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Molecular Mechanisms of Bacterial Resistance to Antimicrobial Peptides

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Abstract Cationic antimicrobial peptides (CAMPs) are integral compounds of the antimicrobial arsenals in virtually all kinds of organisms, with important roles in microbial ecology and higher organisms' host defense. Many bacteria have developed countermeasures to limit the efficacy of CAMPs such as defensins, cathelicidins, kinocidins, or bacteriocins. The best-studied bacterial CAMP resistance mechanisms involve electrostatic repulsion of CAMPs by modification of cell envelope molecules, proteolytic cleavage of CAMPs, production of CAMP-trapping proteins, or extrusion of CAMPs by energy-dependent efflux pumps. The repertoire of CAMPs produced by a given host organism and the efficiency of microbial CAMP resistance mechanisms appear to be crucial in host–pathogen interactions, governing the composition of commensal microbial communities and the virulence of bacterial pathogens. However,

all CAMP resistance mechanisms have limitations and bacteria have never succeeded in becoming fully insensitive to a broad range of CAMPs. CAMPs or conserved CAMP resistance factors are discussed as new mediators and targets, respectively, of novel and sustainable anti-infective strategies.

1 Introduction

One of nature's most ancient strategies for combating unwelcome bacteria is the production of membrane-damaging antimicrobial peptides (Zasloff 2002; Hancock and Chapple 1999). Such molecules are produced by certain bacterial or archaeal strains (bacteriocins) (Riley and Wertz 2002), by plants (plant defensins) (Lay and Anderson 2005), by protozoons (Leippe and Herbst 2004), and by virtually all classes of animals (Zasloff 2002). In order to equip these molecules with a high affinity for bacterial membranes most of them have cationic properties and are referred to as CAMPs (cationic antimicrobial peptides). The antimicrobial activity of CAMPs depends on an ionic milieu comparable to the conditions found in mammalian body fluids (Dorschner et al. 2006). CAMPs include linear, usually α-helical peptides such as the amphibian magainin, the murine CRAMP, and the human LL-37 (Nizet and Gallo 2003), disulfide bridge-stabilized peptides with β-sheet structures such as the α-, β-, and θ-defensins (Ganz 2003; Lehrer 2004), and large chemokines or chemokine-derived molecules with antimicrobial activity named kinocidins (Yang et al. 2003; Dürr and Peschel 2002), to mention only a few typical classes of vertebrate CAMPs. Bacteriocins often contain unusual modifications such as thioether bridges (Guder et al. 2000).

CAMPs have been shown to play crucial roles in microbial ecology and in higher organisms' host defense. However, microorganisms have also found many ways to limit the efficacy of CAMPs (Groisman 1994; Ernst et al. 2001; Peschel 2002; Nizet 2005). Bacteriocin-producing bacteria are resistant to the produced peptides, which enable them to survive while competing microorganisms are inhibited (Riley and Wertz 2002). Bacterial commensals and pathogens of higher organisms, on the other hand, use CAMP resistance mechanisms as a prerequisite to invade and colonize host tissues (Peschel 2002). Unlike antibiotic resistance genes, most CAMP resistance genes are usually not found on plasmids, transposons, or other laterally transferable genetic elements but on the bacterial chromosome in the vicinity of housekeeping genes. At least some of them are considered to have appeared rather early in evolution and seem to be integral parts of the genomes of bacteria whose habitats involve the frequent exposure to CAMPs. As another conse-

quence of their long presence in bacteria, some of the cell wall modifications leading to CAMP resistance affect other bacterial functions such as the attachment and activity of cell wall proteins (Peschel et al. 2000), biofilm formation (Gross et al. 2001), or interaction with epithelial cells (Weidenmaier et al. 2004). Extensive research activities have led to a very large number of studies on bacterial CAMP resistance (Table 1; Fig. 1). This review focuses on established molecular principles of CAMP resistance rather than giving a complete overview on all publications concerning this topic. The ecological aspects of CAMP resistance along with their relevance in microbial biofilm formation and biofilm-associated infections are discussed elsewhere (Otto 2005).

As one would expect, CAMPs seem to be subjected to a very rapid and active evolution (Maxwell et al. 2003), probably as a means to react to the equally fast evolving bacterial resistance mechanisms (Patil et al. 2004). Accordingly, the various mammalian genera are highly variable in the sequences and structures of produced antimicrobial peptides. It can be assumed that the pattern of antimicrobial molecules of a given species is one of the factors that govern the spectrum of its commensal and pathogenic microorganisms. For instance, the production of antiretroviral θ-defensins is discussed as a crucial factor determining resistance (in monkeys) or susceptibility (humans) to HIV (Nguyen et al. 2003). The human gut is particularly rich in bacterial colonizers, which is probably the reason why specialized cells in the crypts of Liberkühn produce an extraordinarily large spectrum of antimicrobial peptides ranging from CRS peptides (mice) (Hornef et al. 2004) to various α-defensins (most mammalian species including humans) (Lehrer 2004; Ganz 2003). Elucidating the basis of microbial CAMP resistance mechanisms will be crucial for understanding, monitoring, and interfering with bacterial colonization and infection.

2 How Widespread Is CAMP Resistance?

Considering the fact that probably each bacterial species encounters CAMP-producing competing microorganisms or host cells, one would expect that most bacteria have evolved at least some strategies to evade CAMP-mediated killing. In fact, increasing research activities have clearly demonstrated that this is true for many microbial habitats. Skin bacteria such as staphylococci, oral bacteria such as streptococci, and intestinal bacteria such as salmonellae have been described to resist high concentrations of locally produced CAMPs (Peschel 2002; Ernst et al. 2001; Nizet 2005) (Table 1). Soil bacteria such as *Bacillus subtilis* also have CAMP resistance mechanisms, probably as a means

Table 1 Mechanisms and prevalence of bacterial CAMP resistance

Resistance mechanism	Species	Reference
Proteolytic cleavage		
PgtE	*Salmonella enterica*	(Guina et al. 2000)
OmpT	*Escherichia coli*	(Stumpe et al. 1998)
Aureolysin, serin protease V8	*Staphylococcus aureus*	(Sieprawska-Lupa et al. 2004)
Unidentified proteases	*Pseudomonas aeruginosa, Enterococcus faecalis, Proteus mirabilis, Porphyromonas gingivalis, Prevotella* spp.	(Schmidtchen et al. 2002)
Production of external CAMP-binding molecules		
SIC protein, M1 protein	*Streptococcus pyogenes*	(Frick et al. 2003; Nizet 2005)
Staphylokinase	*Staphylococcus aureus*	(Jin et al. 2004)
CAMP-specific drug exporters		
MtrCDE	*Neisseria gonorrhoeae*	(Shafer et al. 1998)
EpiFEG	*Staphylococcus epidermidis*; many antibiotic producers	(Peschel and Götz 1996; Jack et al. 1998)
RosA/B	*Yersinia* spp.	(Bengoechea and Skurnik 2000)
Alteration of the electrostatic properties of the bacterial cell surface		
Modification of lipid A with aminoarabinose	*Salmonella enterica*, many Gram-negative spp.	(Ernst et al. 2001b; Miller et al. 2005)
Alanylation of teichoic acids	*Staphylococcus aureus*; many Gram-positive bacteria	(Peschel et al. 1999; Abachin et al. 2002; Poyart et al. 2003; Perego et al. 1995)
Lysinylation of phospholipids	*Staphylococcus aureus*; many Gram-positive and Gram-negative bacteria	(Peschel et al. 2001; Staubitz and Peschel 2002; Ratledge and Wilkinson 1988)
Further mechanisms		
Additional fatty acid in lipid A	*Salmonella enterica*, many Gram-negative spp.	(Guo et al. 1998; Miller et al. 2005)
Modification of mycolic acid	*Mycobacterium tuberculosis*	(Gao et al. 2003)
Reduced cytoplasmic membrane potential	*Staphylococcus aureus*	(Yeaman et al. 1998)

Table 1 (continued)

Resistance mechanism	Species	Reference
Slime and capsule polymers, biofilm formation	*Klebsiella pneumoniae, Staphylococcus epidermidis*, many other bacteria	(Campos et al. 2004; Otto 2005)
Inhibition of CAMP production	*Shigella* spp.	(Islam et al. 2001)

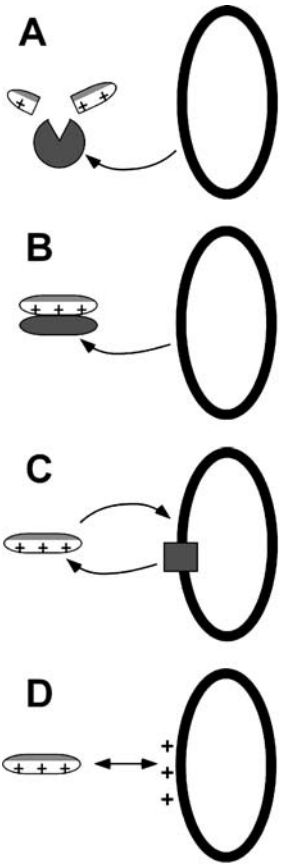

Fig. 1A–D Mechanisms of bacterial CAMP resistance by proteolytic cleavage of CAMPs (**A**), CAMP-trapping molecules (**B**), CAMP extruding transport proteins (**C**), or electrostatic repulsion of CAMPs (**D**)

to achieve protection against bacteriocins frequently produced by other soil microorganisms and fungi (Staubitz and Peschel 2002; Cao and Helmann 2004). The available bacterial genome sequences reveal the presence of CAMP resistance genes in the majority of microbial species, indicating that CAMP resistance is in fact a very widespread bacterial trait (Weidenmaier et al. 2003; Miller et al. 2005). Extensive investigations in some prototype species such as *Salmonella enterica* and *Staphylococcus aureus* have revealed the presence of several resistance mechanisms in one bacterial species, which seem to complement each other in order to achieve high-level resistance to a broad spectrum of CAMPs (Ernst et al. 2001; Peschel 2002). However, different isolates of one particular bacterial species may vary widely in their susceptibility to CAMPs (Midorikawa et al. 2003; Joly et al. 2004) indicating that the various mechanisms may be differently expressed or functional in different clones.

3 Proteolytic Cleavage of CAMPs

The most straightforward way for a bacterial species to inactivate antimicrobial peptides is the production of peptidases and proteases that cleave CAMPs (Fig. 1A). Such enzymes have been described in Gram-negative and Gram-positive bacteria. *S. enterica* produces the outer membrane protease PgtE, which is capable of cleaving the cathelicidin LL-37 and other alpha helical CAMPs (Guina et al. 2000). *S. aureus* expresses several proteases; the metalloprotease aureolysin and the serine protease V8 can cleave LL-37 and the in vitro resistance to LL-37 has been associated with aureolysin production (Sieprawska-Lupa et al. 2004). Many other bacterial species including *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Proteus mirabilis*, *Porphyromonas gingivalis*, and *Prevotella* spp. also produce proteases that cleave linear CAMPs (Schmidtchen et al. 2002).

4 Production of External CAMP-Binding Molecules

Some bacterial species express secreted or surface-anchored proteins that bind certain CAMPs with a very high affinity and thereby prevent their access to the cytoplasmic membrane (Fig. 1B). *Streptococcus pyogenes* secretes the SIC protein, which binds and thereby inactivates LL-37 (Frick et al. 2003). Its production has been correlated with the invasiveness of *S. pyogenes* strains.

A similar approach is used by *S. aureus* in order to achieve resistance to α-defensins. The fibrinolytic exoprotein staphylokinase does not only bind to plasminogen, but also has a high affinity for human α-defensins (Jin et al. 2004). Staphylokinase thereby contributes significantly to α-defensin resistance and staphylokinase production correlates with the in vitro resistance of *S. aureus* isolates to defensins.

The *S. pyogenes* M proteins are covalently attached to the peptidoglycan. Several functions have been assigned to the various M protein domains, which are highly variable in structure and size (Bisno et al. 2003). One of the M protein serotypes, the globally disseminated M1 clone, seems to play a critical role in resistance to LL-37 by binding this peptide with the hypervariable M1 C-terminus (Nizet 2005). Inactivation of the M1 gene or its heterologous expression leads to reduced susceptibility and increased resistance to LL-37 respectively.

5 CAMP-Specific Drug Exporters

Protection against small amphiphatic drugs is often mediated by extrusion of the molecules by energy-dependent export proteins in the cytoplasmic membrane. Many of these resistance factors have a broad substrate spectrum and are referred to as multiple drug resistance exporters (van Veen and Konings 1997). As CAMPs also have amphiphatic, membrane-damaging properties, it is not surprising that some of the known bacterial MDRs confer resistance to certain CAMPs (Fig. 1C). The *Neisseria gonorrhoeae* MtrCDE MDR, a member of the resistance/nodulation/division (RND) class of microbial efflux pumps, contributes to resistance to the small porcine β-sheet CAMP protegrin 1 and to the α-helical human peptide LL-37 (Shafer et al. 1998). Attenuated virulence of *mtr*-mutated *N. gonorrhoeae* suggests a considerable role of the MtrCDE system in evasion of CAMP-mediated killing (Jerse et al. 2003). The QacA efflux pump contributes to *S. aureus* resistance to platelet-derived CAMPs (tPMPs, thrombin-induced microbicidal proteins) (Kupferwasser et al. 1999). However, this mechanism appears to be independent of the transport function of QacA and to result from another activity of this membrane protein.

Bacterial producers of lanthionine-containing bacteriocins usually bear ABC transporters that provide resistance against the produced antimicrobial peptide. These systems always seem to be very specific for the produced peptide and do not protect against a larger spectrum of CAMPs (Riley and Wertz 2002; Peschel and Götz 1996).

6
CAMP Resistance by Altering the Electrostatic Properties of the Bacterial Cell Surface

CAMPs share positive net charges with most antimicrobial molecules and enzymes such as lysozyme, secretory group IIA phospholipase A_2 (PLA2), RNase 7, and myeloperoxidase. These cationic properties are in a striking contrast to the generally anionic net charge of the molecules forming the bacterial cell envelopes such as peptidoglycan, most phospholipids, lipid A (Gram-negatives) and teichoic acids (Gram-positives) (Weidenmaier et al. 2003). In contrast, the outer leaflets of human cell membranes are usually composed of uncharged or zwitterionic lipids such as phosphatidylcholine and sphingolipids (Devaux and Morris 2004), which are unfavorable for binding and integration of CAMPs. It is assumed that host defense factors have evolved cationic properties in order to impart a high and selective affinity for bacterial cell surface molecules (Weidenmaier et al. 2003). Most of the anionic bacterial cell envelope molecules are very ancient and invariable, and it seems to be impossible for microorganisms to replace these molecules with different structures that would be less favorable for interactions with CAMPs. However, many bacteria are able to modify their cell surfaces in order to reduce their negative net charge and thus acquire protection against inactivation by CAMPs (Fig. 1D). Detailed studies of this phenomenon are again available for *S. aureus* and *S. enterica*.

The teichoic acids of staphylococci and other Gram-positive bacteria are composed of alternating glycerolphosphate or ribitolphosphate groups and are substituted with N-acetylglucosamine or D-alanine (Neuhaus and Baddiley 2003). These polymers are anchored to the cytoplasmic membrane (lipoteichoic acids) or connected to the peptidoglycan (wall teichoic acids). The great number of phosphate groups impart polyanionic properties on teichoic acids. D-alanine incorporation introduces positively charged amino groups into teichoic acids, leading to a partial neutralization of the polymers (Peschel et al. 1999). This modification limits the interactions of CAMPs with the staphylococcal cell wall and decreases the susceptibility to a broad variety of cationic host factors ranging from defensins (Peschel et al. 1999) and PLA2 (Koprivnjak et al. 2002) to myeloperoxidase (Collins et al. 2002). In addition to staphylococci, this resistance mechanism has also been described in *Listeria monocytogenes* (Abachin et al. 2002), *Streptococcus agalactiae* (Poyart et al. 2003), *S. pyogenes* (Kristian et al. 2005), and *B. subtilis* (Wecke et al. 1997). The *dltABCD* operon responsible for D-alanine transfer into teichoic acids occurs in the genomes of most bacteria of the low G+C branch of Gram-positive bacteria, indicating that teichoic acid alanylation repre-

sents a very widespread CAMP resistance mechanism (Weidenmaier et al. 2003).

Most of the bacterial phospholipids such as phosphatidylglycerol, cardiolipin, and others share anionic properties with cell wall polymers (Huijbregts et al. 2000). Many bacterial species, however, including staphylococci, enterococci, listeriae and *P. aeruginosa*, are able to modify a considerable amount of phosphatidylglycerol with L-lysine (Ratledge and Wilkinson 1988), which leads again to neutralization of the cell surface net charge and, consequently, to reduced binding of CAMPs and other cationic host defense molecules. MprF, a novel membrane enzyme, is responsible for the synthesis of lysylphosphatidylglycerol (Staubitz et al. 2004; Oku et al. 2004), and its inactivation leads to a considerably increased susceptibility of *S. aureus* to a large variety of CAMPs (Peschel et al. 2001; Kristian et al. 2003a; Koprivnjak et al. 2002; Weidenmaier et al. 2005). *mprF* homologs are found in the genomes of many Gram-positive and Gram-negative bacteria, among them many human, animal, and plant pathogens, and even in some archaeal species, suggesting that these bacteria employ very similar mechanisms to achieve protection against CAMPs (Weidenmaier et al. 2003).

Many Gram-negative bacteria have similar CAMP resistance strategies. Modifications of lipid A, the conserved integral membrane part of the lipopolysaccharide, are responsible for CAMP resistance in *S. enterica* and *P. aeruginosa* (Ernst et al. 1999, 2001). The anionic character of lipid A can be reduced, for instance, by incorporation of cationic aminoarabinose (Nummila et al. 1995; Gunn et al. 1998). Many Gram-negative species bear the *pmr* genes responsible for amioarabinose transfer into lipid A in their genomes (Miller et al. 2005), suggesting that this modification is a widespread trait in Gram-negative bacteria.

Other cell wall modifications such as synthesis of the neutral phospholipid phosphatidylethanolamine (Cao and Helmann 2004), the neutralization of peptidoglycan muropeptides by iso-D-glutamate amidation (Gustafson et al. 1994), and the transfer of positively charged ethanolamine into lipopolysaccharide (Nummila et al. 1995) may also have the purpose of reducing the efficacy of CAMPs. Obviously, many of the bacterial mechanisms of CAMP resistance reflect the same molecular strategy, even though the modified target molecules and the involved genes are unrelated.

7
Further Bacterial Mechanisms of CAMP Resistance

In order to kill bacteria, CAMPs need to integrate into bacterial membranes, diffuse laterally, and form complexes with other CAMP molecules, which

leads to pore formation and efflux of protons and small molecules (Sahl et al. 2004). Membrane fluidity is thus a critical aspect in CAMP-mediated killing and, in some cases, changes in the composition of lipid fatty acids have been implicated in CAMP resistance. Introduction of an additional fatty acid into the lipid A of *S. enterica* mediated by the PagA protein reduces the susceptibility to LL-37 and protegrin PG1 (Guo et al. 1998). Related genes that may play similar roles are found in several Gram-negative pathogens' genomes (Miller et al. 2005) and increased acylation of lipid A has been implicated in adaptation of *P. aeruginosa* during persistent lung infection in cystic fibrosis patients (Ernst et al. 1999). The occurrence of shorter acyl chains in mycolic acids of a *Mycobacterium tuberculosis kasB* mutant leads to increased susceptibility to defensins and lysozyme (Gao et al. 2003). Mycolic acids form an outer membrane-like shield on the mycobacterial surface and the altered acyl chains of the mutant increase the permeability for several antibiotics and CAMPs. *S. aureus* resistance to platelet microbicidal proteins has also been associated with changes in the composition of lipid fatty acids and concomitantly altered membrane fluidity (Bayer et al. 2000). Inactivation of the major cold shock gene *cspA* leads to susceptibility of *S. aureus* to a cathepsin G-derived CAMP for unclear reasons (Katzif et al. 2003). Since mutation of *cspA* also led to deficiency in the yellow membrane carotinoid staphyloxanthine, altered composition and fluidity of the cytoplasmic membrane may be the reason for CAMP susceptibility in this mutant.

CAMPs need a certain threshold membrane potential to integrate into lipid bilayers. Bacterial cytoplasmic membranes usually have a strong potential since they contain the respiratory chain generating a proton-motive force. Eukaryotic cytoplasmic membranes, in contrast, are much less energized, which is one of the factors for the relative insensitivity of eukaryotic cells for CAMPs. Spontaneous mutations in genes encoding bacterial respiratory chain components often lead to small colony phenotypes since these mutants show a strongly attenuated growth behavior. *S. aureus* small colony variants (SCVs), however, have a better capacity to persist in human cells and they are often responsible for recurrent infections (Proctor et al. 1998). SCVs have a lower membrane potential and they are less susceptible to many CAMPs (Yeaman et al. 1998). Accordingly, the SCV phenotype can be regarded as a CAMP resistance mechanism and CAMP resistance may be one of the reasons for the increased ability of SCVs to persist in host tissues.

In some instances, capsular polymers have been shown to contribute to CAMP resistance (Campos et al. 2004; Vuong et al. 2004). Bacterial capsules are usually considered as an antiopsonic and antiphagocytotic virulence factor. They do usually not represent a major diffusion barrier for small molecules such as CAMPs. In some cases, however, the extracellular slime matrix of

capsules and biofilms have been shown to provide protection against certain CAMPs (Campos et al. 2004; Vuong et al. 2004). This phenomenon may depend on the net charge of capsule polymers, as the exopolymer PIA involved in CAMP resistance in *Staphylococcus epidermidis* has a positive net charge and may thus contribute to repulsion of cationic antimicrobial molecules. Slime polymers and the special metabolic adaptations of bacteria in biofilms seem to play important roles in evasion of CAMP-mediated killing. Their relevance in CAMP resistance is reviewed in detail elsewhere in this book (Otto 2005).

Another elegant method of CAMP resistance is used by *Shigella* species, which inhibit expression of LL-37 and β-defensin 1 in human colonic epithelia cells. This event involves *Shigella* plasmid DNA (Islam et al. 2001). The underlying mechanisms are not yet understood.

8 Regulation of CAMP Resistance Mechanisms

Most of the regulatory mechanisms involved in resistance of bacteria against CAMPs are not well understood yet, both in terms of regulating signals and of regulatory proteins. However, there are some well-characterized regulatory pathways in Gram-negative bacteria, which have been shown to play crucial roles in CAMP resistance. The PhoP/PhoQ two-component system plays a key role in the virulence of *S. enterica*, *P. aeruginosa*, and *Yersinia pseudotuberculosis* (Groisman 2001). PhoP/PhoQ-controlled genes such as PagP are necessary for lipid A modification leading to CAMP resistance, as shown in *S. enterica* (Guo et al. 1998). PhoP/PhoQ responds to changes in the magnesium and calcium ion concentrations (García Véscovi et al. 1996), and it has been shown to be activated by the presence of subinhibitory concentrations of CAMPs in *S. enterica* (Bader et al. 2003). The sensor kinase PhoQ directly recognizes CAMPs, thereby displacing PhoQ-bound divalent cations and leading to activation of the response regulator PhoP (Bader et al. 2005). A second two-component regulatory system, PmrA/PmrB, responds to extracellular iron (Wosten et al. 2000), and it is also controlled by PhoP/PhoQ in *S. enterica* (Groisman 2001). It confers resistance to several CAMPs by transcriptional activation of two loci, *pmrE* and *pmrHFIJKLM*, which are required for the biosynthesis of a lipid A variant with 4-aminoarabinose modification (Gunn et al. 2000). This modification leads to a reduction of the anionic character of the bacterial lipid A and, consequently, to CAMP resistance, as discussed above. A related system seems to respond directly to CAMP exposure in *P. aeruginosa* (McPhee et al. 2003).

Much less is known about the regulation mechanisms and stimuli involved in CAMP resistance of Gram-positive bacteria. The global virulence regulatory system *agr* of *S. aureus* is involved in the regulation of the *dlt*-operon, responsible for the alanylation of techoic acids (Dunman et al. 2001). Another two-component regulation system, DltRS, controls expression of the *dlt* operon in *S. agalactiae* (Poyart et al. 2003). Inactivation of regulatory genes has led to increased CAMP susceptibility in *S. pyogenes* (Nizet et al. 2001) and *L. monocytogenes* (Cotter et al. 2002), but the genes controlled by these regulators have remained unknown.

9 Role of CAMP Resistance in Host Colonization and Infection

In addition to obligate pathogens, several bacterial commensals or opportunistic pathogens have been shown to resist high concentrations of CAMPs (Sahly et al. 2003; Shelburne et al. 2005; Brissette et al. 2004; Nishimura et al. 2004). This ability is generally considered as a prerequisite for the colonization of human epithelia whose secretions in the airway as well as in the gastrointestinal and genitourinary tracts contain high amounts of CAMPs such as β-defensins and LL-37. Only a few animal studies have addressed the role of CAMP resistance in bacterial colonization. *S. aureus* colonizes the anterior nares in 30%–40% of the human population (Peacock et al. 2001), which is one of the crucial risk factors for developing severe wound and skin infections or life-threatening systemic infections such as endocarditis and sepsis (von Eiff et al. 2001; Wertheim et al. 2004). A CAMP-susceptible *S. aureus dlt*A mutant has recently been shown to have a strongly reduced capacity to colonize the nares of cotton rats, which represent a good model of human nasal colonization (Weidenmaier et al. 2004). However, since the *dlt*A mutation leads to altered teichoic acids and since teichoic acid structure is critical in *S. aureus* binding to nasal epithelial cells, it is not yet clear whether the abrogated capacity of this mutant to colonize cotton rat nares is a result of reduced binding to epithelial cells, increased killing by nasal CAMPs, or both. Further in vivo studies will be necessary to elucidate the relevance of CAMP resistance in colonization.

The importance of CAMP resistance in localized infections of various organ systems has been demonstrated for many different pathogens and in many animal models. Skin infections caused by *S. pyogenes* (Nizet et al. 2001), *S. aureus* abscess-like tissue cage infections (Kristian et al. 2003b), *Legionella pneumophila* lung infections (Edelstein et al. 2003), *S. enterica* gastrointestinal infections (Gunn et al. 2000), and *N. gonorrhoeae* genital tract

infections (Jerse et al. 2003), to name but a few examples, are strongly affected if CAMP susceptible mutants are used. Increased bacterial killing by CAMPs produced by epithelial cells of infected organs or released by phagocytes upon contact with bacteria is most probably the reason for the observed virulence attenuation. In line with this notion, CAMP-susceptible bacterial mutants are inactivated faster and more efficiently by CAMP-producing phagocytes (Collins et al. 2002; Kristian et al. 2003a, 2005).

CAMP-susceptible *S. agalactiae* and *S. aureus* mutants are also less virulent in blood stream infections studied in mouse sepsis or rabbit endocarditis models (Poyart et al. 2003; Collins et al. 2002; Weidenmaier et al. 2005). Depending on the particular pathogen and the animal model used, alleviated killing by blood phagocytes, inactivation by microbicidal proteins released by activated platelets, or both seems to be the reason for the reduced virulence of CAMP-susceptible mutants under these conditions.

10 Perspectives

The production of CAMPs is a very ancient and still successful strategy of to inhibit microorganisms. Considering the short half-life of the effectiveness of modern antibiotics it seems to be a mystery how CAMPs remained so efficient during evolution. Even the great variety of bacterial CAMP resistance mechanisms has not led to microorganisms with complete resistance to all kinds of CAMPs. It seems that evolution has always found new ways to circumvent the microbial CAMP resistance mechanisms, for instance by rendering CAMPs protease-resistant or by combining two or more antimicrobial mechanisms in one molecule, as shown for the highly versatile bacteriocin nisin (Pag and Sahl 2002). The extraordinary success of CAMPs may be based on the fact that bacteria cannot completely change the composition and properties of their cytoplasmic membrane. The high metabolic costs of becoming resistant to CAMPs, for instance by the energy-consuming, extensive modifications of the cell envelope, may be another reason why it is so difficult for bacteria to develop totally efficient CAMP resistance mechanisms. Nevertheless, some CAMP resistance mechanisms seem to date back to a very early origin, as *mprF*-related genes, for instance, are found in both bacterial and some archaeal genomes (Staubitz and Peschel 2002).

The amazing effectiveness of CAMPs suggest a use of such molecules in antimicrobial therapy. In fact, several CAMPs have yielded promising results in clinical trials (Andres and Dimarcq 2004). The lactococcal bacteriocin nisin has been used as a food preservative for decades (Pag and Sahl 2002) and

daptomycin, a noncationic membrane-damaging antimicrobial lipopeptide with activity against multidrug-resistant staphylococci and enterococci has recently been approved for the use in human infections (Steenbergen et al. 2005), underscoring the therapeutic potential of membrane-active antimicrobial compounds such as CAMPs. On the other hand, highly conserved bacterial CAMP resistance proteins such as MprF or DltABCD may represent interesting new targets for novel anti-infective compounds that would not kill the bacteria but render them susceptible to innate antimicrobial host molecules (Weidenmaier et al. 2003). A deeper understanding of CAMPs and CAMP resistance mechanisms will help to exploit both innate human host defenses and bacterial evasion strategies.

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