking study.* Biochemistry 2001, 40(41): 12395-9.

132. Giacometti, A., Cirioni, O., Barchiesi, F., Del Prete, M.S., Scalise, G. *Antimicrobial activity of polycationic peptides.* Peptides 1999, 20(11): 1265-73.

133. Wang, X.Q., Su, C.X., Zhou, B., Cao, R.B., Chen P.Y. *[Synthesis of hybrid antimicrobial peptide CecA-mag gene and its secretion expression in Pichia pastoris].* Wei Sheng Wu Xue Bao 2007, 47(1): 75-8.

134. Park, Y., Lee, D.G., Jang, S.H., Woo, E.R., Jeong, H.G., Choi, C.H., Hahm, K.S. *A Leu-Lys-rich antimicrobial peptide: Activity and mechanism.* Biochim Biophys Acta 2003, 1645(2): 172-82.

135. Park, Y., Lee, D.G., Hahm, K.S. *HP(2-9)-magainin 2(1-12), a synthetic hybrid peptide, exerts its antifungal effect on Candida albicans by damaging the plasma membrane.* J Pept Sci 2004, 10(4): 204-9.

136. Avrahami, D., Shai, Y. *Bestowing antifungal and antibacterial activities by lipophilic acid conjugation to D,L-amino acid-containing antimicrobial peptides: A plausible mode of action.* Biochemistry 2003, 42(50): 14946-56.

137. Simmaco, M., Barra, D., Chiarini, F., Noviello, L., Melchiorri, P., Kreil, G., Richter, K. *A family of bombinin-related peptides from the skin of Bombina variegata.* Eur J Biochem, 1991, 199(1): 217-22.

138. Rozek, T., Waugh, R.J., Steinborner, S.T., Bowie, J.H., Tyler, M.J., Wallace, J.C. *The maculatin peptides from the skin glands of the tree frog Litoria genimaculata: A comparison of the structures and antibacterial activities of maculatin 1.1 and caerin 1.1.* J Pept Sci 1998, 4(2): 111-5.

139. Simmaco, M., Kreil, G., Barra, D. *Bombinins, antimicrobial peptides from Bombina species.* Biochim Biophys Acta 2009, 1788(8): 1551-5.

140. Mangoni, M.L., Maisetta, G., Di Luca, M. et al. *Comparative analysis of the bactericidal activities of amphibian peptide analogues against multidrug-resistant nosocomial bacterial strains.* Antimicrob Agents Chemother 2008, 52(1): 85-91.

141. Mangoni, M.L., Grovale, N., Giorgi, A., Mignogna, G., Simmaco, M., Barra, D. *Structure-function relationships in bombinins H, antimicrobial peptides from Bombina skin secretions.* Peptides 2000, 21(11): 1673-9.

142. Simmaco, M., Mangoni, M.L., Miele, R., Borro, M., Barra, D. *Defence peptides in the amphibian immune system*. In: Bacterial, Plant, and Animal Toxins. P. Ascenzi, F. Polticelli, P. Visca (Eds). Kerala, Research Signpost (India) 2003.

143. Mangoni, M.L., Marcellini, H.G., Simmaco, M. *Biological characterization and modes of action of temporins and bombinins H, multiple forms of short and mildly cationic anti-microbial peptides from amphibian skin.* J Pept Sci 2007, 13(9): 603-13.

144. Mignogna, G., Simmaco, M., Kreil, G., Barra, D. *Antibacterial and haemolytic peptides containing D-alloisoleucine from the skin of Bombina variegata.* EMBO J 1993, 12(12): 4829-32.

145. Mor, A., Hani, K., Nicolas, P. *The vertebrate peptide antibiotics dermaseptins have overlapping structural features but target specific microorganisms.* J Biol Chem 1994, 269(50): 31635-41.

146. Lorin, C., Saidi, H., Belaid, A. et al. *The antimicrobial peptide dermaseptin S4 inhibits HIV-1 infectivity in vitro.* Virology 2005, 334(2): 264-75.

147. Hernandez, C., Mor, A., Dagger, F., Nicolas, P., Hernandez, A., Benedetti, E.L., Dunia, I. *Functional and structural damage in Leishmania mexicana exposed to the cationic peptide dermaseptin.* Eur J Cell Biol 1992, 59(2): 414-24.

148. Mor, A., Nicolas, P. *Isolation and structure of novel defensive peptides from frog skin.* Eur J Biochem 1994, 219(1-2): 145-54.

149. Savoia, D., Guerrini, R., Marzola, E., Salvadori, S. *Synthesis and antimicrobial activity of dermaseptin S1 analogues.* Bioorg Med Chem 2008, 16(17): 8205-9.

150. Osusky, M., Osuska, L., Kay, W., Misra, S. *Genetic modification of potato against microbial diseases: In vitro and in planta activity of a dermaseptin B1 derivative, MsrA2.* Theor Appl Genet 2005, 111(4): 711-22.

151. Nijnik, A., Hancock, R.E. *The roles of cathelicidin LL-37 in immune defences and novel clinical applications.* Curr Opin Hematol 2009, 16(1): 41-7.

152. Bals, R. *Epithelial antimicrobial peptides in host defense against infection.* Respir Res 2000, 1(3): 141-50.

153. Frohm, M., Agerberth, B., Ahangari, G., Stâhle-Bäckdahl, M., Lidén, S., Wigzell, H., Gudmundsson, G.H. *The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders.* J Biol Chem 1997, 272(24): 15258-63.

154. Cowland, J.B., Johnsen, A.H., Borregaard, N. *hCAP-18, a cathelin/probactenecin-like protein of human neutrophil specific granules.* FEBS Lett 1995, 368(1): 173-6.

155. Gudmundsson, G.H., Agerberth, B., Odeberg, J., Bergman, T., Olsson, B., Salcedo, R. *The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes.* Eur J Biochem 1996, 238(2): 325-32.

156. Agerberth, B., Charo, J., Werr, J. et al. *The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations.* Blood 2000, 96(9): 3086-93.

157. Schauber, J. Gallo, R.L. *The vitamin D pathway: A new target for control of the skin's immune response?* Exp Dermatol 2008, 17(8): 633-9.

158. Segaert, S. *Vitamin D regulation of cathelicidin in the skin: Toward a renaissance of vitamin D in dermatology?* J Invest Dermatol 2008, 128(4): 773-5.

159. López-García, B., Lee, P.H., Yamasaki, K., Gallo, R.L. *Anti-fungal activity of cathelicidins and their potential role in Candida albicans skin infection.* J Invest Dermatol 2005, 125(1): 108-15.

160. Benincasa, M., Scocchi, M., Pacor, S. et al. *Fungicidal activity of five cathelicidin peptides against clinically isolated yeasts.* J Antimicrob Chemother 2006, 58(5): 950-9.

161. Kanthawong, S., Nazmi, K., Wongratanacheewin, S., Bolscher, J.G., Wuthiekanun, V., Taweechaisupapong, S. *In vitro susceptibility of Burkholderia pseudomallei to antimicrobial peptides.* Int J Antimicrob Agents 2009, 34(4): 309-14.

162. Zanetti, M. *Cathelicidins, multifunctional peptides of the innate immunity.* J Leukoc Biol 2004, 75(1): 39-48.

163. Filewod, N.C., Pistolic, J., Hancock, R.E. *Low concentrations of LL-37 alter IL-8 production by keratinocytes and bronchial epithelial cells in response to proinflammatory stimuli.* FEMS Immunol Med Microbiol 2009, 56(3): 233-40.

164. Schittek, B., Paulmann, M., Senyürek, I., Steffen, H. *The role of antimicrobial peptides in human skin and in skin infectious diseases.* Infect Disord Drug Targets 2008, 8(3): 135-43.

165. Wollenberg, A., Klein, E. *Current aspects of innate and adaptive immunity in atopic dermatitis.* Clin Rev Allergy Immunol 2007, 33(1-2): 35-44.

166. Lawyer, C., Pai, S., Watabe, M., Borgia, P., Mashimo, T., Eagleton, L., Watabe, K. *Antimicrobial activity of a 13 amino acid tryptophan-rich peptide derived from a putative porcine precursor protein of a novel family of antibacterial peptides.* FEBS Lett 1996, 390(1): 95-8.

167. Selsted, M.E., Novotny, M.J., Morris, W.L., Tang, Y.Q., Smith, W., Cullor, J.S. *Indolicidin, a novel bactericidal tridecapeptide amide from neutrophils.* J Biol Chem 1992, 267(7): 4292-5.

168. Falla, T.J., Karunaratne, D.N., Hancock, R.E. *Mode of action of the antimicrobial peptide indolicidin.* J Biol Chem 1996, 271(32): 19298-303.

169. Yang, S.T., Yub Shin, S.Y., Kim, Y.C., Kim, Y., Hahm, K.S., Kim, J.I. *Conformation-dependent antibiotic activity of tritrpticin, a cathelicidin-derived antimicrobial peptide.* Biochem Biophys Res Commun 2002, 296(5): 1044-50.

170. Ahmad, I., Perkins, W.R., Lupan, D.M., Selsted, M.E., Janoff, A.S. *Liposomal entrapment of the neutrophil-derived peptide indolicidin endows it with in vivo antifungal activity.* Biochim Biophys Acta 1995, 1237(2): 109-14.

171. Yang, S.T., Shin, S.Y., Hahm, K.S., Kim, J.I. *Design of perfectly symmetric Trp-rich peptides with potent and broad-spectrum antimicrobial activities.* Int J Antimicrob Agents 2006, 27(4): 325-30.

172. Muñoz, A., López-García, B., Pérez-Payá, E., Marcos, J.F. *Antimicrobial properties of derivatives of the cationic tryptophan-rich hexapeptide PAF26.* Biochem Biophys Res Commun 2007, 354(1): 172-7.

173. Li, G.P., Chen, Y.B. *[Cloning, expression and characterization of a new hybrid AMP gene of Hex-Mag].* Wei Sheng Wu Xue Bao 2007, 47(1): 115-20.

174. Stark, M., Liu, L.P., Deber, C.M. *Cationic hydrophobic peptides with antimicrobial activity.* Antimicrob Agents Chemother 2002, 46(11): 3585-90.

175. Burrows, L.L., Stark, M., Chan, C., Glukhov, E., Sinnadurai, S., Deber, C.M. *Activity of novel non-amphipathic cationic antimicrobial peptides against Candida species.* J Antimicrob Chemother 2006, 57(5): 899-907.

176. Fehlbaum, P., Bulet, P., Chernysh, S. et al. *Structure-activity analysis of thanatin, a 21-residue inducible insect defense peptide with sequence homology to frog skin antimicrobial peptides.* Proc Natl Acad Sci U S A 1996, 93(3): 1221-5.

177. Simmaco, M., Mignogna, G., Barra, D., Bossa, F. *Antimicrobial peptides from skin secretions of Rana esculenta. Molecular cloning of cDNAs encoding esculentin and brevinins and isolation of new active peptides.* J Biol Chem 1994, 269(16): 11956-61.

178. Daly, N.L., Koltay, A., Gustafson, K.R., Boyd, M.R., Casas-Finet, J.R., Craik, D.J. *Solution structure by NMR of circulin A: A macrocyclic knotted peptide having anti-HIV activity.* J Mol Biol 1999, 285(1): 333-45.

179. Shafer, W.M., Qu, X., Waring, A.J., Lehrer, R.I. *Modulation of Neisseria gonorrhoeae susceptibility to vertebrate antibacterial peptides due to a member of the resistance/nodulation/division efflux pump family.* Proc Natl Acad Sci U S A 1998, 95(4): 1829-33.

180. Lehrer, R.I., Lichtenstein, A.K., Ganz, T. *Defensins: Antimicrobial and cytotoxic peptides of mammalian cells.* Annu Rev Immunol 1993, 11: 105-28.

181. Miyata, T., Tokunaga, F., Yoneya, T. et al. *Antimicrobial peptides, isolated from horseshoe crab hemocytes, tachyplesin II, and polyphemusins I and II: Chemical structures and biological activity.* J Biochem 1989, 106(4): 663-8.

182. Nakamura, T., Furunaka, H., Miyata, T. et al. *Tachyplesin, a class of antimicrobial peptide from the hemocytes of the horseshoe crab (Tachypleus tridentatus). Isolation and chemical structure.* J Biol Chem 1988, 263(32): 16709-13.

183. Yasin, B., Lehrer, R.I., Harwig, S.S., Wagar, E.A. *Protegrins: Structural requirements for inactivating elementary bodies of Chlamydia trachomatis.* Infect Immun 1996, 64(11): 4863-6.

184. Yasin, B., Harwig, S.S., Lehrer, R.I., Wagar, E.A. *Susceptibility of Chlamydia trachomatis to protegrins and defensins.* Infect Immun 1996, 64(3): 709-13.

185. Tamamura, H., Murakami, T., Horiuchi, S. et al. *Synthesis of protegrin-related peptides and their antibacterial and anti-human immunodeficiency virus activity.* Chem Pharm Bull (Tokyo) 1995, 43(5): 853-8.

186. Yasin, B., Pang, M., Turner, J.S. et al. *Evaluation of the inactivation of infectious Herpes simplex virus by host-defense peptides.* Eur J Clin Microbiol Infect Dis 2000, 19(3): 187-94.

187. Tam, J.P., Wu, C., Yang, J.L. *Membranolytic selectivity of cystine-stabilized cyclic protegrins.* Eur J Biochem 2000, 267(11): 3289-300.

188. Cho, Y., Turner, J.S., Dinh, N.N., Lehrer, R.I. *Activity of protegrins against yeast-phase Candida albicans.* Infect Immun 1998, 66(6): 2486-93.

189. Owen, S.M., Rudolph, D.L., Wang, W., Cole, A.M., Waring, A.J., Lal, R.B., Lehrer, R.I. *RC-101, a retrocyclin-1 analogue with enhanced activity against primary HIV type 1 isolates.* AIDS Res Hum Retroviruses 2004, 20(11): 1157-65.

190. Ganz, T. *Biosynthesis of defensins and other antimicrobial peptides.* Ciba Found Symp 1994, 186: 62-71.

191. Zimmermann, G.R., Legault, P., Selsted, M.E., Pardi, A. *Solution structure of bovine neutrophil beta-defensin-12: The peptide fold of the beta-defensins is identical to that of the classical defensins.* Biochemistry 1995, 34(41): 13663-71.

192. Scott, M.G., Hancock, R.E. *Cationic antimicrobial peptides and their multifunctional role in the immune system.* Crit Rev Immunol 2000, 20(5): 407-31.

193. Lehrer, R.I., Ganz, T., Szklarek, D., Selsted, M.E. *Modulation of the in vitro candidacidal activity of human neutrophil defensins by target cell metabolism and divalent cations.* J Clin Invest 1988, 81(6): 1829-35.

194. Ganz, T., Selsted, M.E., Lehrer, R.I. *Defensins.* Eur J Haematol 1990, 44(1): 1-8.

195. Ganz, T., Liu, L., Valore, E.V., Oren, A. *Posttranslational processing and targeting of transgenic human defensin in murine granulocyte, macrophage, fibroblast, and pituitary adenoma cell lines.* Blood 1993, 82(2): 641-50.

196. Wilson, C.L., Ouellette, A.J., Satchell, D.P. et al. *Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense.* Science 1999, 286(5437): 113-7.

197. Selsted, M.E., Miller, S.I., Henschen, A.H., Ouellette, A.J. *Enteric defensins: Antibiotic peptide components of intestinal host defense.* J Cell Biol 1992, 118(4): 929-36.

198. Jones, D.E., Bevins, C.L. *Defensin-6 mRNA in human Paneth cells: Implications for antimicrobial peptides in host defense of the human bowel.* FEBS Lett 1993, 315(2): 187-92.

199. Quayle, A.J., Porter, E.M., Nussbaum, A.A., Wang, Y.M., Brabec, C., Yip, K.P., Mok, S.C. *Gene expression, immunolocalization, and secretion of human defensin-5 in human female reproductive tract.* Am J Pathol 1998, 152(5): 1247-58.

200. Lehrer, R.I., Barton, A., Daher, K.A., Harwig, S.S., Ganz, T., Selsted, M.E. *Interaction of human defensins with Escherichia coli. Mechanism of bactericidal activity.* J Clin Invest 1989, 84(2): 553-61.

201. Brogden, K.A. *Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria?* Nat Rev Microbiol 2005, 3(3): 238-50.

202. Levitz, S.M., Selsted, M.E., Ganz, T., Lehrer, R.I., Diamond, R.D. *In vitro killing of spores and hyphae of Aspergillus fumigatus and Rhizopus oryzae by rabbit neutrophil cationic peptides and bronchoalveolar macrophages.* J Infect Dis 1986, 154(3): 483-9.

203. Alcouloumre, M.S., Ghannoum, M.A., Ibrahim, A.S., Selsted, M.E., Edwards, J.E., Jr. *Fungicidal properties of defensin NP-1 and activity against Cryptococcus neoformans in vitro.* Antimicrob Agents Chemother 1993, 37(12): 2628-32.

204. Ganz, T., Selsted, M.E., Szklarek, D., Harwig, S.S., Daher, K., Bainton, D.F., Lehrer, R.I. *Defensins. Natural peptide antibiotics of human neutrophils.* J Clin Invest 1985, 76(4): 1427-35.

205. Bensch, K.W., Raida, M., Mägert, H.J., Schulz-Knappe, P., Forssmann, W.G. *hBD-1: A novel beta-defensin from human plasma.* FEBS Lett 1995, 368(2): 331-5.

206. Weinberg, A., Krisanaprakornkit, S., Dale, B.A. *Epithelial antimicrobial peptides: Review and significance for oral applications.* Crit Rev Oral Biol Med 1998, 9(4): 399-414.

207. Tollin, M., Bergman, P., Svenberg, T., Jörnvall, H., Gudmundsson, G.H., Agerberth, B. *Antimicrobial peptides in the first line defence of human colon mucosa.* Peptides 2003, 24(4): 523-30.

208. Cole, A.M., Dewan, P., Ganz, T. *Innate antimicrobial activity of nasal secretions.* Infect Immun 1999, 67(7): 3267-75.

209. Valore, E.V., Park, C.H., Quayle, A.J., Wiles, K.R., McCray, P.B. Jr., Ganz, T. *Human beta-defensin-1: An antimicrobial peptide of urogenital tissues.* J Clin Invest 1998, 101(8): 1633-42.

210. Sahasrabudhe, K.S., Kimball, J.R., Morton, T.H., Weinberg, A., Dale, B.A. *Expression of the antimicrobial peptide, human beta-defensin 1, in duct cells of minor salivary glands and detection in saliva.* J Dent Res 2000, 79(9): 1669-74.

211. Garcia, J.R., Krause, A., Schulz, S. et al. *Human beta-defensin 4: A novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity.* FASEB J 2001, 15(10): 1819-21.

212. Yamaguchi, Y., Nagase, T., Makita, R. et al. *Identification of multiple novel epididymis-specific beta-defensin isoforms in humans and mice.* J Immunol 2002, 169(5): 2516-23.

213. Shi, J., Zhang, G., Wu, H., Ross, C., Blecha, F., Ganz, T. *Porcine epithelial beta-defensin 1 is expressed in the dorsal tongue at antimicrobial concentrations.* Infect Immun 1999, 67(6): 3121-7.

214. Schroder, J.M., Harder, J. *Human beta-defensin-2.* Int J Biochem Cell Biol 1999, 31(6): 645-51.

215. Harder, J., Bartels, J., Christophers, E., Schröder, J.M. *A peptide antibiotic from human skin.* Nature 1997, 387(6636): 861.

216. Jia, H.P., Schutte, B.C., Schudy, A. et al. *Discovery of new human beta-defensins using a genomics-based approach.* Gene 2001, 263(1-2): 211-8.

217. Diamond, G., J.P. Russell, and C.L. Bevins, *Inducible expression of an antibiotic peptide gene in lipopolysaccharide-challenged tracheal epithelial cells.* Proc Natl Acad Sci U S A, 1996. 93(10): 5156-60.

218. Schonwetter, B.S., Stolzenberg, E.D., Zasloff, M.A. *Epithelial antibiotics induced at sites of inflammation.* Science 1995, 267(5204): 1645-8.

219. Russell, J.P., Diamond, G., Tarver, A.P., Scanlin, T.F., Bevins, C.L. *Coordinate induction of two antibiotic genes in tracheal epithelial cells exposed to the inflammatory mediators lipopolysaccharide and tumor necrosis factor alpha.* Infect Immun 1996, 64(5): 1565-8.

220. Singh, P.K., Jia, H.P., Wiles, K. et al. *Production of beta-defensins by human airway epithelia.* Proc Natl Acad Sci U S A 1998, 95(25): 14961-6.

221. Zasloff, M. *Inducing endogenous antimicrobial peptides to battle infections.* Proc Natl Acad Sci U S A 2006, 103(24): 8913-4.

222. Joly, S., Maze, C., McCray, P.B., Jr., Guthmiller, J.M. *Human beta-defensins 2 and 3 demonstrate strain-selective activity against oral microorganisms.* J Clin Microbiol 2004, 42(3): 1024-9.

223. Lawyer, C., Pai, S., Watabe, M., Bakir, H., Eagleton, L., Watabe, K. *Effects of synthetic form of tracheal antimicrobial peptide on respiratory pathogens.* J Antimicrob Chemother 1996, 37(3): 599-604.

224. Diamond, G., Zasloff, M., Eck, H., Brasseur, M., Maloy, W.L., Bevins, C.L. *Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: Peptide isolation and cloning of a cDNA.* Proc Natl Acad Sci U S A 1991, 88(9): 3952-6.

225. Tran, D., Tran, P., Roberts, K., Osapay, G., Schaal, J., Ouellette, A., Selsted, M.E. *Microbicidal properties and cytocidal selectivity of rhesus macaque theta defensins.* Antimicrob Agents Chemother 2008, 52(3): 944-53.

226. Harwig, S.S., Swiderek, K.M., Kokryakov, V.N. et al. *Gallinacins: Cysteine-rich antimicrobial peptides of chicken leukocytes.* FEBS Lett 1994, 342(3): 281-5.

227. van Dijk, A., Veldhuizen, E.J., Kalkhove, S.I., Tjeerdsma-van Bokhoven, J.L., Romijn, R.A., Haagsman, H.P. *The beta-defensin gallinacin-6 is expressed in the chicken digestive tract and has antimicrobial activity against food-borne pathogens.* Antimicrob Agents Chemother 2007, 51(3): 912-22.

228. Tam, J.P., Lu, Y.A., Yang, J.L., Chiu, K.W. *An unusual structural motif of antimicrobial peptides containing end-to-end macrocycle and cystine-knot disulfides.* Proc Natl Acad Sci U S A 1999, 96(16): 8913-8.

229. Feigin, A.M., Takemoto, J.Y., Wangspa, R., Teeter, J.H., Brand, J.G. *Properties of voltage-gated ion channels formed by syringomycin E in planar lipid bilayers.* J Membr Biol 1996, 149(1): 41-7.

230. Zhang, L., Takemoto, J.Y. *Mechanism of action of Pseudomonas syringae phytotoxin, syringomycin. Interaction with the plasma membrane of wild-type and respiratory-deficient strains of Saccharomyces cerevisiae.* Biochim Biophys Acta 1986, 861(1): 201-4.

231. Sorensen, K.N., Kim, K.H., Takemoto, J.Y. *In vitro antifungal and fungicidal activities and erythrocyte toxicities of cyclic lipodepsinonapeptides produced by Pseudomonas syringae pv. syringae.* Antimicrob Agents Chemother 1996, 40(12): 2710-3.

232. Sorensen, K.N., Wanstrom, A.A., Allen, S.D., Takemoto, J.Y. *Efficacy of syringomycin E in a murine model of vaginal candidiasis.* J Antibiot (Tokyo) 1998, 51(8): 743-9.

233. Tomita, M., Bellamy, W., Takase, M., Yamauchi, K., Wakabayashi, H., Kawase, K. *Potent antibacterial peptides generated by pepsin digestion of bovine lactoferrin.* J Dairy Sci 1991, 74(12): 4137-42.

234. Minn, I., Kim, H.S., Kim, S.C. *Antimicrobial peptides derived from pepsinogens in the stomach of the bullfrog, Rana catesbeiana.* Biochim Biophys Acta 1998, 1407(1): 31-9.

235. Nibbering, P.H., Ravensbergen, E., Welling, M.M., van Berkel, L.A., van Berkel, P.H., Pauwels, E.K., Nuijens, J.H. *Human lactoferrin and peptides derived from its N terminus are highly effective against infections with antibiotic-resistant bacteria.* Infect Immun 2001, 69(3): 1469-76.

236. Lupetti, A., Paulusma-Annema, A., Welling, M.M., Senesi, S., van Dissel, J.T., Nibbering, P.H. *Candidacidal activities of human lactoferrin peptides derived from the N terminus.* Antimicrob Agents Chemother 2000, 44(12): 3257-63.

237. Kirkpatrick, C.H., Green, I., Rich, R.R., Schade, A.L. *Inhibition of growth of Candida albicans by iron-unsaturated lactoferrin: Relation to host-defense mechanisms in chronic mucocutaneous candidiasis.* J Infect Dis 1971, 124(6): 539-44.

238. Zarember, K.A., Sugui, J.A., Chang, Y.C., Kwon-Chung, K.J., Gallin, J.I. *Human polymorphonuclear leukocytes inhibit Aspergillus fumigatus conidial growth by lactoferrin-mediated iron depletion.* J Immunol 2007, 178(10): 6367-73.

239. Bellamy, W., Takase, M., Yamauchi, K., Wakabayashi, H., Kawase, K., Tomita, M. *Identification of the bactericidal domain of lactoferrin.* Biochim Biophys Acta 1992, 1121(1-2): 130-6.

240. Bellamy, W.R., Wakabayashi, H., Takase, M., Kawase, K., Shimamura, S., Tomita, M. *Role of cell-binding in the antibacterial mechanism of lactoferricin B.* J Appl Bacteriol 1993, 75(5): 478-84.

241. Viejo-Diaz, M., Andres, M.T., Fierro, J.F. *Different anti-Candida activities of two human lactoferrin-derived peptides, Lfpep and kaliocin-1.* Antimicrob Agents Chemother 2005, 49(7): 2583-8.

242. Ueta, E., Tanida, T., Osaki, T. *A novel bovine lactoferrin peptide, FKCRRWQWRM, suppresses Candida cell growth and activates neutrophils.* J Pept Res 2001, 57(3): 240-9.

243. Bellamy, W., Wakabayashi, H., Takase, M., Kawase, K., Shimamura, S., Tomita, M. *Killing of Candida albicans by lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin.* Med Microbiol Immunol 1993, 182(2): 97-105.

244. Newman, S.L., Gootee, L., Gabay, J.E., Selsted, M.E. *Identification of constituents of human neutrophil azurophil granules that mediate fungistasis against Histoplasma capsulatum.* Infect Immun 2000, 68(10): 5668-72.

245. Elsbach, P., Weiss, J. *The bactericidal/permeability-increasing protein (BPI), a potent element in host-defense against gram-negative bacteria and lipopolysaccharide.* Immunobiology 1993, 187(3-5): 417-29.

246. Horwitz, A., Motchink, P., Nadell, R. *Fungicidal properties from bactericidal/permiability-increasing protein (BPI) act synergistically with fluconazole on a variety of Candida strains.* 37th Intersci Conf Antimicrob Agents Chemother (ICAAC) (Sept 28-Oct 1, Toronto) 1997, Abst F-102.

247. Ammons, S., Aardalen, K., Froebel, S., Little, R. *Efficacy of domain III peptide from bactericidal/permeability-increasing protein (BPI) in murine disseminated aspergillosis.* 37th Intersci Conf Antimicrob Agents Chemother (ICAAC) (Sept 28-Oct 1, Toronto) 1997, Abst B-16.

248. Giacometti, A., Cirioni, O., Ghiselli, R. et al. *Potential therapeutic role of cationic peptides in three experimental models of septic shock.* Antimicrob Agents Chemother 2002, 46(7): 2132-6.

249. Nikawa, H., Fukushima, H., Makihira, S., Hamada, T., Samaranayake, L.P. *Fungicidal effect of three new synthetic cationic peptides against Candida albicans.* Oral Dis 2004, 10(4): 221-8.

250. Wade, D., Andreu, D., Mitchell, S.A., Silveira, A.M., Boman, A., Boman, H.G., Merrifield, R.B. *Antibacterial peptides designed as analogs or hybrids of cecropins and melittin.* Int J Pept Protein Res 1992, 40(5): 429-36.

251. Mosca, D.A., Hurst, M.A., So, W., Viajar, B.S., Fujii, C.A., Falla, T.J. *IB-367, a protegrin peptide with in vitro and in vivo activities against the microflora associated with oral mucositis.* Antimicrob Agents Chemother 2000, 44(7): 1803-8.

252. Chen, J., Falla, T.J., Liu, H. et al. *Development of protegrins for the treatment and prevention of oral mucositis: Structure-activity relationships of synthetic protegrin analogues.* Biopolymers 2000, 55(1): 88-98.

253. Andreu, D., Ubach, J., Boman, A., Wåhlin, B., Wade, D., Merrifield, R.B., Boman, H.G. *Shortened cecropin A-melittin hybrids. Significant size reduction retains potent antibiotic activity.* FEBS Lett 1992, 296(2): 190-4.

254. Gordon, Y.J., Romanowski, E.G., McDermott, A.M. *A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs.* Curr Eye Res 2005, 30(7): 505-15.

255. Chen, H., Xu, Z., Peng, L., Fang, X., Yin, X., Xu, N., Cen, P. *Recent advances in the research and development of human defensins.* Peptides 2006, 27(4): 931-40.

256. Hong, I.P., Lee, S.J., Kim, Y.S., Choi, S.G. *Recombinant expression of human cathelicidin (hCAP18/LL-37) in Pichia pastoris.* Biotechnol Lett 2007, 29(1): 73-8.

257. Huang, L., Leong, S.S., Jiang, R. *Soluble fusion expression and characterization of bioactive human beta-defensin 26 and 27.* Appl Microbiol Biotechnol 2009, 84(2): 301-8.

258. Jin, F., Xu, X., Wang, L., Zhang, W., Gu, D. *Expression of recombinant hybrid peptide cecropinA(1-8)-magainin2(1-12) in Pichia pastoris: Purification and characterization.* Protein Expr Purif 2006, 50(2): 147-56.

259. Chen, H., Xu, Z., Cen, P. *High-level expression of human beta-defensin-2 gene with rare codons in E. coli cell-free system.* Protein Pept Lett 2006, 13(2): 155-62.

260. Kim, H.K., Chun, D.S., Kim, J.S., Yun, C.H., Lee, J.H., Hong, S.K., Kang, D.K. *Expression of the cationic antimicrobial peptide lactoferricin fused with the anionic peptide in Escherichia coli.* Appl Microbiol Biotechnol 2006, 72(2): 330-8.

261. De Samblanx, G.W., Goderis, I.J., Thevissen, K. et al. *Mutational analysis of a plant defensin from radish (Raphanus sativus L.) reveals two adjacent sites important for antifungal activity.* J Biol Chem 1997, 272(2): 1171-9.

262. Chebolu, S., Daniell, H. *Chloroplast-derived vaccine antigens and biopharmaceuticals: Expression, folding, assembly and functionality.* Curr Top Microbiol Immunol 2009, 332: 33-54.

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

264. Bessette, P.H., Aslund, F., Beckwith, J., Georgiou, G. *Efficient folding of proteins with multiple disulfide bonds in the Escherichia coli cytoplasm.* Proc Natl Acad Sci U S A 1999, 96(24): 13703-8.

265. Peng, L., Xu, Z., Fang, X., Wang, F., Yang, S., Cen, P. *Preferential codons enhancing the expression level of human beta-defensin-2 in recombinant Escherichia coli.* Protein Pept Lett 2004, 11(4): 339-44.

266. Beckloff, N., Laube, D., Castro, T. et al. *Activity of an antimicrobial peptide mimetic against planktonic and biofilm cultures of oral pathogens.* Antimicrob Agents Chemother 2007, 51(11): 4125-32.

267. Genco, C.A., Maloy, W.L., Kari, U.P., Motley, M. *Antimicrobial activity of magainin analogues against anaerobic oral pathogens.* Int J Antimicrob Agents 2003, 21(1): 75-8.

268. La Rocca, P., Biggin, P.C., Tieleman, D.P., Sansom, M.S. *Simulation studies of the interaction of antimicrobial peptides and lipid bilayers.* Biochim Biophys Acta 1999, 1462(1-2): 185-200.

269. La Rocca, P., Shai, Y., Sansom, M.S. *Peptide-bilayer interactions: Simulations of dermaseptin B, an antimicrobial peptide.* Biophys Chem 1999, 76(2): 145-59.

270. Massimiliano, M., Colombo, G. *Molecular simulation of peptides: A useful tool for the development of new drugs and for the study of molecular recognition*. Methods Mol Biol 2009, 570: 77-153.

271. Altman, H., Steinberg, D., Porat, Y., Mor, A., Fridman, D., Friedman, M., Bachrach, G. *In vitro assessment of antimicrobial peptides as potential agents against several oral bacteria.* J Antimicrob Chemother 2006, 58(1): 198-201.

272. Balaban, N., Gov, Y., Giacometti, A. et al. *A chimeric peptide composed of a dermaseptin derivative and an RNA III-inhibiting peptide prevents graft-associated infections by antibiotic-resistant staphylococci.* Antimicrob Agents Chemother 2004, 48(7): 2544-50.

273. Helmerhorst, E.J., Hodgson, R., van 't Hof, W., Veerman, E.C., Allison, C., Nieuw Amerongen, A.V. *The effects of histatin-derived basic antimicrobial peptides on oral biofilms.* J Dent Res 1999, 78(6): 1245-50.

274. Ryan, R.P., Fouhy, Y., Garcia, B.F. et al. *Interspecies signalling via the Stenotrophomonas maltophilia diffusible signal factor influences biofilm formation and polymyxin tolerance in Pseudomonas aeruginosa.* Mol Microbiol 2008, 68(1): 75-86.

275. Sims, C.R., Ostrosky-Zeichner, L., Rex, J.H. *Invasive candidiasis in immunocompromised hospitalized patients.* Arch Med Res 2005, 36(6): 660-71.

276. Bulet, P. Stocklin, *Insect antimicrobial peptides: Structures, properties and gene regulation.* Protein Pept Lett 2005, 12(1): 3-11.

277. Yuan, Y., Gao, B., Zhu, S. *Functional expression of a Drosophila antifungal peptide in Escherichia coli.* Protein Expr Purif 2007, 52(2): 457-62.

278. Troxler, R.F., Offner, G.D., Xu, T., Vanderspek, J.C., Oppenheim, F.G. *Structural relationship between human salivary histatins.* J Dent Res 1990, 69(1): 2-6.

279. Boman, H.G. *Antibacterial peptides: Basic facts and emerging concepts.* J Intern Med 2003, 254(3): 197-215.

280. Munoz, A., Lopez-Garcia, B., Marcos, J.F. *Studies on the mode of action of the antifungal hexapeptide PAF26.* Antimicrob Agents Chemother 2006, 50(11): 3847-55.

281. Bulet, P., Hetru, C., Dimarcq, J.L., Hoffmann, D. *Antimicrobial peptides in insects; Structure and function.* Dev Comp Immunol 1999, 23(4-5): 329-44.

282. Kokryakov, V.N., Harwig, S.S., Panyutich, E.A. et al. *Protegrins: Leukocyte antimicrobial peptides that combine features of corticostatic defensins and tachyplesins.* FEBS Lett 1993, 327(2): 231-6.

283. Lehrer, R.I. *Primate defensins.* Nat Rev Microbiol 2004, 2(9): 727-38.

284. Gonzalez-Chavez, S.A., Arevalo-Gallegos, S., Rascon-Cruz, Q. *Lactoferrin: Structure, function and applications.* Int J Antimicrob Agents 2009, 33(4): 301 e1-8.