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Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies

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Short cationic amphiphilic peptides with antimicrobial and/or immunomodulatory activities are present in virtually every life form, as an important component of (innate) immune defenses. These host-defense peptides provide a template for two separate classes of antimicrobial drugs. Direct-acting antimicrobial host-defense peptides can be rapid-acting and potent, and possess an unusually broad spectrum of activity; consequently, they have prospects as new antibiotics, although clinical trials to date have shown efficacy only as topical agents. But for these compounds to fulfill their therapeutic promise and overcome clinical setbacks, further work is needed to understand their mechanisms of action and reduce the potential for unwanted toxicity, to make them more resistant to protease degradation and improve serum half-life, as well as to devise means of manufacturing them on a large scale in a consistent and cost-effective manner. In contrast, the role of cationic host-defense peptides in modulating the innate immune response and boosting infection-resolving immunity while dampening potentially harmful pro-inflammatory (septic) responses gives these peptides the potential to become an entirely new therapeutic approach against bacterial infections.

Short cationic amphiphilic peptides are present in virtually every life form as Nature's antibiotics[1,2]. In mammals, these peptides often have rather weak antimicrobial activity under physiological conditions, and their ability to modulate the immune response through a variety of mechanisms may be more important[3,4] (**Fig. 1**). Both functions are an integral part of the process of innate immunity, which itself has many of the hallmarks of successful anti-infective therapies, namely rapid action and broad-spectrum antimicrobial activities. Consequently cationic peptides are being considered as a new generation of antibiotics, as well as innate immune modulators. We refer to these peptides by the group name host-defense peptides, to capture their broader functions in innate immunity and as cationic antimicrobial peptides when they have direct antimicrobial activity under physiologically meaningful conditions.

As antibiotics, cationic antimicrobial peptides have a mixed history. The cationic peptides polymyxin B and gramicidin S have been used in the clinic and as topical over-the-counter medicines for a long time, and the cationic lantibiotic nisin is used as an antimicrobial food additive. In contrast, despite several series of clinical trials, only one of the new-generation (designer) cationic antimicrobial peptides has demonstrated efficacy in phase 3a clinical trials. Nevertheless, given their exceptionally broad activity spectra—which for a single peptide can include activity against Gram-negative and Gram-positive bacteria, fungi as well as viruses and parasites—substantial interest remains in exploiting the potential of these molecules.

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In this review we describe the promise and challenges for this intriguing and diverse set of molecules, and describe the routes to maximizing their potential. We then describe how their activity in regulating innate immunity may provide an entirely new approach to therapy of infections—one that is sorely needed in this 'antibiotic-resistance era'.

Properties and natural roles of host-defense peptides

Cationic host-defense (antimicrobial) peptides are gene-encoded and derive from precursor peptides through one or more proteolytic activation steps. They can be defined as being short (10–50 amino acids), with an overall positive charge (generally +2 to +9) and a substantial proportion (≥30%) of hydrophobic residues[1,2]. These properties permit the peptide to fold into an amphiphilic structure in three dimensions, often upon contact with membranes, so they form separate patches rich in positively charged and hydrophobic amino acids. Folded peptides fall into four broad structural groups: β-sheet peptides stabilized by two to four disulfide bridges (for example, human α- and β-defensins, plectasin or protegrins); α-helical peptides (for example, LL-37, cecropins or magainins); extended structures rich in glycine, proline, tryptophan, arginine and/or histidine (for example, indolicidin); and loop peptides with one disulfide bridge (for example, bactenecin).

There is an enormous diversity of sequences, and similarity often is found only within defined groups of host-defense peptides from closely related species. A database for eukaryotic host-defense peptides with 895 entries (as of November 2004) has been established[5], and selected examples of primary and spatial structures of some of these peptides are given in **Figure 2**. It has been argued that the immense diversity of cationic peptides arises from their antimicrobial function as well as the different pathogenic microbe challenges they face in each host organism. It is worth noting, however, that several proteins involved in immunity and reproduction show similar 'rapid evolution' to the host-defense (antimicrobial) peptides[6]. Any given mammalian organism

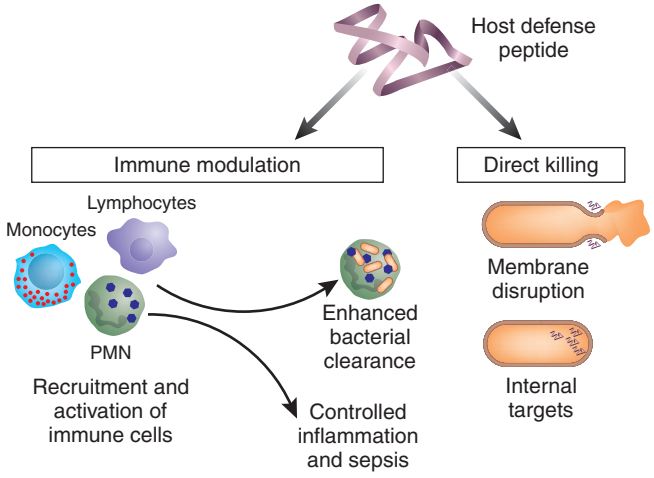

Figure 1 Biological roles of host defense peptide. Both direct antimicrobial killing and innate immune modulation occur with such peptides although certain peptides have one or the other activity preferentially. For a more complete outline of direct killing and immune modulation see refs. 1–4.

might have dozens of different peptides, and these peptides presumably have overlapping roles with respect to both their antimicrobial and innate immune–modulating activities. Thus, it is perhaps unsurprising that mouse gene knockouts deleting any single peptide have rather mild phenotypes, and there are rather few human deficiencies in such peptides (and those that exist such as specific granule deficiencies and Morbus-Kostmann syndrome are complicated in having other underlying defects).

Host-defense peptides vary substantially even among mammalian species[7]. For example, the cathelicidin peptides mouse CRAMP and human LL-37 share only 67% homology, and both species lack the substantial variety of cathelicidins in cattle; mice lack neutrophil α-defensins that are the most prevalent protein in human neutrophils; and cattle completely lack α-defensins in the gut and neutrophils. Indeed, there are substantial variations in defensins even among primate species[8], and these genes are considered to be subject to positive selection and to be among the most rapidly evolving group of mammalian proteins. It seems possible that the rapid coevolution of host-defense peptides, of innate immune systems of which they are a part, as well as of corresponding microbial counter strategies, including resistance and virulence (anti-immunity) strategies, has helped to drive diversity in all biological kingdoms[4,9].

Some themes among the structures of host-defense peptides do exist, however. Mammalian gene structures for the so-called cathelicidins and defensins are relatively conserved, and sequences are conserved in their precursor (pre-pro) regions. Also within the disulfide bridge–containing peptides, a three-dimensional structural motif, the so-called gamma core, has been conserved throughout 2.5 billion years of evolution, and is also present in membrane-active toxins and chemokines, indicating possible evolutionary relationships and the potential role of such a motif in the effector functions that govern host-pathogen relationships[10].

Unlike the β-sheet peptides, the α-helical and extended peptides (which include many cathelicidins) tend to be highly flexible in solution, and adopt amphipathic structures only upon contact with membranes and membrane-mimicking environments. This also holds true for many bacterial peptides (termed bacteriocins), even when they contain one or two disulfide bonds. Among the bacteriocins of Gram-positive bacteria, there is a particular group, the lantibiotics (lanthionine-containing peptide antibiotics), which are characterized by thioether-based intramolecular rings resulting from post-translational modifications of serine (or threonine) and cysteine residues (for example, nisin and mersacidin; **Fig. 2**)[11]. Lanthionine rings, some of which represent conserved binding motifs for recognition of specific targets, create segments of defined spatial structures in the peptides[12]. These ring structures also provide stability against proteases, possibly including the antigen-processing machinery, because antibodies against highly cross-bridged antibiotics, such as mersacidin, are very difficult to obtain.

In addition to ribosomally synthesized antimicrobial peptides, microbes also produce peptide antibiotics of broad structural diversity using large multifunctional enzymes, the so-called nonribosomal-peptide synthetases[13]. These enzymes have modular structures with each module incorporating (and modifying) a specific amino acid, accommodating residues in D configurations as well as many other nonprotein amino acids, ring formation, glycosylation and acylation. Some prominent examples of nonribosomally synthesized peptides include the cationic peptides polymyxin B and gramicidin S that are used in the topical treatment of infections, as well as the noncationic glycopeptide vancomycin and the lipopeptide daptomycin, which have become important reserve antibiotics against multiply resistant Gram-positive bacteria.

Because of the potent antibiotic activity, in the low-nanomolar concentration range, of many bacterially derived peptides, it is often taken for granted that these peptides are effective weapons in the fight for ecological niches. As with many other antibiotic compounds, however, experimental data have yet to be obtained showing that, for example, lantibiotics are produced in complex ecosystems such as the human gastrointestinal tract or skin, or that exogenous producer strains can effectively compete with preexisting normal flora because of their ability to produce an antibiotic compound. In contrast, the knockout of a staphylococcal lantibiotic gene cluster resulted in growth attenuation in a mouse abscess model[14], indicating that there may be additional ecological roles for bacteriocins.

Mechanism of action of cationic antimicrobial peptides

Virtually every peptide sequence with a net positive charge and a few hydrophobic residues will have antimicrobial activity if assayed in buffer or dilute medium as often used in the literature. It is worth noting, however, that the antimicrobial activity of cationic host-defense peptides is antagonized to variable extents by divalent cations like Mg^{2+} and Ca^{2+} (at physiological concentrations of 1–2 mM), monovalent cations such as Na^+ and K^+ (100 mM), and polyanions such as glycosaminoglycans (heparin and others) and mucins. Thus, the term antimicrobial peptides should be reserved for those peptides that are convincingly demonstrated to have an ability to directly kill microbes under such physiological conditions.

The cationic and amphiphilic nature of antimicrobial peptides is associated with their activity. The overall positive charge ensures accumulation at polyanionic microbial cell surfaces that contain acidic polymers, such as lipopolysaccharide, and wall-associated teichoic acids in Gram-negative and Gram-positive bacteria, respectively. They transit the outer membrane of the former via self-promoted uptake[1]. Subsequently these peptides contact the anionic surface of the cytoplasmic membrane and insert in a manner such that they initially straddle the interface of the hydrophilic head groups and the fatty acyl chains of membrane phospholipids. After insertion into the membrane, antimicrobial peptides act by either disrupting the physical integrity of the bilayer, via membrane thinning, transient poration and/or disruption of the barrier function, or translocate across the membrane and act on internal targets (**Fig. 1**).

Several complex and controversial models describe these subsequent events, including the reorientation of peptide molecules perpendicular

to the membrane to form either barrel-stave or toroidal channels, the breakdown of membrane integrity as a result of the swamping of membrane charge by a 'carpet' of peptides at the interface, the detergent-like dissolution of patches of membrane and the formation of peptide-lipid aggregates within the bilayer[15]. Each of these successfully predicts the ability of cationic antimicrobial peptides to break down the cytoplasmic membrane, but only the toroidal channel and aggregate models explain the action of certain peptides on cytoplasmic targets. Indeed, the action of many peptides cannot be explained by disruption of membrane permeability barriers, as discussed in several reviews[9,15,16]. Other highly charged peptides may interfere with the activity of the cell-wall lytic enzymes on the outside of the cell[17]. A similar complexity of mechanism also applies to the antiviral and anti-fungal action of such peptides[15].

The development of clinically useable conventional antibiotics has been often biased toward the concept of each antibiotic having a single primary target and a single mode of action, although this may not be entirely correct[18]. Nature appears to have chosen a different concept for the evolution of the innate host-defense peptides, favoring the design of 'dirty' drugs that disturb many biological functions with modest potency rather than blocking a specific high-affinity target[9]. Peptides have been effective for billions of years, so such an approach might represent a method of extending the half-life of these antimicrobials, beyond the 1–2 decades enjoyed by most conventional antibiotics. There is no question that such peptides will eventually induce resistance[9,19], and although resistance is clearly more difficult to attain than for conventional antibiotics, it has been suggested that it might have more severe connotations if it led to cross-resistance to innate human antimicrobial peptides[19]. However, several issues mitigate these concerns—including the fact that to date, (i) all knockout animals lacking host-defense peptides are quite healthy with only modest alteration in susceptibility to infection, (ii) the cross-resistance of laboratory-selected mutants to other peptides seems to be limited[20]—and the importance of immunomodulatory properties of these peptides, which would not be affected by antimicrobial resistance, has been increasingly recognized.

In the case of the lantibiotics, the concept of combining several antibiotic activities in one molecule has permitted the achievement of unprecedented potencies as demonstrated by, for example, nisin, gallidermin and lacticin 3147 (ref. 21). In addition to moderate affinity targets that these peptides use to antagonize a broad spectrum of Gram-positive bacteria, they also interact with high-affinity pyrophosphate binding sites on the membrane-bound cell wall precursor Lipid II (ref. 21), leading to more effective formation of pores and/or inhibition of cell wall peptidoglycan biosynthesis. When these activities operate together, which is usually the case with bacteria closely related to the producer strain, subnanomolar concentrations are sufficient for killing.

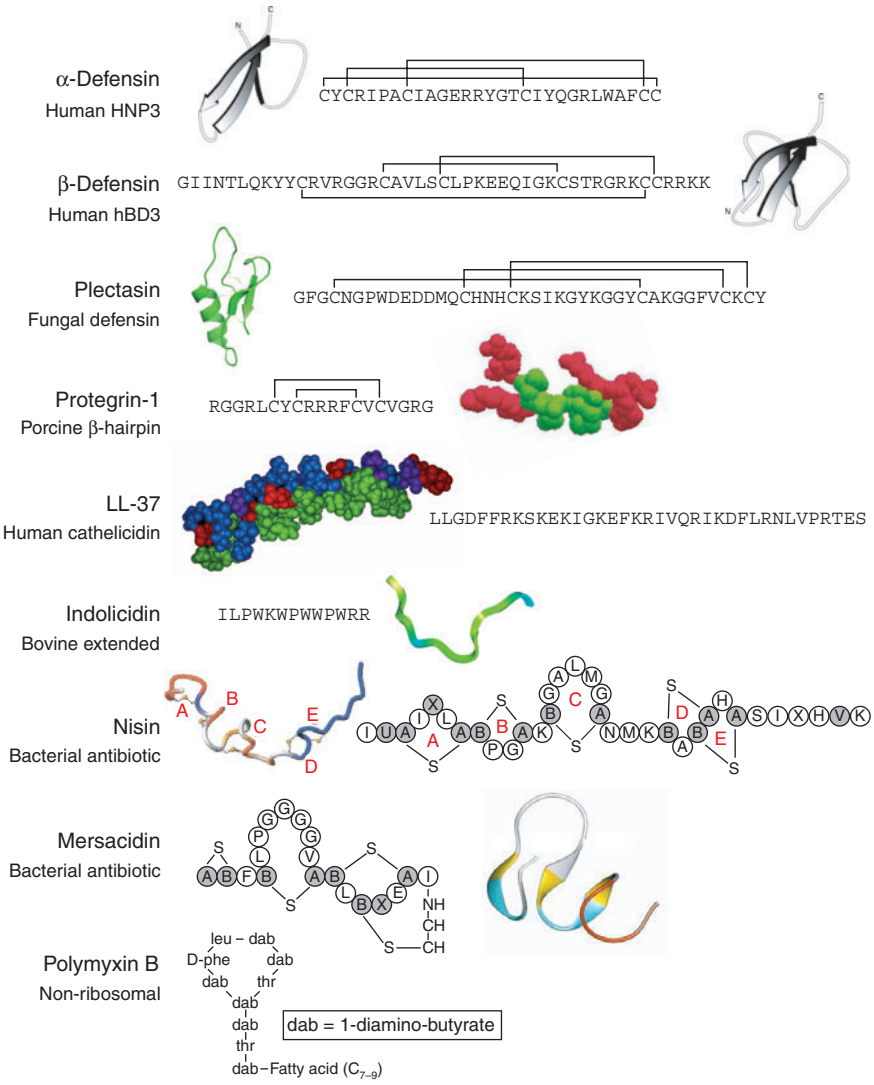

Figure 2 Selected structures and sequences of host-defense peptides.

Clinical experience to date

Nature has taught us that cationic peptides have tremendous structural diversity and an impressive array of clinically meaningful activities. This has provided a huge impetus to the development of new synthetic peptides. Even so, despite nearly two decades of serious design efforts, there has been limited success in the clinic[22].

To date, four cationic peptides or proteins have advanced into phase 3 clinical-efficacy trials. These have been indicated for curing or preventing impetigo and diabetic foot ulcers (the frog magainin derivative MSI-78; Pexiganan), oral mucosaitis (the pig protegrin derivative IB-367; Iseganan), sepsis (the human bactericidal permeability protein derivative $rBPI_{23}$; Neuprex) and catheter-associated infections (the cattle indolicidin variant CP-226; Omiganan). Only two peptides demonstrated efficacy.

Pexiganan, topically administered to sufferers of diabetic foot ulcer, was as effective as oral antibiotic treatment with ofloxacin, leading to a clinical cure or improvement in 90% of patients. Even so, the US Food and Drug Administration (FDA) did not approve this drug for medical use. In phase 3a clinical trials, Omiganan missed its primary clinical target, the prevention of catheter-associated infections, because of a low

number of enrolled patients; however, it did achieve statistically significant success both in reducing infections that tunnel in from the catheter insertion site and in reducing catheter colonization, and confirmatory phase 3b trials are currently under way.

In addition to the above peptides, many other molecules are proceeding through discovery, development and clinical trials (**Table 1**). Thus, it is worth considering the potential limitations of this class of drugs and what can be done to overcome these issues (**Table 2**).

Limitations of host-defense peptides

One possible disadvantage of drugs with such complex mechanisms of action is their potential for toxicity. All clinical trials to date have used topical applications to address surface infections, rather than the more lucrative systemic (parenteral and oral) antibiotic market. Thus, it seems evident that there is a possible toxicity issue limiting systemic application although the issue of toxicity has been little addressed in the past. Although many peptides are membrane-active in prokaryotes, they seem to have a lesser ability to disrupt membranes composed of eukaryotic components owing to the absence of negatively charged lipids on the surface, the lack of a strong membrane potential gradient (oriented internal negative) in most eukaryotic membranes and the presence of cholesterol in eukaryotes[1,9,16], and it is indeed not difficult to design peptides with virtually no acute human-cell toxicity. This may be a little deceiving, however, because antimicrobial peptides share features with eukaryotic nuclear localization signal peptides, and, for example, human LL-37 can freely translocate into cells and even carry passenger molecules into the nucleus[23,24]. Thus, the antimicrobial peptide field urgently needs studies that evaluate more subtle toxicities associated with systemic peptide application including, for example, apoptosis induction and mast-cell degranulation[4].

A second and obvious disadvantage of natural peptides is their potential lability to proteases, creating potentially unfavorable pharmacokinetics. In particular, chymotrypsin-like enzymes attack proteins at basic residues, which are an obligate feature of antimicrobial peptides. This issue has been addressed, and several solutions have been proposed[25], including the use of unusual or D- (rather than natural L-) amino acids (which renders peptides protease-resistant), the use of nonpeptidic backbones (peptidomimetics, as discussed below), formulation to improve stability (for example, in liposomes) or the chemical modification of peptides to create protease-resistant (and/or less toxic) prodrug molecules (as used in the drug Colymycin (colistimethate), which is the methane-sulfonated derivative of polymyxin E). Unfortunately, there is a

Table 1 Peptides and peptidomimetics in commercial development

Company (location)	Drug	Stage of development	Medical use
AM-Pharma (Bilthoven, The Netherlands)	hLF-1-11 (small peptide derived from human lactoferrin)	Phase 2	Allogeneic bone marrow stem cell transplantation–associated infections
BioLineRx (Jerusalem)	BL2060 (a synthetic compound comprising fatty acid and lysine copolymers)	Lead optimization	Anti-infective
Ceragenix (Denver)	CSA-13 (cationic steroid (ceragenin) that mimics host-defense peptides)	Preclinical	Anti-infective
Helix Biomedix (Bothell, Washington, USA)	HB-50 (synthetic natural peptide mimetic of cecropin)	Preclinical	Anti-infective
	HB-107 (19-amino-acid fragment of cecropin B)	Preclinical	Wound healing
Inimex (Vancouver, BC, Canada)	IMX942 (5-amino-acid peptide)	Lead optimization	Immunomodulation; treatment of fevers and neutropenia in chemotherapy patients
Lytix Biopharma (Tromso, Norway)	Not available	Discovery	Anti-infective, antitumor
Migenix (Vancouver, BC, Canada)	Omiganan pentahydrocholoride/ CP-226/MX-226/CLS001 (12-mer analog of bactolysin)	Phase 3b/phase 2	Prevention of catheter-related infections; dermatology-related infections
Novacta Biosystems Ltd. (Hatfield, England)	Mersacidin (bacteriocin)	Preclinical	Gram-positive infections
Novobiotics (Cambridge, Massachusetts, USA)	Not available	Discovery	Nail fungus; methicillin-resistant *S. aureus*
Novozymes A/S (Bagsvaerd, Denmark)	Plectasin (fungal defensin)	Preclinical	Systemic anti–Gram positive, especially pneumococcal and streptococcal infections
Pacgen (Vancouver, BC, Canada)	PAC113 (based on the active segment of histatin 5 protein found in human saliva)	Investigational New Drug (IND) approval	Oral candidiasis
PepTx (St. Paul, MN, USA)	PTX002 (33-mer peptide) PTX005 (12-mer peptide), PTX006 (*N*-acylated analog of PTX005) and PTX007 (a nonpeptidic structural analog of PTX005)	Discovery	Broad-spectrum antimicrobial antiendotoxin
Polymedix (Philadelphia)	Peptidomimetics (derived from the arylamide, calixarene, hydrazide and salicylamide series)	Discovery/preclinical	Anti-infectives; antimicrobial polymers and coating materials
Zengen (Woodland Hills, CA, USA)	CZEN-002 (synthetic 8-mer derived from α-melanocyte–stimulating hormone)	Phase 2b	Vulvovaginal candidiasis

This is a listing of known antimicrobials in development and/or clinical trials in private companies. Most of this information is based on a review by Zhang & Falla[22], as gleaned by them and updated by us primarily from company press releases, public presentations and the AdisInsight 'R&D Insight' database (http://www.adisinsight.com). We do not include in this table several peptides that went through clinical trials but were not approved.

dearth of studies investigating cationic antimicrobial peptide pharmacokinetics or the effects that modifications, such as those mentioned above have on lifetime, activity and toxicity of these peptides.

The single largest issue in the field is arguably the high cost of manufacturing peptides. This has limited both the testing and development of large numbers of variants and the potential clinical targets to which these molecules can be applied. Peptides tend to be very expensive drugs, costing between $100 and $600 per gram (an average daily dose for most systemic therapeutics) or more to manufacture by solid-phase chemical synthesis. Thus, all strategies moving forward have to take this into account, and there is a growing need for a less expensive peptide-production platform. Many attempts have been made to produce designer peptides by a variety of recombinant DNA methods using bacteria and fungi as well as plant and animal production systems; however, none of these expression systems have proven commercially feasible to date. One recent breakthrough in this regard is the use, as a template, of a natural fungal peptide, plectasin, combined with a fungal expression system to produce an antimicrobial peptide at the scale and purity required for therapeutic administration[26].

Table 2 Design and development strategies for antimicrobial and host-defense peptides

Activity	Critical need	Strategies
Screening	Larger variety of peptides	Peptide arrays for increased diversity
		New natural lead molecules
		Peptide-like (mimetic) approaches
		Non-natural amino acids
		Screen for both antimicrobial and immune-modulating activities
Toxicity	Understand mechanisms of toxicity	Toxicology in animal models
		Assess subtle toxicities
		Toxicogenomics
Pharmacology	Improve half-life *in vivo*	Peptidomimetics
		Modified and/or D-amino acids
		New formulations (for example, liposomal)
		Immune-modulating peptides may not require regular dosing
Cost of goods	Lower the cost of expensive therapeutics	Make shorter analogs that work
		Recombinant manufacturing processes
		Natural sources (for example, lantibiotics)
		Immune-modulating peptides may require smaller doses
		Local administration
Efficacy	Improve activities in the context of model infections	Realistic animal models of disease
		In vitro assays should match *in vivo* realities (for example, physiological conditions)
		Develop *in vitro* methods of predicting effective immune modulation

Bacteriocins overcome some of these issues as they can be produced by recombinant means in bacteria, and large-scale fermentation and purification schemes have been developed. Additionally, the lantibiotics, which have unusual structures and amino acids, are relatively resistant to proteases. It can be assumed that these molecules are relatively safe, at least when taken orally, because bacteriocins of lactic acid bacteria, in particular nisin, have a long and impressive history in food preservation[27]. For such purposes, cost-effective semi-purified preparations of nisin, such as Nisaplin, are available; otherwise, producer strains can be included directly in the food production process. Various clinical applications have also been considered[26], including the topical treatment of skin infections, such as juvenile acne (gallidermin), bovine mastitis (nisin, lacticin 3147) and eradication of methicillin-resistant *Staphylococcus aureus* nasal colonization.

New strategies and tools for developing peptide-based antibiotics

To date, peptide discovery efforts have been limited by the expense of producing them by conventional solid-phase peptide synthesis methods, which has tended to limit peptide optimization campaigns to a few dozen peptides. Recently, a new method was introduced[28], using arrays of peptides robotically spot-synthesized on cellulose sheets, and combined with a highly sensitive antimicrobial assay measuring the ability of peptides when released from the cellulose to decrease ATP-dependent luminescence in a luciferase-expressing reporter strain of *Pseudomonas aeruginosa*. This system permits the production and screening of up to an estimated 50,000 peptides per pipetting robot per year and has been used to analyze the effects of substitution of each amino acid in a 12-amino acid peptide by all 19 alternative amino acids. Extrapolating these data on the most favorable substitutions to smaller peptides has led to the discovery of an eight amino acid peptide with excellent broad spectrum activity. The use of shorter peptides, which has also been addressed through combinatorial library strategies[29], would also provide a solution to the issue of cost of goods.

The development of antibiotic peptides has also stimulated research toward isolation of natural peptides with non standard amino acids (so-called peptaibols; http://www.cryst.bbk.ac.uk/peptaibol/home.shtml) as well as the development of peptidomimetic compounds[30] and *N*-alkylglycine polymers (peptoids)[31] as novel antibiotics. Synthetic strategies have been based on antimicrobial peptide templates such as protegrins[32] or magainins[33], whereas other mimetics have been designed that incorporate amino acid analogs that can block essential enzymes[34]. Such molecules also solve the issue of stability as they tend to be protease resistant. If created as simple polymers using repeating subunits, these mimetics may also solve the cost of goods issue.

A major breakthrough in the development of improved lantibiotics was resolving the chemistry of dehydration of hydroxyamino acids and subsequent thioether ring formation. Using the corresponding purified biosynthesis enzyme LctM, production of thioether-containing peptides *in vitro* has been achieved[35]. This should enable the construction of new lantibiotics in which, for example, the target binding modules are conserved and combined with more suitable structural features to enlarge the activity spectra such that at least strains of a certain species or genus (for example, staphylococci) will be reliably killed at therapeutically relevant concentrations. Statistical analysis of lantibiotic gene clusters identified to date has clearly identified residues that are amenable to modification[36].

Potential of immunomodulatory host-defense peptides

Recent work has indicated that cationic peptides are modulators of innate immunity, a property that may allow the development of a novel anti-infective therapeutic strategy[4,37]. Innate immunity is triggered when conserved bacterial signature molecules (for example, lipopolysaccharide) interact with host pattern recognition receptors including Toll-like receptors (TLRs)—discovered originally because they have a role in induction of anti-fungal peptides in *Drosophila melanogaster*,

although now these receptors have been identified in all mammals. After TLR binding, effector mechanisms are stimulated that assist in the prevention or resolution of modest infections. As mentioned above, innate immunity has some of the basic hallmarks of an ideal antimicrobial therapy in that it is rapidly acting (within hours), relatively nonspecific and involves a package of effector mechanisms making resistance-development unlikely. Overstimulation of innate immunity, however, leads to a syndrome termed sepsis, which afflicts 750,000 individuals and kills 140,000 in North America annually. Thus, stimulation of innate immunity by natural mechanisms, for example using TLR agonists, creates the risk of inducing or exacerbating potentially harmful proinflammatory responses. Nonetheless, certain TLR agonists, such as CpG oligonucleotides, are currently being developed[37,38].

Cationic host-defense peptides have been shown to have a broad range of immunomodulatory properties, including the modulation of expression of hundreds of genes in monocytes, epithelial cells and others, direct chemoattraction of immune cells, induction of chemokines and differentiation responses, promotion of angiogenesis and wound-healing responses, and resolution of infections[3,4,37]. Some of these properties would be considered proinflammatory, but host-defense peptides actually suppress TLR signaling responses, the lipopolysaccharide-stimulated production of proinflammatory cytokines like tumor necrosis factor α (TNFα), and septic shock in animal models[4,39]. And although the situation is a bit clouded for the natural host-defense peptides like the well-studied human LL-37, small peptides have now been developed that have absolutely no antibacterial activity, but that are nevertheless able to protect against infection in animal models[39] (Scott, M.G. & R.E.W.H., unpublished data). Thus, the selective upregulation of innate immunity potentially provides a new means of treating infections.

Conclusions

Cationic antimicrobial peptides comprise potent and broad-spectrum molecules that have many desirable properties. There seems to be a strong likelihood that the first cationic antimicrobial peptide will find its way into medical practice within the next year or so, in the clinical context of catheter-associated infections, where there is a real need for such a solution. Although there are relatively few follow-up products in the clinic at this time, it can be anticipated that the applications of peptides to other topical uses will be more extensively explored. One particularly interesting future direction would be to explore aerosol formulations[40] because, although delivery to the lung by this methodology can be considered a topical application, lung infections constitute one of the serious areas of concern for antibiotic-resistance development and are often addressed by using parenteral antibiotics. Thus peptides may be able to overcome resistance to conventional antibiotics in life-threatening infections when applied by aerosol.

Another objective that seems to be within reach is the issue of *in vivo* stability (pharmokinetics), as the refinement of peptidomimetic technologies makes the prospect of a protease-resistant mimic of cationic antimicrobial peptides quite feasible. The issue of cost could also be addressed through mimetic technology, especially when combined with combinatorial peptide array methods (also applicable to building diverse mimetics), which are realizing the goal of making smaller, broadly active peptides. A third objective, that is perhaps more long term, is that of addressing methods of formulation or application for systemic or oral use. To achieve this, a primary goal would be to understand in more detail the nature of prospective toxicities that might limit systemic applications of peptides. Despite these uncertainties, it seems likely that peptides will find a niche in the antibiotics market.

Similar approaches may also enhance the development of immunomodulatory host-defense peptides. In this case, the upregulation of certain innate immune mechanisms while suppressing proinflammatory cytokine responses offers an exciting and novel approach to anti-infective therapy, and one that should not incite resistance because the peptides act through a diverse innate immune system rather than direct action on bacteria. Whereas cationic peptides are not necessarily going to solve all of the issues created by the growing problem of antibiotic resistance, they do offer a very exciting approach to addressing this concern.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Biotechnology* website for details).

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