Antimicrobial Peptide Resistance Mechanisms of Human Bacterial Pathogens

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Abstract
The critical role played by antimicrobial peptides (AMPs) in mammalian innate immunity is increasingly recognized. Bacteria differ in their intrinsic susceptibility to AMPs, and the relative resistance of some important human pathogens to these defense molecules is now appreciated as an important virulence phenotype. Experimental analysis has identified diverse mechanisms of bacterial AMP resistance including altered cell surface charge, active efflux, production of proteases or trapping proteins, and modification of host cellular processes. The contribution of these resistance mechanisms to pathogenesis is confirmed through direct comparison of wild-type bacteria and AMP-sensitive mutants using *in vivo* infection models. Knowledge of the molecular basis of bacterial AMP resistance may provide new targets for antimicrobial therapy of human infectious diseases.

Introduction
In a complex environment, higher organisms face the constant threat of microbial infection. An important component of mammalian innate immunity is recognized to be the production of small, cationic antimicrobial peptides (AMPs). Recent discoveries using knockout mice and transgenic expression systems have confirmed that AMPs play a crucial role in restricting microbial proliferation to skin and mucosal surfaces, and preventing spread to the deep tissues where serious infection may develop. Studies of human AMP expression have revealed sophisticated systems of regulation: skin keratinocytes and mucosal epithelial cells produce very low levels of AMP under baseline conditions, but their expression of AMPs can be induced dramatically in response to injury or infectious stimuli. These epithelial barrier functions are further supplemented by AMPs produced by leukocytes resident in the tissues (macrophages, dendritic cells, mast cells) or recruited in the acute inflammatory response (neutrophils).

Throughout evolution, each member of the human microflora seeks to maintain a survival advantage. Microbial adaptation is appreciated to come more quickly, with replication times often measured in minutes and hours rather than decades between human generations. As is increasingly the case with pharmaceutical antibiotics, it appears that bacteria exposed to human AMPs have evolved under selective pressure to develop mechanisms of resistance. However, even though these selective pressures have existed for countless centuries, human AMPs still possess a broad-spectrum of potent activity against a diverse array of Gram-positive and Gram-negative bacterial species, fungi, as well as certain protozoan parasites and enveloped viruses. Though the ability to resist AMP killing appears to be a formidable challenge for microbial evolution, AMP resistance is increasingly recognized as a discriminating feature of some important human pathogens. When AMPs are rendered ineffective as a component of barrier defense or phagocytic killing, remaining host microbicidal functions maybe insufficient to prevent the risk of invasive infections produced by the AMP-resistant organism.

Here we review our current understanding of mechanisms that human pathogens have evolved to resist AMP killing. In particular, research studies on bacterial disease agents have uncovered a great diversity of viable strategies for avoiding AMP killing. These include decreased affinity through cell surface alterations, external trapping mechanisms, membrane efflux pumps, peptidases, and downregulation of host AMP production. Experimental evidence linking the degree of bacterial AMP resistance to pathogenic potential will be discussed, as these findings corroborate the crucial role of AMP in host innate immune defense. Bacterial resistance to AMP has implications for a number of human disease conditions, and improved understanding of these mechanism may provide novel targets for antibiotic drug design.

AMP resistance through cell surface modification
The antibacterial action of the defensin and cathelicidin classes of human AMPs is derived from their unique amphipathic structure and charge characteristics. Not unlike individual tissue cells of higher organisms, the surface of microbes tends to display a preponderance of negative charge. The cationic nature of AMPs allows them to be drawn electrostatically to engage a microbe threatening to breech an epithelial barrier, or in the case of phagocytic cells, to effect the killing of a microbe following endocytotic uptake. When a critical concentration of AMPs accumulate on the microbial surface, intercolation and assembly of their hydrophobic faces within the cytoplasmic membrane leads to formation of ion channels or aqueous pores, leading to microbial death through hypoosmotic lysis (Gutsmann *et al*., 2001; Oren *et al*., 1999). Certain cathelicidins may additionally reach intracellular targets to disrupt microbial protein synthesis (Boman *et al*., 1993).

In bacteria, the principal site of AMP action is the cytoplasmic membrane. To reach this target, AMPs must negotiate and traverse enveloping structures of varying complexity. Gram-positive bacteria lack an outer membrane, but possess a thick cell wall composed of heavily crosslinked polymers of teichoic or lipoteichoic acids and peptidoglycan. Gram-negative bacteria do

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not produce teichoic acids, but have a multilayered surface structure including a peptidoglycan matrix in the periplasmic space beneath an outer membrane. This outer membrane contains the bound complex of lipid A, core polysaccharide and specific side-chain (O) polysaccharides known as lipopolysaccharide (LPS). Additional complexity to bacterial surfaces is generated by proteins anchored to the cell wall or membrane, and many human bacterial pathogens are notable for their production of surface polysaccharide capsules.

The Gram-positive human pathogen *Staphylococcus aureus* is a prominent cause of wound infections, cellulitis, abscesses, osteomyelitis, septic arthritis, endocarditis and septicemia. *S. aureus* exhibits significantly higher minimum inhibitory concentrations (MICs) to human AMPs than observed in similar organisms (Peschel and Collins, 2001). It appears that specific modifications of teichoic acid in the *S. aureus* cell wall go a long way to explaining this enhanced AMP resistance. Teichoic acids are polyol phosphate polymers, e.g. polyglycerol phosphate and polyribitol phosphate, forming a backbone that can be further modified by substitutions with amino sugars (e.g. *N*-acetylglucosamine) or the amino acid D-alanine. Generally, teichoic acids are polyanionic because of the abundance of phosphate groups in the repeating structure. However, incorporation of D-alanine is accomplished by an ester bond that leaves the positively charged amino group free and exposed (Fig. 1).

S. aureus incorporates significant quantities of D-alanine into its teichoic acid, reducing cell wall negative charge, and serving to repel the cationic AMPs before they can reach their target of action (Peschel, 2002). D-alanylation of teichoic acid in the *S. aureus* cell wall is accomplished by the gene products of a four gene operon, *dlt*ABCD. *S. aureus* harboring mutations in the *dlt* operon have increased cell-surface negative charge and are more sensitive to killing by human α-defensins and cathelicidin as well as variety of other cationic AMPs (Kristian *et al*., 2003b; Peschel *et al*., 1999). Conversely, overexpression of the *dlt* genes in wild-type *S. aureus* by introducing additional copies of the operon on a plasmid led to increased D-alanylation of teichoic acid, decreased cell surface negative charge, and increased levels of AMP resistance (Peschel *et al*., 1999). These discoveries prompted analysis of *dlt* operons that are present in other Gram-positive bacterial pathogens of humans. Group B *Streptococcus* (GBS) and *Listeria monocytogenes* each produce septicemia and meningitis in human neonates and immunocompromised individuals. Mutation of the GBS *dlt*A gene blocked D-alanylation of teichoic acid and increased the susceptibility of the organism to killing by human defensins and other mammalian AMPs

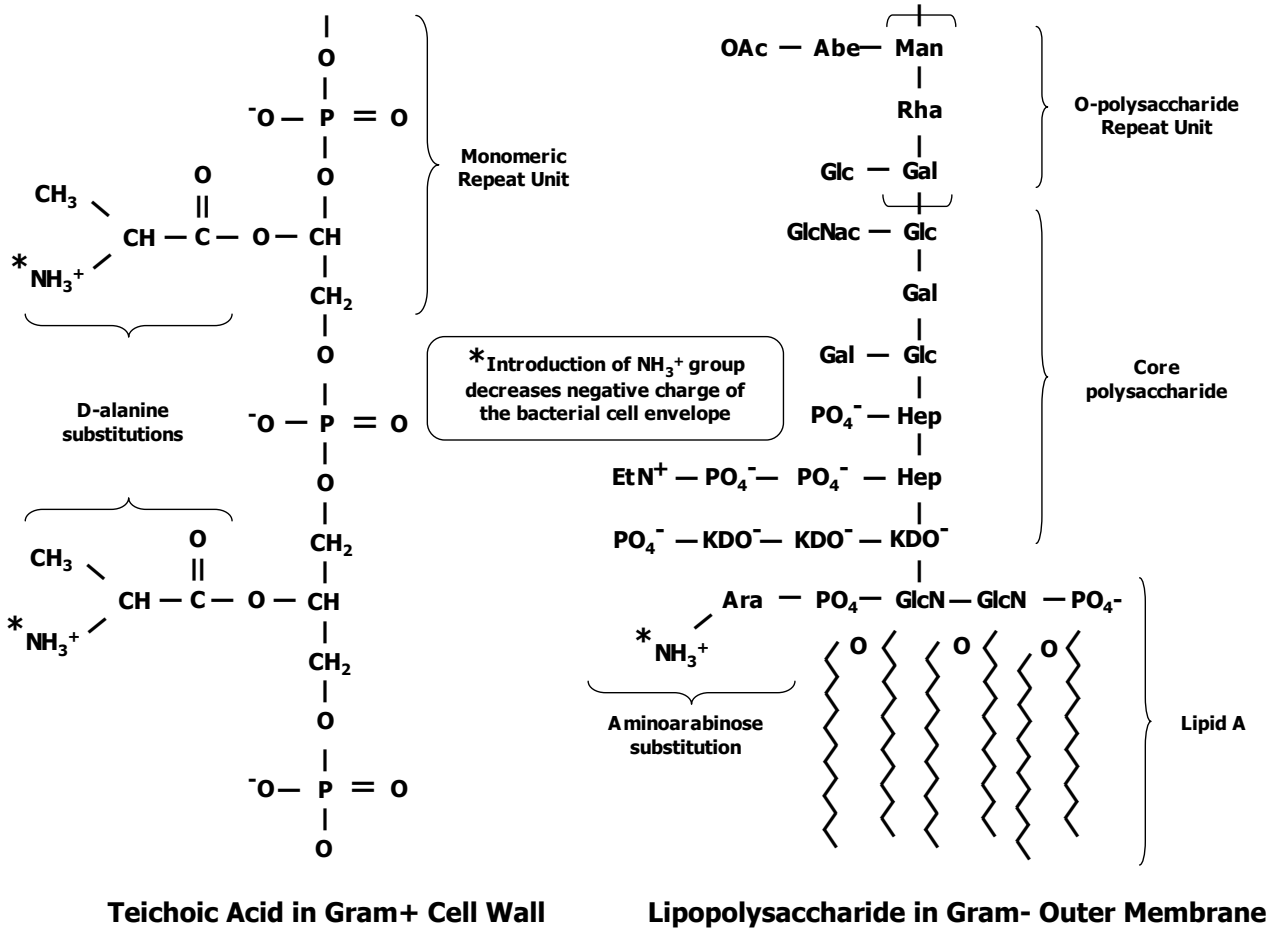


Fig. 1. Some specific surface modifications associated with decreased negative charge and increased resistance to cationic antimicrobial peptides.

(Poyart *et al*., 2003). Similarly, inactivation of *dlt*A in *L. monocytogenes*, an intracellular pathogen, led to decreased interaction with phagocytic and nonphagocytic cells and increased susceptibility to cationic AMPs (Abachin *et al*., 2002). The Gram-positive rod *Bacillus subtilis* also uses *dlt*A modification of techoic acid to reduce AMP sensitivity (Cao and Helmann, 2004).

Screening of an *S. aureus* mutant library for increased susceptibility to AMPs recently led to identification of another gene, *mpr*F, that encodes a novel function for modification of cell surface charge. The *mpr*F mutant was more sensitive to killing by neutrophil defensins, and was found to lack a positively-charged membrane phospholipid derivative enriched in the amino acid L-lysine (Peschel *et al*., 2001). Absence of this unusual phospholipid leaves only the highly anionic phosphatidylglcerol and diphosphatidylglycerol components in the cell membrane resulting in a high attraction for cationic AMPs. Expression of the *mpr*F gene in *Escherichia coli* was sufficient to confer production of the cationic lysine-substituted phosphatidylglcerol (L-PG), indicating that MprF indeed represents the L-PG synthase enzyme (Staubitz *et al*., 2004). Reduced lysyl-phosphatidylglycerol content in the cell membrane of *S. aureus* can also be seen with mutation of the *lys*C gene, which is involved in the biosynthesis of lysine from aspartic acid, with resulting sensitivity to human β-defensin and cathelicidin killing (Nishi *et al*., 2004).

In Gram-negative bacteria, the permeability and charge characteristics of the outer membrane influence the ability of cationic AMPs to reach their target on the cytoplasmic membrane. The outer leaflet of the outer membrane serves as the site of attachment for lipid A, an anionic dimer of glucosamine linked to fatty acid chains and flanked by polar phosphate groups. Covalently bonded to lipid A is a core polysaccharide and specific side-chain "O" polysaccharides that vary from species to species (Fig. 1a). The lipid A-polysaccharide complex is referred to as lipopolysaccharide (LPS) or endotoxin because of its proinflammatory and toxic effects in animals including humans. Modifications of the lipid A component of LPS appear to reduce overall negative charge appear to represent an important mechanism of AMP resistance in several Gram-negative human pathogens.

Salmonella species produce gastroenteritis and systemic infections including enteric fever in humans. The relative resistance of *Salmonella* to AMP killing has been studied extensively in the model organism *S. enterica* serovar *typhimurium*. A prominent *S. enterica* modification conferring resistance is the addition of 4-aminoarabinose (Ara4N) to the phosphate group of the lipid A backbone, for which two genetic loci are required: *pmr*E and *pmr*HFIJKLM. Nonpolar mutagenesis studies have demonstrated that all genes except pmrM are essential for Ara4N addition to lipid A and AMP resistance. Expression of these genes can be upregulated 3,000-fold by the actions of a two-component regulator system, PmrAB (Gunn *et al*., 2000). Ara4N modification of lipid A also contributes to AMP resistance in *Proteus mirabilis*, a frequent isolate in urinary tract infections including pyelonephritis. *P. mirabilis* mutants with loss of these Ara4N side groups demonstrate increased sensitivity to AMPs (McCoy *et al*., 2001). Similar function and regulation of the Salmonella *pmr* AMP resistance locus is appreciated in the homologous operon of *Yersinia pseudotubercolosis*, an agent of food poisoning and enterocolitis (Marceau *et al*., 2003). Inducible AMP resistance under control of a PmrAB-like signal transduction system has also been documented in *Pseudomonas aeruginosa* and linked to Ara4N modification of lipid A (Moskowitz *et al*., 2004). During its adaptation to chronic colonization of the airway of cystic fibrosis patients, *P. aeruginosa* has been shown to synthesize a unique hexa-acylated lipid A containing palmitate and aminoarabinose conferring increased resistance to AMPs and increased inflammatory responses (Ernst *et al*., 1999b).

Another mechanism linked to AMP resistance in *S. enterica* has been mapped to the *pag*P gene, which functions to increase acylation of lipid A. Mutants with interruption of *pag*P demonstrate increased outer membrane permeability in response to AMPs (Guo *et al*., 1998). A *pag*P mutation in the intracellular respiratory tract pathogen *Legionella pneumophila* also leads to increased sensitivity to cationic AMPs and decreased replication (Robey *et al*., 2001). Acylation of lipooligosaccharide in the Gram-negative coccobacillus and human pathogen *Haemophilus influenzae* encoded by the *htr*B gene is a key factor in resistance to human β-defensin 2. Mutation of *htr*B resulted in a greater than 45-fold increase in *H. influenzae* sensitivity to the AMP, a phenotype that was reversed by restoration of *htr*B in trans (Starner *et al*., 2002). *H. influenzae* also contains a phase-variable structure called phosphorylcholine (ChoP) on the oligosaccharide portion of LPS. Constitutive ChoP-expressing mutants exhibited 1,000-fold greater survival upon exposure to human cathelicidin LL-37 than mutants lacking ChoP (Lysenko *et al*., 2000). When the wild-type phase-variable *H. influenzae* strain was exposed to increasing concentrations of LL-37, progressive selection for the ChoP-positive phase was documented.

In summary, one common pattern of AMP resistance in both Gram-positive and -negative bacteria involves modification of normally anionic cell surface constituents with cationic molecules; the net effect of these substitutions is to repel positively-charged AMPs before they can reach the cytoplasmic membrane and disrupt its integrity (Fig. 2). Recently, additional studies have linked enzymes involved in biosynthesis and crosslinking of cell envelope components to AMP resistance in manners not necessarily involving altered charge. The *pon*A gene of GBS encodes penicillin-binding protein 1a (PBP1a), a high molecular weight bifunctional molecule with transglycosylase and transpeptidase activity. GBS *pon*A mutants are more sensitive to killing by both human cathelicidin and defensins (Hamilton *et al*., 2004). Phosphoglucomutase (*pgm*) is required for lipopolysaccharide production in *Bordetella bronchiseptica* and cell-wall integrity in *Streptococcus iniae*; mutations in the *pgm* gene in either species lead to increased sensitivity to cationic AMP killing (Buchanan *et al*., 2005; West *et al*., 2000). *Mycobacterium marinum* mutants harboring transposon insertions in the beta-ketoacyl-acyl carrier protein synthase B gene (*kas*B) grew normally *in vitro* but were inhibited in their growth in human macrophages (Gao *et al*., 2003). Chemical

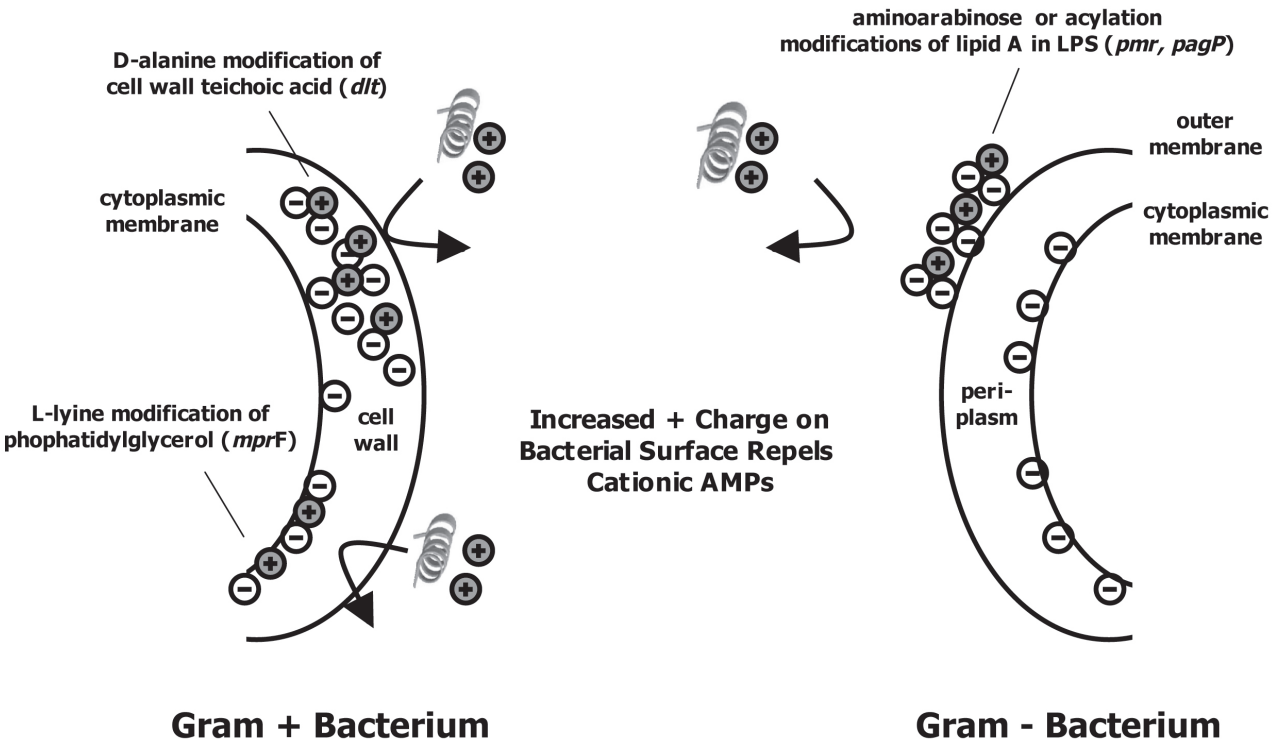


Fig. 2. Bacterial resistance to cationic antimicrobial peptides mediated by alterations in surface charge.

analysis revealed the *kas*B mutants synthesized unusually short mycolic acids, a unique family of fatty acids that contributes the low permeability of mycobacterial cell walls. The *M. marinum kas*B mutant was markedly more susceptible to killing by human defensins. Normal levels of AMP resistance could be restored to the *M. marinum* mutant by transformation with an expression vector containing *kas*B from *Mycobacterium tuberculosis*. These findings suggest that *kas*B may function similarly in *M. tuberculosis* to increase AMP resistance, and therefore could represent a novel target for treatment of a worldwide infection responsible for nearly 3 million deaths annually.

External trapping of AMPs

Another group of adaptations that allows certain pathogenic bacteria to avoid killing by AMPs involves binding or neutralization of the AMP. This form of resistance can be accomplished directly through the actions of a bacterial surface-associated or secreted protein, or indirectly by the induced release of AMP binding molecules from host cell surface (Fig. 3). When a significant proportion of the AMP released through the innate immune response is bound to the interfering molecules, insufficient quantities of the AMP reach the bacterial cell membrane to effect bacterial killing.

S. aureus produces an exoprotein called staphylokinase (SK), which has been studied extensively due to its ability to activate host plasminogen. A recent study discovered that SK is able to directly bind the α-defensins produced by human neutrophils. The consequence of the SK-AMP complex was a near complete inhibition of bactericidal effects on the *S. aureus* bacterium (Jin *et al*., 2004). The neutralizing effect of SK on the AMP was independent of plasminogen-binding site. Testing of a panel of *S. aureus* strains found that those producing SK were resistant to α-defensins, and that addition of purified SK to SK-negative *S. aureus* cultures rescued them from α-defensin killing (Jin *et al*., 2004). It is suggested that AMP-binding by SK may work together with *dlt* and *mpr*F-mediated cell-surface charge modifications for neutrophil defensin resistance during *S. aureus* infections.

Group A *Streptococcus* (GAS) is an important Gram-positive human pathogen associated with a wide range of mucosal and invasive infections, from simple pharyngitis and impetigo to potentially life-threatening necrotizing fasciitis and toxic shock syndrome. Strain variability in GAS is the result of hypervariable domains at the N-terminus of the surface M protein. M protein is anchored to the GAS cell wall by its C-terminus, then extends outward as a coiled-coil dimer to forms a fimbrial coating over the bacterial surface. M protein is recognized as a virulence factor with antiphagocytic and host cell adherence properties encoded in its conserved C-terminal and central domains, but the function of the hypervariable domain has been less well studied. Of the more than 150 GAS M-protein serotypes, a globally disseminated M1 serotype clone has been associated with a resurgence of severe, invasive GAS infections documented since the mid-1980s (Efstratiou, 2000). Analysis of a collection of GAS clinical isolates from the Centers from Disease Control found that M1 strains exhibited significantly higher levels of resistance to human cathelicidin LL-37 than strains belonging to other M serotypes associated only with superficial infections (Lauth *et al*., 2004). A knockout of the *emm*1 gene encoding M protein increased the sensitivity of M1 GAS to LL-37, while heterologous expression of the M1 protein in M49 GAS or *Lactococcus lactis* caused the transformed bacteria to gain cathelicidin resistance.

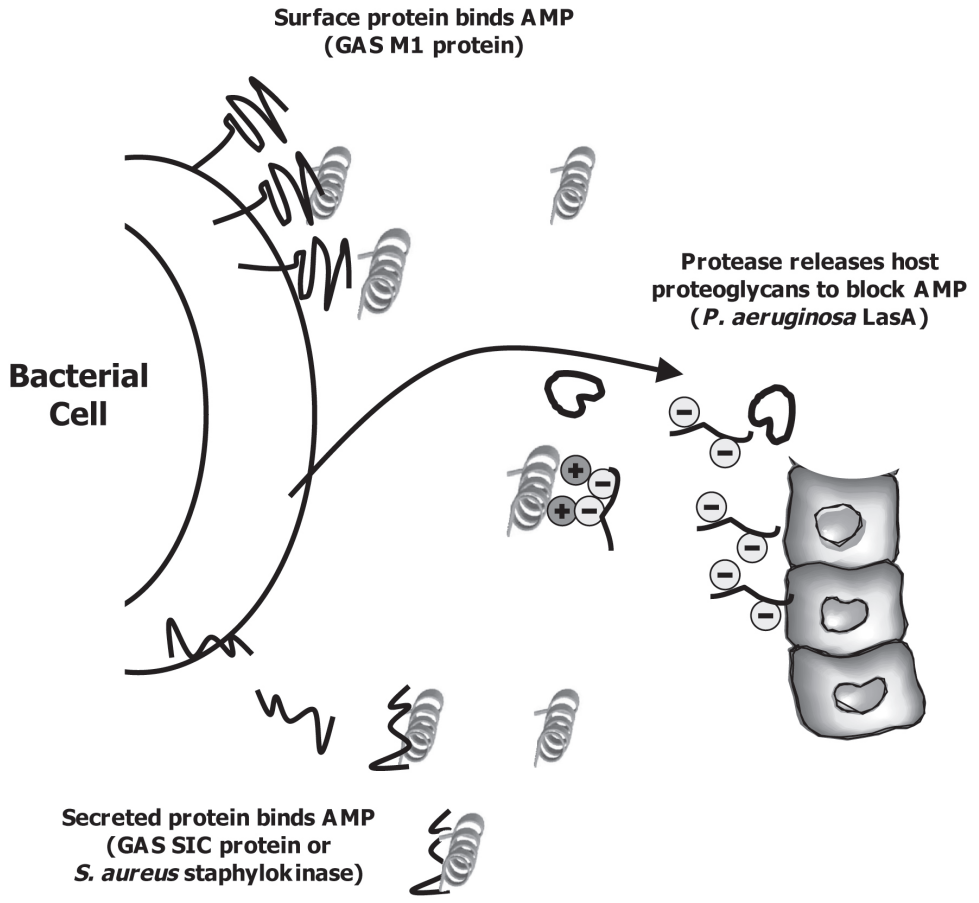


Fig. 3. Bacterial resistance to cationic antimicrobial peptides mediated by mechanisms for trapping and inactivation.

Studies with recombinant peptide fragments mapped the AMP binding property to the hypervariable C-terminus of M1 protein, a function lacking in the same domain from GAS M49 protein (Lauth *et al*., 2004). Virulent GAS M1 strains secrete a protein called SIC is absent in the vast majority of noninvasive GAS strains. SIC was found to bind and inactivate LL-37 and human α-defensin, protecting the bacterium from their antimicrobial action (Frick *et al*., 2003). In these studies, a SIC-negative M1 GAS mutant showed increased sensitivity to AMP killing.

Another mechanism by which bacteria appear to trap and inactivate AMPs is by exploitation of negatively charged proteoglycan molecules that decorate the surfaces of host epithelial cells. For example, extracellular proteases secreted by the human pathogens GAS, *Enterococcus faecalis* and *P. aeruginosa* degrade decorin and other cell-surface proteoglycans, thereby releasing dermatan sulfate. Free dermatan sulfate can then in turn bind and inactivate human α-defensins (Schmidtchen *et al*., 2001). *P. aeruginosa* also enhances shedding of the heparan sulfate proteoglycan syndecan-1 from various host cells; this phenotype can serve to neutralize cationic AMPs and has been attributed to the actions of a 20-kDa protein virulence factor LasA (Park *et al*., 2000). Given these observations regarding the AMP neutralizing effects of host proteoglycans, it is interesting to speculate whether surface exposed bacterial carbohydrates could play a role in AMP resistance. For example, certain human pathogens possess exopolysaccharide capsules composed of hyaluronan (e.g. GAS) or chondroitin (e.g. *Pasteurella multocida*), or enriched in sialic acid moieties (e.g. GBS, *Neisseria meningitides*). Acapsular mutants in all these species loose virulence, a phenomenon classically attributed to interference with complement-mediated opsonphagocytosis. It will be interesting in future studies to explore a potential role of acidic sugar capsules in AMP trapping as an additional selective advantage. Such a correlation has recently been suggested for the pathogen *Klebsiella pneumoniae*, as mutants lacking capsular polysaccharide were demonstrated to be more susceptible to a variety of cationic AMPs including human α- and β-defensins (Campos *et al*., 2004).

Active efflux of AMPs

Energy-driven drug efflux systems are increasingly recognized as mechanisms of bacterial resistance to conventional pharmaceutical antibiotics. The major categories include pumps energized by ATP and those driven by proton motive force I (Levy, 2002). The efflux pumps may recognize a limited number of substrates, such as *mef*A in GAS for macrolide efflux. or a broad range of antimicrobials, for example the *mex*CD-*opr*J multidrug efflux pump in *P. aeruginosa*. Exposure to antibiotics promotes the mutational overexpression of active or silent multidrug transporters, leading to increased antibiotic resistance without a requirement for acquisition

of multiple, specific resistance determinants (Zgurskaya, 2002). Evidence indicates that in a few cases, efflux systems may also contribute to the intrinsic resistance of certain human pathogens to the action of cationic AMPs of the innate immune system (Fig. 4).

Neisseria gonorrhoeae is a major agent of sexually transmitted infections in humans including urethritis and cervicitis. *N. gonorrhoeae* express an energy-dependent efflux system MtrCDE that expels hydrophobic antimicrobial agents and increases resistance to antibiotics such as penicillin and erythromycin (Veal *et al*., 2002). Genetic and biochemical evidence has demonstrated that gonococcal resistance to protegrin-1 and human cathelicidin LL-37 is modulated by overexpression of the *mtr* genes (Shafer *et al*., 1998). *S. enterica* mutants with a defect in the *sap* locus sharing homology with potassium-linked efflux systems in *E. coli* are more susceptible to the cationic AMP protamine (Parra-Lopez *et al*., 1994), while a homologous locus in H. influenzae promotes resistance to β-defensin (Mason *et al*., 2005). Certain *S. aureus* strains harbor a multiresistance plasmid pSK1 that encodes the QacA efflux pump. *S. aureus* positive for *qac*A exhibit higher levels of resistance to a cationic rabbit platelet AMP, tPMP, but not to other structurally distinct AMPS including human α-defensin and protamine (Kupferwasser *et al*., 1999).

Proteolytic degradation of AMPs

Many pathogenic bacterial species produce proteases or peptidases that contribute to disease pathogenesis by altering host substrates or by inactivating key components of the host immune response. Examples include cleavage of IgA by *S. pneumoniae* (Weiser *et al*., 2003), inactivation of complement C5a by GBS (Hill *et al*., 1988), or degradation of chemokine interleukin-8 by GAS (Hidalgo-Grass *et al*., 2004). One of the reasons that mammalian AMPs have remained such an effective and broad-spectrum component of innate immune defense is their relative resistance to proteolytic degradation, for example proline-rich sequences in many cathelicidin peptides resist attack by serine proteases because proline prevents cleavage of the scissile bond (Shinnar *et al*., 2003). Nevertheless, certain important pathogens of humans secrete, or express on their surface, proteases that recognize and cleave cationic AMPs, and therefore allow the bacteria avoid the microbicidal action of the host peptide (Fig. 4).

The human cathelicidin LL-37 is the target of proteases from the important human pathogens GAS, *P. aeruginosa*, *P. mirabilis* and *Enterococcus faecalis* (Schmidtchen *et al*., 2002). In some experiments, cell culture supernatants prepared from GAS expressing cysteine proteinase, *P. mirabilis* expressing a 50 kDa

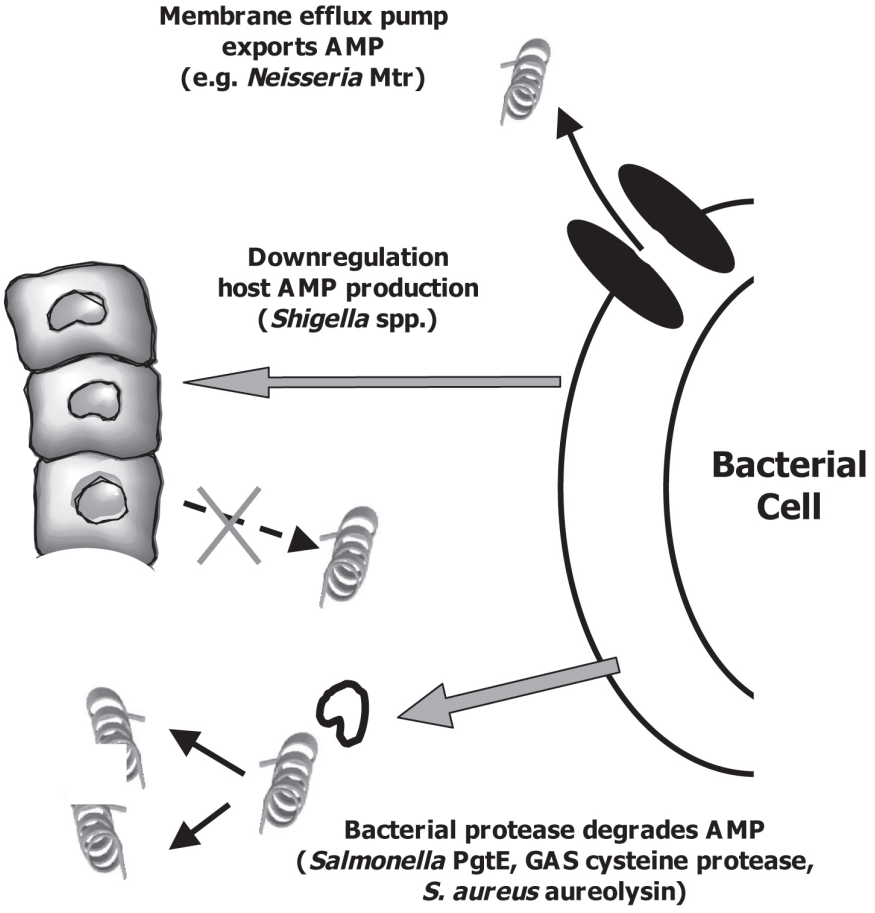


Fig. 4. Bacterial resistance to cationic antimicrobial peptides mediated by efflux pumps, proteolytic activation, or downregulation of host production.

metalloprotease, or *E. faecalis* expressing gelatinase were found to degrade LL-37, while LL-37 was preserved when specific inhibitors of each protease were added to the assay. More extensive mass spectrometry analysis on LL-37 degradation by *P. aeruginosa* elastase mapped the proteolytic activity to initial cleavages at Asn-Leu and Asp-Phe, and *ex vivo* studies showed that expression of the bacterial elastase enhanced survival of *P. aeruginosa* in a wound fluid environment (Schmidtchen *et al*., 2002). The metalloprotease aureolysin of *S. aureus* cleaves and inactivates LL-37 in a concentration and time-dependent manner, and a inverse correlation can be established between the level of aureolysin production of *S. aureus* strains and their susceptibility to LL-37 killing (Sieprawska-Lupa *et al*., 2004).

An outer membrane protease of *S. enterica* known as PgtE can specifically cleave C18G, an α-helical cationic AMP. Mutagenesis of the pgtE rendered the *S. enterica* strain more susceptible to C18G and the human cathelicidin LL-37 (Guina *et al*., 2000). Proteases produced by *E. coli* and *S. aureus* inactivate lactoferricin B, a cationic AMP derived from the N-terminus of mammalian lactoferrin (Ulvatne *et al*., 2002). Culture supernatants or purified proteases of the human periodontal pathogens *Porphyromonas gingivalis* and *Prevotella* spp. are known to inactivate cationic AMPs such as cecropin B and brevinin (Devine *et al*., 1999), but their effect on human AMPs has not been determined.

Bacterial gene regulation and AMP resistance

A number of important human bacterial pathogens have global regulatory networks for coordinate upregulation or downregulation of virulence factors dependent on growth phase or upon environmental cues encountered in different tissue niches of their host. Well-studied examples include the PhoP/PhoQ two-component regulatory system of *S. enterica*, the *agr*-encoded quorom sensing locus of *S. aureus*, the response regulator ArcA of *Vibrio cholerae* or the *cov*RS locus of GAS. Recent evidence indicates that some of the complex phenotypes involved in determining bacterial resistance to AMPs appear to have come under transcriptional control by such global regulation systems.

The PhoP/PhoQ two-component system of *S. enterica* governs transcription of at least 25 genetic loci in response to changes in the extracellular concentration of magnesium and calcium, such as those encountered within the phagolysome of host macrophages. The PhoP/PhoQ regulators promote remodeling of the bacterial envelope, including upregulation of enzymes that modify LPS charge (Ernst *et al*., 1999a; Ernst *et al*., 2001). Mutations in the *pho*PQ operon decrease *S. enterica* survival within macrophages and increase susceptibility to cationic AMPs and acid pH (Garcia Vescovi *et al*., 1994). One of the targets of PhoP/PhoQ upregulation is the PgtE outer membrane protease that degrades α-helical AMPs (Guina *et al*., 2000), while another is the *pag*P gene required for lipid A acylation (Guo *et al*., 1998). A homologous PhoP/PhoQ regulatory system in *P. aeruginosa* regulates resistant to cationic AMPs including cathelicidin LL-37 in response to low magnesium conditions (Macfarlane *et al*., 2000; McPhee *et al*., 2003). In both *S. enterica* and *P. aeruginosa* a second two-component regulatory system called PmrA/PmrB is involved in upregulating AMP resistance through an increase in the gene expression for Ara4N incorporation in LPS (Gunn *et al*., 2000; McPhee *et al*., 2003).

The extracytoplasmic sigma factor RpoE is required for *Salmonella* resistance to the murine α-defensin cryptdin-4, and the expression of the *rpo*E gene is induced adaptively when the bacterium is exposed to the peptide (Crouch *et al*., 2005). A number of other *S. enterica* genes with homology to transcriptional regulators (*vir*K, *som*A, *rcs*C) have recently been identified and linked to resistance against killing by the cationic AMP polymixin B. It is hypothesized but not proven that these loci contribute to remodeling of the bacterial outer membrane in response to the host environment (Detweiler *et al*., 2003). The thermoregulated transcription factor PrfA of *L. monocytogenes* controls the expression of as-yet-undefined phenotypic properties that influence the susceptibility of the organism to defensins (Lopez-Solanilla *et al*., 2003). Lastly, the *dlt* operon encoding D-alanylation of techoic acid in is among the *agr*-regulated genes identified in transcriptional profiling of *S. aureus* (Dunman *et al*., 2001), but a link between the *agr* locus and AMP resistance is not conclusively established.

It is likely that future studies will identify more pathways and levels of transcriptional control for genes encoding AMP resistance. Intuitively, a bacterial species must seek to strike a balance between surviving innate immune clearance and the potential metabolic expenditures required to undergo the resistance-conferring biosynthetic modifications. An exciting observation in examination of these mechanisms is the ability of the bacteria to upregulate the PhoP/PhoQ or PmrA/PmrB signaling cascades upon sensing the presence of subinhibitory concentrations of cationic AMPs (Bader *et al*., 2003; McPhee *et al*., 2003). In effect, these pathogenic bacteria can adapt themselves in real time to survive against the challenges of AMP-mediated innate immune clearance mechanisms.

Bacterial regulation of host AMP production

One of the elegant features of AMPs in mammalian innate immunity are their inducibility. Only low levels of the defense peptides are generated by epithelia and leukocytes at baseline, yet their expression can be dramatically induced in response to infectious challenge. For example, immunostaining demonstrates that subcutaneous infections with the Gram-positive pathogen GAS provoke marked increases in local expression of cathelicidin both mice and humans (Dorschner *et al*., 2001; Lauth *et al*., 2004). In porcine and bovine models, leukocyte expression of cathelicidin is specifically induced in response to Gram-negative bacterial infection or LPS (Tomasinsig *et al*., 2002; Wu *et al*., 2000). Recent studies of human airway epithelial cells indicate that β-defensin expression can be induced by cell wall lipoteichoic acid in a process involving Toll-like receptor-2 recognition and activation of NFκB signal transduction pathways (Birchler *et al*., 2001; Wang *et al*., 2003), and the epidermal β-defensin-2 response to LPS is further augmented by mononuclear cell production of interleukin-1 (Liu *et al*., 2003).

Interference with or suppression of such host pathways for AMP induction appears to be another mechanism by which certain pathogenic bacteria can avoid innate immune clearance. For example, while several bacterial species strongly induce human β-defensin-2 expression from skin epithelial cells, the common skin pathogen GAS is a very poor and variable inducer of the AMP (Dinulos *et al*., 2003). This observation suggests that GAS avoids the initial pattern recognition receptors or somehow actively downregulates the β-defensin-2 induction pathway. An elegant study of the human gastrointestinal pathogen *Shigella dysenteriae* found that expression of the AMPs LL-37 and β-defensin-1 is downregulated at the transcriptional level in the early stages of infection (Islam *et al*., 2001) (Fig. 4). The downregulation was first observed in biopsies from humans with bacillary dysentery then confirmed in epithelial and monocyte cell culture experiments; plasmid DNA from *S. dysenteriae* appeared to be one molecule contributing to the inhibition.

In contrast to inhibiting host AMP production, an alternative bacterial survival strategy can involve the stimulation of host mechanisms that function to counterregulate the AMP response. Such a process may take place in the lungs of patients with cystic fibrosis, where the pathogen *P. aeruginosa* stimulates the accumulation of the cysteine protease cathepsins B, L and S in the airway fluid. These proteases degrade and inactivate human β-defensins 2 and 3, favoring the chronic bacterial infection and colonization characteristic of the disease (Taggart *et al*., 2003).

Finally, it is becoming apparent that regulation of skin and mucosal AMP defenses will only be fully understood in the greater context of the normal (nonpathogenic) microflora. Over the years, much interest has been focused on how loss of tolerance to normal gut microflora may play a role in the pathogenesis of inflammatory bowel disease (Landers *et al*., 2002), or conversely, how oral administration of commensal bacteria may have therapeutic immunomodulatory ("probiotic") effects (Gionchetti *et al*., 2002). The recent discovery of the antimicrobial properties of angiogenins, cationic peptides produced in Paneth cells of the intestinal crypts, is interesting in this regard. In the murine model, angiogenin-4 production is strongly induced by the normal microflora constituent *Bacteroides thetaiotaomicron* (Hooper *et al*., 2003), a property which distinguishes it from other intestinal AMPs. Though the *B. thetaiotaomicron* bacterium itself is relatively resistant to angiogenin-4 action, the peptide shows killing activity against a number of Gram-positive and -negative pathogens. The fascinating implication of these studies is that bacteria of the normal flora may play a critical role in establishing AMP defenses, such that perturbations in the host-commensal relationship could adversely impact the innate immune barrier of the gut mucosa.

Correlation of AMP resistance with virulence

This review has identified several phenotypic properties of pathogenic bacteria that serve directly or indirectly to increase their resistance to cationic AMPs *in vitro*. In the realm of innate immunity, these phenotypes would mean little without evidence that bacterial AMP resistance contributes to disease potential. Such a link has firmly been established, and represents a byproduct of two lines of investigations: (a) animal studies that establish an essential role of mammalian AMP production in innate defense against bacterial infection, and (b) studies with isogenic mutant bacteria that associate *in vitro* AMP sensitivity or resistance to *in vivo* pathogenicity.

Definitive proof that the endogenous expression of AMPs contributes to mammalian innate immunity was achieved only recently through the generation and analysis of knockout mice. A mouse deleted for the Cnlp gene encoding the sole mouse cathelicidin mCRAMP exhibits normal growth and development and produces normal numbers of circulating leukocytes (Nizet *et al*., 2001). However, blood and isolated mast cells from Cnlp–/– mice are notably deficient in killing the pathogen *S. pyogenes,* and knockout mice challenged with this bacterium develop much more severe and persistent necrotizing skin infections than their wild-type littermates (Di Nardo *et al*., 2003; Nizet *et al*., 2001). Compared to normal littermates, Cnlp–/– mice are more susceptible to intestinal colonization, epithelial cell damage, and systemic dissemination of the murine intestinal pathogen *Citrobacter rodentium*, establishing cathelicidin as an important component of innate immune defense in the colon (Iimura *et al*., 2005). A mCRAMP-sensitive mutant of the intracellular pathogen *S. typhimurium* showed enhanced survival in macrophages from Clnp–/– mice, indicating cathelicidin contributes to killing within the phagolysosome (Rosenberger *et al*., 2004). Defb1, a murine homologue to human β-defensins, is expressed on respiratory epithelium. The Defb1–/– mouse showed delayed clearance of *H. influenzae* from the lung and increased bladder colonization with *S. aureus*. (Morrison *et al*., 2002; Moser *et al*., 2002). Similarly, mice deficient in the metalloproteinase matrilysin required for cryptdin (α-defensin) activation possess decreased enteric microbicidal activity against orally administered *Salmonella* (Wilson *et al*., 1999).

Complementary investigations indicate that augmentation of AMP levels on host mucosal surfaces can retard bacterial colonization and enhance resistance to infection. In these experiments, increased AMP levels are achieved through gene therapy or by exogenous administration of purified or recombinant peptide. For example, the airway surface fluid of cystic fibrosis xenografts failed to kill *P. aeruginosa* or *S. aureus*; however, exposure of the xenografts to an adenovirus expressing human cathelicidin restored bacterial killing to normal levels. Mice treated intratracheally with this adenoviral-AMP vector had a lower bacterial load and diminished inflammatory response after *P. aeruginosa* challenge. In lambs with pneumonia, intratracheal administration of cathelicidin AMP reduced the concentration of bacteria in pulmonary tissues and fluid (Brogden *et al*., 2001). Topical administrations of a gel containing a cationic AMP has also been shown to decrease *Trichomonas vaginalis* colonization of vaginal epithelium in mice (Lushbaugh *et al*., 2000), block *P. aeruginosa* superinfection of experimental burns in rats (Chalekson *et al*., 2002), and inhibit development of wound infections in pigs (Ceccarelli *et al*., 2001). Transgenic mice expressing human intestinal defensin-5 show marked resistance to oral challenge with

Salmonella (Salzman *et al*., 2003); similarly, transgenic mice expressing porcine cathelicidin are less susceptible to GAS skin infection (Lee *et al*. 2005). Lastly, dermal wounds in nude mice can be treated with grafting of a genetically-engineered epidermal sheet expressing human β-defensin 3, which functions to produce augmented levels of the defense peptides (Sawamura *et al*., 2005).

Given the mounting direct evidence that AMPs play a critical role in innate mucosal defense, the correlation of *in vivo* bacterial AMP resistance phenotypes to pathogenic potential is afforded considerable credence. Direct comparisons of resistant wild-type bacteria with isogenic, AMP-sensitive mutants have been performed for each of the major classes of resistance mechanisms, providing validation of the adaptive benefit of these phenotypes for survival of the organism in the mammalian host.

Dlt– and MprF– mutants of *S. aureus* lack D-alanine and L-lysine cell wall modifications, respectively, and are more sensitive to AMP activity *in vitro*. Both types of mutants are more readily phagocytosed by neutrophils and less able to establish systemic infection in mice (Kristian *et al*., 2003a; Kristian *et al*., 2003b; Peschel *et al*., 2001). A Dlt– mutant of *L. monocytogenes* possesses a 10,000-fold greater LD50 than the wild-type strain in murine infection (Abachin *et al*., 2002), and a Dlt– GBS strain was severely impaired in both mouse and neonatal rat models (Poyart *et al*., 2003). *S. enterica* mutants in PmrA lack the aminoarabinose modification of LPS associated with AMP resistance and are less virulent than wild-type following oral administration in a murine enteric infection model; however, the mutants are equally virulent when inoculated systemically (Gunn, 2001; Gunn *et al*., 2000), indicating a specific role for the phenotype in resistance to mucosal defenses. Lipid A acylation encoded by a *pag*P homologue in *Legionella* is required for AMP resistance and for efficient lung colonization in mice (Robey *et al*., 2001). Thus in both Gram-positive and -negative bacteria, specific cell surface modifications resulting in decreased negative charge and enhanced resistance to cationic AMPs are associated with greater pathogenic potential.

Bacterial resistance achieved through external trapping of AMPs can also be correlated to increased pathogenicity. GAS mutants lacking the surface-associated M1 protein or secreted SIC protein are more easily cleared *in vivo* upon mouse challenge (Frick *et al*., 2003), however these proteins have additional properties of complement interference. Staphylococcal strains producing AMP-binding staphylokinase were protected against the bactericidal effect of α-defensins in murine arthritis model (Jin *et al*., 2004). The pathogen *P. aeruginosa*, through its virulence factor LasA, enhances *in vitro* shedding of the proteoglycan syndecan-1 which interferes with AMP function (Park *et al*., 2000). Newborn mice deficient in syndecan-1 resist *P. aeruginosa* lung infection but become susceptible when treated with syndecan-1 derived heparan sulfate chains (Park *et al*., 2001).

N. gonorrhoeae lacking the MtrCDE efflux system for AMP resistance were deficient in their ability to establish genital tract infection in mice (Jerse *et al*., 2003). Nontypeable *H. influenzae* with a deficiency in the *sap* AMP transport locus had attenuated survival in a chinchilla model of otitis media (Mason *et al*., 2005). The PhoP/PhoQ regulatory system controls multiple virulence phenotypes in *Salmonella spp*., including a K^+-coupled AMP efflux pump and the AMP protease, PgtE. *S. enterica* mutants in the PhoP/PhoQ regulatory system are more sensitive to AMP killing and significantly less virulent in the murine gastrointestinal infection model (Parra-Lopez *et al*., 1994). The inner membrane protein Mig-14 inhibits binding of CRAMP to *Salmonella* by an unknown mechanism (Brodsky *et al*., 2002). *Salmonella* mutants deficient in Mig-14 are more susceptible to murine cathelicidin mCRAMP, impaired in their survival within activated macrophages, and unable to establish chronic splenic and hepatic infection after orogastric administration (Brodsky *et al*., 2005).

The studies above have used mutagenesis to target bacterial phenotypes associated with AMP resistance *in vitro* and determined experimentally that loss of the resistance phenotype translates to decreased virulence *in vivo*. Recently, a study of GAS pathogenicity approached the issue from the opposite perspective. This bacterium is normally sensitive to cathelicidin killing, but resistant mutants can be selected by serial exposure to cathelicidin *in vitro*. Upon subcutaneous inoculation of mice, the AMP-resistant *S. pyogenes* mutant was found to induce more severe necrotizing skin infections than the wild-type parent strain. While the sensitive wild-type strain was killed in whole blood from normal mice, the resistant mutant proliferated. As predicted, no difference between wild-type and AMP-resistant mutant bacteria were seen for growth in blood from cathelicidin-deficient mice (Nizet *et al*., 2001).

Altered AMP production and opportunistic infection

The contribution of intrinsic bacterial AMP resistance to infectious disease pathogenesis is exaggerated when local AMP production is impaired. Recent studies have determined that decreased AMP production or local interference with AMP function may explain the increased infectious risks associated with certain chronic diseases or epidermal injuries. In such settings, a lower effective concentration of AMP allows survival or proliferation of bacterial species that would normally be eradicated, creating a potential for opportunistic infection.

Kostmann syndrome is a rare inherited disorder characterized by severe congenital neutropenia. These patients are treated with granulocyte-colony stimulating factor to increase neutrophil counts within the normal range; nevertheless, they still experience frequent infections and develop periodontal disease. Functional analysis of neutrophils isolated from these patients demonstrated normal oxidative burst function, but revealed a lack of measurable cathelicidin and diminished levels of α-defensins (Putsep *et al*., 2002). The chronic inflammation of cystic fibrosis is associated with increased levels of AMP in respiratory tract secretions. However, the cystic fibrosis airway surface fluid is diminished in its ability to kill bacteria. This defect is reflected in chronic high level bacterial colonization and recurrent pneumonia with low virulence organisms such as *P. aeruginosa*. The bacterial killing ability of cystic fibrosis airway fluid is

restored when its salt concentration is lowered to normal levels, suggesting the abnormally high salt concentrations produced by the defective cystic fibrosis transmembrane conductance regulator may be responsible (Smith *et al*., 1996). The bacterial killing ability of epithelial-derived AMPs such as the human β-defensins and cathelicidin are inactivated by high salt concentrations (Bals *et al*., 1998; Goldman *et al*., 1997), suggesting a defect in this component of innate immune defense may be responsible for the chronic pulmonary infections seen in cystic fibrosis patients.

Crohn's disease (CD) is a chronic disease of the intestinal mucosa characterized by granulomatous inflammation. Abnormal response to intestinal bacteria has been postulated to play a role in development of CD, including culture studies identifying *Mycobacterium avium*, subsp. *paratuberculosis* in the blood of CD patients (Naser *et al*., 2004). The inflamed colonic mucosa in CD is characterized impaired of constitutive and inducible production of β-defensins (Wehkamp *et al*., 2005). When CD affects the small intestine, diminished defensin expression is observed in Paneth cells of the ileum, and this deficiency is even more pronounced in the subset of CD patients harboring a mutation in the gene encoding the Nod2 protein (Wehkamp *et al*., 2005). Nod2 is a pattern recognition receptor for bacterial muramyl dipeptide that is required for the expression of the cryptdin group of intestinal β-defensins. The importance of Nod2 in innate mucosal immunity of the intestine has recently been explored using Nod2-deficient mice. These animals are susceptible to bacterial infection via the oral route but not through intravenous or intraperitoneal challenge (Kobayashi *et al*., 2005).

Differential expression of AMPs appears to play a determinative role in the susceptibility of chronic inflammatory skin disorders to infectious complications. In psoriasis, cathelicidin and β-defensin levels are elevated and secondary infection is rare, whereas in atopic dermatitis cathelicidin expression of the same AMPs is deficient and bacterial or viral superinfection is common. Especially relevant in this regard is the proven activity of human cathelicidin against *S. pyogenes* (Dorschner *et al*., 2001) and the synergistic activity of human cathelidicin and β-defensin against *S. aureus*, the leading agents of superficial and invasive skin infection in humans. Expression of β-defensin is absent in full-thickness burn wounds and blister fluid from partial thickness burns (Ong *et al*., 2002), evidence of an innate immune defect that may be contribute in the greatly increased risk of burn wound infection and sepsis. In chronic ulcers, cathelicidin levels are low and absent in the ulcer edge epithelium (Heilborn *et al*., 2003). Cathelicidin AMPs are important in successful wound closure and defects in their production can be correlated with development of chronic ulcers.

Conclusions and future perspective

AMPs represent an evolutionarily ancient defense strategy now recognized to play an important role in human innate immunity. Not all bacteria are created equal when it comes to AMP susceptibility, and it appears that the ability to resist AMP killing is a discriminating feature of several important human pathogens. Bacteria such as *S. aureus* and *Salmonella* spp. that generally exhibit AMP resistance would be predicted to possess a survival advantage on compromised epithelium, in deeper body tissues, and in the phagolysosomes of neutrophils and macrophages. This concept is supported by the fact that *S. aureus* is the most common cause of human wound infections and deep-tissue abscesses, while *Salmonella* spp. are leading agents of chronic systemic infections (enteric and typhoid fever) that can develop in otherwise healthy individuals. In contrast, bacteria more sensitive to AMPs, such as *E. coli*, can occupy a niche on mucosal surfaces with local or toxin-mediated disease effects, generally invading deep tissues only in groups with broader defects in innate or acquired immunity (e.g. neonates, the elderly or chemotherapy patients).

The selective pressure toward bacterial AMP resistance is reflected in the broad diversity of AMP resistance mechanisms that have been elucidated in recent molecular microbiologic investigations (summarized in Table 1). One path to resistance shared by several bacterial species and achieved in various fashions involves modification of the normal anionic constituents of their cell surfaces to repulse rather than attract cationic AMPs. Alternative resistance mechanisms include the binding, active efflux or proteolytic activation of AMPs before they reach their cell membrane target of action. Corroborating the importance of AMPs in host defense, isogenic bacterial mutants with decreased AMP resistance are less virulent than their wild-type parent strains in animal models of invasive bacterial infection.

Why do so many bacterial species remain sensitive to AMP action? One may speculate that some mutations conferring increased AMP resistance could involve biochemical modifications that are metabolically too expensive for maintenance. Additionally, such mutations may not prove advantageous to the organism in epithelial colonization or host-to-host transmission, where even greater selective pressures exist. The balance between surviving innate immune clearance and metabolic expenditure is reflected in the adaptations of several bacteria toward inducible resistance, in which exposure to low levels of the AMPs leads to upregulation of resistance phenotypes (Bader *et al*., 2003; McPhee *et al*., 2003; Nizet *et al*., 2001; Shelburne *et al*., 2005).

It is interesting to ponder why bacterial species that are normally sensitive to AMP killing sometimes produce serious infections in humans without known immunodeficiency. Certain pathogenic species (e.g. *Streptococcus pneumoniae*, *Neisseria meningitides*) possess mechanisms for epithelial cell invasion and transcytosis that could allow bloodstream and deep tissue access without causing the barrier injury that would trigger host AMP production in the local tissues. For these and many other human pathogens, surface proteins and/or polysaccharide capsules impede phagocytic uptake, thus avoiding a second site of AMP action within the phagolysosome. For most agents of human bacterial infection, the number of individuals colonized asymptomatically greatly exceeds the low incidence of invasive disease. Is it possible that in rare events a quantum "phase shift" to higher AMP resistance allows invasion? Alternatively, do some patients have subtle congenital or acquired defects in their specific ability to mount an appropriate AMP response to minor

Table 1. Summary of antimicrobial peptide resistance mechanisms of human bacterial pathogens.

AMP Resistance Phenotype	Gene(s)	AMPs Affected	Organism(s)	Virulence Role * (model system)	Selected Reference
Cell Surface Alterations					
D-alanylation of teichoic acid in bacterial cell wall	*dlt* operon	Defensins, Caths	*Staphylococcus aureus* Group B *Streptococcus* *Listeria monocytogenes*	Sepsis and septic arthritis (mice) Lung or systemic infection (mice) Bloodstream infection (mice)	(Collins *et al*., 2002) (Poyart *et al*., 2003) (Abachin *et al*., 2002)
L-lysine to phosphatidylglycerol	*mprF* *lysS*	Defensins, Caths	*Staphylococcus aureus*	Systemic infection (mice)	(Peschel *et al*., 2001) (Nishi *et al*., 2004)
Addition of aminoarabinose to lipid A in LPS	*pmr* genes	Defensins, Caths	*Salmonella enterica* *Proteus mirabilis* *Pseudomonas aeruginosa*	Gastrointestinal infection (mice) Unknown Unknown	(Gunn *et al*., 2000) (McCoy *et al*., 2001) (Moskowitz *et al*., 2004)
Acylation of lipid A in LPS	*pagP* *rcp* *htrP*	Defensins, Caths	*Salmonella enterica* *Legionella pneumophila* *Haemophilus influenzae*	Unknown Lung colonization (mice) Unknown	(Guo *et al*., 1998) (Robey *et al*., 2001) (Starner *et al*., 2002)
Phosphorylcholine in LPS	*glpQ*	Caths	*Haemophilus influenzae*	Unknown	(Lysenko *et al*., 2000)
PCN-binding protein PbP1	*ponA*	Defensins, Caths	Group B *Streptococcus*	Lung or systemic infection (rats)	(Hamilton *et al*., 2004)
Mycolic acid synthesis	*kasB*	Defensins	*Mycobacterium marinum*	Intracellular survival (macrophages)	(Gao *et al*., 2003)
AMP Trapping Mechanisms					
Staphylokinase	*sak*	Defensins	*Staphylococcus aureus*	Unknown	(Jin *et al*., 2004)
M1 surface protein	*emm*1	Caths	Group A *Streptococcus*	Skin or systemic infection (mice)	(Lauth *et al*., 2004)
SIC protein	*sic*	Defensins, Caths	Group A *Streptococcus*	Skin infection (mice)	(Frick *et al*., 2003)
Shedding of host proteoglycans that in turn neutralize AMPs	*lasA*	Defensins, Caths	*Pseudomonas aeruginosa* *Enterococcus faecalis* Group A *Streptococcus*	Pulmonary infection (mice) Unknown Unknown	(Park *et al*., 2001) (Schmidtchen *et al*., 2001) (Schmidtchen *et al*., 2001)
Active Efflux of AMPs					
ATP-dependent efflux system	*mtr* genes	Caths	*Neisseria gonorrhoeae*	Genital tract infection (mice)	(Jerse *et al*., 2003)
K^+-linked efflux pump	*sap* operon *sapA*	Protamine Defensins	*Salmonella enterica* *Haemophilus influenzae*	Gastrointestinal infection (mice) Otitis media (chinchillas)	(Parra-Lopez *et al*., 1994) (Mason *et al*. 2005)
Plasmid-encoded efflux pump	*qacA*	Rabbit tPMP	*Staphylococcus aureus*	Endovascular infection (rabbits)	(Kupferwasser *et al*., 1999)
Proteolytic Degradation of AMPs					
Elastase	*lasB*	Caths	*Pseudomonas aeruginosa*	Corneal infection (mice)	(Schmidtchen *et al*., 2002)
Gelatinase	*gelE*	Caths	*Enterococcus faecalis*	Peritonitis (mice)	(Schmidtchen *et al*., 2002)
Metalloproteinase	*zapA*	Caths	*Proteus mirabilis*	Urinary tract infection (mice)	(Schmidtchen *et al*., 2002)
Cysteine protease	*speB*, *ideS*	Caths	Group A *Streptococcus*	Skin infection (mice)	(Schmidtchen *et al*., 2002)
Metalloproteinase (aureolysin)	*aur*	Caths	*Staphylococcus aureus*	Unknown	(Sieprawska-Lupa *et al*., 2005)
Surface protease	*pgtE*	Caths	*Salmonella enterica*	Unknown	(Guina *et al*., 2000)
Metalloprotease	*degP*	Lactoferricin	*Escherichia coli*	Unknown	(Ulvatne *et al*., 2002)
Gingipains (serine proteases)	*rgpA/B*	Cecropin B	*Porphyromonas gingivalis*	Abscess formation (mice)	(Devine *et al*., 1999)

Table 1. Continued

AMP Resistance Phenotype	Gene(s)	AMPs Affected	Organism(s)	Virulence Role * (model system)	Selected Reference
Alteration of Host Processes					
Downregulate AMP transcription	Unknown	Caths	*Shigella dysenteriae*	*Shigella* dysentery (humans)	(Islam *et al*., 2001)
Stimulation of host cathepsins	Unknown	Defensins	*Pseudomonas aeruginosa*	Unknown	(Taggart *et al*., 2003)
Regulatory Networks					
Two-component regulator	*pho*P/*pho*Q	Defensins, protamine	*Salmonella enterica* *Pseudomonas aeruginosa*	Gastrointestinal infection (mice)	(Ernst *et al*., 2001) (Macfarlane *et al*., 2000)
Two-component regulator	*pmr*A/*pmr*B	Defensins, Polymyxin B	*Salmonella enterica* *Pseudomonas aeruginosa*	Gastrointestinal infection (mice)	(Gunn *et al*., 2000) (McPhee *et al*., 2003)
Extracytoplasmic sigma factor	*rpoE*	Defensins	*Salmonella enterica*	Gastrointestinal infection (mice)	(Crouch *et al*., 2005)
Thermoregulated transcription factor	*prfA*	Defensins	*Listeria monocytogenes*	Gastrointestinal infection (mice)	(Lopez-Solanilla *et al*., 2003)

Abbreviation: Cath = cathelicidin. * Virulence roles demonstrated in animal models with isogenic mutants often performed in independent studies and not conclusively linked *per se* to antimicrobial peptide resistance.

injury? Cofactors such as intercurrent viral infection could produce a transient defect in an otherwise effective AMP barrier to invasive disease.

In summary, human medicine must contend with the fact that some bacterial pathogens have developed their own form of "innate immunity": a resistance to the AMP component of our epithelial and cellular defense. Improved knowledge of the molecular basis of these bacterial escape mechanisms may identify novel targets for antimicrobial therapy. Such drugs could act to block bacterial cell wall modifications, efflux pumps, or inactivating factors, thereby rendering the organisms sensitive to the natural innate immune defense provided by AMPs.

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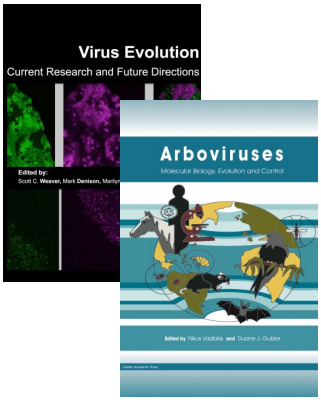
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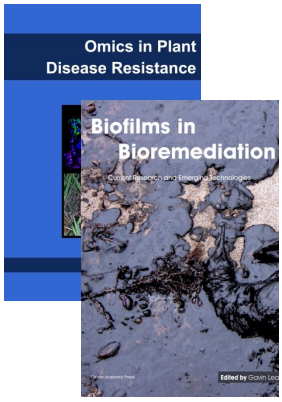
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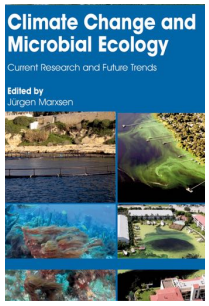
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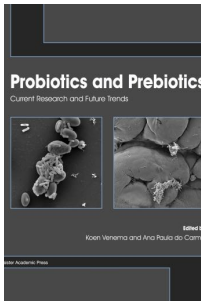
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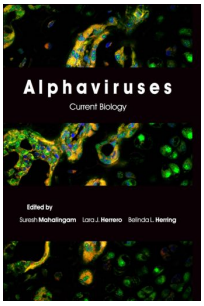
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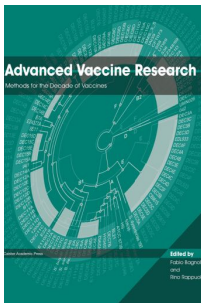


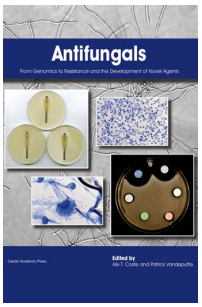












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