Cationic peptides: effectors in innate immunity and novel antimicrobials

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Cationic antimicrobial peptides are produced by all organisms, from plants and insects to human beings, as a major part of their immediately effective, non-specific defences against infections. With the increasing development of antibiotic resistance among key bacterial pathogens, there is an urgent need to discover novel classes of antibiotics. Therefore, cationic peptides are being developed through clinical trials as anti-infective agents. In addition to their ability to kill microbes, these peptides seem to have effector functions in innate immunity and can upregulate the expression of multiple genes in eukaryotic cells. One such function might involve the dampening of signalling by bacterial molecules such as lipopolysaccharide and lipoteichoic acid.
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Since their introduction into human medicine, antibiotics have had an enormous impact on treatment of infectious diseases and the success of invasive medical procedures, such as surgery and chemotherapy. However, the rise in antibiotic resistance threatens to reverse some of these gains.[1] One reason for this development is the paucity of truly novel antibiotics since the introduction of quinolones in the early 1960s. Indeed, for more than 30 years until the release of the streptogramins, synercid, and the oxazolidinone linezolid during the past 18 months, there were no new antibiotic chemical structures. Unfortunately, these antibiotics are niche drugs developed for antibiotic-resistant Gram-positive pathogens, and their restricted activity ranges and toxicity concerns somewhat limit their impact. Thus, it is important to consider new classes of antibiotics. One source is "nature's antibiotics,"[2] the cationic peptides. In this review, I discuss the role of these peptides in innate immunity, and their use as templates in development of a new class of antibiotics that are in phase III clinical trials of topical therapy.

About 20 years ago, the lymph of insects, the granules of human neutrophils, and the skin of frogs were shown to contain peptides that could kill bacteria in culture. Since then, more than 600 cationic peptides have been observed in virtually all species, including bacteria, fungi, insects, tunicates, amphibians, crustaceans, birds, fish, mammals, and human beings.[3] They have generally been referred to as cationic antimicrobial peptides, but in addition to their ability to kill microorganisms directly,[4,5] these substances seem to be able to recruit and promote other elements of host immunity, particularly innate immunity.[6–9] The term cationic amphiphilic peptides, abbreviated to cationic peptides, is therefore used here.

Nature and distribution

Cationic peptides have an enormous variety of sequences and structures,[3] but certain features are common.[2,3,5] The natural cationic peptides are generally 12–50 aminoacids in length, have a net positive charge due an excess of basic lysine and arginine residues over acidic residues, and contain around 50% hydrophobic aminoacids. They fold, owing to the presence of disulphide bridges or contact with membranes, into three-dimensional amphiphilic structures in which the positively charged and hydrophilic domain(s) are well separated from the hydrophobic domain(s). Such a molecule is well suited to interacting with membranes, especially bacterial membranes with their negatively charged and hydrophilic head groups and hydrophobic cores. Nevertheless, both the secondary structures of the cationic peptides, which fit into four classes, and their aminoacid sequences, even within a given class of secondary structures, are quite heterogeneous. The four structural classes include β-sheet molecules stabilised by two or three disulphide bonds, amphipathic α-helices, extended molecules, and loops due to a single disulphide bond (figure 1; the last three classes form upon membrane interaction). The β-sheet and α-helical molecules are by far the most common in nature. Table 1 describes a few representative molecules from nature and related synthetic molecules.

Antimicrobial activities

In the past, with very few exceptions, antibiotics did not have activity against fungi and antifungal drugs did not act against bacteria. However, cationic peptides have a startling range of antimicrobial activities that can include action against most Gram-negative and Gram-positive bacteria, fungi, enveloped viruses, and eukaryotic parasites (table 2). Table 1 presents the minimum inhibitory concentrations (MIC) of representative peptides for a Gram-negative bacterium (*Escherichia coli*), a Gram-positive bacterium (*Staphylococcus aureus*), and a fungal pathogen (*Candida albicans*). Various methods are routinely used to assess the ability of peptides to kill bacteria. However, the gold standard is becoming the National Committee for Clinical Laboratory Standards broth dilution method,[11] modified slightly to avoid binding of peptides to plastic surfaces. Generally, the best cationic

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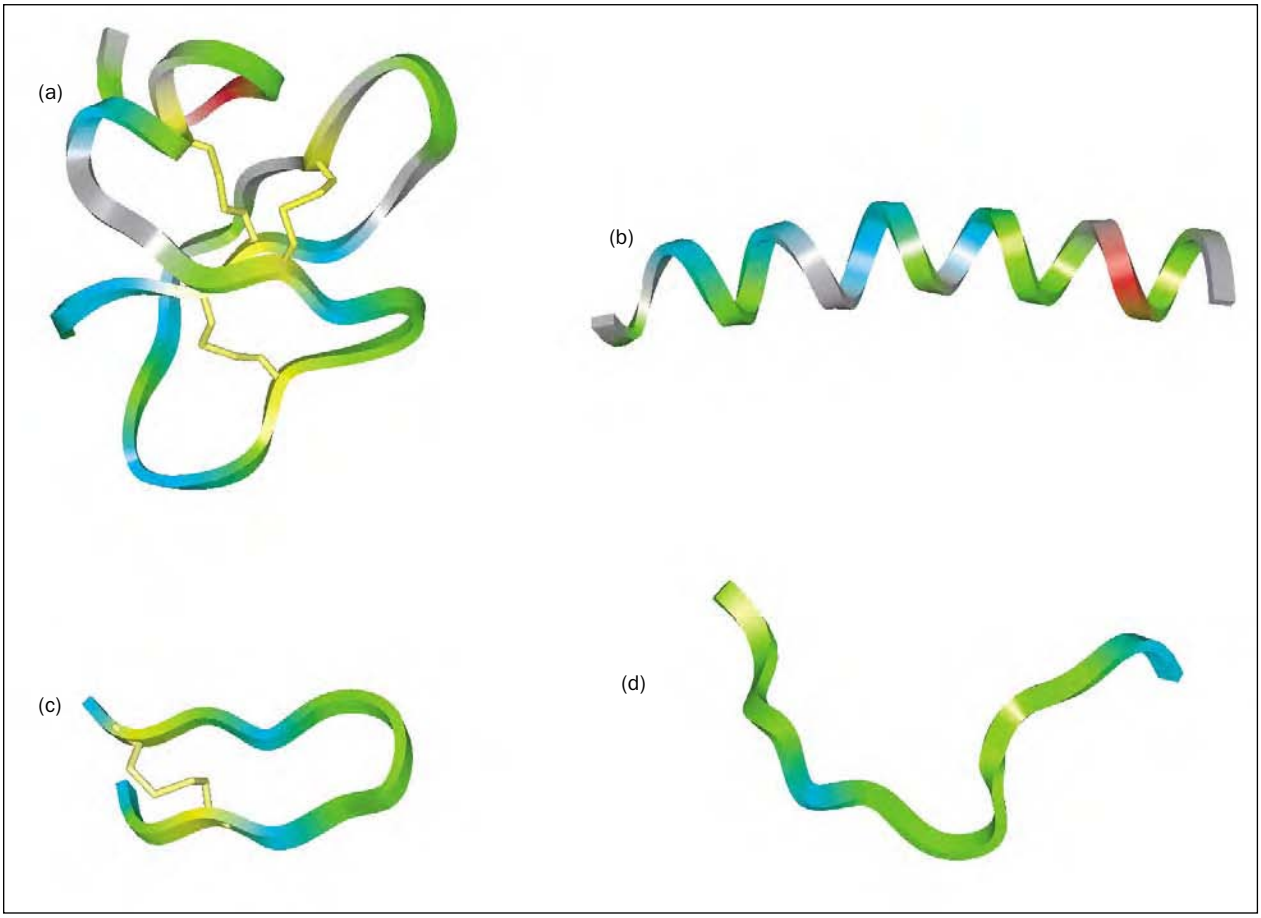

Figure 1. Molecular models of the different structural classes of cationic peptides. These models (taken from the NMR structural database) are based on two-dimensional nuclear magnetic resonance spectroscopy of the peptides in aqueous solution for human β-defensin-2 (HBD-2) or a membrane mimetic condition (other peptides). (a) HBD-2 (PDB code 1FQQ), which forms a triple-stranded β-sheet structure (containing a small α-helical segment at the N-terminus) stabilised by three cysteine disulphide bridges. (b) The amphipathic α-helical structure of magainin 2 (PDB code 2MAG). (c) The β-turn loop structure of bovine bactenecin (model based on the published structure[10]). (d) The extended boat-shaped structure of bovine indolicidin (PDB code 1G89). The backbone structures are shown with the charged regions in blue and the hydrophobic residues in green.

peptides have good activities against most bacteria, and excellent activities (MIC of 1–4 μg/mL) against highly resistant bacteria such as multidrug-resistant *Pseudomonas aeruginosa*, meticillin-resistant *S aureus*, and *Stenotrophomonas maltophilia*.[15,17,18] The cationic peptides are not affected by antibiotic-resistance mechanisms that are limiting the use of other antibiotics; for example, they are as active against meticillin-resistant *S aureus* as they are against meticillin-sensitive strains. A few antibiotic-resistance mechanisms that affect antimicrobial peptides have been described,[19–21] but most seem to have only a moderate (two to four fold) effect on MIC. Indeed, selection of resistant mutants against most peptides is quite difficult, with more than 12 passages on 50% MIC of antibiotic being required to increase resistance by two fold. However, there are a few resistant bacterial species, including *Burkholderia cepacia* (by virtue of its unique outer membrane[14]) and *Serratia* spp.

Cationic peptides are bactericidal, with the MIC and minimum bactericidal concentration coinciding or differing by no more than two fold. They kill bacteria very rapidly (figure 2) compared with conventional bactericidal antibiotics.[15,17]

A large number of, but by no means all, cationic peptides have useful antifungal activities. Indeed, given the importance of fungal diseases of plants, it is perhaps not surprising that many plant peptides are selective for fungi,[5] as are certain insect peptides such as drosomycin.[6] Few studies have been done to study the antifungal spectrum of cationic peptides, and we know little about the specific mechanism of action, although various processes have been described, including morphological distortions, rapid ion fluxes,[23] and inhibition of energised mitochondria.[24]

Another target that has been even more poorly defined is eukaryotic parasites. Selected peptides have activity against protozoa, including trypanosomes, malaria parasites, and nematodes.[25–27] Activity against cancer cells has also been reported,[28,29] although there is doubt as to whether such peptides have the necessary selectivity for malignant over normal cells, and some peptides can be quite toxic. Some peptides, including defensins, indolicidin, polyphemusin, and melittin, also have activity against viruses including HIV, herpes simplex virus, influenza A virus, and vesicular stomatitis virus.[30] Mechanisms have been reported to include blockage of virus-cell fusion and the activity of HIV long terminal repeats.

Table 1. Sequences and properties of selected natural and synthetic cationic peptides

Peptide	Class*	Derivation	Sequence†	MIC (mg/mL)‡ *Escherichia coli*	*Staphylococcus aureus*	*Candida albicans*
HNP1	β3(α)	Human neutrophils	AC_1YC_2**R**IPAC_3IAGE**RR**$YGTC_3$IYQGRLWAFC_2C_1	>50	3·1§	
HBD-3	β3(β)	Human skin	GIINTLQ**K**YYC_1**R**V**R**GG**R**C_2AVLSC_3LP**K**EEQIG**K**C_2 ST**R**G**RK**C_1C_3**RRKK**	~6‖	~12	~6
Polyphemusin I	β2	Horseshoe crab	**RR**WC_1F**R**VC_2Y**R**GFC_2Y**RK**C_1**R**-NH_2	0·13	0·5	1
Protegrin	β2	Pig	**R**GG**R**LC_1YC_2**RR**FC_2VC_1VG**R**-NH_2	0·5	2	4
IB-367	β2	Synthetic	**R**GGLC_1YC_2**R**G**R**FC_1VC_2VG**R**-NH_2	1	4	32
Magainin II	α	Frog	GIG**K**FLHSA**KK**FG**K**AFVGEIMNS	50	>100	
MSI-78	α	Synthetic	GIG**K**FL**KK**A**KK**FG**K**AFVKIL**KK**-NH2	2	16	
Cecropin B	α	Silk moth	**K**W**K**VF**KK**IE**K**MG**R**NI**R**NGIV**K**AGPAIAVLGEA**K**AL-NH_2	5	>200	
CP-α2	α	Synthetic	**K**W**KK**FI**KK**IGIGAVL**K**VLTTGLPAL**K**LT**KK**	2	16	64
Indolicidin	E	Bovine neutrophils	ILPW**K**WPWWPW**RR**-NH_2	16	8	4
CP-11CN	E	Synthetic	IL**KK**WPWWPW**RRK**-NH_2	4	16	16
CP-10A	α	Synthetic	ILAW**K**WAWWAW**RR**-NH_2	8	4	16
Bactenecin	C	Bovine neutrophils	**R**LC_1**R**IVVI**R**VC_1**R**	8	32	64
BacW2R	C	Synthetic	**RR**LC_1**R**IVWVI**R**VC_1**R**	2	2	>64
Gramicidin S	βC	Bacteria	Cyclic (L**O**VPF^dL**O**VPF^d)	8	2	2
Polymyxin B	CL	Bacteria	Isooctanoyl **B**T**BB**(**B**F^dL**BB**T) cyclised	0·5	32	32

*Classes are: β, beta-structured (number refers to the number of disulphide bridges; α or β after the number refers to the family of mammalian α or β defensins from which the peptides come); α, amphipathic α-helical; E, extended structure; C, cyclic; L (polymyxin only), lipopeptide. †One-letter aminoacid code with the following additions. Residues positively charged at neutral pH are in bold. Parentheses indicate aminoacids that are cyclised. Superscript d represents the D-enantiomer; all other aminoacids are L-form. The subscript numbers represent aminoacids that are joined by cysteine disulphides. O, ornithine; B, diaminobutyrate; ×, 2,3-didehydrobutyrine; U, 2,3-didehydroalanine; Z, a-aminobutyrate. ‡MICs were generally determined by modified NCCLS broth dilution assays,[11] and results from the our laboratory are generally used for consistency. Inhibitory concentrations are greatly affected by the method used and the salt content of the assay medium. By the radial diffusion assay method of Lehrer and colleagues, killing at much lower concentrations can be demonstrated. §MICs were not done for *S aureus* but were done for another Gram-positive bacterium *Enterobacter cloacae*,[12] and I assumed here that they are similar. ‖Concentrations resulting in 100% killing.[13]

Peptides are generally found at quite low concentrations in the normal tissues of mammals, and several different peptides can be found in a single tissue.[8] Indeed, their natural role may involve synergy both with each other and with other agents in the host. In frogs, magainin 2 shows synergistic killing with the peptide PGLa,[31] and this finding has been extended by checkerboard titration studies with various cationic peptides.[12] Synergy has also been shown with lysozyme,[12] with various antibiotics against selected wild-type and mutant bacteria,[14] and with antifungal agents, antiprotozoal agents, and the anticancer drug doxorubicin against fungi, protozoa, and cancer cells, respectively.

Mechanism of antibacterial action

An enormous amount of work has been invested in model membrane studies.[32–35] However, although the findings of such studies are consistent with the central observation that the interaction of cationic peptides with the bacterial cytoplasmic membrane is an essential step in the peptides' bactericidal activity, they have also led to a broad variety of hypotheses to explain bacterial killing. Gram-negative bacteria have an additional outer membrane barrier to cross, and the self-promoted uptake hypothesis[36] appears to describe how such uptake occurs (figure 3). According to this hypothesis, the cationic peptides interact with the highly negatively charged surface of the outer membrane and displace magnesium ions that normally partly neutralise this charge. The high negative charge is carried by the anionic glycolipid lipopolysaccharide, which fills the outer monolayer of the outer membrane. The cationic peptide then distorts the outer membrane either by strongly binding to the lipopolysaccharide or by neutralising charge over a patch of the outer membrane. The peptide is then proposed to insert into and translocate across this bilayer. Since many cationic peptides are selective for Gram-negative over

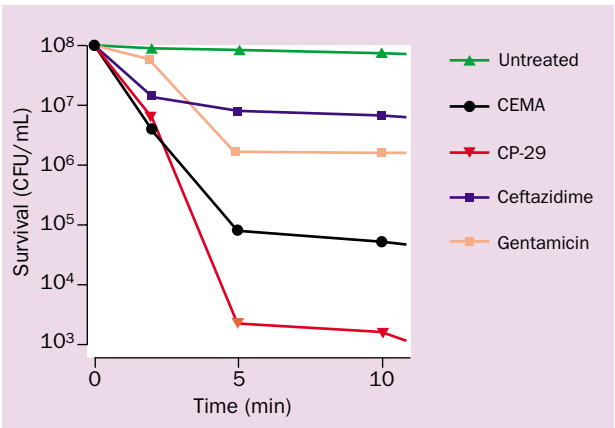

Figure 2. Rate of killing of E coli *UB1005 in Mueller-Hinton broth by four fold the MIC of cationic peptides compared with conventional antibiotics Adapted from published data.[22] Ceftazidime at 2 μg/mL, gentamicin at 0·5 μg/mL, CEMA at 4 μg/mL, and CP-29 at 2 μg/mL.*

Table 2. Activities of cationic antimicrobial peptides and some examples of peptides with those activities

Activities of antimicrobial peptides	Example peptides*
Broad-spectrum antibacterial	Protegrin, IB-367, MSI-78, indolicidin, CEMA, gramicidin S, polyphemusin,
Anti Gram-negative bacteria	Polymyxin B
Anti Gram-positive bacteria	HNP1
Synergy with conventional antibiotics	CEMA, magainin II, MSI-78, IB-367
Antifungal	Protegrin, CEMA, indolicidin, gramicidin S, polyphemusin
Synergy with conventional antifungals	Indolicidin
Antiviral (HIV, HSV)	Indolicidin, polyphemusin, protegrin
Anticancer	CEMA, indolicidin
Synergy with conventional anticancer agents	Indolicidin
Antiparasite	Magainin II, indolicidin
Antiendotoxin	CEMA, polyphemusin variants
Wound healing	Magainins, PR39
Chemotactic	HNP-1

*In addition to the peptides described in table 1, CEMA (previously termed CP28 or MBI-28) is an α-helical peptide.[14] Polyphemusin is a β-hairpin peptide from horseshoe crabs, structurally related to protegrin,[15] and PR39 is an extended peptide.[16]

Gram-positive bacteria, and since their action on the outer membrane causes protrusion of the outer membrane or blebs at discrete points on the cell surface (figure 4), we can assume that this interaction with the outer membrane focuses the peptide to attack discrete areas of the cytoplasmic membrane. Also, the distortion of the outer membrane appears to provide a partial explanation for the synergistic activities of antimicrobial peptides described above. However, these outer-membrane interactions do not result in cell death, because peptides that interact well with the outer membrane, but do not kill cells well, have been demonstrated.[37]

Having crossed the outer membrane (or the thick cell wall in the case of Gram-positive bacteria), the peptides approach the cytoplasmic membrane. Model studies have clearly shown, and virtually all researchers agree, that the peptides interact electrostatically with the anionic surface of the bacterial cytoplasmic membrane and this interaction induces insertion of the peptide into a position parallel to the membrane at the interface of the hydrophilic head groups and hydrophobic fatty acyl chains of the membrane phopholipids.[32–35] During insertion, the peptide folds into a membrane-bound structure, if not already folded as a result of disulphide bridging or passage across the outer membrane. After parallel membrane insertion, four outcomes have been proposed on the basis of model membrane studies and to some extent intact cell studies (figure 4). Although several reviewers have suggested lysis as an outcome, there is little evidence for complete dissolution of the majority of bacterial cells at the minimum effective concentration. The left-hand inset of figure 4 shows intact *E coli* treated with 32 times the MIC of the peptide CEMA, without apparent loss of underlying cell shape. There is, however, a striking change in outer-membrane morphology, which adopts a blistered appearance. The right hand panel of figure 4 shows no loss of integrity in *S epidermidis* despite treatment with ten times the MIC of

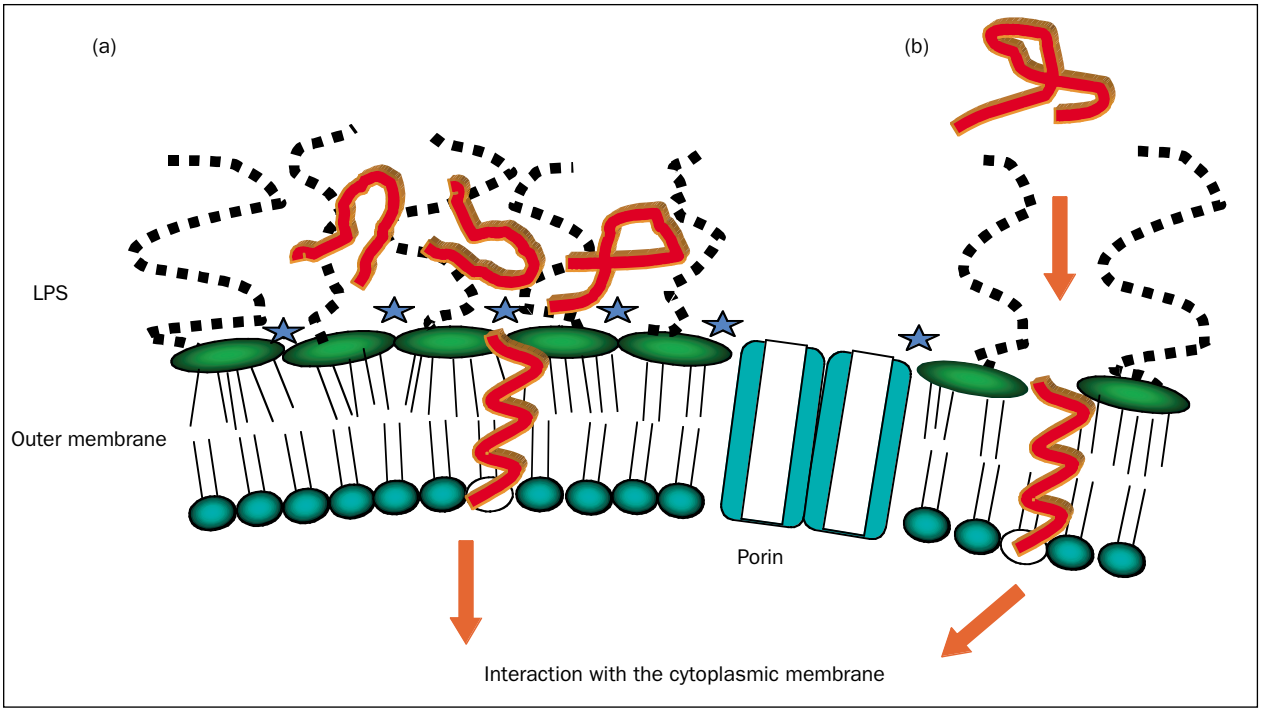

Figure 3. Self-promoted uptake of cationic peptides across the outer membrane. Unfolded cationic peptides are proposed to associate with the negatively charged (mainly due to the presence of highly anionic lipopolysaccharide [LPS]) surface of the outer membrane. They then either neutralise the charge over a patch of outer membrane, creating cracks through which the peptide can cross the outer membrane (a) or actually bind to the divalent cation binding sites on lipopolysaccharide, and disrupt the membrane (b). Once the peptide has crossed the outer membrane, it will interact with the negatively charged surface of the cytoplasmic membrane.

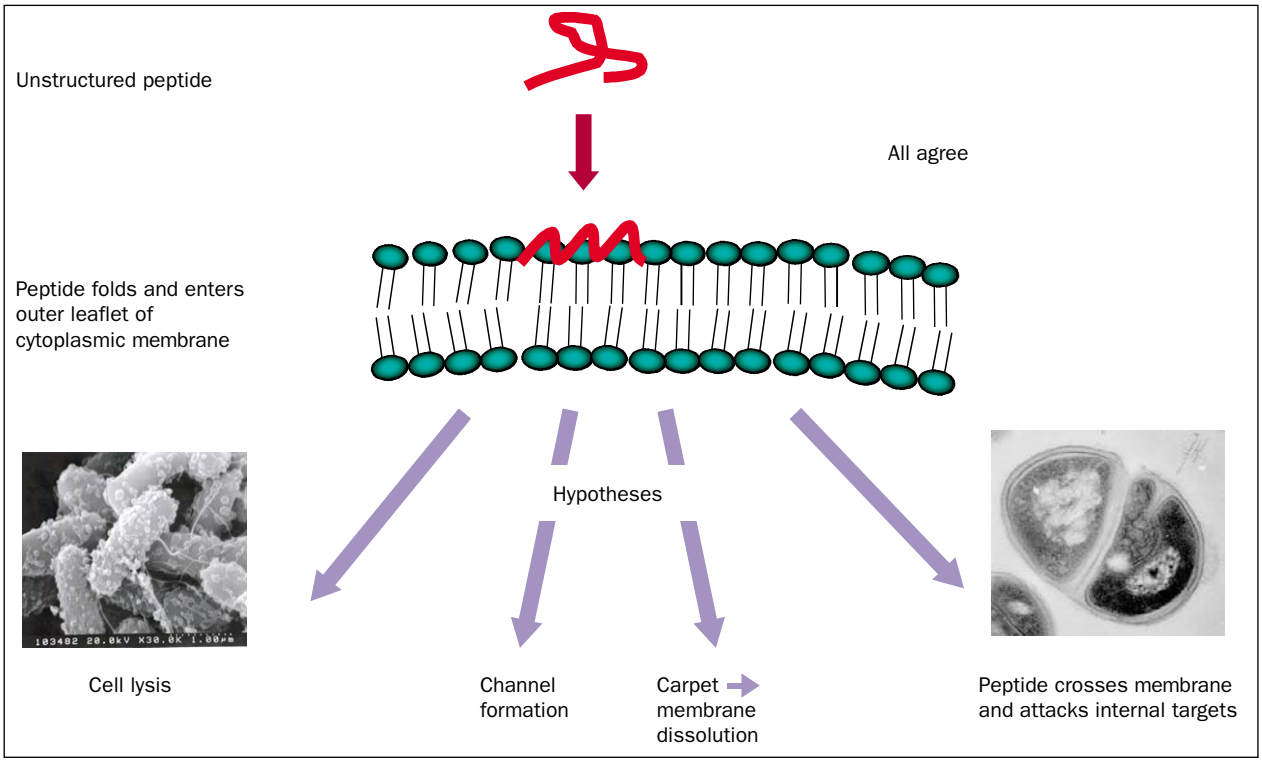

Figure 4. Peptide interaction with the cytoplasmic membrane of bacteria. The mechanism of entry of peptides to cells is undisputed, but there are four hypotheses for how they kill cells. One view is that they lyse cells, but E coli *is not lysed by treatment with 64 µg/mL CEMA for 30 min (inset on left). Another model suggests that when many peptide molecules insert into the membrane interface, they aggregate into a micelle that spans the membrane or flip-flop across the membrane under the influence of the large transmembrane electrical potential gradient;[30,36] inset on right represents the interaction of a linear bactenecin variant Bac2A (with the cysteines changed to alanines) with* Staphylococcus epidermidis*. Some of the events that can be seen are condensation of the DNA indicating uptake of the peptide into the cytoplasm, and cell division defects including an aberrant septum and the initiation of a false septum.*

Bac-2A, and obvious changes to the cell (DNA condensation and a false septum).

Two other hypotheses suggest that the peptides reach a high concentration at the outer surface of the cytoplasmic membrane and reorient to a position perpendicular to the cytoplasmic membrane to form channels with regular structure[33] or cause catastrophic breakdown of cytoplasmic-membrane integrity (the "carpet" model).[35] In these models, the mechanism of action is thought to be breakdown of cytoplasmic-membrane integrity. However, although virtually all cationic amphiphilic peptides cause cytoplasmic-membrane permeabilisation if applied at high enough concentrations, many do not depolarise (break down the membrane potential gradient of) intact cells at concentrations leading to cell killing.[38] Indeed, the toad histone-derived peptide buforin can translocate across lipid bilayers without affecting the membrane barrier function,[39] and all peptides active against Gram-negative organisms are by definition capable of translocation across at least one bilayer, the outer membrane.[32] For this reason, and to explain the results of studies on model membranes and bacterial cytoplasmic-membrane interaction for a wide variety of peptides, we proposed the micellar aggregate channel hypothesis,[32,38] which postulates that the peptides reorient according to concentration and possibly the cytoplasmic-membrane electrical potential gradient (−140 mV oriented as internal negative) to form micelle-like aggregates that provide informal channels for the movement of ions across the membrane. According to planar bilayer studies,[38] such channels can vary in both size and duration but can last as little time as microseconds; they are proposed to collapse in such a way that the peptide can move to the outer or inner monolayer in a parallel configuration, with the inner monolayer peptide having been translocated. In this model, various targets are possible, including the cytoplasmic-membrane barrier, cell-wall synthesis or degradation, cell division, macromolecular synthesis, or even selective enzyme targets (eg, figure 4, inset on left). Individual peptides might "prefer" a particular target, but

Table 3. Influence of selected cationic antimicrobial peptides given intraperitoneally as a single dose of 8 mg/kg to neutropenic mice infected with *P aeruginosa* and to galactosamine-sensitised mice treated with endotoxin

	Survival (%)	
Peptide*	*P aeruginosa* infection	Endotoxin
None	6	0
CEMA	43	78
CPα2	80	ND
Polyphemusin	20	10
PV5	40	50

*Peptides are the cecropin-melittin hybrid α-helical peptides CEMA and CPa2[14] and horseshoe crab polyphemusin and a variant PV5.[15] ND=not determined.

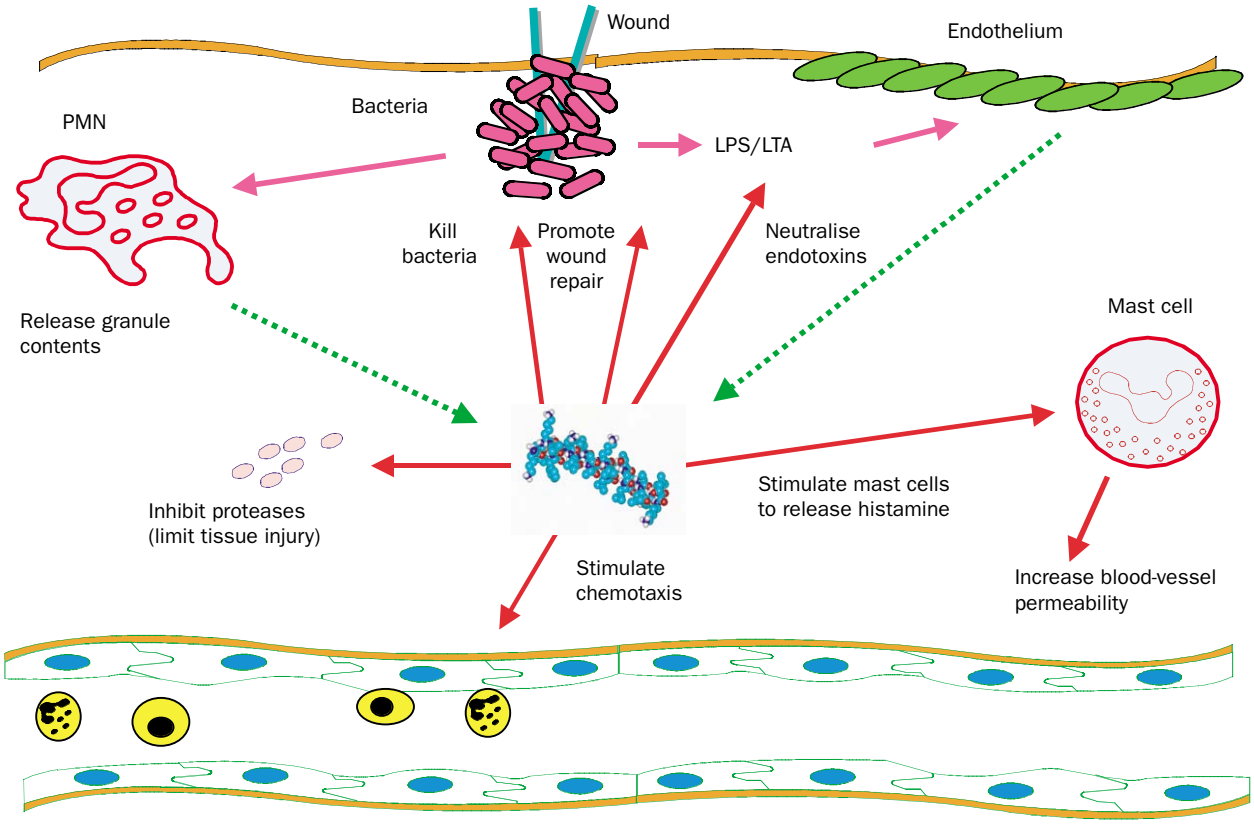

Figure 5. Scheme illustrating the proposed role of cationic peptides in innate immunity with specific reference to events that occur in chronic inflammation. Dotted arrows represent events that lead to increased production of extracellular cationic peptides, solid red lines actions of the peptides, and solid pink lines events due to the bacteria. The overall scheme presented is a mosaic of the separate effects.[7,8] LPS=lipopolysaccharide; LTA= lipoteichoic acid; PMN=polymorphonuclear leucocytes.

the existence of secondary targets and the proposed dependence of these targets on physical interactions (charge–charge and hydrophobic interactions) might help to explain why development of resistance against cationic peptides is difficult. The net effect is that some monomers will be translocated into the cytoplasm[39] and can dissociate from the membrane and bind to cellular polyanions such as nucleic acids.[22]

Role in innate immunity

There is much evidence that cationic peptides have an important role in living hosts.[6–9,40] Since a single host can contain up to about 35 different antimicrobial peptides from all structural classes,[3] elimination of all of these peptides at once is not possible, so clever manipulations must be made to assess in-vivo importance. For example, in drosophila, mutations in regulatory or signalling genes can affect the expression of many peptides and increase susceptibility to bacterial or fungal infections.[41] In mice, Wilson and colleagues[42] identified the enzyme matrilysin, which brings about processing of intestinal preprodefensins to mature defensins. Knocking out the matrilysin gene denuded the small intestine of mature defensins and increased susceptibility to infection with ingested organisms by ten fold. Cole and colleagues[43] similarly applied protease inhibitors specific for the protegrin-processing enzyme to wounds on the skin of pigs, decreasing the amounts of active protegrin and the ability to resist a bacterial challenge. Although similar experiments cannot be done in healthy human beings, patients with specific granule deficiency syndrome lack α-defensins and have severe and frequent bacterial infections.[5] Another way of assessing activity in vivo is to increase the amount of cationic peptides. This has been done in two ways. Bals and co-workers[44] introduced an adenovirus carrying the transgene for the human peptide LL-37 into the lungs of mice, leading to protection against *E coli* infections and endotoxin. Many other studies have shown that exogenously introduced peptides can protect against endotoxaemia and bacterial and fungal infections (table 3).[8,9]

There is no doubt that cationic peptides can be found in high (bactericidal) concentrations at certain sites in the host.[8,9] For example, the concentration of defensins in the azurophilic granules of neutrophils can be as high as 10 mg/mL, whereas various insect peptides when induced can circulate in the lymph at concentrations of up to 100 μg/mL or more. In these cases, we can assume that such peptides function in innate immunity to kill infectious agents directly. On the other hand, certain body sites in human beings contain quite low concentrations of peptides (eg, airway surface fluids contain 0·3 to 8 μg/mL of human β-defensin-2[45,46] and 2 μg/mL of the α-helical cathelicidin LL-37[47]). These concentrations can be increased for some peptides by infection, but except in pathological,

inflammatory diseases, concentrations still seem to be below those needed to kill infectious agents. Thus, the fact that these peptides have various activities that are relevant to innate immunity is of great interest.

In particular, peptides can neutralise host responses to conserved bacterial signalling molecules such as endotoxic lipopolysaccharide from Gram-negative bacteria,[48] lipoteichoic acid from Gram-positive bacteria,[9] and unmethylated CpG DNA from all bacteria (MG Scott, REW Hancock, unpublished). Such molecules interact with Toll-like receptors on the surface of host cells to trigger signalling cascades and cause upregulation of cytokines, such as tumour necrosis factor (TNF) and interleukin 6, chemokines like macrophage inflammatory protein 1α and 1β, and dozens of other gene products.[48] Although low concentrations of these signalling molecules cause beneficial proinflammatory responses and fever, too sustained or vigorous a response can lead to systemic circulation problems, organ failure, and even death.[49] Cationic peptides can neutralise these responses, for example by suppressing the upregulation by lipopolysaccharide of TNF expression both in macrophages in culture and in sensitised mice.[14] This action results in protection against endotoxaemia and death.[50] The mechanism of suppression involves both inhibition of binding of lipopolysaccharide to a serum factor lipopolysaccharide-binding protein, in addition to a proposed direct action on host cells.[50] Microarray experiments showed that the effects of the cationic peptides CEMA[48] and LL-37[50] are selective, in that of the 52 genes observed to be upregulated by lipopolysaccharide, only about 35 were suppressed to differing extents in the presence of cationic peptides. Since the natural bacterial flora of animals can conceivably release small amounts of bacterial signalling molecules, one role of cationic peptides at the surface of cells may thus be to prevent the induction of inflammatory responses by these bacteria.

Cationic peptides also have various interactions that relate to innate immunity, including stimulation of the chemoattraction of monocytes and neutrophils, promotion of histamine release from mast cells, inhibition of tissue proteases, and stimulation of wound healing (figure 5). Microarray experiments have confirmed the ability of cationic peptides to upregulate selectively the expression of more than 30 genes.[48] Furthermore, there is strong circumstantial evidence for involvement of specific receptors in the chemotactic response stimulated by peptides.[47] Nevertheless, to date these results remain fragmentary and there are as yet no data in animal models to confirm that such interactions are important.

Clinical development

The general proof of principle for the use of cationic antimicrobial peptides as therapeutic agents has already been established.[2] Two bacterium-derived, non-ribosomally synthesised cationic peptides, gramicidin S and polymyxin B,[32] have already found use in topical creams and solutions. However, these molecules tend to be toxic and this characteristic limits their potential for systemic use. Interestingly, neutralisation of the amino groups of polymyxin E with methane sulphonate creates a prodrug, colomycin, that can be used systemically.

Cationic peptides have had a chequered history in the clinic and currently only five clinical trials of topical treatment are underway. These include a phase III trial for therapy, by a protegrin-like molecule, IB-367, of oral mucositis, a painful ulcerative polymicrobial infection most commonly associated with radiotherapy or chemotherapy for cancer. There were also phase II clinical trials of IB-367 in aerosol formulation for *P aeruginosa* lung infections in people with cystic fibrosis. An indolicidin, MBI-226, is undergoing phase III clinical trials for sterilisation of insertion sites for central venous catheters; these trials have been fast-tracked by the US Food and Drug Admistration. Other indolicidin-like peptides are being investigated for therapy of acute acne (in phase II clinical trials).

However, until cationic amphiphilic peptides can be used systemically they will not achieve their true potential, and the barriers that must be overcome are discussed below.

Barriers

Any new class of pharmaceuticals faces a series of tests that must be overcome to achieve success in the clinic. In general, these include demonstration of good activity, appropriate formulation, an appropriate manufacturing method, sufficient stability in vivo, and low toxicity. Since there are virtually no published data on many of these topics for cationic peptides, the following discussion is somewhat speculative.

There is no doubt that cationic amphiphilic peptides have excellent antimicrobial activity in vitro and, in principle, represent almost ideal candidate drugs. There is some evidence that this good in-vitro activity can translate to in-vivo activity in animals, but studies in which protection is complete are rare, probably owing to formulation or stability issues. An example is observed for polyphemusin I, a β-hairpin peptide from horseshoe crabs, which in vitro is the most active peptide we have studied to date, but in animal models has no activity against infections.[15] By contrast, modest sequence modifications can create peptides with slightly lower in-vitro activity but reasonable (although incomplete) protection in infections of animals.[15] The most obvious cause of poor or incomplete in-vivo activity is lack of stability due to the action of host proteases. Ways of overcoming this instability might be improved formulation (eg, in liposomes, masking the peptide), use of the prodrug approach as discussed above for colomycin, development of cyclic peptides with strained peptide bonds that are more resistant to proteolysis, and sequence modifications. In the last case,[51] cationic peptide precursors can be protease inhibitors,[52] so moderate changes in sequence might convert a protease substrate to a protease inhibitor.

Search strategy and selection criteria

I have followed the field of antimicrobial peptides closely for the past 15 years with an automated monthly search of *Current Contents* and *Biological Abstracts*, using key words that include general terms such as antimicrobial peptides and the names of specific peptides of note. In addition, manual searches of Medline were carried out, and the press releases of the major companies involved in the field were examined. The amount of literature in this field is becoming voluminous, so the references used were highly selected to present specific points of note, and many general review articles rather than the first paper presenting a given topic. I recommend that readers also examine review articles referenced herein to find many other excellent papers.

Conclusion

Antimicrobial peptides offer an enormous range of useful activities ranging from antimicrobial to immunomodulation. They are proceeding to the clinic as topical antibiotic agents. However, elucidation of their biological importance in innate immunity and realisation of their full clinical potential will require much more effort.

Acknowledgments

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