Department of Bacteriology

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General Summary

We are studying bacterial pathogenesis and host immune responses to bacteria. In particular, we focus on elucidating the molecular mechanisms of biofilm formation of *Staphylococcus aureus* and the pathogenesis of enterohemorrhagic *Escherichia coli*. We also conduct research for finding molecular targets to prevent biofilm-associated infections and for development of new pneumococcal vaccines. Active collaborative researches with several basic and clinical laboratories have been conducted.

Research Activities

Analysis of protective effect of a novel pneumococcal vaccine against pneumococcal infection

Streptococcus pneumoniae is a major cause of community-acquired pneumonia and occasionally causes invasive pneumococcal diseases (IPDs), such as bacteremia and sepsis, especially in young children and older adults. *Streptococcus pneumoniae* has about 100 serotypes based on the difference in the components of capsular polysaccharides. Current pneumococcal vaccines contain polysaccharides of a limited number of serotypes as antigens. The introduction of the current pneumococcal vaccine for young children as a routine immunization has decreased the incidence of IPD. However, patients who have IPD with nonvaccine serotypes are gradually increasing. Thus, development of a novel vaccine that covers most serotypes is desired. Our laboratory generated a novel protein-based vaccine with a glycolipid adjuvant that has potent immune-stimulatory activities. This novel vaccine induced long-term protection against various serotypes, including the nonvaccine serotypes.

Development of a universal pneumococcal vaccine

Current polysaccharide-based pneumococcal vaccines are effective for preventing IPD with vaccine serotypes. However, patients who have IPD with nonvaccine serotypes are gradually increasing (serotype replacement). Thus, development of a novel vaccine that covers most serotypes is desired. In collaboration with other academic institutions and a vaccine company, we have worked on the development of a universal pneumococcal vaccine. A new vaccine that we have developed contains pneumococcal surface protein A antigens that cover a majority of pneumococcal strains. Our results suggest that the new vaccine provides protective effects against infection with various pneumococcal strains. The new vaccine that we have developed would be useful as a universal pneumococcal vaccine.

Basic research against biofilm formation of S. aureus
We found new insights regarding *S. aureus* biofilms and discovered the potential for new treatments. First, we demonstrated that RNA is a new component in biofilms. RNA localized in the biofilm by binding to the polysaccharide and wall teichoic acids which is ubiquitous in the cell wall of Gram-positive bacteria. In addition, RNA extracted from human blood promoted biofilm formation in a catheter-related flow model. Second, we demonstrated that *S. aureus* surface protein G promotes biofilm formation by a mechanism different from those previously reported. Third, we found *Bacillus subtilis natto* has an inhibitory effect on *S. aureus*. Transcriptome analysis revealed that sporulation and motility might be important for the expression of the effect. Fourth, we discovered a derivative of glucose inhibited biofilm formation of methicillin-resistant *S. aureus* (MRSA) and improved the susceptibility of some antibiotics.

Effect of transglycosylase gene deletion on antimicrobial susceptibility of MRSA
The functions of lytic transglycosylases (LTs) in relation to cell division, biofilm formation, and antibiotic-resistance have been determined for several bacteria. The only known *S. aureus* LTs are immunodominant staphylococcal antigen A (IsaA) and *Staphylococcus epidermidis* D protein (SceD), both of which have been shown to possess cell wall hydrolytic activity. In this study, we aimed to characterize the roles of LTs in MRSA by investigating their effects on antibiotic susceptibility. In immunodominant staphylococcal antigen A gene (*isaA)*-deleted strains, β-lactam resistance was significantly decreased compared with that of wild-type strains. Plasmid-based expression of penicillin binding protein 2 prime gene (*mecA*), a major determinant of β-lactam resistance in MRSA, in an *isaA*-deleted strain did not restore β-lactam resistance, demonstrating that the β-lactam susceptibility phenotype is exhibited by *isaA* mutant regardless of the expression level of *mecA*. Overall, our results suggest that IsaA is a potential therapeutic target for MRSA infections.

Periplasmic oxidative burst-mediated cell death in dormant bacteria
Stress is known to induce bacterial dormancy, which is an unculturable state, but the details, including underlying mechanisms, are poorly understood. We found that stressed Gram-negative bacteria, including enterohemorrhagic *Escherichia coli* O157, entered a dormant state depending on the activity of the sigma factor σs. These stressed bacteria exhibited outer membrane disintegrity and periplasmic redox imbalance which led to the periplasmic oxidative burst and cell death. On the basis of these findings, we developed a culture method that isolates dormant *E. coli* O157 from contaminated food sources. This study provides evidence of the novel stress response and cell death pathway in dormant Gram-negative bacteria, including food-borne pathogens, which are related to public health and food safety.

Distinct stage of biofilm dispersed bacteria from planktonic lifestyles
Bacteria have 2 growth modes, switching between planktonic and biofilm lifestyles. Biofilm dispersals release free-living bacteria that can lead to bacterial spread in a new location. We discovered that *S. aureus* causes biofilm dispersal by nuclease and dispersed

bacteria evaded polymorphonuclear neutrophil phagocytosis. In a mouse model of infection, dispersed bacteria showed greater survival in the blood than did planktonic bacteria and caused a lethal infection within 24 hours. Dispersed bacteria showed decreased sensitivity against bactericidal agents, such as hydrogen peroxide and aminoglycoside antibiotics. These results indicate that biofilm-dispersed bacteria differ from planktonic bacteria and have greater virulence.

Publications

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