Commentary

Fatal Attraction Evaded: How Pathogenic Bacteria Resist Cationic Polypeptides

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Antimicrobial polypeptides are widely distributed effectors of host defense in animals and plants. They include enzymes that digest vital microbial structures (e.g., lysozyme, neutrophil elastase, phospholipase A2), substances that bind and sequester iron or other essential nutrients (e.g., lactoferrin), and polypeptides that insert into and disrupt microbial membranes (e.g., bactericidal permeability-inducing protein, defensins, cathelicidins; references 1 and 2). Despite their diverse sizes, structures, and mechanisms of action, nearly all antimicrobial proteins and peptides have a net cationic (positive) charge. It is thought that the electrostatic attraction of cationic polypeptides increases the deposition of the polypeptides onto the negatively charged microbial surfaces and thereby promotes their effectiveness. In support of this model, the activity of most but not all antimicrobial polypeptides is competitively inhibited by increasing the ionic strength of the medium (3–5) whose solute anions and cations shield the opposing charges of the cationic polypeptides and anionic microbial surfaces and diminish their mutual attraction.

Microbes have evolved a repertoire of countermeasures that limit the effectiveness of antimicrobial polypeptides. Earlier clues that bacterial resistance to cationic polypeptides could be due to the loss of negative surface charges came from exposing *Pseudomonas fluorescens* to phosphate limitation. Under these conditions, anionic phospholipids of bacterial membranes were largely replaced by positively charged ornithine-modified lipids (6) and the bacteria became completely resistant to the cationic peptide antibiotic polymyxin. In other studies, acquired resistance of enteric bacteria to the cationic antibiotic polymyxin and the antimicrobial peptide cecropin was associated with increased content of aminoarabinose and decreased anionic charge of lipopolysaccharide (7, 8).

The strategy of transposon-mediated gene disruption in pathogenic bacteria has proven particularly fruitful in identifying bacterial genes essential for bacterial survival despite host defense. The inserted transposon allows rapid sequencing and identification of the disrupted gene in bacterial mutants that lost resistance to host defense mechanisms. The method identified the PhoP-PhoQ two-component regulator of *Salmonella typhimurium* as an environmental sensor and master switch that promotes survival in macrophages and activates resistance to antimicrobial peptides (9–11). Among the many processes regulated by PhoP-PhoQ are several that covalently modify the lipid A moiety of lipopolysaccharide and diminish its negative charge by adding aminoarabinose, palmitate, and 2-hydroxymyristate (12). In *Staphyloccus aureus*, disruption of the dlt operon increased bacterial susceptibility to a number of cationic polypeptides, including human defensins human neutrophil peptide 1–3, animal-derived protegrins, tachyplesins, and magainin II, and even to the bacteria-derived gallidermin and nisin (13). The dlt operon mediates the multiple steps required for the modification of negatively charged cell wall component teichoic acid by D-alanine, a modification that normally decreases the negative charge of the cell wall. Dlt mutants bind higher amounts of cationic polypeptides and lower amounts of a control anionic polypeptide than the wild-type bacteria, further supporting the importance of surface charge for bacteria–polypeptide interactions.

The study in this issue by Peschel et al. (14) provides the most convincing evidence to date that charge modifications of bacterial membranes are an important mechanism to decrease their vulnerability to cationic polypeptides. By transposon mutagenesis in *Staphyloccus xylosus*, Peschel et al. identified a gene, *mprF*, that conferred resistance to the cationic peptide antibiotic gallidermin. The homologous *S. aureus mprF* mutant showed at least 8–30-fold higher susceptibility to human neutrophil defensin, porcine neutrophil protegrins, and horseshoe crab hemocyte peptide tachyplesin and bound much more cationic peptide than the wild-type. Comparison of the lipid composition of the wild-type and *mprF* strains revealed a dramatic change: the major membrane lipid lysylphosphatidylglycerol was lost in the mutant but could be restored by complementation with the *mprF* gene. Lysylphosphatidylglycerol results from the addition of the cationic amino acid lysine to the anionic phospholipid phosphatidylglycerol, the principal phospholipid of the *S. aureus* membrane (15, 16). In the

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wild-type, this modification changes the net charge of up to 38% of phosphatidylglycerol from negative to positive. The shear magnitude of this effect, involving the major components visible on paper chromatograms of bacterial lipids, suggests that this is a critically important bacterial adaptation. It is notable that artificial membranes composed of unmodified phosphatidylglycerol are particularly vulnerable to cationic antimicrobial peptides (17).

The *mprF* lesion affected the susceptibility of the mutant to host defense mechanisms and its pathogenicity. The *mprF* mutant was killed much faster than wild-type *S. aureus* by human neutrophils and was less virulent in the mouse models of sepsis and septic arthritis. The *mprF* defect was specific for cationic polypeptides since the mutant bacteria had unchanged susceptibility to reactive oxygen intermediates generated by the myeloperoxidase–hydrogen peroxide–chloride system, or to the uncharged antibiotic gramicidin. The proposed role of *mprF* in the bacterial arsenal of countermeasures is further supported by its location near other genes implicated in microbial resistance. Unfortunately, the neighbors or the structure of *mprF* itself give no clues to its specific function. The gene encodes a large membrane protein with homologues in other bacteria pathogenic in plants and animals. It will be important to determine whether the mprF protein is the lysylphosphatidylglycerol synthase or if it supplies an essential synthetic intermediate, or exerts a regulatory function. Until then, caution is required in attributing the pathogenic effect of the gene solely to lysylation of phosphatidylglycerol and its effects on bacterial surface charge.

S. aureus has much more lysylphosphatidylglycerol than other less pathogenic *Staphylococci* (18). This raises the question of whether the relatively small amounts of this lipid in *S. xylosus* account for its resistance to gallidermin compared with the *mprF* mutant, or if additional mechanisms contribute. Conversely, as suggested by the authors, the large amounts of lysylphosphatidylglycerol in *S. aureus* may selectively protect it in such cationic polypeptide-rich niches as nasal fluid (19) or inflamed skin (20).

Surface charge modifications are not the only mechanisms that contribute to resistance of bacteria to antimicrobial polypeptides. Some bacteria employ proteases to digest and inactivate antimicrobial polypeptides (21). PgtE, a posttranscriptionally regulated outer membrane protease of *S. typhimurium,* is also induced by the PhoP-PhoQ system, and degrades α-helical antimicrobial peptides but not the more protease-resistant β-sheet containing defensins or protegrins. *Neisseria gonorhoeae*, a pathogen well known for its ability to survive in neutrophil-rich environments, employs an efflux system, mtr, that removes various structurally unrelated antimicrobial substances from exposed bacteria. Protegrins and LL-37, abundant cationic peptides of porcine and human neutrophils, respectively (22), are partially neutralized by the mtr system. Bacteria with disabled mtr efflux are $\sim$10-fold more sensitive to protegrin than wild-type strains and accumulate substantially higher amounts of the peptide. Peptide removal is blocked by proton ionophores that depolarize the bacterial cytoplasmic membrane and is restored by glucose, indicating that it is an energy-dependent process.

Although the specific mechanisms of resistance to cationic polypeptides have been described in a small number of laboratory bacterial strains, they are widely distributed with homologues of the resistance genes already identified in many pathogenic bacteria. If the emerging view of the biological role of these mechanisms is correct, many of the bacterial resistance mechanisms will lack mammalian counterparts and could be pharmacologically inhibited without adverse effects on the host. The bacterial countermeasures to host defense mechanisms may yet prove to be the Achilles' heel of pathogens increasingly refractory to conventional antibiotics.

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References

1. Ganz, T., and R.I. Lehrer. 1997. Antimicrobial peptides of leukocytes. *Curr. Opin. Hematol.* 4:53–58.
2. Ganz, T., and J. Weiss. 1997. Antimicrobial peptides of phagocytes and epithelia. *Semin. Hematol.* 34:343–354.
3. Hirsch, J.G. 1958. Bactericidal action of histone. *J. Exp. Med.* 108:925–944.
4. Odeberg, H., and I. Olsson. 1975. Antibacterial activity of cationic proteins from human granulocytes. *J. Clin. Invest.* 56:1118–1124.
5. Selsted, M.E., D. Szklarek, T. Ganz, and R.I. Lehrer. 1985. Activity of rabbit leukocyte peptides against *Candida albicans*. *Infect. Immun.* 49:202–206.
6. Dorrer, E., and M. Teuber. 1977. Induction of polymyxin resistance in *Pseudomonas fluorescens* by phosphate limitation. *Arch. Microbiol.* 114:87–89.
7. Shafer, W.M., S.G. Casey, and J.K. Spitznagel. 1984. Lipid A and resistance of *Salmonella typhimurium* to antimicrobial granule proteins of human neutrophil granulocytes. *Infect. Immun.* 43:834–838.
8. Vaara, M., and T. Vaara. 1994. Ability of cecropin B to penetrate the enterobacterial outer membrane. *Antimicrob. Agents Chemother.* 38:2498–2501.
9. Fields, P.I., E.A. Groisman, and F. Heffron. 1989. A *Salmonella* locus that controls resistance to microbicidal proteins from phagocytic cells. *Science*. 243:1059–1062.
10. Miller, S.I., W.S. Pulkkinen, M.E. Selsted, and J.J. Mekalanos. 1990. Characterization of defensin resistance phenotypes associated with mutations in the phoP virulence regulon of *Salmonella typhimurium*. *Infect. Immun.* 58:3706–3710.
11. Groisman, E.A. 2001. The pleiotropic two-component regulatory system PhoP-PhoQ. *J. Bacteriol.* 183:1835–1842.
12. Guo, L., K.B. Lim, J.S. Gunn, B. Bainbridge, R.P. Darveau, M. Hackett, and S.I. Miller. 1997. Regulation of lipid A modifications by *Salmonella typhimurium* virulence genes phoP-phoQ. *Science*. 276:250–253.
13. Peschel, A., C. Vuong, M. Otto, and F. Gotz. 2000. The D-alanine residues of *Staphylococcus aureus* teichoic acids alter the susceptibility to vancomycin and the activity of autolytic enzymes. *Antimicrob. Agents Chemother.* 44:2845–2847.
14. Peschel, A., R.W. Jack, M. Otto, L.V. Collins, P. Staubitz, G. Nicholson, H. Kalbacher, W.F. Nieuwenhuizen, G. Jung, A. Tarkowski, et al. 2001. *Staphylococcus aureus* resistance to

human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with L-lysine. *J. Exp. Med.* 193:1067–1076.

15. Haest, C.W., J. de Gier, J.A. den Kamp, P. Bartels, and L.L. van Deenen. 1972. Changes in permeability of *Staphylococcus aureus* and derived liposomes with varying lipid composition. *Biochim. Biophys. Acta.* 255:720–733.
16. Short, S.A., and D.C. White. 1971. Metabolism of phosphatidylglycerol, lysylphosphatidylglycerol, and cardiolipin of *Staphylococcus aureus*. *J. Bacteriol.* 108:219–226.
17. Matsuzaki, K., M. Fukui, N. Fujii, and K. Miyajima. 1991. Interactions of an antimicrobial peptide, tachyplesin I, with lipid membranes. *Biochim. Biophys. Acta.* 1070:259–264.
18. Nahaie, M.R., M. Goodfellow, D.E. Minnikin, and V. Hajek. 1984. Polar lipid and isoprenoid quinone composition in the classification of *Staphylococcus*. *J. Gen. Microbiol.* 130: 2427–2437.
19. Cole, A.M., P. Dewan, and T. Ganz. 1999. Innate antimicrobial activity of nasal secretions. *Infect. Immun.* 67:3267–3275.
20. Harder, J., J. Bartels, E. Christophers, and J.-M. Schroeder. 1997. A peptide antibiotic from human skin. *Nature.* 387: 861–862.
21. Guina, T., E.C. Yi, H. Wang, M. Hackett, and S.I. Miller. 2000. A PhoP-regulated outer membrane protease of *Salmonella enterica* serovar *typhimurium* promotes resistance to α-helical antimicrobial peptides. *J. Bacteriol.* 182:4077–4086.
22. Shafer, W.M., X. Qu, A.J. Waring, and R.I. Lehrer. 1998. Modulation of *Neisseria gonorrhoeae* susceptibility to vertebrate antibacterial peptides due to a member of the resistance/nodulation/division efflux pump family. *Proc. Natl. Acad. Sci. USA.* 95:1829–1833.