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MULTI-AUTHOR REVIEW

Bacterial resistance mechanisms against host defense peptides

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Abstract Host defense peptides and proteins are important components of the innate host defense against pathogenic microorganisms. They target negatively charged bacterial surfaces and disrupt microbial cytoplasmic membranes, which ultimately leads to bacterial destruction. Throughout evolution, pathogens devised several mechanisms to protect themselves from deleterious damage of host defense peptides. These strategies include (a) inactivation and cleavage of host defense peptides by production of host defense binding proteins and proteases, (b) repulsion of the peptides by alteration of pathogen's surface charge employing modifications by amino acids or amino sugars of anionic molecules (e.g., teichoic acids, lipid A and phospholipids), (c) alteration of bacterial membrane fluidity, and (d) expulsion of the peptides using multi drug pumps. Together with bacterial regulatory network(s) that regulate expression and activity of these mechanisms, they represent attractive targets for development of novel antibacterials.

Keywords Pathogens · Cationic peptides · Teichoic acids · Lipid A · D-alanylation · Aminoarabinose · Ethanolamine · Multidrug efflux pumps · Proteases

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Abbreviations

CAMPs	Cationic antimicrobial peptides
gIIA PLA2	group IIA phospholipase A2
HBD3	Human β-defensin 3
HNPs	Human neutrophil peptides
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
MDR	Multidrug resistance
PAMPs	Pathogen-associated molecular patterns
PGA	Anionic poly-γ -glutamic acid
TA	Teichoic acids
PG	Phosphatidylglycerol
PIA	Polysaccharide intercellular adhesin
SIC	Streptococcal inhibitor of complement
WTA	Wall teichoic acid

Introduction

In order to survive on and within the host, bacterial pathogens have evolved numerous mechanisms to combat host immune system. Among these are secreted molecules that interfere with recognition of bacterial pathogens by host immune system and pathogen-associated components that make the invader more resistant to the arsenal of host antimicrobial molecules such as lysoyzme, group IIA phospholipase A2, and small cationic antimicrobial peptides (CAMPs) [1]. Bacterial resistance mechanisms to the latter will be the focus of this brief.

Infection is typically initiated when a breach of the skin or mucosal barriers allows bacterial pathogen access to adjoining tissues or the bloodstream. Whether an infection is contained or spreads depends on a complex interplay between the pathogen's virulence determinants and host

defense mechanisms. The effective host immune response is based on quick recognition, isolation, and elimination of the pathogen. Innate immunity is the first line of defense against the invading microbial pathogens [1, 2]. The swift action of cellular (e.g., phagocytes) and secreted compounds (e.g., CAMPs) of innate immunity is in part due to the ability of innate immunity to recognize invariant structures on pathogens, so-called pathogen-associated molecular patterns or PAMPs [3]. While the killing by phagocytes occurs by combined action of reactive oxygen species and CAMPs, CAMPs are also secreted by various types of epithelial cells and therefore represent an important component of host defense on their own [1, 4, 5]. Many CAMPs have additional immunomodulatory roles that contribute to antimicrobial host defense [6].

Host defense peptides and their role in innate immune defense

CAMPs represent an evolutionary ancient part of immune response that is found in all kingdoms of life, from bacteria to plants and animals [5, 7]. These are small (12–50 amino acids) hydrophobic molecules that usually have a net positive charge of +2 or more and display a broad spectrum antimicrobial activity against bacteria as well as fungi [5, 8]. In humans, CAMPs can be placed in three distinct groups based on their peptide structure, amino acid composition, and number of disulfide bonds [5, 9]. Cathelicidins are CAMPs with α-helical structure, which do not contain cysteine residues. The only human representative of cathelicidin is LL-37 (see Fig. 1) [9]. Defensins have a *β*-sheet structure that is stabilized by three disulphide bridges. The pattern of disulphide bridges determines whether defensins belong to the group of α- or *β*-defensins. Defensins belonging to the two groups also differ by which cell type they are produced. While α-defensins [e.g., human neutrophil peptides (HNPs)] are mainly produced by neutrophil granulocytes and the intestinal Paneth cells, *β*-defensins are produced by epithelial tissues such as skin and epithelia of gastrointestinal, respiratory, and genitourinary tracts (Fig. 1) [5, 8, 10–12]. Kinocidins are the third group of CAMPs related to chemokines that are released from platelets upon contact with bacteria (thrombocidins) or other cells [13, 14]. Thrombocidins have been shown to participate in endovascular infections, e.g., in elimination of bacteria in endocardial vegetations [15–17]. In addition to the three above-mentioned major groups of CAMPs, further human antimicrobial peptides have been described including anionic dermcidins produced in human sweat, the iron-regulatory hormone hepcidin [18], and split products form human proteins such as α-melanocyte-stimulating hormone [19].

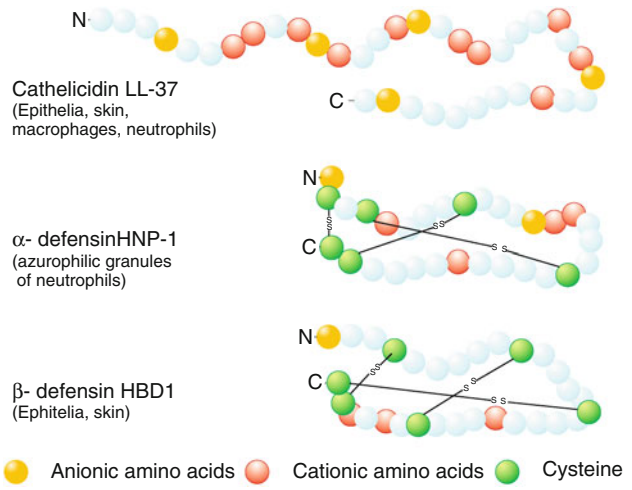

Fig. 1 Structure of selected human cationic antimicrobial peptides. The only representative of cathelicidins in humans is α-helical LL-37. *S S* Disulphide bonds between cysteines. *HNP-1* human neutrophil peptide 1. *HBD-1* human *β*-defensin 1. Modified from [121]

There are several proposed mechanisms of how CAMPs kill bacteria. Whether CAMPs induce pore formation or disrupt the membrane in any other way is still a matter of debate and may be different for different CAMPs. The modes of action may include cytoplasmic leakage and additional antibacterial mechanisms such as inhibition of membrane-bound cell wall biosynthetic steps (e.g., interaction of human-*β* defensin-3 (HBD3) and plectasin with lipidII, or binding of friulimicin B to bactoprenol phosphate carrier C_{55}–P) [20–22], all ultimately leading to death of bacteria [7, 23, 24]. CAMPs mechanism of action can be described in at least three steps [5, 25, 26]. In order to reach the bacterial membrane, CAMPs need to initially bind to the bacterial cell surface and traverse the bacterial cell wall. The third step is insertion of CAMPs in the bacterial membrane that ultimately leads to cell membrane disruption and cell death [5, 8, 23]. The cationic nature of CAMP allows the peptides to distinguish between largely neutrally charged host membranes and mostly anionic bacterial membranes. Besides bacterial membrane damage, CAMPs can assist in killing of microorganisms by modulating host immune responses. To this end, CAMPs can bind lipopolysaccharides (LPS), the outer leaflet of the outer membrane of all Gram-negative bacteria with high proinflammatory capacity, to dampen host inflammatory response [27, 28]. In addition, many CAMPs can act as chemokines, can induce apoptosis, or play a role in activation of autoimmune response [7, 28, 29]. CAMP-derived synthetic peptides lacking antimicrobial activity have been shown to modulate immune responses and contribute to the clearance of infection [30], which underscores the importance of immunomodulatory CAMP activities.

How do bacteria defend against host defense peptides?

Extracellular mechanisms

Bacteria can defend themselves against host defense peptides using several different strategies. Some pathogens produce extracellular molecules that bind and trap CAMPs and thereby prevent their antimicrobial action. An example of such a protein is *Staphylococcus aureus* (*S. aureus*) staphylokinase. Besides binding of plasminogen that may facilitate *S. aureus* invasion of tissues, staphylokinase also forms complexes with α-defensins (HNP 1-3) resulting in over 80% reduced activity of these CAMPs against *S. aureus* [31]. Streptococcal inhibitor of complement (SIC) closely resembles the protective action of staphylokinase against the CAMPs. Even though SIC has been initially discovered as a complement-binding protein, Pence et al. [32] recently found that SIC-deficient *Streptococcus pyogenes* is more sensitive to LL-37. It has been proposed that SIC protects *S. pyogenes* by direct binding and inactivation of CAMPs [33].

Alternatively, bacteria can intercept host defense peptides before they reach their target—the bacterial cytoplasmic membrane—using cell envelope-associated or secreted proteases. Several pathogenic microbes use proteases that cleave CAMPs, thereby abolishing their antibacterial activity. Examples of such secreted proteases can be found in *S. aureus* (e.g., V8 and aureolysin proteases [34]), *Proteus mirabilis* (ZapA [35]), *S. pyogenes* (streptopain SpeB [35]), or *Pseudomonas aeruginosa* (elastase [35]). Alternatively, CAMP-degrading proteases can also be associated with the bacterial cell envelope either anchored in the cell wall or in the outer membrane such as PgtE of *Salmonella enterica* [36] and *E. coli* OmpT [37]. It has been suggested that CAMP evolution led to introduction of multiple cysteine bridges, resulting in a form that is substantially more resistant to proteolysis [7].

Modifications of cell surface

Synthesis of capsular polysaccharides

To protect themselves from host immune response including host defense peptides, bacteria can encase themselves by producing elaborate extracellular matrices. *S. epidermidis*, for example, produces cationic exopolymer polysaccharide intercellular adhesin (PIA) and anionic poly-γ-glutamic acid (PGA), which were both shown to play a role in resistance to cationic LL-37 and HBD3 as well as anionic defense peptide dermcidin [38–40]. Because these polysaccharide matrices protect bacteria from both cationic and anionic host defense peptides, the resistance mechanism probably involves both electrostatic and mechanical (charge-independent) sequestration of host defense peptides, far from their ultimate target—bacterial cytoplasmic membrane.

Role of teichoic acids (TA) in resistance to host defense peptides

Host evolution of defense peptides led to selection of mostly positively charged compounds. For measuring bactericidal effects of defense peptides, it seems that the rule of 'the more cationic the better' may be applied [5, 41, 42]. Net positive charge may be beneficial in targeting of bacterial surfaces that are generally negatively charged. Molecules that contribute to the net negativity of bacterial surface include peptidoglycan, anionic polymers such as TA, teichuronic acid, lipopolysaccharide (LPS) and anionic phospholipids in the bacterial cytoplasmic membrane. In the last decade, substantial advances in uncovering novel bacterial resistance mechanism that reduce the net negative charge of bacterial surfaces have been shown to confer resistance to CAMPs. These mechanisms differ between Gram-positive and Gram-negative bacteria due to the different composition of their cell surfaces (see Fig. 2). The envelope of Gram-negative bacteria consists of a thin layer of peptidoglycan and an extra outer membrane with inner phospholipid layer and outer LPS layer [43]. In contrast, Gram-positive bacteria lack the outer membrane, but have a thick, multiple-peptidoglycan layer cell wall. Analogous to LPS, the cell envelope of Gram-positive bacteria also contains various glyco- or glycolipid polymers, namely TA, which are attached to either peptidoglycan (wall teichoic acid, WTA) or cell membrane (lipoteichoic acid, LTA) [44, 45]. Both LPS and teichoic acids are negatively charged and contribute to the net negativity of bacterial surface [43–46].

Structurally, WTA and LTA differ depending on bacterial species. For example in *S. aureus*, they consist of repeating units of ribitol and glycerol phosphates, respectively [47, 48]. The length of the glycerol phosphate polymers of LTA can exceed 40 U [49], extending through the peptidoglycan layers. Together, LTA and WTA form a negatively charged lattice that bridges bacterial cell membrane and cell wall. The biochemical role of TA in bacterial physiology has been a focus of many studies. TA have been implicated in maintaining proton gradient, cationic homeostasis in bacterial cell wall, regulating enzymatic activity of autolysins and assembly of division site [50]. The biochemical properties of TA have been tightly linked to the substitutions of TA by the amino acid D-alanine. D-alanine's positively charged free amino group partially influences the net negativity of TA [44, 50, 51]. In turn, D-alanyl esters of TA modulate functions of TA that significantly increase resistance of Gram-positive bacteria to CAMPs and proteins [52–58].

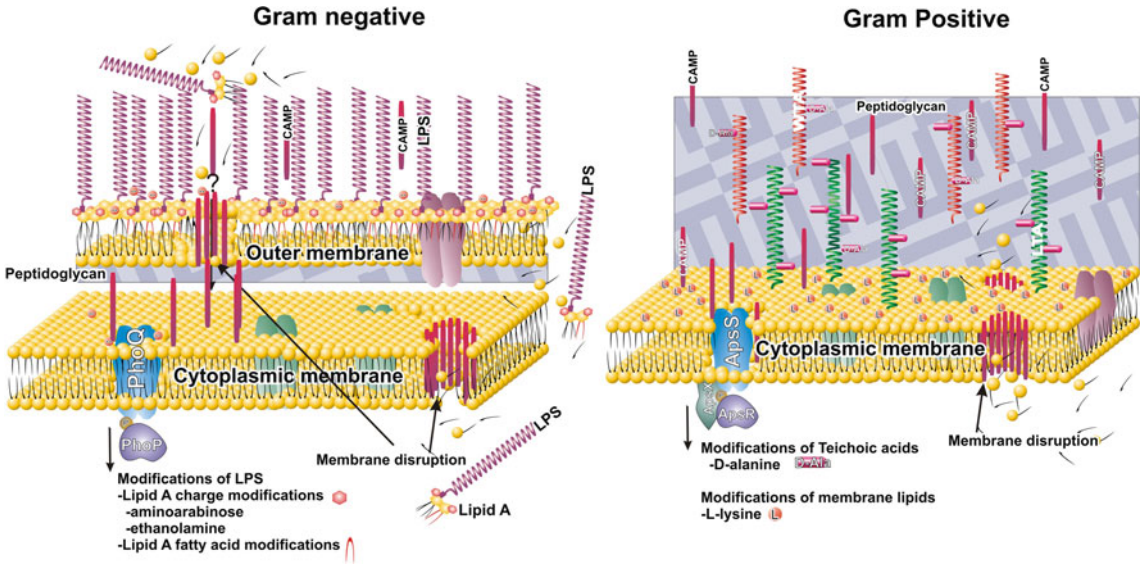

Fig. 2 Sensing and responding to CAMPs in Gram-negative and Gram-positive bacteria. Initial binding of CAMPs is dependent on electrostatic interactions between cationic surface of CAMPs and negatively charged moieties in bacterial cell envelope (lipid A and teichoic acids: WTA, wall teichoic acid, LTA, lipoteichoic acid). Before disruption of the cytoplasmic membrane, CAMPs need to penetrate the outer membrane (Gram-negative bacteria) or cell wall composed of thick layers of peptidoglycan (Gram-positive bacteria). PhoQ and ApsS are sensor histidine kinases that respond to CAMPs or CAMPs-mediated membrane perturbation. PhoP and ApsR are transcriptional regulators that upregulate expression of CAMPs resistance genes. Substitutions of lipid A by aminoarabinose and ethanolamine, modification of phosphatidylglycerol by L-lysine, and D-alanine modification of WTA and LTA, act to neutralize the net negative charge of bacterial cell envelope. In addition, increased resistance is achieved by changing membrane fluidity (e.g., by changes of fatty acid acylation pattern of lipid A)

D-Alanylation of TA

At least four proteins, encoded by a single operon *dltABCD,* are necessary for D-alanylation of both WTA and LTA. D-alanyl carrier protein ligase (Dcl; *dltA*) activates D-alanine using ATP. With assistance of DltD (*dltD*), this activated complex is delivered to the D-alanine carrier protein (Dcp) encoded by *dltC*. DltB (*dltB*) is predicted to be a transmembrane protein and is thought to be involved in passage of the D-alanyl-Dcp complex across the cytoplasmic membrane, where D-alanine is transferred to the glycerol phosphate backbone of LTA and WTA [44]. The glycerol phosphate backbone is derived from phosphatidyl-glycerol (PG), the major phospholipid on growing *S. aureus*. Transfer of D-alanine most likely occurs at the base of the growing chain of LTA and WTA [59]. An additional open reading frame (*"dltX"*) present within the 5′ end of the *dlt* operon of *S. aureus* [60] and other Gram-positive bacteria has been recently identified, however, its function is not yet fully understood.

Inactivation of any of *dltA–D* genes results in complete loss of D-alanine esters from TA [52, 61–63]. Such *dlt* mutants have been created in numerous Gram-positive organisms including *S. aureus*, Streptococcal species, *Listeria*, *Bacillus*, and *Enterococcus* [52–58, 64]. In vitro studies have shown that *dltA S. aureus* is more sensitive to host cationic peptides (defensins) and proteins, especially group IIA PLA_2 [41, 52] and is defective in adherence to artificial surfaces and formation of biofilms [65]. In addition, this mutant strain is virulence-attenuated in several animal models [53, 54, 66, 67]. Similar phenotypes have been observed in other bacterial species (see Table 1 for details). Of note, D-alanylation may also regulate pro-inflammatory activity of LTA [68], which may be an important determinant in establishing persistent infections (e.g., biofilms) and colonization of special niches. D-alanylation of TA in *Lactobacillus* have been show to be crucial for establishing successful colonization of the gastrointestinal tract [69].

The mechanism of D-alanyl-TA resistance to cationic peptides

CAMPs killing action is accomplished in three steps: (1) binding of CAMPs to the cell wall that depends on electrostatic interaction between cationic surfaces of defense peptides and negatively charged bacterial surfaces, (2) penetration of the cell wall, and, (3) insertion of CAMPs in the cell membrane leading to membrane disruption [4, 5]. In the absence of D-alanine esters, increased binding of CAMPs has been observed, suggesting that initial binding of CAMPs may be the critical step controlled by D-alanine esters [52]. However, additional binding sites for CAMPs may be revealed secondary to CAMPs' inflicted cell damage that may mask the additional effect of D-alanine esters on CAMP

Table 1 Summary of bacterial resistance mechanisms to CAMPs

Mechanism of resistance	Product name/gene	Organism	Virulence of the mutant	Reference
CAMP binding and inactivation	Staphylokinase	*S. aureus*	↑ systemic infection	[122, 123]
	SIC	*S. pyogenes*	↓ systemic infection	[32]
CAMP proteolytic cleavage	V8 protease	*S. aureus*	ND	[34]
	Aureolysin	*S. aureus*	ND	[34]
	ZapA	*P. mirabilis*	↓ colonization of urinary tract	[35, 124]
	Streptopain SpeB	*S. pyogenes*	ND	[35, 125]
	Elastase LasA	*P. aeruginosa*	↓ chronic infection in rat	[35, 126, 127]
Capsular polysaccharides	PIA, PGA	*S. epidermidis*	↓virulence	[38–40, 128, 129]
	Capsule synthesis gene cluster	*N. meningitidis*	↓virulence	[130–132]
		K. pneumoniae	↓ virulence in murine model of pneumonia	[133–135]
Surface charge neutralization		*S. aureus*	↓ virulence in mouse, ↓ virulence in rabbit model of endocarditis	[53, 54, 136]
D-alanine modification of teichoic acids	*dltABCD*	*S. pyogenes*	ND	[58, 137]
		L. monocytogenes	↓ mouse model of infection	[55]
		L. reuteri	↓ colonization of mouse	[69]
L-Lysine modification of PG	*mprF*	*S. aureus*	↓ virulence in mouse model of infection ↓ virulence rabbit model of endocarditis	[53, 85, 138]
Lipid A modifications Aminoarabinose Ethanolamine	*pmrAB, pmrE, pmrFHIJKL pmrC,*	*S. enterica ser. typhimurium*	↓ virulence in mice	[98, 100, 139, 140]
Decrease in membrane fluidity				
Modification of lipid A acylation	*pagP, pagL,*	*S. enterica ser. typhimurium*	No phenotype	[141, 142]
Production of carotenoids	*crtOPQMN*	*S. aureus*	↓virulence in subcutaneous abscess model	[143–145]
Presence of efflux pumps	*qacA*	*S. aureus*	ND	[16, 119, 146]
CAMP expulsion	*mtrCDE*	*N. gonorrhoeae*	↓ virulence in murine model of infection	[117, 147, 148]

↑ increased virulence, ↓ decreased virulence or virulence attenuated, *ND* not determined

action. Additional insights on CAMPs action may be gained from studies of another cationic protein that is part of mammalian innate immune response, human group IIA phospholipase A2 (gIIA PLA2). Antibacterial action of gIIA PLA2 is besides enzymatic degradation of phospholipids closely similar to the smaller CAMPs, but each of the steps can be controlled by manipulation of the presence of Ca^{2+} in the medium [41, 70, 71]. The results of these studies suggest that lack of D-alanine esters in TA may promote the penetration and release of autolytic enzymes that enhance the activity of cell wall bound gIIA PLA2 [41].

Regulation of D-alanylation of TA

Environmental factors such as pH, temperature, and salt (e.g., NaCl) concentration [72–74] are known to affect the degree to which TA are substituted by D-alanines. For example, the degree of D-alanylation of LTA is 0.77 mol D-alanine/mol glycerol phosphate when bacteria are grown under low-salt conditions (0.2% NaCl), but decreased to only 0.3 mol D-alanine/mol glycerol phosphate when bacteria are grown in medium with much higher salt (7.5% NaCl) concentration [75]. D-alanylation of TA could be further modulated by regulating the activity of the proteins encoded by *dlt* operon [76] or their abundance by regulation of transcription of *dlt* genes.

In *Bacillus subtilis*, the *dlt* operon is part of the σ^x regulon and is regulated by the global transcriptional regulators AbrB and Spo0A [61]. In *Streptococcus agalactiae*, a two-component system, *dltRS,* that is part of the *dlt* operon, is presumably involved in transcriptional regulation of *dlt* expression [77]. In *S. aureus*, transcription

profiling studies have demonstrated increased *dlt* mRNA in an accessory gene regulator (*agr*) mutant and decreased *dlt* mRNA in a *rot* (repressor of toxins) mutant suggesting a role for the *agr* and *rot* global regulators in negative and positive regulation of *dlt*, respectively [78, 79]. In addition, *S. aureus* represses *dlt* transcription in response to increased concentration of mono and divalent cations that is in part dependent on ArlRS two-component system [60]. Most recently, studies aimed to identify genome-wide responses of *S. epidermidis* to HBD3 revealed a novel three-component system ApsRSX also termed GraRSX in *S. aureus* [80–84]. ApsRSX is an unusual three components system, composed of the classical combination of sensor histidine kinase and response regulator plus an additional protein, the function of which has yet to be determined. Most significantly, ApsS sensor kinase has been shown to be activated in the presence of CAMPs, in turn upregulating expression of resistance genes against CAMPs: *dlt* operon, *mprF* for modification of phospholipids involved in resistance to CAMPs [85] (see Fig. 2 and below), and *vraFG* operon encoding a transporter conferring resistance to glycopeptide vancomycin [86].

Modification of bacterial cell membrane

Charge neutralization

The ultimate step in CAMPs is integration into bacterial membrane leading to bacterial destruction [5]. This final step has been shown to be driven by electrostatic interactions between cationic defense peptides and anionic lipids in bacterial cell membrane [24, 87]. Neutralization mechanisms similar to charge compensation of anionic TA by D-alanine described above have also been described for bacterial membrane lipids [53, 82, 85]. In many Gram-positive and Gram-negative pathogens, phosphatidylglycerol (PG), a major component of bacterial cell membrane, is modified by positively charged amino acid lysine. The synthesis of lysyl-PG is dependent on the MprF protein [85]. MprF is highly conserved in Gram-positive and Gram-negative pathogens and is a 97-kDa integral membrane protein composed of two functional domains. A C-terminal domain is responsible for synthesis of lysyl-PG at the inner leaflet of cytoplasmic membrane using PG and lysyl-tRNA as substrates [85, 88, 89], while the N-terminal—flippase domain—is responsible for translocation of lysyl-PG to the outer leaflet of cytoplasmic membrane [89]. In addition to increased sensitivity to CAMPs, *mprF* mutants are more sensitive to killing by neutrophils and virulence-attenuated in multiple animal models (Table 1) [53, 85]. MprF has also been shown to be under control of ApsRSX regulator [81, 83]. Increased lysyl-PG content and point mutation resulting in MprF gain of function have been recently reported to increase resistance to daptomycin in vitro and in vivo [90–92]. Daptomycin is an anionic antibiotic but has CAMP-like properties in the presence of calcium ions [93] and is used as one of the last resort drugs in treatment of multiple drug resistant *S. aureus* (MRSA). In addition, *mprF* affects sensitivity to several other antibiotics such as gentamicin, vancomycin, and moenomycin [94–96].

Gram-negative bacteria can repel CAMPs by regulating anionic surface charge. Lipid A, the anionic component of LPS, consists of two glucosamine units with free phosphate groups linked to four or more acyl chains [43]. *Salmonella* can modify lipid A and LPS core sugars by incorporation of aminoarabinose or phosphoethanol amine (Fig. 2), leading to reduction in the net negative charge of lipid A and increased resistance to the CAMP polymyxin B [97–99]. The genes responsible for decoration of lipid A by aminoarabinose (*pmrEHFIJKL*) and ethanolamine (*pmrC*) are under control of PmrAB two-component system. The activity of PmrA transcriptional regulator is controlled by two different systems: (a) a sensor kinase PmrB that senses iron, zinc and mild acidic conditions, and (b) PhoPQ two-component system that regulates PmrA activity through PmrD protein [97, 100].

Changes in membrane fluidity

Salmonella typhimurium PhoPQ is one of the most studied bacterial two-component systems that controls several groups of genes important for bacterial survival in the host [101–103]. PhoPQ is a Mg^{2+} sensor, responds to changes in pH [104–106] and to the presence of CAMPs [107]. Crystal structure of PhoQ sensor kinase has revealed a patch of acidic amino acids that are responsible for binding of divalent cations and CAMPs (Fig. 2) [107]. PhoP has been shown to directly or indirectly influence expression >100 genes. Among them are Mg^{2+} transporters (MgtA and MgtCB), SlyA, which regulates genes important for survival inside macrophages, RpoS that regulates genes important for resistance to oxidative stress, and PmrAB, a two-component system that regulates genes for modifications of LPS by amino arabinose [104, 108, 109]. One of the PhoPQ-mediated responses is also upregulation of *pagP* that is responsible for additional acylation of the lipid A and *pagL* that is involved in deacylation of lipid A (Fig. 2). Such modified acylation of lipid A in turn reduces fluidity and permeability of the bacterial outer membrane and renders bacteria more resistant to CAMPs [36, 110, 111]. PhoPQ homologs can be found in many Gram-negative pathogens including *Shigella flexneri*, *Yersinia pestis*, and *P. aeruginosa*, suggesting this two-component system and its function may be one of the evolutionary adaptations to pathogen life within the host [112].

Changes in membrane fluidity have also been shown to influence CAMP resistance of Gram-positive organisms. For example, incorporation of longer chain unsaturated fatty acids in membrane lipids results in an increased membrane fluidity and resistance of *S. aureus* to platelet-derived CAMP [113]. Several studies also suggested that pigment production in *S. aureus* may influence sensitivity to CAMPs. Just recently, Mishra et al. [114] discovered that an *S. aureus* carotenoid staphyloxanthin increases *S. aureus* resistance to human neutrophil defensin 1, platelet-derived CAMPs, and polymyxin B. However, the mechanism of resistance proposed in this study is that staphyloxanthin increases membrane rigidity [114]. Why extreme increase and extreme decrease in membrane fluidity lead to increased resistance to CAMPs is still subject of investigation, however, it appears that the mechanism of resistance may be specific for different CAMPs.

Expulsion of host defense peptides

CAMPs that after damaging the membrane end up inside the cytoplasm, can also be actively exported from the cell by certain multi drug resistance exporters (MDR). Such examples have been found in several bacterial species including *Neisseria gonorrhoeae* [115–117]. *N. gonorrhoeae*, utilizes the MtrCDE MDR exporter to expel diverse antibiotics and confer resistance to the CAMPs protegrin PG1 and LL-37. However, in a recent study using *E. coli* overexpressing certain MDR exporters s of *S. aureus*, *P. aeruginosa,* and *E. coli* failed to show increased resistance to several CAMPs, indicating that most MDR exporters do not mediate broad CAMP resistance and that only some MDR exporters can expel certain CAMPs [118]. Further more, resistance mediated by *S. aureus* MDR exporter QacA to rabbit tPMP has been suggested not to be associated with the ability of QacA active transport of CAMP but rather attributed to the secondary QacA mediated changes on membrane fluidity [119]

Conclusions

Several advances in bacterial host defenses against host antimicrobial peptides have been recently reported. They include secreted proteins or cell surface-associated proteins that irreversibly bind or cleave CAMPs or glycopolymeric matrices that trap CAMPs to prevent their access to bacterial cytoplasmic membrane (see Table 1). Perhaps the biggest advances were made in identification of cell wall-associated mechanisms, the primary action of which appears to be electrostatic repulsion of CAMPs (Fig. 2). Whether it is modification of lipid A of Gram-negative LPS by aminoarabinose or ethanolamine, modification of phosphatidylglycerol by lysine, or esterification of TA by D-alanine, the common denominator to all is a reduction of bacterial net negative surface charge. In the era of increasing numbers of bacterial infections resistant to multiple antibiotics, novel antibiotics are in dire need. Even though CAMPs can be designed in such a way that they are more resistant to proteolytic degradation, high concentrations are needed for their effectiveness and their potentially harmful immunomodulatory effect may not make them suitable for further considerations [7]. In contrast, drugs that target bacterial responses to CAMPs that appear to be conserved among pathogens may be much more suitable. Inhibition of D-alanylation of TA offers a premium target due to the multiple effects that lack of D-alanine esters have on pathogenicity of several Gram-positive pathogens (see Table 1). To this end, inhibitors of DltA, a protein involved in the first step of D-alanylation have shown remarkable success as potential therapeutics in vitro and in vivo, especially when used in combination with other antibiotics [120]. MprF may be an even better target, since it is present in both Gram-positive as well as Gram-negative pathogens. Furthermore, because of involvement of MprF in resistance to daptomycin, inhibitors of MprF could be of even greater value.

Perhaps the newest notion to exploit is a discovery that bacteria can sense and respond to the presence of CAMPs by upregulation of genes responsible for their resistance. These bacterial sensing systems are well conserved among pathogens and may present attractive targets for developing new antimicrobials.

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