Department of Bacteriology

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General Summary

Research projects of our department have focused on: 1) the role of fibronectin-binding proteins (FNBP) A and B in staphylococcal infection, 2) the analysis of *Staphylococcus aureus* biofilm formation and detachment, and 3) the molecular mechanisms of bacterial ATP secretion.

Research Activities

Role of FnBPA and FnBPB in in vitro cellular infections and in vivo septic infections by S. aureus

FnBPA and FnBPB are important adhesins for *S. aureus* infection. We constructed strains with mutations of *fnbA* or *fnbB* or both from *S. aureus* SH1000, which possesses intact *rsbU*, and studied the role of these adhesins in *in vitro* and *in vivo* infections. In intravenous infection, all *fnb* mutants caused a marked reduction in the colonization rate and the mortality rate of mice. The *fnbB* mutant caused a more severe decrease in body weight than did the *fnbA* mutant. Serum levels of interleukin 6 and nuclear factor (NF)-κB activation in spleen cells were markedly reduced in *fnbA* or *fnbA/B* mutant infections; however, there was no significant reduction in *fnbB* mutant infections. In *in vitro* cellular infection, FnBPA was shown to be indispensable for adhesion to and internalization by nonprofessional phagocytic cells upon ingestion by inflammatory macrophages and NF-κB activation. However, both FnBPs were required for efficient cellular responses. The results showed that FnBPA is more important for *in vitro* and *in viv*o infections; however, cooperation between FnBPA and FnBPB is indispensable for the induction of severe infection resulting in septic death.

Analysis of biofilm detachment factor secreted by S. aureus

The bacteria within the biofilm matrix are protected from the host immune system and from antibiotic attack. Therefore, finding the biofilm-disassembling substance might prove widely useful in medical and industrial applications for preventing or eradicating biofilms. The biofilm matrix formed by *S. aureus* is composed of protein, polysaccharides, and DNA. We found that *S. aureus* secreted the factor that detaches its own biofilm. The culture supernatant of *S. aureus* also detached the biofilms of *Staphylococcus epidermidis*, methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa*, and *Escherichia coli*, which are causative bacteria of biofilm infections. The molecular weight of the factor responsible for the detachment effect is less than 500 Da. On-going study focuses on identifying the factor.

Identification and spatiotemporal dynamics of S. aureus biofilm matrix proteins
All biofilms contain an extracellular matrix that holds cells together. This matrix is often composed of polysaccharide biopolymers along with extracellular DNAs and proteins. Although proteinaceous components of the *S. aureus* biofilm matrix could be targets for vaccine development, they are largely unknown. Here, we surveyed *S. aureus* biofilm matrix proteins with proteomic approaches. Cell surface proteins were extracted from biofilms formed by a clinically isolated MRSA strain and subjected to top-down proteome analysis. A total of 133 proteins, including adhesins required for attachment to host cells, biofilm-related proteins, cell wall-anchored proteins, cytoplasmic proteins, and proteins of unknown function, were identified. A green fluorescent protein reporter assay revealed that MHC class II analog protein (Map), a predominant protein among them, formed extracellular biofilm matrix structures. In addition, purified recombinant Map stimulated biofilm formation by *S. aureus* in a dose-dependent manner, indicating that Map plays a crucial role in *S. aureus* biofilm formation. Roles of the other identified in the staphylococcal biofilm formation proteins are under investigation.

Studies on characteristics of S. epidermidis against S. aureus
Last year we reported that the serine protease Esp secreted by a subset of the commensal bacterium *S. epidermidis* inhibits biofilm formation and nasal colonization by *S. aureus*, a pathogen of humans.
We have addressed the ability of Esp-secreting *S. epidermidis* to prevent colonization by MRSA. MRSA has emerged in recent decades as a leading cause of infections worldwide and predisposes to infection, but available regimens are ineffective at preventing MRSA colonization. Studies of human nasal flora suggest that resident bacteria play a critical role in limiting *S. aureus* growth and prompted us to ask whether application of commensal resident bacteria can prevent nasal colonization with MRSA. We established a murine model system to study this question and showed that nasal precolonization with *S. epidermidis* is enhanced by prior application of streptomycin. Once nasally colonized with *S. epidermidis*, the mice became more resistant to colonization with MRSA. Our study suggests that application of commensal bacteria with antibiotics could represent a more effective strategy to prevent MRSA colonization.

Characterization of ATP-secreting bacteria from mice and humans
We have reported that ATP-secreting bacteria are present in the intestines of mice and humans. However, the mechanisms of ATP secretion in bacteria are not completely understood. To investigate the mechanisms, we have generated mutants that cannot secrete ATP. We are investigating the mechanisms using these mutants.

Publications

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