Department of Bacteriology

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General Summary

Research projects of our department have focused on: 1) the mechanism of inhibition of *Staphylococcus aureus* colonization by commensal *Staphylococcus epidermidis*; 2) the role of beta-hemolysin in the inhibition of interleukin (IL)-8 production by human umbilical endothelial cells; 3) fibronectin-mediated colonization via fibronectin binding proteins (FnBPs) in *S. aureus* infection; 4) induction of fibroblast apoptosis by intracellular *S. aureus*; 5) the mechanism of bacterial biofilm formation; and 6) molecular analysis of viable but nonculturable bacteria.

Research Activities

Characterization of a biofilm destruction factor of S. epidermidis

To clarify the mechanism by which *S. epidermidis* inhibits *S. aureus* colonization, we performed an epidemiological survey and *in vitro* studies. The studies revealed the presence of 2 types of *S. epidermidis*: one that inhibits colonization of *S. aureus* (inhibitory type) and the other that does not (non-inhibitory type). The prevalence of *S. aureus* decreased significantly in the human nasal cavity in the presence of a inhibitory *S. epidermidis* strain. Inhibitory type of strain secretes a factor that inhibits *S. aureus* colonization and disrupts preexisting *S. aureus* biofilm. This factor, biofilm destruction factor, is a 27-kDa protein that belongs to a serine protease family.

Biofilm formation of clinical isolated staphylococcus species

Biofilm infections caused by staphylococcus species are associated with indwelling medical devices, such as intravenous catheters and prosthetic joints. Such infections are difficult to treat and often necessitate removal of the implants. A precise analysis of biofilm formation by clinically isolated staphylococci would be useful for establishing methods to prevent and treat biofilm infection. We analyzed the capacity for biofilm formation and the biofilm component *in vitro* using strains isolated from patients of The Jikei University Hospital. Biofilm formation was observed in 29.2% (7 of 24) of methicillin-sensitive *S. aureus* (MSSA) strains, 29.2% (7 of 24) of methicillin-resistant *S. aureus* (MRSA) strains, and 25.0% (7 of 28) of *S. epidermidis* strains. Among staphylococci, 2 of the 7 biofilm-forming strains were induced by NaCl, and 5 of the strains were induced by glucose. Only 1 biofilm formed by an MRSA strain was destroyed by a polysaccharide-degrading enzyme (dispersin B), but 4 biofilms formed by *S. epidermidis* were susceptible to dispersin B. On the other hand, a protein- degrading enzyme (proteinase K) destroyed 4 biofilms formed by MSSA strains and 4 biofilms formed by MRSA strains but only 2 biofilms formed by *S. epidermidis* strains. Seven of these 10

biofilms susceptible to proteinase K were destroyed by a DNA-degrading enzyme (DNase I). There were no marked differences in the frequency of biofilm formation among the clinically isolated MSSA, MRSA, and *S. epidermidis* strains.
The biofilms of *S. epidermidis* were dependent on polysaccharides; on the other hand, the biofilms of *S. aureus* were dependent on proteins. A large amount of extracellular DNA was contained in the proteinaceous biofilms.

Restoration of culturability of starvation-stressed and low-temperature—stressed Escherichia coli O157 cells by using H_2O_2-degrading compounds
Late-exponential—phase cells of *E. coli* O157 became nonculturable in sterilized distilled water microcosms at 4°C. Plate counts declined from 3 million to less than 0.1 colony-forming units (CFU)/mL in about 21 days. However, when samples of microcosms at 21 days were inoculated onto an agar medium amended with catalase or nonenzyme peroxide-degrading compounds, such as sodium pyruvate and alpha-ketoglutaric acid, plate counts increased to 10^4 to 10^5 CFU/ml within 48 hours. The proposed mode of action of the catalase or pyruvate is via degradation of the metabolic by-product H_2O_2 rather than through supplementation of a required nutrient in the recovery of nonculturable cells. Our studies were based on the assumption that the E32511/HSC strain responds to starvation and low temperature by entering a nonculturable state and that the correction of oxidative stress upon the inoculation of bacteria on agar plates promotes the recovery of nonculturable cells.

Inhibition of endothelial IL-8 production and neutrophil transmigration by S. aureus beta-hemolysin
Neutrophils play a crucial role in the host response to infection with *S. aureus*, which is a major human pathogen capable of causing life-threatening disease. IL-8 is a potent chemoattractant and activator of neutrophils. We have previously reported that *S. aureus* secretes a factor that suppresses IL-8 production by human endothelial cells. Here we describe our isolation of an inhibitor of IL-8 production from the supernatant and identification of it as staphylococcal beta-hemolysin. Beta-hemolysin reduced IL-8 production without cytotoxicity to endothelial cells. Pretreatment with beta-hemolysin decreased the expression of both IL-8 mRNA and protein induced by tumor necrosis factor alpha (TNF-α). Migration of neutrophils across TNF-α—activated endothelium was also inhibited by beta-hemolysin. In contrast, beta-hemolysin had no effect on the expression of intercellular adhesive molecule 1 in activated endothelial cells. These results show that beta-hemolysin produced by *S. aureus* interferes with inflammatory signaling in endothelial cells and may help *S. aureus* evade the host immune response.

Contribution of FnBPs in the infection and biofilm formation of S. aureus
S. aureus employs a variety of adhesins to colonize host tissues and organs by binding to extracellular matrix proteins or plasma proteins. Among them, FnBPs are thought to be the most important adhesin for interacting with host cells. As we have already found by using an *fnbA* mutant strain derived from the parental SH1000 strain, FnBPA

is important for *in vitro* and *in vivo* infections by *S. aureus* because of the effective colonization of host tissues.
To clarify the role of FnBPB in infection and the advantage for *S. aureus* to maintain such similar factors simultaneously, we constructed an *fnb*B-deficient mutant and an *fnb*A/*fnb*B double-deficient mutant from a wild-type strain, SH1000. The experiments for *in vitro* and *in vivo* infection are now under way using these mutant strains.
Furthermore, the role of these adhesins in biofilm formation is being studied. A series of experiments indicate that FnBPB likely plays a significant role in biofilm formation by *S. aureus*.

Growth-phase—dependent interaction between fibroblasts and S. aureus
Staphylococci of the clinically isolated OK11 strain that had been grown in brain-heart infusion broth at 37°C for 2 hours (exponential phase) and 18 hours (stationary phase) were treated with purified fibronectin to examine the participation of fibronectin in the ingestion of bacteria by L929 fibroblasts. We have shown that exponential-phase staphylococci express a large amount of FnBP on their surfaces, whereas stationary phase cells do not. The L929 fibroblasts, which have networks of fibronectin on their surfaces, ingested a large amount of bacteria in the exponential phase. The L929 fibroblasts formed microfilaments at the edges so as to ingest bacteria rapidly.

Publications

O-Uchi J, Sasaki H, Morimoto S, Kusakari Y, Shinji H, Obata T, Hongo K, Komukai K, Kurihara S. Interaction of α_1-adrenoceptor subtypes with different G proteins induces opposite effects on cardiac L-type Ca^{2+} channel. *Circ Res* 2008; **102:** 1378–88.

Tajima A, Iwase T, Shinji H, Seki K, Mizunoe Y. Inhibition of endothelial interleukin-8 production and neutrophil transmigration by *Staphylococcus aureus* beta-hemolysin. *Infect Immun* 2009; **77:** 327–34.