Inhibition of Staphylococcal Wound Infection and Potentiation of Antibiotic Prophylaxis by a Recombinant Fragment of the Fibronectin-Binding Protein of Staphylococcus aureus

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Barbara E. Menzies,1,2,a Yordanka Kourteva,2 Allen B. Kaiser,2 and Douglas S. Kernodle1,2

Adherence of Staphylococcus aureus to host tissues is a critical step for colonization and initiation of infection. The fibronectin-binding proteins (FnBPs) of S. aureus have been implicated in adherence and internalization in nonprofessional phagocytes. A recombinant fragment of the fibronectin-binding domains (rFnBF) that potently inhibits S. aureus entry into host cells was generated. To test the hypothesis that rFnBF may attenuate the establishment of infection, the ability of intermuscularly administered rFnBF to prevent abscess formation was determined in a guinea pig model of wound infection. rFnBF exhibited dose-dependent inhibition of abscess formation and, at a 100-μg dose, raised the median infective dose ~170-fold, compared with the control. In addition, rFnBF potentiated the benefit of prophylaxis with cefazolin. Thus, exogenous administration of the fibronectin-binding domain of FnBP reduces the risk of staphylococcal abscess formation and should be investigated further as a novel agent for prevention of wound infection.

Staphylococcus aureus is a major pathogenic organism that causes minor superficial infections and serious systemic infections such as pneumonia, septicemia, deep-tissue abscesses, and endocarditis [1]. Adherence of S. aureus to host tissues is presumed to be a critical step in colonization and subsequent establishment of infection. Although S. aureus is considered to be a classic extracellular pathogen, evidence for an intracellular existence continues to emerge from in vitro studies that use nonprofessional phagocytes, including endothelial cells, epithelial cells, and osteoblasts [2–4]. This capacity of S. aureus to invade host cells and to persist in a viable state may shield this pathogen from host defense systems and most antimicrobials. Indeed, intracellular invasion would provide an explanation for the high prevalence of tissue metastasis and chronic persistence of S. aureus after episodes of bacteremia.

The bacterial determinants of S. aureus tissue adherence and intracellular invasion are being intensively investigated. Cell-wall adhesins of the MSCRAMM (microbial surface components recognizing adhesive matrix molecule) family recognize target ligands on host cells or within the extracellular matrix [5]. Fibronectin (Fn)–binding MSCRAMMs exist on the cell surface of S. aureus and other pathogenic gram-positive bacteria. The S. aureus Fn-binding proteins (FnBPs) contribute to adherence of this organism to immobilized Fn, to plasma clots, and to explanted medical devices [6–8].

The laboratory S. aureus strain 8325–4 possesses 2 closely linked genes, fnbA and fnbB, that encode FnBPA and FnBPB, respectively [9, 10]. Studies that used isogenic mutants of one or both genes showed that both surface proteins contribute to the ability of S. aureus to adhere to Fn-coated surfaces [6]. The Fn-binding domains of both FnBPA and FnBPB exhibit ~94% amino acid identity and share homologous repeats with the Fn-binding MSCRAMMs expressed by several streptococcal species [11, 12]. The structural organization of the Fn-binding domains of S. aureus FnBPA and FnBPB consists of 4 units repeated in tandem (D1–D4) and a fifth repeat (Du) in ~100 amino acid residues of N-terminal to D1. Each can bind to the 29-kDa Fn N-terminal fragment. Synthetic peptides and recombinant protein fragments of the Fn-binding domain inhibit the binding of S. aureus organisms to immobilized Fn [13].

Although Fn-induced agglutination of S. aureus has been correlated with invasiveness in the clinical setting [14], animal model studies that used strains isogenic for FnBP have yielded conflicting data regarding its impact on virulence in experimental endocarditis [15, 16], and there are few published reports that address its role in virulence in other animal models of infection. Recent in vitro studies, however, have demonstrated that theFnBPs have a role in mediating adherence and entry into cultured mammalian cells. An S. aureus strain in which both fnbA and fnbB had been inactivated exhibited reduced adherence and entry into mammalian epithelial cells and human umbil-
ical vein endothelial cells, compared with its parent [17–20]. Further confirmation of the contributory role of FnBP in adherence and invasion in vitro has been demonstrated by studies that show reduced \textit{S. aureus} adherence and/or invasion in various mammalian cells after pretreatment with recombinant or synthetic fragments of the Fn-binding domain of FnBPA of \textit{S. aureus} [18, 19] and of the FnBP of \textit{Streptococcus dysgalactiae} [20].

In this study, we generated a recombinant fragment of the Fn-binding domain of FnBP (rFnBF) from \textit{S. aureus} 8325-4 that exhibits potent inhibition of \textit{S. aureus} adherence and entry into human umbilical vein endothelial cells. We hypothesized that if FnBP-mediated adherence of host cells is important in the establishment of \textit{S. aureus} infection, then exogenous rFnBF might effectively compete with staphylococcal organisms for adhesion to host tissues and thus reduce the likelihood of infection. To test this hypothesis, we determined the ability of rFnBF to prevent abscess formation in a small-inoculum guinea pig model of wound infection.

**Materials and Methods**

**Bacterial strains and cultivation.** \textit{S. aureus} 8325-4, a standard laboratory strain, is a derivative of NCTC 8325 that has been cured of prophages. DU5883 (provided by T. Foster, Trinity College, Dublin) is a derivative of 8325-4 in which the genes \textit{fnbA} and \textit{fnbB} were inactivated by insertion of drug-resistance markers [6]. DK2076 is a clinical \textit{S. aureus} isolate of phage group 94/96. Other clinical \textit{S. aureus} isolates from cases of bacteremia and endocarditis (M19, M20, S. aureus a clinical isolate of phage group 94/96. Other clinical \textit{S. aureus} isolates from cases of bacteremia and endocarditis (M19, M20, M21, M25, and M27; W. Wilson, Mayo Clinic) were used. All strains were grown in brain-heart infusion (BHI) broth (Difco Laboratories) at 37°C with aeration and shaking. For the invasion experiments, an overnight culture was diluted 1:20 in fresh BHI and incubated for 1.5–2 h. The bacterial cells were then collected by centrifugation, washed twice with sterile PBS, and resuspended in medium 199 (M199; LifeTechnologies).

**Construction of expression plasmids and purification of rFnBFs.** To express and purify the Fn-binding domains of FnBP, DNA was amplified from \textit{S. aureus} 8325-4 and linked to the gene encoding for IgG binding in the phagemid pEZZ 18 (Amersham Pharmacia Biotech). This synthetic IgG-binding site (ZZ) is based on the binding site in protein A. Proteins are expressed as fusions with the ZZ peptide and secreted into the extracellular medium under the direction of the protein-A signal sequence. A 491-bp fragment of the 8325-4 \textit{fnbA} gene encoding D1–D4 was amplified by polymerase chain reaction. A factor Xa cleavage site was engineered upstream from the \textit{fnb} reading frame, so that the ZZ component could be cleaved from the rFnBF protein.

\textit{Escherichia coli} harboring the recombinant vector was cultivated overnight in tryptic soy broth (Difco) supplemented with 0.4% glycerine. The fusion protein was purified from the supernatant of the culture by use of IgG Sepharose 6 Fast Flow (Amersham Pharmacia Biotech), to which the ZZ domain binds. The rFnBF was cleaved from the fusion protein by factor Xa (New England Biolabs) at 4°C for 48 h, and the preparation was applied to the same column to allow removal of the ZZ peptide from the preparation. The rFnBF in the flowthrough was concentrated in PBS and filter sterilized over a 0.2-μm membrane. The preparation yielded a single protein band of ~35 kDa, as determined by SDS-PAGE. Protein concentration was determined by a Bradford-based assay (Bio-Rad).

An rFnBF encompassing D1–D4 and containing an N-terminal extension of 6 histidine residues (rFnBF-6×His) was constructed by amplifying the \textit{fnbA} region of \textit{S. aureus} (nt 2275–2760). The amplified fragment was cloned into a pQE30 vector (Qiagen), expressed in \textit{E. coli} M15[pREP4], and purified by nickel–nitrilotriacetic acid affinity chromatography under denaturing conditions according to the instructions of the manufacturer.

**SDS-PAGE and Western ligand affinity blotting.** Proteins of the bacterial culture supernatants and purified recombinant protein fractions were separated by SDS-PAGE according to the standard methods of Laemmli [21] by using 12% polyacrylamide gels and were stained with Coomassie blue. For immunodetection, proteins were transferred onto nitrocellulose membranes with a buffer system, as described by Towbin et al. [22]. The FnBP fragment was detected by overlay of the blot with pure human Fn (1 μg/mL) for 60 min, followed by probing with anti–human Fn IgG (Sigma). We used an alkaline phosphatase–conjugated anti-rabbit IgG (Sigma) as the secondary antibody and 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium (Sigma) as substrates.

**Endothelial-cell cultures.** Human umbilical vein endothelial cells were purchased from Clonetics and propagated in M199 supplemented with 15% fetal bovine serum (Hyclone), endothelial-cell mitogen (Biomedical Technologies; 50 μg/mL), penicillin G (100 U/mL), and streptomycin sulfate (100 μg/mL) on gelatin-coated 24-well plates (Corning). All experiments were performed with second-to-fourth–passage cells that had reached confluence.

**Invasion assay.** Endothelial cells in confluent 24-well plates were washed 3 times with M199 to remove antibiotics and then incubated with or without rFnBF in M199. After 30 min, \textit{S. aureus} was added to achieve an MOI of 10:1. After a 60-min coincubation, the wells were washed and treated with 2.5 μg/mL recombinant lysoptatin (Sigma) for 30 min to remove extracellular bacteria. The wells were washed again to remove traces of lysoptatin, and sterile water was added to hypotonically lyse endothelial cells. The well contents were then serially diluted and plated on agar plates for counting of colony-forming units.

**In vivo procedures.** Details of this small-inoculum prophylaxis model have been described elsewhere [23]. Guinea pigs were allowed to acclimate for at least 24 h before the initiation of the experimental protocol.

\textit{S. aureus} strains were maintained at ~70°C in tryptic soy broth containing 10% glycerol. Bacterial colonies were harvested after overnight growth on tryptic soy agar and suspended in PBS (pH 6.0) to achieve a standard turbidity. Serial 10- and 2-fold dilutions were made to prepare a range of inocula that produced abscesses 0%–100% of the time. Each dilution was mixed in a 1:1 (vol/vol) ratio with sterile dextran microbeads (Cytodex; Sigma). Back counts were made in triplicate and averaged to determine the precise size of the bacterial inoculum.

On the day of in vivo experimentation, dorsal hair was removed from albino Hartley guinea pigs (weight, 500 ± 50 g; Harlan Sprague Dawley) of both sexes, and a grid designating 12 sites
Figure 1. A. Structural organization of fibronectin (Fn)–binding protein A of Staphylococcus aureus 8325-4. Block S, N-terminal signal sequence; block A, fibrinogen-binding region; blocks B1 and B2, homologous repeats of unknown function; blocks Du and D1–D4, Fn-binding repeats; block W, cell wall–spanning region; block M, membrane-spanning region. Recombinant protein constructs used in this study (a fragment of the recombinant Fn-binding protein [rFnBF] and rFnBF expressed as a histidine-tagged protein [rFnBF-6-His]) are indicated. B, lane M, low–molecular-weight markers (Bio-Rad); lane 1, Coomassie-stained SDS-PAGE, using 12% polyacrylamide gel, of the expressed fusion protein ZZ-rFnBF (rFnBF with ZZ peptide) in the culture supernatant; lane 2, eluted ZZ-rFnBF off of the IgG-Sepharose (Amersham Pharmacia Biotech) column; lane 3, ZZ and rFnBF after factor Xa cleavage; lane 4, cleaved rFnBF isolated after a second run on the IgG-Sepharose column; lane 5, cleaved ZZ peptide.

was drawn. The potential space between fascia that surrounded the skin-related and trunk muscle groups underlying each site was inoculated with 0.2 mL of one of the bacterium-bead suspensions containing either rFnBF, PBS (control), or the cleaved ZZ portion of the original fusion protein (control). In some animals, cefazolin (Eli Lilly) at 100 mg/kg was administered subcutaneously 15 min before intermuscular inoculation. After inoculation, the guinea pigs were returned to their cages. Three days later, the guinea pigs were sacrificed, and the new-growth dorsal hair was removed by depilation. By use of a biopsy punch and sterile technique, the microbeads, with or without adherent abscess material, were removed from each of the 12 sites and inoculated onto sheep blood agar plates. The plates were incubated at 37°C for 24 h, and the presence or absence of bacterial growth was recorded.

Wound histopathology. Wound sites were excised by punch biopsy and placed in 10% buffered formalin. Tissue sections were stained with hematoxylin-eosin.

Whole-blood bactericidal assay. The effect of rFnBF on killing of S. aureus in whole blood was determined by an in vitro bactericidal assay. S. aureus DK2076 was cultured overnight in tryptic soy broth and then diluted in fresh RPMI 1640 medium containing 1-glutamine and 6.25% fetal bovine serum. The bacteria were grown to the middle of the logarithmic phase (2–3 h) in a shaking incubator at 37°C. Bacteria were washed twice in RPMI 1640 medium, resuspended in RPMI 1640 medium, and adjusted by spectrophotometric absorbance at 600 nm to achieve a concentration of 2 × 10^8 organisms/mL. From this suspension, dilutions in RPMI 1640 medium were made to achieve numbers of 10^3 and 10^5 bacteria in a 100-μL suspension. Whole blood was taken from a guinea pig via cardiac puncture and anticoagulated with 10 U/mL heparin immediately before the assay. For measurement of the effect of rFnBP on bactericidal activity, tubes containing heparinized blood (500 μL) were pretreated with rFnBF (or bovine serum albumin [BSA] control) for 15 min with end-over-end rotation at 37°C, followed by addition of staphylococci (100 μL) for another 1 h of incubation. Aliquots were taken for colony-count plating on sheep blood agar. Assay conditions were performed in triplicate and repeated in 2 separate experiments. Identical assays were made with human blood.

Statistical analysis. An inoculum-response (dose-response) curve was calculated, using binary logistic regression, from the percentage of growth at various bacterial inoculum levels, and the differences between the inoculum-response data for the control lesions and the data for the rFnBF-treated lesions were determined [24]. The ID_{so} was determined by the equation exp(−intercept/slope of log of back count). All analyses were done with JMP software (version 3.1.6; SAS Institute) with a level of significance set at P < .05.

Results

Analysis of the rFnBF of S. aureus FnBP. The Fn-binding activity of the FnBPs in S. aureus 8325-4 has been localized to 4 tandem repeats of 37 or 38 residues (D1–D4) and a fifth repeat N-terminal to D1 (Du) [9, 10] (figure 1A). rFnBF encompassing the domains D1–D4 was expressed as a fusion protein on pEZZ 18 in E. coli, purified from overnight culture supernatants, and was used in various assays to determine its bactericidal and other activities.

Figure 2. Dose-response effect of recombinant fibronectin-binding protein (rFnBF) on internalization of Staphylococcus aureus DK2076 by human umbilical vein endothelial cells. rFnBF was added to confluent endothelial cells in 24-well plates for 30 min, and then S. aureus at an MOI of 10:1 was added for 1 h. The total nos. of internalized staphylococci were determined by the lysostaphin protection assay with colony plating. Data are mean nos. of internalized bacteria per well. The experiment was repeated at least 3 times, with similar results.
cleaved from the ZZ peptide. In later work, rFnBF was expressed as a histidine-tagged protein (rFnBF-6×His). Application of 10 μg of the cleaved rFnBF to an SDS-PAGE gel yielded a single band of ~35 kDa (figure 1B). rFnBF-6×His yielded similar findings on SDS-PAGE analysis. That the migration of this protein on SDS-PAGE was higher than would be predicted from the DNA insert is consistent with prior observations; it is thought that this higher migration is related to the high content of acidic amino acids [25]. When they were blotted onto nitrocellulose membranes, both rFnBF and rFnBF-6×His bound human Fn in an overlay method, as detected by anti-Fn antibody and as seen when Fn was immobilized onto microtiter plates (not shown).

**Exhibition by rFnBF of potent inhibition of S. aureus entry into endothelial cells.** Confluent monolayers of human umbilical vein endothelial cells in 24-well plates were pretreated with or without rFnBF for 30 min before the addition of S. aureus DU2076 at a ratio of bacterial to endothelial cells of 10:1. We used the lysostaphin protection assay to determine the numbers of internalized S. aureus. Pretreatment of endothelial cells with rFnBF strongly reduced S. aureus internalization within endothelial cells in a dose-dependent manner (figure 2). Doses of rFnBF as low as 10 ng/well reduced the number of S. aureus organisms internalized to <1% of the number obtained in the absence of rFnBF. A dose-related decrease in S. aureus entry was maximal at 10 μg/well. Larger doses of rFnBF (20 and 50 μg) caused endothelial-cell detachment. Similar inhibition of S. aureus entry was observed with laboratory strain 3825-4 and clinical S. aureus isolates taken from cases of bacteraemia and endocarditis (figure 3). The results show that rFnBF is potent in its ability to inhibit entry of S. aureus into cultured endothelial cells. Another recombinant protein, rFnBF-6×His, showed a similar dose-response inhibition of S. aureus internalization within cultured endothelial cells (data not shown).

**Prevention of S. aureus abscess formation by rFnBFs.** To determine whether rFnBF inhibits staphylococcal abscess formation, we used a guinea pig model of muscular wound infection to gauge the effect of rFnBF on the ID₅₀. The recombinant protein was administered exogenously within the inoculum, which contained staphylococci and microcarrier beads. Initially, we added FnBF in doses of 10 and 20 μg to serial 2-fold inoculum preparations of S. aureus–microbead suspensions. Controls consisted of PBS and the ZZ peptide. A benefit of rFnBF was observed with both doses; the benefit associated with the 20-μg dose was slightly higher, corresponding to a 25-fold higher increase in ID₅₀ compared with ZZ peptide–treated control lesions (P < .0001; table 1, experiment 1).

Subsequent ID₅₀ measurements in this model were done with rFnBF-6×His in doses as high as 100 μg. Again, dose-related increases in ID₅₀ were observed as the amount of this recombinant protein increased (table 1, experiment 2). A dose of 100 μg increased the ID₅₀ ~170-fold beyond that for the PBS control (P < .0001).

**Additive effect of rFnBF and cefazolin in preventing S. aureus abscess formation.** We next determined whether rFnBF alters the prophylactic anti-infective property of cefazolin, an antimicrobial commonly used in surgical wound prophylaxis. Guinea pigs receiving both rFnBF and subcutaneous cefazolin had ID₅₀ levels that were 9.5-fold higher (P < .0001) than those of guinea pigs receiving cefazolin alone (table 2).

**ID₅₀ comparisons between an fnbA/fnbB-inactivated S. aureus strain and its wild-type parent.** S. aureus DU5883, in which both fnb genes are inactivated, binds poorly to immobilized Fn and has reduced adherence and intracellular uptake by a variety of cultured mammalian cells, compared with its parent, 8325-4. To determine the effect of Fn binding by staphylococci on the establishment of infection, we evaluated 8325-4 and DU5883 in the guinea pig wound abscess model. The ID₅₀ levels for S. aureus 8325-4 and DU5883 were 2.4 and 0.8 cfu, respectively (not statistically significant).

**Table 1.** ID₅₀ assessments in lesions inoculated with *Staphylococcus aureus* with coadministration of recombinant fibronectin-binding fragment (rFnBF).

<table>
<thead>
<tr>
<th>Experiment no., regimen</th>
<th>ID₅₀ of strain, cfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PBS control</td>
<td>0.6</td>
</tr>
<tr>
<td>ZZ peptide control</td>
<td>1.1</td>
</tr>
<tr>
<td>rFnBF, 10 μg</td>
<td>16.4*</td>
</tr>
<tr>
<td>rFnBF, 20 μg</td>
<td>27.2*</td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>PBS control</td>
<td>0.8</td>
</tr>
<tr>
<td>rFnBF-6×His, 10 μg</td>
<td>9.1*</td>
</tr>
<tr>
<td>rFnBF-6×His, 20 μg</td>
<td>27*</td>
</tr>
<tr>
<td>rFnBF-6×His, 100 μg</td>
<td>137*</td>
</tr>
</tbody>
</table>

**NOTE.** rFnBF-6×His, rFnBF expressed as a histidine-tagged protein.

*P < .0001, vs. PBS control.
Table 2. ID$_{50}$ assessments for lesions inoculated with *Staphylococcus aureus* with recombinant fibronectin-binding fragment (rFnBF) in guinea pigs receiving cefazolin.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>ID$_{50}$, cfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>rFnBF, 10 µg</td>
<td>16.4</td>
</tr>
<tr>
<td>Cefazolin*</td>
<td>753</td>
</tr>
<tr>
<td>Cefazolin* with rFnBF, 10 µg</td>
<td>7130$^b$</td>
</tr>
</tbody>
</table>

* Cefazolin (100 mg/kg) was administered subcutaneously 15 min before intermuscular inoculation of the staphylococcal inoculum with or without rFnBF.

* $^b$ P < .0001, vs. cefazolin regimen and rFnBF regimen.

**Intermuscular wound histology in response to rFnBF.** A few lesions were harvested by punch biopsy and submitted for histopathologic study in addition to culture. A hematoxylin-eosin preparation of a culture-positive high-inoculum lesion (85 × ID$_{50}$; ~50 organisms) into which BSA or PBS had been coadministered was characterized by an extensive collection of polymorphonuclear leukocytes (PMNL) with infiltration into the muscular zones (figure 4A). Microscopical examination of culture-negative lesions into which 100 µg of rFnBF-6×His was coadministered with the same amount of *S. aureus* inoculum revealed an intact muscular architecture that was void of infiltration by PMNL. There was, however, a mild degree of infiltration by mononuclear inflammatory cells along the periphery of the intermuscular zone (figure 4B). This sparse mononuclear-cell infiltration was also observed in lesions inoculated with microbeads containing rFnBF-6×His, BSA, or PBS in the absence of staphylococci (not shown).

**Discussion**

Several studies have demonstrated that the FnBPs of *S. aureus* has a major contribution to staphylococcal adherence and invasion of a variety of cultured epithelial and endothelial cells [17–19]. To our knowledge, our results are the first in vivo data that introduce the Fn-binding domains of *S. aureus* FnBP as novel prophylactic agents in wound infection. The rFnBF that is common to both FnBPA and FnBPB exhibited potency in blocking *S. aureus* entry into cultured endothelial cells and also prevented abscess formation in a guinea pig model. Given that the rFnBF was expressed as a fusion protein containing the synthetic ZZ peptide based on the IgG-binding site of *S. aureus* protein A, we were concerned that this component may confound interpretation of the effects of the rFnBF fusion protein. Therefore, the fusion protein was designed so that cleavage of the

**Figure 4.** Photomicrographs of hematoxylin-eosin–stained histologic sections from intermuscular lesions in the guinea pig wound infection model. Each lesion was inoculated with a suspension containing the same amount of *Staphylococcus aureus* DK2076 inoculum (85 × ID$_{50}$; ~50 organisms), dextran microbeads (MC), and either bovine serum albumin (BSA) control (A) or the recombinant fibronectin-binding fragment (rFnBF) (B). The lesion treated with BSA was culture positive for *S. aureus*, and histologic examination (A) revealed extensive polymorphonuclear leukocyte infiltration and erythrocyte accumulation in the muscle layers. The lesion treated with histidine-tagged rFnBF (B) was culture negative, and histologic examination indicated sparse infiltration with mononuclear leukocytes along the periphery of the intermuscular zone, adjacent to the MCs. Original magnification, ×400.
Table 3. Bactericidal activity (percentage of staphylococci killed) of guinea pig whole blood pretreated with recombinant fibronectin-binding fragment (rFnBF) of Staphylococcus aureus.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Inoculum of 236 organisms</th>
<th>Inoculum of 2400 organisms</th>
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<tbody>
<tr>
<td>rFnBF, 2 µg/mL</td>
<td>89.8</td>
<td>91.2</td>
</tr>
<tr>
<td>rFnBF, 10 µg/mL</td>
<td>94.9</td>
<td>91.0</td>
</tr>
<tr>
<td>BSA, 10 µg/mL</td>
<td>92.0</td>
<td>92.4</td>
</tr>
</tbody>
</table>

NOTE. For a description of experimental methods, see the section “Whole-blood bactericidal assay” in Materials and Methods. Data are shown as the mean percentage of staphylococci killed at 1 h vs. time inoculum was added. The experiment was repeated twice, with similar results.

rFnBF from the “ZZ” could be achieved. When used as a control in the wound model, the ZZ peptide yielded results similar to those for the PBS control. Thus, we conclude that the prophylactic anti-infective efficacy of the preparation was entirely attributable to rFnBF. Furthermore, the in vivo prophylactic capacity was reproducible by use of another recombinant protein (tagged with 6 histidine residues) encompassing the Fn-binding domains and was dose dependent, suggesting involvement of receptor-ligand interactions.

The prevention of S. aureus abscess formation in this model by rFnBF is similar to our observations of the action of antimicrobials, including the β-lactams [23]. Moreover, rFnBF potentiates the ability of cefazolin to prevent infection, attesting again to its prophylactic efficacy.

Although the FnBP analogue was effective as a recombinant prophylactic agent in this model, it was previously difficult to support the role of FnBP in virulence by use of S. aureus strains deficient in the expression of one or both fnbA and fnbB genes. Using a transposon-insertion mutant defective in Fn-binding, Kuypers and Proctor [15] found that the ability of S. aureus to bind Fn in vitro correlated with its ability to establish endocarditis in vivo but did not correlate with the numbers of bacteria in other tissues. A subsequent study that used a different pair of strains, in which the mutant strain carried allelic-inactivated fnb genes, failed to show a difference in virulence in experimental endocarditis [16].

In the guinea pig wound infection model, we also were unable to show a difference in the ID₅₀ of the wild-type strain and the strain deficient in fnbA and fnbB. One possible explanation is that the expression of functionally redundant factors by a complex pathogen such as S. aureus makes it difficult to discern the contribution of a single factor during the disease process. This possibility is supported by a recent study in which fnbA was cloned into the less pathogenic host Lactococcus lactis [26]. The recombinant fnbA-expressing organism was rendered more virulent in experimental endocarditis, thereby identifying FnBP as a critical adhesin in endovascular infection.

The elucidation of the mechanisms by which this FnBP analogue prevents S. aureus wound infection in this animal model will require further detailed studies. Clearly, the results of the in vitro studies shown here and of other studies demonstrate that rFnBF and similar recombinant fragments and synthetic peptides of the Fn-binding domains of FnBP block S. aureus adherence and uptake into a variety of mammalian cells of epithelial and endothelial origin [18–20]. We theorize that similar mechanisms are operative within the tissue environment of the host. Specifically, we propose that rFnBF competes with S. aureus for binding to Fn molecules or other ligands in the extracellular matrix. Sinha et al. [19] proposed that Fn serves as a bridging ligand between FnBP and host-cell receptors such as the α5β1 integrin and showed that this integrin is involved in uptake of S. aureus by host cells.

It is also possible that FnBP and analogues such as rFnBF may be capable of mediating these cellular events by direct binding to host-cell receptors. In this scenario, Fn, as a bridging ligand, may promote or facilitate FnBP-mediated adherence and invasion but may not be necessary for these events. Blocking these receptors from participating in S. aureus adherence and/or entry of host cells may permit these organisms to be more easily killed by extracellular defenses, including cationic antimicrobial peptides [27] and phospholipase A2 [28]. Gresham et al. [29] recently showed that manipulations to limit the migration of neutrophils into the site of infection reduce the bacterial burden in S. aureus infection, presumably by diminishing the number of staphylococci that are sheltered by the neutrophil from extracellular host defenses. Thus, it appears that the circumstance of staphylococci gaining access to an intracellular location without being killed, whether within neutrophils or nonprofessional phagocytes, might actually be deleterious to the host and contribute to the pathogenesis of infection.

In summary, the distinct use of rFnBF as a prophylactic anti-infective agent in vivo introduces a novel use of a bacterial adhesin. This opens the way for comparative studies of smaller peptides or derivatives of FnBP as prophylactic pharmaceutical weapons. Furthermore, this novel use of rFnBF as a prophylactic agent should promote similar investigation of other adhesins of S. aureus and of other bacteria. In the present era of emerging multiantimicrobial resistance in S. aureus, new approaches to combating this pathogen should be explored.

Acknowledgment

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