Emerging Themes and Therapeutic Prospects for Anti-Infective Peptides

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Abstract

Pathogens resistant to most conventional anti-infectives are a harbinger of the need to discover and develop novel anti-infective agents and strategies. Endogenous host defense peptides (HDPs) have retained evolution-tested efficacy against pathogens that have become refractory to traditional antibiotics. Evidence indicates that HDPs target membrane integrity, bioenergetics, and other essential features of microbes that may be less mutable than conventional antibiotic targets. For these reasons, HDPs have received increasing attention as templates for development of potential anti-infective therapeutics. Unfortunately, advances toward this goal have proven disappointing, in part owing to limited understanding of relevant structure-activity and selective toxicity relationships in vivo, a limited number of reports and overall understanding of HDP pharmacology, and the difficulty of cost-effective production of such peptides on a commodity scale. However, recent molecular insights and technology innovations have led to novel HDP-based and mimetic anti-infective peptide candidates designed to overcome these limitations. Although initial setbacks have presented challenges to therapeutic development, emerging themes continue to highlight the potential of HDP-based anti-infectives as a platform for next-generation therapeutics that will help address the growing threat of multidrug-resistant infections.

INTRODUCTION

Infectious diseases remain one of most serious health threats facing the global community (1). Moreover, the rise of multidrug-resistant pathogens has prompted an urgent need for novel anti-infective agents. Host defense peptides (HDPs) are endogenous antibiotics with high potency and efficacy against broad spectra of pathogens, including those that are refractory to conventional antibiotics. In addition, over the course of an evolutionary time span, these peptides have remained effective defenses against which little resistance has emerged. Owing to these properties, numerous laboratory and commercial groups have begun development of various HDPs as potential therapeutics. This review focuses on recent advances in development of HDPs as potential novel anti-infective agents.

DIVERSITY, DEPLOYMENT, AND IMMUNE COORDINATION

The field of HDP research has seen exponential growth over the past 30 years; more than 1,000 peptides are listed in various antimicrobial peptide databases. HDPs are traditionally classified by structural elements and are composed of four major peptide groups: (*a*) α-helical, (*b*) β-sheet, (*c*) loop (one cysteine bond), and (*d*) rich in certain amino acids. In mammals, the major HDP families are represented by α-, β-, and θ-defensins, cathelicidins, and histatins. Defensin family peptides are typically composed of three antiparallel β-sheets connected by three or more disulfide bonds. Categorization of defensins as α, β, or θ is based on their gene structures—which, when translated, lead to peptides with different precursor domains and disulfide connectivities. α-Defensins, the primary HDPs of leukocytes and the human gut, are characterized as having a relatively long precursor structure and a C1-C6, C2-C4, C3-C5 disulfide connectivity (2). β-Defensins, which have undergone extensive gene duplication events in many mammals (3), are the predominant HDPs of epithelia and are characterized by a C1-C5, C2-C4, C3-C6 disulfide bonding pattern. θ-Defensins, which are found exclusively in leukocytes of Old World monkeys and orangutans (4), are generated by a ligation event of two truncated α-defensin peptides to form a circular molecule.

Cathelicidins, which constitute the other major HDP family of mammals, are characterized as having a highly conserved precursor domain but a widely divergent mature peptide domain. The only cathelicidin identified in human is LL-37, which is broadly expressed in epithelia and leukocytes. LL-37 has been structurally characterized to have a primarily α-helical (helix-break-helix) conformation. Cathelicidins have also been isolated from other mammals (and vertebrates), and although all contain the conserved prodomain sequence, the mature peptides are divergent between species. Families of cathelicidins from nonhuman mammals include peptides that have α-helical or single-disulfide loop structures and those enriched in certain amino acids (e.g., proline, glycine, arginine, tryptophan, histidine).

Defensins and cathelicidins also exist in nonmammalian organisms. Representatives of the cathelicidin peptide family that contain the conserved propeptide domain have been isolated from birds, reptiles, teleost fish, and the primitive protovertebrate hagfish (5). With respect to the defensin family, peptides homologous to mammalian defensins have been characterized in birds and reptiles. Furthermore, peptides with a defensin-like cysteine array have been isolated from invertebrate sources such as insects, mollusks, plants, and fungi. Whether the vertebrate and invertebrate defensin families are genetically related has yet to be established. Recent studies have identified significant primary-sequence and three-dimensional identity between certain peptide toxins and many defensins, suggesting that they may have derived from a common genetic ancestor (6).

Recently Discovered Novel Host Defense Peptides

As the field of HDP biology becomes more active, newly isolated peptides are increasingly found to be members of known family groups. Therefore, most newly discovered HDPs represent variations on a theme and are genetic homologs of peptides that have previously been characterized in other species. Given this trend, the discovery of truly novel HDPs has become increasingly rare; however, here we highlight several novel HDP sequences that have been isolated during the past few years.

In vertebrates, several newly identified HDPs share homology with known antimicrobial peptide families yet are unique at the mature peptide sequence level. In the mouse, a novel cDNA related to classical defensins—termed murine β-defensin-like small peptide—has been isolated from brain tissue. Interestingly, this peptide shares significant identity with the murine β-defensin precursor. However, the mature peptide domain contains no cysteines, which are the hallmark of defensin peptides. Instead, this domain is composed of 17 residues that share limited homology with the noncysteine residues of murine β-defensins. A synthetic version of the mature peptide was shown to be antimicrobial in vitro and α-helical in a lipid mimetic environment (7). Within the cathelicidin family, several novel mature peptide sequences have been isolated from the donkey. These peptides—termed EA-CATH-1 and EA-CATH-2—are 25 and 26 residues in length, respectively, and show limited homology with the mature domains of the horse cathelicidins. Synthetic forms of EA-CATH-1 and EA-CATH-2 are stable in serum, have marked antimicrobial activity in vitro, and adopt α-helical conformation in 50%-lipid systems (8). In amphibians, two 18-residue peptides termed shuchin-1 and shuchin-2 have been isolated from skin secretions of the frog *Rana shuchinae*. These peptides show no homology with other frog antimicrobial peptide sequences, are not hemolytic, and have antimicrobial activity against bacteria and fungi in vitro (9).

In invertebrates and lower eukaryotes, several new peptide families have recently been characterized. In arthropods, two antimalarial peptides—termed meucin-24 and meucin-25—have been isolated from scorpion venom. Meucin-24 is α-helical and shares some homology with frog magainins and scorpion long-chain potassium-channel toxins (10). Meucin-25 is of variable structure and has minimal homology with other HDPs (10). Sequencing the genome of another arthropod, the *Tribolium castaneum* (red flour beetle), has revealed a novel proline-, tyrosine-, and glutamine-rich antimicrobial peptide that bears little homology with any previously characterized HDP (11). In other invertebrates, two peptides isolated from sea urchin—termed centrocins—have been characterized (12). These peptides are approximately 4 kDa, and each is composed of a heavy chain and a light chain connected by an intramolecular disulfide linker. Centrocins share some homology with distinctins in frogs and some homology with dicynthaurin in tunicates; each of these peptides exhibits in vitro activity against gram-positive and gram-negative bacteria. In fungi, a 10-kDa defensin-like peptide with broad antifungal activity has been isolated from *Helminthosporium victoriae* (13). In plants, an unusual linear cyclotide derivative—termed kalata B20-lin—has been characterized from *Oldenlandia affinis* (14). The linear form of this molecule arises from a premature stop codon that removes the residues essential for the cyclization characteristic of this family of antimicrobial peptides.

The Expanding Host Defense Repertoire

HDPs make up a crucial effector arm of the innate immune system and represent a first line of defense against infection. The overall pattern of HDP expression corresponds to host tissues that are most often exposed to microorganisms (e.g., nearly all external and internal epithelia, such as those found in the skin and in the respiratory, gastrointestinal, reproductive, and urinary tracts) or to cells that are most likely to engage in direct interaction with potential pathogens (e.g., granulocytes, monocytes/macrophages, platelets, lymphocytes, and dendritic cells) (15). Host

defense at particular sites is context specific, with individual tissues expressing unique subsets of HDPs that may be optimally suited to defending against cognate pathogens. This recursive correspondence may reflect a coevolutionary relationship between a given peptide and specific pathogen(s).

Most mammalian HDPs have been isolated from either epithelial or myeloid sources. Yet, recent investigations have documented HDP expression in numerous novel tissues. For example, human β-defensin-2 (hBD-2) expression has been detected in human umbilical vein endothelial cells in culture (16) after stimulation with interleukin-1β (IL-1β) and transforming growth factor β1. Similarly, hBD-2 mRNA has also been detected in a cell line derived from human brain capillary cells after exposure to *Chlamydophila pneumoniae* (17). Beyond the more well-known HDPs, another peptide—dermcidin—has been identified in human tears. Dermcidin, a rare anionic antimicrobial peptide, had previously been characterized only as a component of sweat secretions (18). In nonhuman vertebrates, the ovodefensin peptides of chickens constitute a novel site of HDP expression. Ovodefensins are expressed at a high level in the magnum region of the oviduct, in a context that contributes to the formation of the egg white (19). Ovodefensins are most closely related to the chicken avian β-defensins, although with a different spacing within the cysteine array. These examples highlight the expanding scope of tissues that appear to benefit from protection by HDPs.

Beyond Classical Host Defense Peptide Taxonomy

As described above, the major HDP groups are represented by the defensin and cathelicidin families, which fall into four major structural groups: α-helical, β-sheet, loop, or enriched in particular amino acids. However, in addition to the traditional HDP classification schema, there are now several groups of peptides that could arguably be included under the larger HDP umbrella. Many of these new classes derive from polypeptides or proteins that have previously had an alternate primary classification, such as the chemokines. These polypeptides and proteins, which had primarily been categorized as immunomodulatory and chemoattractant proteins, have repeatedly exhibited potent antimicrobial activities. In addition, it is becoming increasingly recognized that with the finite size of the proteome, many proteins are multifunctional in nature. Therefore, many proteins are being recategorized as having potent secondary and sometimes additional functions. Moreover, examples of HDP domains within larger proteins and HDPs that are proteolytically liberated from a parent molecule have been identified. Finally, careful analysis of HDP processing in situ demonstrates that certain HDPs undergo extensive posttranslational processing to generate a complex mixture of peptide products that have varying efficacies and activities. Several examples are highlighted as illustrations of these concepts in the following section.

It is well established that many HDPs, including the defensins and cathelicidins, have immunomodulating functions; however, certain immunomodulatory proteins may reciprocally have antimicrobial activities. This dual functionality seems true for the chemokine family of peptides. Chemokines have traditionally been characterized as having chemoattractive properties (the term chemokine is derived from the Greek word *kinos*, which means "to take action"). However, in addition to their chemoattractant properties, at least half of the studied chemokines have antimicrobial activities equivalent to and sometimes greater than those of classical HDPs. Chemokines share many structural features with the defensin family of peptides. For example, defensins typically contain three disulfide bonds and have either a triple-β-sheet structure (vertebrates) or a double-β-sheet, single-α-helix structure (invertebrates). In comparison, chemokines contain two disulfide bonds and typically adopt a triple-β-sheet, single-α-helix structure. On the basis of these

structures, the term kinocidin has been coined for chemokines that have antimicrobial properties, reflecting dual chemokine and microbicidal functions of these polypeptides.

Evidence supporting dual functionality of kinocidins as chemoattractants and microbicidal agents has been growing over the past 15 years, with an exponential increase in recent years. Yeaman and colleagues (20, 21) carried out seminal studies demonstrating the potent microbicidal activity of the kinocidin human platelet factor-4 (hPF-4) (CXCL4) isolated from thrombin-stimulated platelets. The authors showed that hPF-4, the fifth most abundant protein of platelets, has in vitro microbicidal efficacy against the human pathogens *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Additional studies demonstrated that kinocidins IL-8 (CXCL8), GRO-α (CXCL1), MCP-1 (CCL2), RANTES (CCL5), and lymphotactin (CL1) were microbicidal for bacteria and fungi (22). IL-8, hPF-4, and lymphotactin had greater activity than the neutrophil defensin human neutrophil peptide 1 (HNP-1) in numerous assays. Kinocidins GCP-2 (CXCL6; 23), MIG (CXCL9; 24), PBP/NAP-2 (CXCL7; 25), CXCL14 (26), and MIP-3α (CCL20; 25) also have exhibited significant antimicrobial activity. Other excellent studies have reported antimicrobial activity for chemokines as well (27, 28).

In several instances, the microbicidal domain within the holokinocidin molecule has been isolated. For hPF-4, a synthetic peptide walk-through library analysis localized the primary microbicidal domain to the C-terminal hemimer, and more specifically to the α-helical domain of the protein (29). Subsequent studies have extended this finding, showing that the microbicidal domains for IL-8 (22) and MIP-3α (25) also localize to their C-terminal α-helices. These findings are not entirely unexpected as the α-helical domain of these and many other chemokines is both cationic and amphipathic in nature. Furthermore, many chemokines have been characterized as having a dimeric or greater multiplex structure, and the α-helical domains lie on the outer solvent/environmentally exposed surface of these multimers (30). A recent study demonstrated a likely evolutionary relationship among kinocidins, classical HDPs, and other peptides—including venoms and toxins not traditionally considered to have antimicrobial activity (31). Together, this body of evidence substantiates potent microbicidal activity for many kinocidins and suggests that this family is an as-yet underappreciated member of the broader family of HDPs.

Cryptic Host Defense Effector Peptides

Another emergent category within the field of HDP biology is the identification of microbicidal peptides and domains within larger proteins. Herein, the nomenclature cryptic host defense effector peptides is proposed for these molecules. In this case, the parent molecule undergoes specific posttranslational processing to liberate one or more antimicrobial peptides. Several examples have come to light recently. This phenomenon occurs for tissue factor pathway inhibitor, a 40-kDa protein that is important for modulation of the coagulation process. Recent studies demonstrate that tissue factor pathway inhibitor undergoes cleavage to generate numerous small antimicrobial peptides with activities similar to those of LL-37 (32). Lysozyme is also subject to proteolytic processing, thereby liberating small potent microbicidal peptides (33). Yet another example occurs with thrombin, which undergoes C-terminal proteolysis in vivo to generate several α-helical antimicrobial peptides (34, 35). These peptides are active against gram-positive and gram-negative bacteria and protective against *Pseudomonas aeruginosa*–induced sepsis (34). Other examples of cryptic antimicrobial peptides include those from complement precursors (36).

Just as proteolytic processing is the mechanism behind the release of HDPs from larger precursors, it is also at the heart of another category of previously underappreciated molecules: HDPs that undergo further processing after deployment. As protein-detection methods have become more sensitive, it has been increasingly observed that certain HDPs may undergo processing at

sites of infection and/or inflammation. For example, dermcidin—the anionic peptide released from eccrine sweat glands—is processed in a way that yields up to 14 variants (18). Similarly, histatins—α-helical peptides secreted from parotid glands into human saliva—generate up to 50 variants (37). Furthermore, LL-37 and other cathelicidins from leukocytes or epithelia undergo a high degree of processing by the enzymes kallikrein-5 and kallikrein-7. In this case, the processing is so extensive that only a small fraction of the generated peptide population corresponds to LL-37 (38, 39).

A new strategy to exploit proteolytic processing of HDPs is also in development. Multimodular propeptides—including proteases and other enzymes—have been designed to activate in response to microbial- or host-derived signals in the context of infection. These molecules, termed context-activated protides, are in preclinical development (31, 40). Protides may have two complementary advantages with respect to their therapeutic potential. First, they have been designed to intensify in the immediate proximity of infection, thus targeting antimicrobial effector modules to sites of microbial density. Second, in contrast to conventional antibiotics, which select for resistant pathogens, protides are designed to provide a survival advantage to organisms that do not express virulence determinant activators. This novel approach to anti-infective strategy has the potential to shift the evolution of pathogens in favor of nonpathogenic phenotypes.

Combinatorial Interplay Among Host Defense Peptide Effector Modules

The significance of such proteolytic events is incompletely understood; however, these events may represent one mechanism by which nature uses a finite genome to generate a proteome with the greatest repertoire for defending against infection. For example, studies of LL-37 suggest that the generated fragments may have pleiotropic effects. The fragments released by kallikrein-5 have a relatively high level of antimicrobial activity, for example, whereas those liberated by kallikrein-7 have a lower level. Furthermore, some of the kallikrein-generated fragments are proinflammatory, whereas others have anti-inflammatory properties. In this respect, it is likely that proteolytic products of antimicrobial peptides modulate the potential host toxicity of certain HDPs (38). Thus, although the functional relevance of these or other multiply processed HDP fragments has yet to be determined, by analogy it is probable that various peptide fragments may have distinct antimicrobial and immunomodulatory effects.

Immune Coordination

Over the past decade, it has become increasingly clear that direct antimicrobial activity may be only one of the functions attributable to many HDPs. Without question, an area of expansive growth within the field of HDP biology has been in the discovery that many classical antimicrobial peptides also have immunomodulatory properties. This concept provides an important link that bridges the innate and adaptive arms of the immune system. Seminal studies initiated more than 10 years ago demonstrated that α- and β-defensins are chemotactic for monocytes, macrophages, T cells, and immature dendritic cells (41–43). One mechanism by which this process occurs was demonstrated for hBD-2, which binds Toll-like receptor 2 (TLR-2) to activate immature dendritic cells as well as facilitate Th1 immune polarization (44). Subsequent studies have reinforced these observations. In particular, the α-defensins HNP-1, HNP-2, and HNP-3 are chemotactic for $CD4^+CD45^+$ and $CD8^+$ T cells. In contrast, the β-defensins hBD-1 and hBD-3 stimulate the migration of memory T cells and immature dendritic cells (45). Furthermore, the human cathelicidin LL-37 also has chemotactic properties (46). For instance, LL-37 is chemotactic for monocytes and neutrophils, thereby increasing the population of phagocytic cells at sites of tissue injury and infection (47).

LL-37 also has chemoattractive effects on keratinocytes in vitro and in vivo (48), which likely play an important role in the process of wound disinfection and healing.

Toll-like receptors, immune modulation, and a bridge to adaptive immunity. Many studies also demonstrate that HDPs function as signal transducers—stimulating cellular release of immunomodulatory effector molecules at sites of injury and infection. For example, LL-37 appears to evoke the release of the cytokine IL-6 and chemokines CXCL8 (IL-8) and CXCL1 (GRO-α) in bronchial epithelial cells through a nuclear factor kappa B (NF-κB)-mediated signaling pathway (49). In keratinocytes, dermcidin upregulates TNF-α, IL-8 (CXCL8), IP-10 (CSCL10), and MIP-3α (CCL20) through G protein and MAPK pathways (50). In mast cells, hBDs and LL-37 can lead to the release of the pruritogenic cytokine IL-31 in a process that is likely mediated through the MAPK pathway (51). In this same report, exposure to hBDs and LL-37 also resulted in mast cell release of IL-2, IL-4, IL-6, GM-CSF, NGF, and PGE_2. As IL-31 is elevated in psoriatic skin mast cells, these HDPs might contribute to this inflammatory process. A related study suggests a more direct role for HDPs in the pathogenesis of psoriasis. In some situations, HDP-mediated signaling appears to initiate an inappropriate immunomodulatory cascade. In psoriasis, LL-37—when complexed with self-DNA or self-RNA—can cause aberrant activation of endosomal TLR-7, TLR-8, and TLR-9 in plasmacytoid and myeloid dendritic cells (52, 53). In turn, such activation may enhance the inflammatory milieu in psoriatic autoimmune disease processes.

As mentioned above, although some reports suggest a proinflammatory role for certain HDPs, alternative studies indicate that some HDPs may also have an important anti-inflammatory function. An emerging body of evidence suggests that the human cathelicidin LL-37 may have anti-inflammatory properties and provide a protective role in sepsis (54). In human monocytic cells, LL-37 was able to dampen the inflammatory response by inhibiting the release of TNF-α (54). Furthermore, LL-37 reduced nuclear translocation of NF-κB, thus altering downstream proinflammatory gene expression. Likewise, in a rat intra-abdominal sepsis model, LL-37 significantly downregulated proinflammatory cytokines, correlating with improved survival rates (55). This anti-inflammatory process relied in part on inhibition of TLR-4 signaling by altering membrane function (33). Together, these studies suggest that certain HDPs may have anti-inflammatory properties under specific environmental conditions.

Potentiation of professional phagocytes. Beyond their microbicidal, chemoattractant, or immunomodulatory functions, an additional property ascribed to certain HDPs is potentiation of professional phagocytes, such as neutrophils and macrophages. Numerous reports suggest that HDPs have direct stimulatory activity on phagocytes and that they function as opsonins, promoting the uptake of microorganisms. For example, Yu et al. (56) showed that LL-37 synergistically acts with IL-1β to enhance production of IL-6, IL-10, MCP-1, and MCP-3 in human monocytes. This report suggests that LL-37 acts directly on monocytes to stimulate release of immunomodulatory agents. Support for a role for HDPs as opsonins comes from several studies. Studies using an engineered peptide modeled on kinocidin helices of the CXCL4 family—termed RP-1—identified a fivefold increase in neutrophil phagocytosis and intracellular killing of *S. aureus* when organisms were preincubated with the peptide (57). Similarly, studies with bacterial permeability increasing protein have shown enhanced phagocytic uptake of an encapsulated and phagocytosis-resistant strain of *E. coli* (58). Finally, cryptdin-2 treatment of macrophages leads to significantly higher levels of intracellular killing of *Salmonella typhimurium* than was observed in untreated macrophages (59). Collectively, these studies suggest that certain HDPs potentiate professional phagocytes as well as enhance pathogen uptake.

In general, HDPs are responsive to host challenges; they are either constitutively expressed or upregulated in response to inflammation and infection. In a broad sense, most α-defensins are expressed in a constitutive manner, whereas β-defensins are often upregulated in response to injury, inflammation, or infection. The cathelicidin LL-37 is constitutively expressed in many cell types, but it also is inducible in keratinocytes and resident skin mast cells (60). One of the primary mechanisms by which HDPs are upregulated in response to tissue injury or inflammation occurs via stimulation of the TLR-class pattern recognition receptor (PRR) family. This family of receptors, known to include more than 19 members, binds pathogen-associated molecular pattern molecules and transduces information through various signaling pathways including AP1 and JAK/STATi (61). The most well-characterized TLRs include TLR-2, TLR-4, and TLR-9, which bind lipoteichoic acid, lipopolysaccharide (LPS), and microbially derived nucleic acid (or CpG sequences), respectively. More recently, numerous intracellular pattern recognition elements—termed NOD-like receptors (NLRs)—have also been characterized. The NLRs are essential for recognition of intracellular pathogen determinants and in particular are important for differentiating microbial versus self-dsDNA. Self-dsDNA is not typically found within the organellar compartments, whereas pathogen-derived nucleic acids are commonly found within lysosomal compartments. The ultimate downstream target for many PRR and NLR signaling events is often the NF-κB transcription factor. Thus, hosts have multiple signal transduction mechanisms to identify and respond to microbial threats with HDPs.

As genomic sequence information becomes increasingly available, the regulatory pathways driving HDP synthesis are being mapped in ever finer detail. This knowledge has confirmed many prior predictions and revealed novel insights regarding HDP constitutive and inducible signaling pathways. For example, human α-defensins HDP-5 and HDP-6, which are primarily expressed in Paneth cells, fall under the regulation of transcription factor-4 (TCF-4) as a part of the Wnt signaling pathway (62). A polymorphism in the promoter region for TCF-4 leads to deficiencies in expression of these defensins and is linked to the incidence of Crohn's disease (63). In contrast, hBDs are responsive to both cell-mediated and infection-stimulated activation pathways (TLR activation pathways). In particular, hBD synthesis is upregulated by the proinflammatory cytokines IL-1β and TNF-α in numerous tissues and models of inflammation (64, 65). Alternatively, the most well-characterized hBD, hBD-2, is upregulated in monocytes and epithelial cells in response to bacterial stimulation of TLRs mediated through NF-κB signaling (66–68). Furthermore, recent evidence supports the concept that certain HDPs may be upregulated by 1,25-dihydroxyvitamin D_3 (VD_3; 69). In particular, DEFB4 (hBD-2) expression is stimulated by IL-1β and VD_3, whereas cathelicidin expression is stimulated by VD_3 only. The DEFB4 promoter has one VD_3 response element and two NF-κB sites, whereas cathelicidin has three VD_3 response element sites and no NF-κB sites. Moreover, synergistic activity of LL-37 and hBD-2 may be important for activity against *Mycobacterium tuberculosis* (70). Together, these findings indicate that the genes encoding HDPs may be responsive to microbial as well as other proinflammatory stimuli.

STRUCTURES, MECHANISMS, AND IMMUNORELATIVITY

Emergence of Unifying Themes

Unifying themes of HDP immunobiology are emerging from a consideration of their evolutionary past and potential biomedical future. From the most primitive prokaryotes and eukaryotes to the most immunologically complex mammals, many HDPs share recurring structural and functional features. These include:

1. Small, cationic, and amphiphilic structure. Nearly all HDPs are relatively small, cationic, and amphiphilic in nature. As these features have been conserved over vast evolutionary distances, they likely represent structural elements that are essential for microbicidal activity. This conservation of functional elements suggests a common mechanism of action, which is thought to occur through disruption of the microbial plasma membrane and rapid energy dissipation for many HDPs.
2. Site-specific expression. Although some HDPs are elaborated systemically, many are expressed as a function of a specific cell type in a specific anatomic context. Examples include histatin expression by parotid glands, cryptdin expression by Paneth cells of the gastrointestinal mucosa, and dermcidin expression by the integument.
3. Ribosomal synthesis and proform storage. Most HDPs are first synthesized by ribosomes and stored as prepro versions for rapid deployment. This mechanism has an advantage over complex metabolite antibiotics, such as the macrolides, which can take considerable time for synthesis and often depend on specific nutritional requirements.
4. Targeting of essential microbial structures and functions. The microbial cell membrane is a structure that is less mutable than specific protein targets. Furthermore, if modified, membranes confer alternate susceptibilities and/or a growth disadvantage. Rapid membrane disruption and energy dissipation are believed to be features of HDP-mediated microbial inactivation that lessen the development of resistance.
5. Modular topology. Emerging recognition of HDP multifunctionality, such as immunomodulatory and antimicrobial functions, has prompted closer evaluation of molecular domains that may confer specific activities. For example, within the kinocidin family of microbicidal chemokines, the extended N-terminal domain is chemotactic, whereas the C-terminal α-helical domain is antimicrobial (22, 29). Connecting these two domains is a disulfide-stabilized, triple-stranded β-sheet motif, termed the γ-core signature motif (6, 31). Analogous γ-core signatures are also found in the immunomodulatory and antimicrobial defensin family and similar peptides, likely mediating unique molecular determinants or modules within them. Furthermore, the growing body of evidence indicating that many HDPs may undergo proteolytic disassembly suggests that specific antimicrobial domains may be liberated at sites of host infection or injury (**Figure 1**).

In the aforementioned respects, HDPs differ from conventional antibiotics that more typically perturb ribosomal and/or enzymatic functions of microorganisms. HDPs are among the few classes of antimicrobial agents for which relatively minimal resistance has been documented in the laboratory. Beyond the evolutionary experiments of nature, most in vitro studies support this concept, revealing little HDP resistance even after many passages in the presence of such peptides. However, the interesting paper by Perron et al. (71) offers insights into artificial conditions that may select for resistance to an HDP analog after 600–700 generations in vitro.

New Insights into Potential Targets of Host Defense Peptides

This review focuses on recent advances in the development of HDPs as potential novel anti-infective agents. Such advances derive from a greater understanding of HDP mechanisms of action and resistance. Examples of emerging insights include clearer understandings of:

- the mechanisms by which HDPs interact with and permeabilize the target cell membrane (72);
- Lipid II as a specific target for HDPs or analogs (73, 74);
- LPS as a specific target for NK-2 and other HDPs or fragments thereof (75, 76);
- DNA or other intracellular targets of HDP activity (77–79);

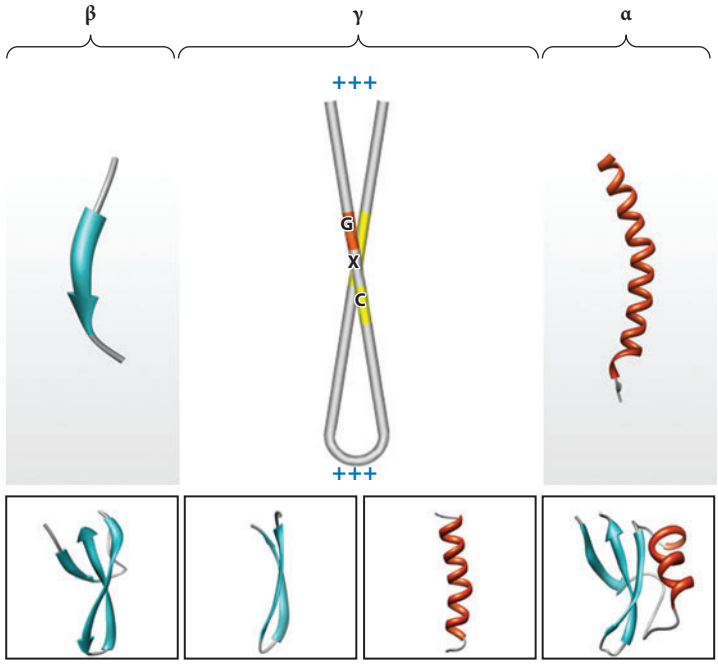

Figure 1

Schematic representation of host defense peptide (HDP) modular topology, illustrating the molecular dissection of a prototypic HDP. HDPs' multiple immunomodulatory and antimicrobial functions are increasingly localized to distinct structural domains. An example typified here is that of the kinocidin family of multifunctional HDPs. Kinocidins are characterized as having an N-terminal chemotactic domain (*aqua*; extended/random coil; β), an interposing disulfide-stabilized motif with positively charged termini (*gray*; γ-core structure; γ), and a C-terminal antimicrobial domain (*red*; helix; α). Iterations of the γ-core motif are present in all classes of disulfide-stabilized HDPs and related molecules, including defensins, protegrins, and similar structures. This evolutionarily conserved GXC motif in the γ-core domain [glycine (G), *orange*; variable (X), *gray*; and cysteine (C), *yellow*] is identified. Recent evidence suggests that many HDPs undergo proteolytic processing, which may liberate specific modules for deployment at sites of host infection or tissue injury. Lower panels, left to right: human neutrophil peptide 1; protegrin-1; magainin; and Ah-AMP-1 (plant defensin).

- the roles of HDPs in cooperation with neutrophil extracellular traps as a novel mechanism of host defense (80);
- degradation by extracellular peptidases or proteases as a mechanism by which pathogens reduce their susceptibility to HDPs (81–84);
- global regulatory functions in HDP susceptibility or resistance (84, 86);
- efflux as a potential HDP resistance mechanism in bacteria (87); and
- fungal cell wall components as specific targets for HDPs such as NaD1 and histatins (88, 89).

Comprehensive reviews of these and related topics are available elsewhere (90, 91). (See also the Visualizing Host Defense Peptides interactive tool in the **Supplemental Material**; to access the **Supplemental Material**, follow the **Supplemental Materials link** from the Annual Reviews home page at **http://www.annualreviews.org**).

Supplemental Material

Molecular Basis for Selective Toxicity

The application of increasingly sophisticated analytical methods, such as nuclear magnetic resonance, Fourier transform infrared spectroscopy, and surface plasmon resonance, to questions of HDP structure-activity relationships has reinforced prior hypotheses regarding HDP mechanisms of action. Moreover, such methods have afforded deeper insights into the molecular basis for selective toxicity of many such molecules. Integrating advances regarding HDP structure-activity relationships suggests that selective toxicity results from a fine balance among overall peptide conformation, cationicity, and amphipathicity—rather than any radical aspects thereof.

Many studies reinforce the hypothesis that HDP cationicity is essential for the initial attraction to, and selectivity for, the relatively electronegative target pathogen. Most microorganisms are significantly more electronegative than neighboring host cells owing to intrinsic structural and physiologic features. First, a majority of microorganisms have a relative abundance of anionic phospholipids, such as phosphatidylglycerol, phosphatidylserine, and cardiolipin (91). These anionic phospholipids are localized largely to the outer leaflet of the microbial plasma membrane, endowing this surface with a net negative charge. In comparison, eukaryotic cell membranes are typically composed of more neutral zwitterionic phospholipids and cholesterol, and they further sequester anionic lipids to the inner leaflet of the plasma membrane. Second, negatively charged components of the cell wall, such as LPS of gram-negative organisms and teichoic and lipoteichoic acids of gram-positive organisms, contribute to the overall anionic nature of the bacterial outer surface. Finally, the average potential difference across microbial plasma membranes (−130 mV to −150 mV) is significantly greater than that of most mammalian membranes (−90 mV to −110 mV) (92), further enhancing the overall electronegativity of the microorganism. Together, these features make the microbial surface markedly more electronegative than that of surrounding host cells and hence more selectively attractive for cationic peptides. Supporting these concepts are the recent observations that net increases in surface charge (e.g., via lysinylation of lipids and related mechanisms) confer somewhat reduced susceptibility to sublethal antimicrobial peptide concentrations in austere conditions in vitro (93–95).

As described above, a certain degree of cationicity is likely essential for selectivity for the pathogen surface. Therefore, one mechanism by which to attenuate the toxicity of candidate HDP therapeutics has been to increase the net cationicity, allowing for increased selectivity and reduced toxicity. In the case of brevinin-2-related peptide, an HDP isolated from frog skin, substituting a Lys for the native Asp at position 4 of the peptide led to a fourfold increase in potency against *E. coli* and twofold increases in activity against *S. aureus* and *C. albicans*. Furthermore, this increased cationicity did not lead to an increase in hemolytic activity (96). Although increasing net cationicity can improve efficacy for certain peptides, studies with model peptides demonstrate that when cationicity or cationic density is excessive (e.g., beyond +10; 97, 98), efficacy or therapeutic index can be reduced. High levels of cationicity may electrostatically trap peptides at the anionic phospholipid bilayer and prevent penetration of the microorganism.

Another HDP property important for microbicidal activity as well as selective toxicity is peptide amphipathicity/hydrophobicity. A growing body of evidence reinforces the hypothesis that the hydrophobic surface of many HDPs is essential for permeabilization of microbial plasma membranes. Although most studies have utilized model membrane lipids, they repeatedly demonstrate that individual hydrophobic residues as well as larger hydrophobic domains are the means by which HDPs penetrate membrane structures (98). One further refinement of this theory suggests that an "imperfect" hydrophobic domain may be more efficacious than a purely nonpolar region. Many natural HDPs include a polar single-residue or multiple-residue "break" in the midst of a hydrophobic domain. Such an "imperfection" may allow for deeper integration into the

phospholipid bilayer as polar elements drag phospholipid head groups into the membrane (72). However, whereas hydrophobic elements are essential for membrane permeabilization, they can also lead to host cytotoxicity. Studies with model HDPs demonstrate that as peptide hydrophobicity increases, indiscriminant binding to mammalian cells increases as well. For example, novicidin, a derivative of the sheep cathelicidin SMAP-29, was found to be toxic in preliminary trials of drug efficacy. However, the toxicity of this peptide was mitigated by reducing net hydrophobicity through replacement of a leucine with the less hydrophobic alanine. This modification reduced nondiscriminant binding while preserving efficacy (99). Ultimately, the design of novel therapeutic HDPs should include careful consideration of net hydrophobicity to maximize peptide efficacy while minimizing host toxicity.

One relatively recent development in the field of HDP selective toxicity comes from the observation that many defensins share a high degree of sequence and structural identity with certain classes of peptide toxins (31). This level of identity is characterized by the near superimposability of certain defensin peptide backbones with those of peptide toxins from mollusks, arthropods, and reptiles. In some cases, the toxin gene is derived from the duplication and modification of a gene for a somatic HDP (100). The mechanism by which most of these peptide toxins work is through the occlusion of eukaryotic ion channels, which often leads to paralysis and sometimes death of the target organism. Given the sequence and structural similarities between certain defensin peptides and peptide toxins, it would seem prudent to consider the possibility that peptide toxicity might arise from partial or limited interaction with ion channels. As the toxin residues that interact with ion channel surfaces have been well characterized, modulation of the corresponding HDP residues should be relatively straightforward. Studies to further this field of investigation are ongoing.

Influence of Microenvironment on Host Defense Peptide Efficacy

As the field of HDP immunobiology has become more sophisticated, numerous investigators have become aware that traditional in vitro assays may be highly artificial and serve as poor models of HDP activity in vivo. HDPs likely interact with other blood and/or tissue components that may significantly impact their activity and efficacy. In general, more representative assay systems are not typically used because of the increased number of variables in physiologically relevant microenvironments. In spite of these obstacles, numerous investigators have modified their HDP assay conditions to more closely reflect a native environment. Gallo and coworkers (101) devised a biologically relevant carbonate-based buffer assay system that contains physiologic levels of NaCl. LL-37 and defensin HDPs, which are normally inhibited by serum and physiologic NaCl (125 mM) concentrations, were shown to be highly efficacious in the presence of both these agents when assayed in a carbonate-containing buffer. Importantly, the carbonate-based buffer system induced global changes in *S. aureus* and *E. coli* gene expression, leading to alterations in cell wall thickness and sigma factor B expression. These changes were correlated with increased susceptibility to HDPs. Yeaman and colleagues (102) have also created an assay system to assess HDP activity in a physiologic context. These investigators developed a plasma-, serum-, and whole-blood-based assay to measure HDP activity. In this study, the HDPs RP-1 and RP-11 (modeled on the kinocidin CXCL4) were assessed for activity in traditional media as well as in complex fluid biomatrices. RP-1 and RP-11 were significantly more active in the complex biomatrices of serum, plasma, or whole blood than in artificial media. Furthermore, preincubation of peptides in plasma or whole blood did not lead to a loss in efficacy, suggesting that these peptides are not subject to serum inactivation. Collectively, the aforementioned studies suggest that artificial-media-based HDP assays may underestimate HDP potency in physiologic settings and indicate a need for continued refinement of HDP assay systems.

Synergistic Interactions Among Host Defense Peptides

The reductionist nature of standard HDP assays typically fails to address the possibility of microbicidal synergism among HDPs. In many well-characterized biological systems, closer scrutiny has revealed the likelihood for physiologically relevant protein-protein interactions. Homo- or heteromeric complexes and seemingly infinite protein-protein contacts are more often the rule than the exception. Given these findings, and the complexity of the host-pathogen environment, it is not surprising that HDP synergism is evident. Tang et al. (21) showed that two platelet microbicidal proteins—platelet factor-4 and connective tissue-activating peptide-3—were synergistic against *E. coli*. More recently, Chen et al. (103) demonstrated that many combinations of hBD-1, hBD-2, and/or hBD-3 with or without LL-37 led to synergistic microbicidal action against *S. aureus* with a similar but less pronounced trend for *E. coli*. This effect was enhanced for hBDs at low pH (4.6), suggesting the possibility for greater efficacy in the phagolysosomal compartment or in tissue sites with reduced pH. A similar synergism has been documented for cationic peptides and anti-HIV activity. For example, the full complement of cervical HDPs was required for efficient anti-HIV activity (104). In another report, synergism was observed for hBD-2 and hBD-4 against a mucoid strain of *P. aeruginosa* (105). In summary, these observations suggest the possibility of synergism among many mammalian HDPs. Perhaps such a combinatorial approach is a means by which the potential repertoire of HDPs can be exponentially increased to address highly mutable microbial targets.

Another important area of investigation has been to assess the potential for HDP synergism with conventional antibiotics. This may allow for rapid development of a novel and potentially efficacious spectrum of cocktail anti-infective therapies. As microbial permeabilization is one of the primary mechanisms by which HDPs exert their effects, perforating microbes with an HDP effector may render them more susceptible to conventional antibiotics. A combinatorial approach that evaluates various HDP/antibiotic pairings has the potential to yield a highly efficacious new class of therapeutics. Numerous studies offer proof-of-principle results that support this approach. Checkerboard studies using the β-defensin peptides HE2-α and HE2-β from the male reproductive tract demonstrated synergy with conventional antibiotics against *E. coli* (106). In particular HE2-α and HE2-β were synergistic with ciprofloxacin and doxycycline, antibiotics that are commonly used to treat reproductive tract infections. Likewise, fractional inhibitory concentration index (FIC_{index}) analyses found high degrees of synergy between the echinocandins caspofungin and anidulafungin and the frog-skin-derived HDP ranalexin (107). These studies also suggest that the efficacy of traditional antimicrobial agents relies in part on favorable interactions with endogenous HDPs.

THERAPEUTIC PROSPECTS

There is an urgent need for novel classes of antimicrobial agents that have distinct mechanisms of action, and many investigative groups have initiated development of HDPs and HDP mimetics as potential anti-infective agents. Herein, prototypic examples of these developments are considered.

Pharmacologic Barriers and Breakthroughs

Initial attempts to apply HDPs as systemic therapeutics have been disappointing, and as of 2011, there is still no HDP-derived anti-infective agent for systemic use. Early studies with bacterially derived polymyxin E and gramicidin S revealed these molecules to be highly active in vitro, but they caused erythrocyte lysis and systemic toxicity in vivo (108, 109). Despite these limitations,

the therapeutic potential of polymyxin and gramicidin has been harnessed in commercially available antibacterial topical ointments. One of the first eukaryotic HDPs tested for systemic use was indolicidin, a tryptophan-rich tridecapeptide cathelicidin isolated from bovine neutrophils. Unfortunately, as with the bacterially derived peptides, even low levels of this peptide caused systemic toxicity and death in experimental animals (110). Several other reports indicated that mammalian neutrophil defensins and bactenecin were cytotoxic for certain subsets of mammalian cells (111, 112).

The aforementioned reports are from the published literature, but much of the work in this area has been proprietary—and relatively little information has been available for review. Nonetheless, these early disappointments, along with clinical-trial failures of topical peptides, have dampened the initial fervor for the development of HDPs as systemic anti-infectives and have led to a search for alternative HDP templates and/or alternate delivery formulations that might render such molecules more druggable and less toxic.

Insights into Host Defense Peptide Pharmacokinetics and Pharmacodynamics

A major technological barrier in the development of HDP-based anti-infectives has been in the area of pharmacokinetics. Conventional antibiotics are typically small molecules that have been optimized for tissue distribution and penetration, plasma half-life, and clearance. In contrast, HDPs are often an order of magnitude larger than conventional antibiotics and are often more structurally flexible. As many HDP-based anti-infectives are either wholly or partially protein based, they are subject to endogenous immune surveillance and degradation mechanisms that may limit their immune tolerance and stability. There has been a striking paucity in formal pharmacologic and toxicologic evaluation of anti-infective peptide candidates and little reported information regarding their half-life, distribution, protein binding, metabolism, or mechanism of clearance.

Pharmacology of antimicrobial peptides. A recent study in animals by Brouwer et al. (113) provides some new insights regarding the pharmacology of anti-infective peptide candidates. Technetium-99m (^{99m}Tc) was used to label a number of HDPs [human lactoferrin-derived peptide 1-11 (hLF1-11), HNP, and synthetic histatin variants], and their pharmacokinetic distribution was monitored by scintigraphic imaging. Importantly, results demonstrated that all of the labeled HDPs localized specifically to the site of infection within a short time period (2 h). Approximately 1.5% of the administered peptide [^{99m}Tc-hLF(1-11)] accumulated at the infection site, indicating an acceptable rate of retention. Beyond localization, this study also verified the efficacy of the labeled HDPs as measured by significant reductions in bacterial colony-forming units (CFUs). In addition, ^{99m}Tc-hLF(1-11) collected in urine at 24 h still had potent microbicidal activity, indicating little if any protease-mediated or other inactivation. With respect to pharmacokinetics, systemically administered ^{99m}Tc-hLF(1-11) was cleared primarily through the liver and through the kidneys and urinary tract, whereas systemic ^{99m}Tc-HNP was cleared primarily through the kidneys. Together, these data demonstrate that three classes of HDPs localized specifically to sites of infection and had good microbicidal activity in vivo. One additional application for this technology may be to utilize labeled HDPs to identify sites of infection, thus discriminating between sterile-inflamed and infected sites.

Formulation and delivery. One approach to deliver HDP-based therapeutics systemically has been to administer HDPs in lipid-based formulations. Liposomal formulations, developed for use with conventional anti-infectives, constitute one of the more well-characterized delivery

systems. Liposomal formulations may offer advantages to enhance drug disposition as they (*a*) afford measured peptide release; (*b*) increase peptide stability, peptide solubility, and plasma half-life; and (*c*) can reduce peptide toxicity by limiting nonspecific interactions. Studies with the antifungal agent amphotericin B demonstrated that liposomal delivery dramatically reduced toxicity and increased plasma half-life with efficacy equivalent to that observed in nonliposomal formulations (114). With respect to protein-based HDP antimicrobials, indolicidin was used in one of the earliest reported attempts to reduce systemic toxicity through this approach. Liposomal delivery of indolicidin led to significantly reduced toxicity in mice, as evidenced by improved systemic tolerance: Maximum tolerable dose with no deaths was 0.4 mg kg^{-1} for free indolicidin versus 40 mg kg^{-1} for indolicidin–palmitoyl oleoyl phosphatidyl choline (110). More recently, liposomal formulations have been used to mitigate the systemic toxicity of polymyxin E, which has been limited to topical applications. Liposomal encapsulation of polymyxin E had significantly reduced toxicity in an animal model (115).

Another delivery substrate evaluated as a means to reduce toxicity and increase systemic exposure is human serum albumin (HSA). As a carrier, HSA provides several advantages, including host compatibility, equilibrium kinetics with endogenous free fatty acids, and, in some cases, increased tissue penetration due to close association with plasma membranes. In a recent study by Hussain & Siligardi (116), lipid-free HSA was used as a carrier for a lipotagged antimicrobial nonapeptide. HSA-nonapeptide complexes exhibited twice the efficacy against *S. aureus* compared with the lipopeptide alone, suggesting increased biostability.

These examples suggest that reductions in toxicity and increased plasma half-life seen for conventional antibiotics can also be applied to anti-infective peptide candidate pharmaceutical agents. Thus, although certain HDPs may have high levels of cytotoxicity when outside their native context, it appears possible to attenuate nonspecific or untoward interactions through delivery of HDPs as either lipid-based HSA or other formulations.

Commercial Perspectives: Evaluation of Anti-Infective Peptides in Ex Vivo and In Vivo Models

Preclinical evaluation of HDP or analog candidates has been a limiting step in the development of anti-infective peptide therapeutics. Limitations have arisen in part from the difficulty in generating quantities that are sufficient for statistically powered evaluation in rodents or larger species. In addition, the lack of pharmacologic studies has limited the interpretation of peptide efficacy versus pharmacology in study outcomes. Moreover, the use of animal models in which bona fide infection is not established, or in which peptide and pathogen are placed out of context, has obscured interpretation of in vivo efficacy of candidate molecules. The following section considers recent advances in preclinical and clinical evaluation of the development of HDPs as therapeutics.

Preclinical studies of native HDP efficacy have been performed in nonsystemic and systemic models. *P. aeruginosa*, a gram-negative pathogen, is one of the leading causes of hospital-acquired ventilator-associated pneumonia. Importantly, *P. aeruginosa* is becoming increasingly multidrug-resistant and hence is associated with significant rates of morbidity and mortality in the hospital setting. Two recent studies assessed the utility of native HDPs given as intranasal formulations in the treatment of lung models of *P. aeruginosa* infection. A study using a truncated form of LL-37 showed that intranasal administration of the peptide led to complete eradication of biofilm and significantly reduced total CFUs in nasal diluent (117). In the second study, the efficacy of colicin, a well-characterized HDP derived from *Bacillus polymyxa*, was assessed in a *P. aeruginosa* model of pneumonia. In this case, administration of colistin in combination with rifampin led to decreased production of inflammatory cytokines and significantly enhanced survival rates (118). Whereas

the aforementioned studies examine intranasal intervention models, an alternate gene therapy mode of HDP administration has also been described. In this case, a plasmid encoding the human β-defensin hBD-2 was transfected into murine lung through the use of polyethylenimine. After 48 h, a challenge of *P. aeruginosa*–coated beads was administered. Plasmid-mediated expression of hBD-2 led to a significant reduction in bacterial load and inflammatory cytokines, as well as a milder infection overall (119). Together, these data suggest that intranasal application of HDPs or HDP-expressing plasmids may be minimally toxic while simultaneously efficacious against *P. aeruginosa* infections in the lung.

Recent studies have also assessed the systemic efficacy of native HDPs in numerous relevant infection models. Multidrug-resistant *S. aureus* infections pose a serious clinical threat and are the cause of a growing number of community- and hospital-acquired infections. The frog-skin-derived HDP ranalexin in combination with lysostaphin has recently been assessed for efficacy against multidrug-resistant *S. aureus* in a murine systemic infection model (120). Doses of 12 mg kg^{-1} of ranalexin and 0.1 mg kg^{-1} of lysostaphin reduced kidney microbial burden by 1 $\log_{10}$ CFU g^{-1} when compared with either treatment alone or with controls. Several recent studies have also investigated the efficacy of systemic native HDP administration versus numerous gram-negative infection models. Two HDPs, bactenecin (a proline-rich bovine cathelicidin) and cryptdin-2 (an α-defensin from murine species), have been tested in *S. typhimurium* infection models. Systemically administered bactenecin (30 mg kg^{-1}) led to significantly increased survival rates and reduced CFU g^{-1} per organ despite rapid clearance (121). Similarly, cryptdin-2 infusion (10 μg per animal) led to clearance of *Salmonella* from the liver, spleen, and intestine (59). A recent study has also demonstrated the capacity of the synthetic peptide RP-1 to exert efficacy against *S. aureus* in an established biofilm model of infection (122). Animals with established (7-day) catheter biofilm infections were treated intravenously with RP-1 alone or with a combination of RP-1 and vancomycin. After four treatment days, RP-1 had efficacy equivalent to that of vancomycin in reducing catheter-associated bacterial densities. Moreover, synergism between RP-1 and vancomycin was demonstrated in this model, and greater efficacy was observed when the two agents were combined. The aforementioned studies offer proof-of-principle evidence that suggests the potential efficacy of systemically delivered HDPs against clinically relevant models of infection.

Progress in Development of Therapeutic Candidates

The studies described above highlight recent developments in the use of native HDPs as therapeutic agents. However, the trend within the field of HDP-based pharmaceuticals is toward development of modified peptides and/or HDP mimetics to address the shortcomings of native HDPs, including uncertain bioavailability, stability, toxicity, and immunologic recognition. Furthermore, the cost and challenges of commodity-scale production of HDPs have been historical barriers to development. Investigators have taken an alternative strategy of developing partially or wholly synthetic HDP analogs that recapitulate the microbicidal potency of native HDPs but are not subject to many of their pharmacokinetic limitations. These synthetic analogs fall into two major categories: peptide-based therapeutics and peptide mimetics (peptidomimetics). Peptide-based therapeutics are usually derived from native HDPs optimized for efficacy and are typically constructed using amino acid–based synthetic chemistry. In contrast, HDP mimetics frequently derive only their basic physicochemical properties from HDPs and use partial or non-peptide-based chemistries to optimize efficacy of minimized structures.

Although progress toward clinical development of anti-infective peptides has been slow, numerous molecules are in preclinical or clinical evaluation. The majority of these compounds are based on naturally occurring HDPs of mammals and other organisms. **Table 1** considers

Table 1 Examples of host defense peptides (HDPs) or HDP-based candidates in therapeutic development

Compound	Template	Structure	Mode	Stage	Indication
Access Pharmaceuticals, Inc.					
Pexiganan	Magainin	22-aa helix	Topical	Phase III	Diabetic foot ulcer
AM-Pharma					
hLF1-11	Lactoferrin	11-aa linear	Systemic	Phase IIa	Transplant infection
Ardea Biosciences, Inc.					
IB-367	Protegrin	17-aa congener	Oral	Phase III	Oral mucositis
BioLineRx Ltd.					
BL-2060	Computational	Oligo-acyl-lysine	Peritoneal	Preclinical	Sepsis
Cadence Pharmaceuticals, Inc.					
Omigard	Indolicidin	Indolicidin congener	Catheter	Phase III	Bacteremia
			Catheter	Phase III	Catheter site infection
Ceragenix Pharmaceuticals, Inc.					
Ceragenins CeraShield/ CSA-13	HDP Cationic amino sterol	Peptidomimetic	Catheter	Preclinical	*Escherichia coli*
Cutanea Life Sciences					
Omiganan	Indolicidin	Indolicidin congener	Skin	Phase II	Rosacea papulopustular
Exponential Biotherapies, Inc.					
EA-230	hCG	hCG congener	Systemic	Phase II	Sepsis/endotoxemia
Helix BioMedix, Inc.					
HB1345 HB1275	Computational	Lipohexapeptide	Topical, Systemic	Preclinical	Antibiotic-resistant infection
Inimex Pharmaceuticals, Inc.					
IMX942	Innate defense regulator	Peptide	Systemic	Preclinical	*Streptococcus pneumoniae*
Lytix Biopharma AS					
LTX-109	HDP	Peptide	Systemic	Preclinical	MRSA, VRE, and *Pseudomonas aeruginosa*
Novozymes					
NZ2114	Plectasin	Congener	Systemic	Preclinical	Gram-positive infection
Pacgen Biopharmaceuticals Corp.					
PAC-113	Histatin	Histatin-5 congener	Oral	Phase IIb	Candidasis
PepTx, Inc.					
PTX002 PTX005	α-Helical HDP	Peptide	Peritoneal	Preclinical	Gram-negative infection
Pharma BAM Spb, Ltd.					
Glutoxim	Unknown	Hexapeptide with disulfide	Systemic	Phase II	Cancer
PolyMedix, Inc.					
PMX-30063	Defensin	Peptidomimetic	Systemic	Phase II	Gram-positive infection
Polyphor Ltd.					
POL7080	Protegrin	Peptidomimetic	–	Preclinical	Gram-negative infection

(*Continued*)

Table 1 (*Continued*)

Compound	Template	Structure	Mode	Stage	Indication
XOMA Ltd.					
XMP.629	BPI	BPI functional domain II	Topical	Phase III	Gram-positive agent of acne
rBPI21	BPI	–	Systemic	Phase III	Gram-negative meningococcemia

Abbreviations: aa, amino acid; BPI, bacterial permeability increasing protein; hCG, human chorionic gonadotropin; HDP, host defense peptide; hLF, human lactoferrin-derived peptide; MRSA, multidrug-resistant *Staphylococcus* aureus; VRE, vancomycin-resistant enterococci.

Supplemental Material

examples of native and mimetic HDP-derived molecules being developed as therapeutic candidates. More detailed information regarding these and related examples can be found in the **Supplemental Material**.

SUMMARY AND PROSPECTUS

HDPs are novel antimicrobial agents that have been isolated from every organism from which they have been sought and have been retained over an evolutionary time span. The unique biophysical properties of HDPs allow for potent, broad-spectrum activity and selective toxicity toward microbial pathogens. HDPs differ from conventional antibiotics in that their primary mechanism of action is thought to be through permeabilization of microbial membranes, a property that may allow for relative immunity to bacteria-resistance mechanisms. On the basis of these properties, HDPs are obvious candidates for pharmaceutical development, as they represent an entirely novel class of anti-infective agents. However, initial pharmacologic endeavors using HDPs were discouraging. Peptide-based therapeutics have pharmacologic shortcomings, including limited bioavailability, poor metabolic stability, inherent toxicity, and the potential for immunologic recognition. Furthermore, the cost and difficulty of synthesis of HDPs at a commercial scale were prohibitive in many cases.

Insights gleaned from early studies in the development of HDPs as pharmaceuticals have led to numerous modifications and iterative refinements in the design of candidate HDPs. As a result, numerous novel HDP-based and mimetic anti-infectives have been designed to overcome the limitations described above. Furthermore, innovations in synthetic and recombinant methods of production have led to cost-effective solutions that allow for large-scale production of candidate pharmaceuticals. Collectively, these efforts have led to commercial development of more than 20 unique HDP-based pharmaceuticals; a number of candidates are entering Phase III clinical trials.

The advent of contemporary antibiotics and vaccines prompted the U.S. Surgeon General William Stewart to suggest that "it was time to close the book on infectious diseases" in the late 1960s. However, even as such words were spoken, microbial evolution was at work, leading to the generation of new organisms that would be resistant to antibiotics not invented until decades later. In the twenty-first century, this same evolutionary plasticity will continue to render some pathogens resistant to most if not all conventional antibiotics. These realities make clear the need for development of novel classes of anti-infectives proven over an evolutionary time frame to have durable efficacy against even the most refractory pathogens. Novel anti-infectives based on HDPs may represent such a class of agents, as they have retained efficacy against even the most hardened microbes. Although initial setbacks may have discouraged or delayed pharmaceutical development to date, recent technological breakthroughs suggest that HDP-based anti-infectives will be a platform for the anti-infectives of tomorrow.

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N.Y.Y. participates in research programs sponsored by NovaDigm Therapeutics, Inc. M.R.Y. is a founder and shareholder of NovaDigm Therapeutics, Inc., which targets antibiotic-resistant infections. N.Y.Y. and M.R.Y. are founders and shareholders of ImmunoTx, Inc., which develops peptide-based and other novel anti-infective technologies. M.R.Y. is also founder of Metacin, Inc., a multimedia and consulting service for research and education. The authors have no specific conflicts of interest with respect to this review and were not paid by the aforementioned entities to write it.

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Errata

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