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Lack of the Extracellular 19-Kilodalton Fibrinogen-Binding Protein from *Staphylococcus aureus* Decreases Virulence in Experimental Wound Infection

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A mutant deficient for the 19-kDa extracellular fibrinogen-binding protein (Fib) from *Staphylococcus aureus* has been constructed. The gene was inactivated by allele replacement. A 2.0-kb fragment from transposon Tn*4001* carrying the gene for gentamicin resistance was inserted into the gene encoding Fib (*fib*). The genotype was verified by PCR analysis, and the loss of Fib was demonstrated by Western blotting (immunoblotting). The mutation has not altered the ability of the strain to bind to fibrinogen or fibronectin compared with that of the isogenic parental strain, FDA486. The mutant, designated K4.3, was compared with strain FDA486 in a wound infection model in rats. Sixty-eight percent of the rats challenged with parental strain FDA486 developed severe clinical signs of wound infection, whereas only 29% of the animals challenged with isogenic mutant K4.3 showed severe symptoms ($P < 0.01$). The weight loss of animals infected with the wild type was also significantly different from that of animals infected with the mutant strain. The result demonstrates that the extracellular 19-kDa fibrinogen-binding protein from *S. aureus* contributes to the virulence in wound infection and delays the healing process.

Staphylococcus aureus is one of the most common etiological agents of wound infection which can lead to septicemia, osteomyelitis, or endocarditis. Several extracellular virulence and pathogenicity factors from *S. aureus*, such as alpha-toxin, leucocidin, lipase, protease, nuclease, enterotoxins, toxic shock syndrome toxin, coagulase, and staphylokinase, have been characterized and are believed to contribute to the outcome of the infectious process. The importance of alpha-toxin has been shown by employing mutants defective in the corresponding gene in experimental infections (23). The role of coagulase, leading to fibrin formation, has been shown to be of little importance in endocarditis by comparing a site-specific mutant with its isogenic parental strain (1). Exactly which of these factors are involved in and contribute to the severity of various types of infections is still unclear.

Numerous proteins from *S. aureus* have been shown to bind to host matrix proteins, proteins in plasma and tissues such as fibronectin (8, 14, 30), fibrinogen (4, 21), collagen (24), elastin (22), plasminogen (17), thrombospondin (12), and vitronectin (25). For each of these binding functions, a corresponding surface-associated protein has been identified. These binding proteins undoubtedly mediate adherence of *S. aureus* and have been proposed to contribute to the colonization of host tissues (9). However, the contribution and importance of each and every one of these binding functions in different infections are unclear. The multitude of binding functions implies a complex multifactorial nature behind adhesion, with several alternative strategies for adherence.

During vascular trauma, different enzymes induce blood clotting through a cascade reaction which converts fibrinogen to fibrin. *S. aureus* interacts with fibrinogen in several ways, producing different proteins that bind specifically to this 340-kDa plasma protein. Several different fibrinogen-binding proteins from *S. aureus* are produced. A cell surface-associated protein, the clumping factor of 92 kDa, is the main mediator of adherence to fibrin (10, 21). Three mainly extracellular fibrinogen-binding proteins from *S. aureus* have been identified (3, 4). An 87-kDa fibrinogen-binding coagulase is produced during the exponential growth phase, a 60-kDa fibrinogen- and prothrombin-binding coagulase is produced during the postexponential growth phase, and a 19-kDa fibrinogen-binding protein (Fib) is produced constitutively. Also, a cell surface-associated coagulase has been characterized (7). Fib has sequence homology with coagulase but not with the clumping factor (5), and it has no enzymatic activity. The exact role of this extracellular fibrinogen-binding protein has not yet been clarified. However, the high incidence of this protein among *S. aureus* isolates (100%) implies an important role for this protein (6).

We have constructed an insertional inactivation mutant deficient for the 19-kDa fibrinogen-binding protein and compared its virulence with that of the isogenic parental strain, FDA486, in an experimental wound infection model in rats. The allele replacement resulted in reduced virulence.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used are listed in Table 1. Shuttle vector pSPT181 was obtained from Lars Janzon (Department of Bacteriology, Karolinska Institute, Stockholm, Sweden) (13). Plasmid pPQ132 (19) was used for generation of the gentamicin resistance gene. Plasmid pGEM-11Zf(−) was obtained from Promega (Madison, Wis.).

Recombinant DNA methods. Restriction enzymes, DNA ligase, and *Taq* polymerase were purchased from Promega and used according to the recommendations of the manufacturer. DNA manipulations were performed as described previously (26).

Isolation of the Gm^r gene. A 2.0-kb DNA fragment encompassing the gene for gentamicin resistance (Gm^r) was amplified from plasmid pPQ132 by PCR. Oligonucleotides (obtained from CyberGene AB, Huddinge, Sweden) GmR1 and GmR2 (5′ AGTCTAGAGGAGCCGTTCTTATGGAC 3′ and 5′ AGTCTAGA ACAGGAGTCTGGACTTGA 3′, respectively) were used. The DNA fragment was digested with *Xba*I (GmR1 and GmR2 have *Xba*I sites incorporated) and was ligated into the *Xba*I site of vector pGEM-11Zf(−).

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Description or construction	Source or reference
Strains		
E. coli XL1-Blue		Stratagene (La Jolla, Calif.)
S. aureus		
FDA486		Clontech Inc. (Palo Alto, Calif.)
RN4220	Restriction-deficient derivative of 8325-4	16
Plasmids		
pB*fib* III	pBluescriptSK plus vector plus *fib* gene	5
pBR*fib*	4.0-kb fragment from pBR322 plus 2.5-kb fragment with *fib* gene	This study
pPQ132	pBR322 plus Gm^r gene	19
pGEMGm^r	pGEM-11Zf(−) plus Gm^r gene	This study
pBR*fib*::Gm^r	pBR322 plus *fib* gene plus Gm^r gene	This study
pSPT181	Shuttle vector consisting of pSP64 and pRN8103	13
pSPT*fib*::Gm^r	pSPT181 plus *fib* gene plus Gm^r gene	This study

Construction of plasmid pSPT*fib*::Gm^r. Plasmids from *Escherichia coli* were extracted by using the Magic Minipreps system, and purification of DNA fragments was performed with Magic PCR preps (Promega). Plasmid DNA from *S. aureus* was extracted by the alkaline method of Birnboim and Doly (2), with lysozyme substituted for 100 μg of lysostaphin per ml.

The *Bam*HI-*Sal*I 4.0-kb fragment from pBR322 containing the ampicillin resistance gene and the origin of replication was ligated with a 2.4-kb *Bam*HI-*Sal*I fragment from pB*fib* III containing the *fib* gene (5) and used to transform *E. coli* XL1 Blue by electroporation. Colonies with a recombinant plasmid with the *fib* gene were detected by PCR using primers S.fib (5′GCGAAGGATACGGT CCAAGAGA 3′) and M.fib (5′ CAATTCGCTCTTGTAAGAACCATT 3′) (5) with positions as shown in Fig. 1B. The plasmid was designated pBR*fib.*

The Gm^r gene was excised from plasmid pGEMGm^r by *Xba*I digestion and introduced into *Xba*I-linearized pBR*fib* (Fig. 1A). The resulting plasmid, pBR*fib*::Gm^r, conferred resistance to both ampicillin and gentamicin (1 μg/ml). pSPT*fib*::Gm^r was constructed by ligating *Bam*HI- and *Sal*I-cleaved pSPT181 with plasmid pBR*fib*::Gm^r cleaved with *Pst*I, *Bam*HI, and *Sal*I restriction enzymes (Fig. 1A). (*Pst*I was used to minimize the risk of reconstituting pBR*fib*::Gm^r during ligation.) pSPT181 has an origin of replication which is functional in *E. coli* and a temperature-sensitive origin of replication which is functional in *S. aureus* at 32°C, whereas at 43°C, replication is suppressed. *E. coli* transformants with pSPT*fib*::Gm^r were detected by their resistance to ampicillin and gentamicin and by PCR analysis using GmR1-GmR2 and S.fib-M.fib primer pairs.

Inactivation of the *fib* gene in FDA486. *S. aureus* restriction-deficient strain RN4220 was transformed with pSPT*fib*::Gm^r. The plasmid was purified from a transformant and retransformed into *S. aureus* FDA486. Transformants were selected on brain heart infusion (BHI) broth agar plates containing 1 μg of tetracycline per ml at 32°C. A transformant was grown overnight in tryptone soy broth with 2 μg of tetracycline per ml and 8 μg of gentamicin per ml at 32°C and then with 8 μg of gentamicin per ml at 43°C for 4 h. The cultures were diluted 1:100 into fresh BHI broth without antibiotic and incubated at 43°C for 8 h. The culture was serially diluted and plated on gentamicin plates. Colonies able to grow at the nonpermissive temperature in the presence of gentamicin have undergone recombination at the *fib* sequences flanking the Gm^r gene, which results in loss of tetracycline resistance and irreversible integration of the Gm^r gene. Tetracycline-sensitive and gentamicin-resistant colonies were detected by replica plating on plates with tetracycline or gentamicin.

Confirmation of the *fib*::Gm^r allele replacement. Genomic DNA from *S. aureus* FDA486 and isogenic mutant K4.3 was extracted as previously described (20). Primer S.fib (5) was used in combination with primer II (5′ AGCTGTAG CTAATGCCAACCTA 3′) (Fig. 1B). Primer II is homologous to a site located downstream of the *fib* gene which is not included in pBfib III, pBR*fib*::Gm^r, or pSPT*fib*::Gm^r. At the initial cloning of the *fib* gene, the λ clone isolated (λfib50) also contained this flanking DNA fragment (2a, 5). The Expand long-template PCR system (Boehringer) was used, and the procedure recommended by the manufacturer was followed. PCR amplification by 25 repeated cycles was performed with an annealing temperature of 52°C and an elongation time at 68°C of 3.5 min for primer pair S.fib and II. The nucleotide sequence of the PCR fragment was determined with primers M, II, and G (Fig. 1B) to prove the genetic linkage between *fib*, the Gm^r gene, and the chromosomal region. Primer G (5′ CGGTAGTGGTTATGATAGTGTG 3′) corresponds to bases 1146 to 1167 of the 2.0-kb fragment of Tn*4001.*

Western blot (immunoblot) analysis. Fibrinogen-binding proteins from a 10-ml culture supernatant were purified on fibrinogen-Sepharose and analyzed by Western affinity blotting as described previously (3). Briefly, proteins separated on polyacrylamide gels were transblotted onto nitrocellulose membranes which were incubated for 1 h at room temperature with 10 μg of human fibrinogen per ml and then with rabbit anti-human fibrinogen conjugated with peroxidase (Dakopatts, Glostrup, Denmark). Western immunoblotting was also performed in parallel, using antibodies against Fib previously raised in a rabbit and then sheep anti-rabbit peroxidase-conjugated antibodies. All incubations were done for 1 h at 37°C with washes in phosphate-buffered saline (PBS) with 0.05% Tween 20 (PBST) between the steps.

Electroporation. Plasmids were electroporated into *S. aureus* RN4220 and FDA486 as described by Schenk and Laddaga (27) and into *E. coli* as described in the manual for Bio-Rad's Gene Pulser electroporation equipment.

Bacterial adherence to fibrinogen and fibronectin. Microtiter plates (Costar) were coated overnight at room temperature with 0.005 to 10 μg of fibrinogen (IMCO Inc., Stockholm, Sweden) per ml or 0.1 to 8 μg of fibronectin (Sigma, St. Louis, Mo.) per ml in serial dilutions. Aftercoating was done with 2% bovine serum albumin (BSA) for 1 h at 37°C. Bacteria (100 μl) were added at 4×10^8/ml in PBST, and the plates were incubated at 37°C for 2 h. The microtiter plates were washed three times with PBST to remove nonadherent bacteria. Adherence was determined with antibodies directed against *S. aureus* raised in rabbits (kindly supplied by R. Möllby, Department of Bacteriology, Karolinska Institute, Stockholm, Sweden), followed by alkaline phosphatase-conjugated antibodies against rabbit immunoglobulin G (Sigma). Both incubations were done for 1 h at 37°C, with washings between. Bound antibodies were measured with paranitrophenylphosphate (Sigma), and A_{405}s were read.

Clumping reaction. The clumping reaction assay system consisted of measurement of agglutination of staphylococci in serially diluted solutions of fibrinogen (10).

Quantification of coagulase activity. Washed cells of strains FDA486 and K4.3 grown overnight in BHI broth were diluted serially in twofold steps, and 20 μl was added to 0.5 ml of coagulase plasma (Difco Laboratories, Detroit, Mich.) at 37°C. Clotting was observed at regular intervals.

Quantification of alpha-toxin. An enzyme-linked immunosorbent assay was used to quantify alpha-toxin. Cultures of *S. aureus* FDA486 and K4.3 were cultivated overnight in BHI broth, and the supernatants after centrifugation were serially diluted in microtiter plates (Costar) for coating overnight. Aftercoating was done with 2% BSA for 1 h at room temperature. The amount of alpha-toxin was determined by using rabbit antibodies against alpha-toxin (supplied by R. Möllby) diluted 1:500 followed by peroxidase-conjugated anti-rabbit goat antibodies, with washings between. All incubations were done at 37°C for 1 h. A color reaction was developed with 1, 2-phenylenediamine tablets (Dako).

Wound infection model. Sixty-eight female, pathogen-free Wistar rats weighing approximately 185 g were used for the main experiment. One group of 10 animals served as a control group. During and before the experiment, the animals received a normal diet and were housed in cages containing five rats each.

The rats were anesthetized with pentobarbitol. Under aseptic conditions, an incision, 3 cm long, was made through the skin of the left portion of the shoulder. Each animal was infected in the submuscular space under the fascia at the site of the incision with 10^4 CFU of either strain FDA486 ($n = 30$) or K4.3 ($n = 28$) or treated with only PBS ($n = 10$), and the incision was then closed. The volume of the bacterial suspension was 0.2 ml, and no foreign body was included in the inoculum. The strains were placed in separate, blindly labelled tubes, the contents of which were unknown during the entire observation period to the person in charge of the wound infection experiments.

In a pilot experiment aiming at optimization of the challenge dose, five animals in each group were given 10^8, 10^7, 10^6, 10^5, 10^4, or 10^3 CFU of strain FDA486. On the basis of the rate of infection, 10^4 CFU was the dose chosen for further studies. In a control experiment, nine rats, divided into three groups of three rats each, were infected subcutaneously in the shoulder area and under the right front leg with 10^6, 10^5, or 10^4 CFU of strain FDA486 without any surgical incisions. No clinical or microbiological signs of an infection were found when the incision was omitted.

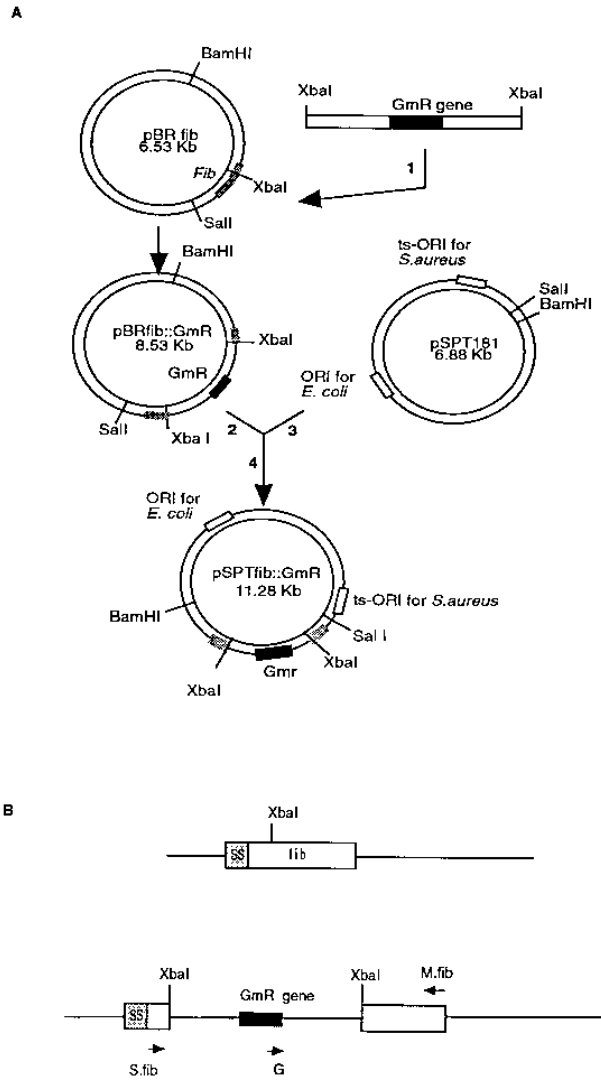

FIG. 1. (A) Construction of plasmid pSPT*fib*::Gm^r. The *fib* gene was first transferred from pB*fib* III (5) to a vector lacking an *Xba*I site, pBR322 (see text), resulting in plasmid pBR*fib*. The numbered steps indicate the following: 1, a 2.0-kb fragment containing the Gm^r gene incorporated into the *Xba*I site of the *fib* gene by *Xba*I cleavage followed by ligation; 2 and 3, *Bam*HI and *Sal*I double cleavage; and 4, ligation. (B) The *fib* gene in strain FDA486 and the *fib*::Gm^r inserted fragment in the mutant strain K4.3. Forward and reverse primers used for PCR amplification are shown. The size of the *fib* gene is 495 bp, and the insert (not drawn to scale) is approximately 2 kbp.

All the procedures were carried out under strictly aseptic conditions, and care was taken to avoid contamination of the instruments and the surgical site with airborne microorganisms.

The rats were observed daily for 1 week. Each rat was weighed and clinically examined every day. Clinical wound symptoms associated with an infectious process, such as edema, pain, abscess formation, delayed healing, color alteration, or decreased activity, were scored as criteria for a fully developed infection. Rats were divided into three groups according to the severity of the wound infection. Rats which showed all of the cardinal symptoms mentioned above were scored as ++, indicating a fully developed wound infection. Rats which had few of the cardinal symptoms were scored as +, indicating weak signs of wound infection. Rats without any symptoms were scored as 0, indicating no signs of developed wound infection (wound completely healed).

After the observation period, the rats were sacrificed with CO_2. The wound site was reentered, and under strictly aseptic conditions, a bacteriological sample was taken from each rat. Blood agar plates were then inoculated with the samples and incubated overnight at 37°C. Five colonies from each animal with positive growth were taken for morphological investigation and determination of gentamicin resistance.

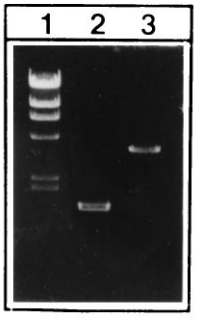

FIG. 2. PCR amplification of the *fib* gene in FDA486 and of *fib*::Gm^r in isogenic mutant K4.3. Lane 1, lambda DNA *Hin*dIII markers; lane 2, PCR product from FDA486 with primers S.fib and II; lane 3, PCR product from K4.3 with primers S.fib and II.

Infective endocarditis model. Thirty female Wistar rats were anesthetized with pentorbital and catheterized via the right carotid artery to induce endocardial vegetations on the aortic valve (11). Twenty-four hours later, the catheters were removed and 10^4 CFU of either strain FDA486 or K4.3 was injected in the tail vein. After another 24 h, the animals were killed, the aortic valves with vegetations were homogenized, and the number of CFU was determined.

Statistical analysis. Values for severity of infection were compared by using the χ^2 test, and weight changes and log CFU were compared by a two-tailed *t* test.

RESULTS

Allele replacement. The plasmid pSPT*fib*::Gm^r was constructed in order to substitute *fib* (the gene encoding the 19-kDa fibrinogen-binding protein) with *fib*::Gm^r. Recombinations taking place at the *fib* sequences flanking Gm^r result in integration of Gm^r and loss of tetracycline resistance residing on plasmid pSPT*fib*::Gm^r. Such recombinants were found after growth at 43°C, a temperature not allowing autonomous replication of the plasmid. One clone, named K4.3, was selected for further analysis.

Genotypic analysis of strain K4.3. PCR analysis with primers S.fib and II was used to verify insertion of a 2.0-kb fragment in the *fib* gene as shown in Fig. 1B. The PCR product from wild-type strain FDA486 showed a band of approximately 1.6 kb (Fig. 2, lane 2). The PCR product from isogenic mutant K4.3 was 2.0 kb larger (lane 3), corresponding to the size of the fragment incorporated into the genome. The nucleotide sequence of this fragment was determined by using primers M.fib, II, and G, showing the fusion between the Gm^r gene and the *fib* gene on the chromosome (data not shown). Furthermore, this fragment was digested with *Xba*I, which resulted in bands corresponding to expected sizes (data not show). The data are consistent with a gene arrangement in mutant K4.3 as shown in Fig. 1B.

Phenotypic analysis of strain K4.3. Western immunoblotting using rabbit antiserum against Fib (reacting also against coagulases of 60 and 87 kDa) was used to detect production of Fib by strain FDA486 and a lack of Fib production by strain K4.3 (Fig. 3A). Similarly, Western ligand affinity blotting using fibrinogen and antifibrinogen antibodies was used to detect Fib (Fig. 3B). In both experiments, fibrinogen-binding proteins were purified on fibrinogen-Sepharose. Fib could be detected in culture supernatant from *S. aureus* FDA486 (Fig. 3, lanes 1). However, strain K4.3 lacked Fib (lanes 2). Fibrinogen-binding coagulases (60 and 87 kDa) (4) were also detected, showing

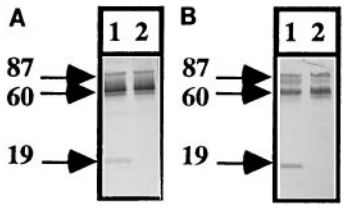

FIG. 3. Western affinity and immunoblotting of fibrinogen-binding proteins from equal amounts of culture supernatants purified on fibrinogen-Sepharose. (A) Detection with rabbit antibodies against fibringen-binding proteins. (B) Detection with human fibrinogen followed by anti-fibrinogen antibodies. Lanes: 1, proteins from FDA 486; 2, proteins from recombinant K4.3. Positions of molecular mass markers (in kilodaltons) are shown on the left.

that these proteins have not been affected by the genetic manipulation.

Cell-bound coagulase (data not shown), the clumping reaction (data not shown), and alpha-toxin production (data not shown) were found to be similar in FDA486 and K4.3.

Bacterial adherence to fibrinogen, collagen, and fibronectin. Isogenic mutant K4.3, deficient in the 19-kDa fibrinogen-binding protein, was compared with wild-type strain FDA486 for the ability to bind to immobilized fibrinogen, collagen types II and III, and fibronectin. Varying the amount of ligand protein with a constant amount of bacteria shows dose responses of similar magnitudes for the two strains (data not shown). Neither of the two strains can bind to collagen.

Virulence comparison in a wound infection model. Rats were subjected to skin wound experimental infection with strains FDA486 and K4.3. The development of the wound, the healing process, and the severity of the infections were monitored for a week. The severity of infection was categorized as follows: noninfected with no signs of infection (0), mild signs of infection (+), and severe infection not healed after 7 days (++). Table 2 shows the distribution of rats in these groups. Sixty-seven percent of animals infected with FDA486 had severe wounds, whereas only 29% of animals infected with K4.3 fell into this category ($P < 0.01$). Control animals that received bacteria subcutaneously without prior incision did not develop infection, nor did control rats given PBS instead of bacteria after incision.

Microbiological samples were taken from all animals on day 7. Bacterial growth was found more often in animals infected with FDA486 than in animals infected with K4.3, and microbial growth was positively correlated with severity of infection ($P < 0.01$).

Each animal was weighed before infection and then daily throughout the experiment. The weight changes in comparison with day 0 were calculated (Fig. 4). All animals lost weight on the first day because of anesthesia and operation. Animals in the control group and those infected with mutant strain K4.3 regained weight from the second day, whereas animals infected with strain FDA486 kept losing weight also on days 2, 3, and 4

TABLE 2. Severity of wound infection in the rat model

Inoculum	No. (%) of rats with the indicated score			Total
	++	+	0	
FDA486	20 (66.7)[a]	4 (13.3)	6 (20)	30
K4.3	8 (28.6)	8 (28.6)	12 (42.8)	28
PBS[b]	0	0	10 (100)	10

[a] Significantly different from result for animals infected with K4.3 ($P < 0.01$).
[b] PBS instead of bacteria injected into wound.

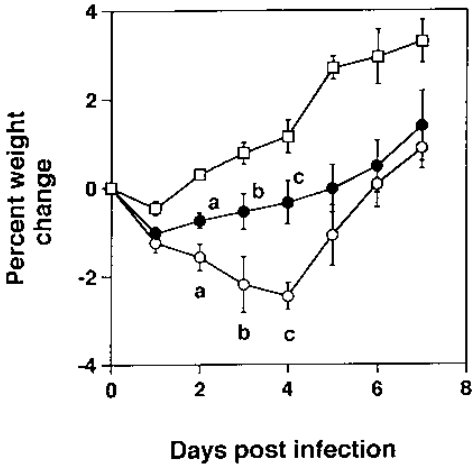

FIG. 4. Weight change of animals during wound infection. The percent weight changes relative to weight at day 0 measured at days 1 to 7 are shown as mean values with standard errors. a, $P = 0.04$; b, $P = 0.007$; c, $P = 0.004$. Open circles, animals infected with strain FDA486; closed circles, animals infected with strain K4.3; and squares, noninfected control rats.

($P = 0.037$, 0.007, and 0.004, respectively, in comparison with animals infected with FDA486 or K4.3 on these days).

Virulence comparison in endocarditis model. Rats were catheterized to induce vegetations on the aortic valve. Strains FDA486 and K4.3 were given intravenously, and the log numbers of CFU were determined 24 h after challenge. Figure 5 shows that no significant difference in CFU between the two strains was apparent, implying similar virulence and abilities to establish and maintain infective endocarditis ($P = 0.2$).

DISCUSSION

We constructed a mutant of *S. aureus* FDA486 lacking the *fib* gene, encoding an extracellular fibrinogen-binding protein. The genotype of this strain, K4.3, was verified by PCR analysis and by sequence determination of the PCR product, demonstrating that (i) *fib* is split by the Gm^r gene and (ii) a genetic linkage between the acquired Gm^r gene and a genomic region

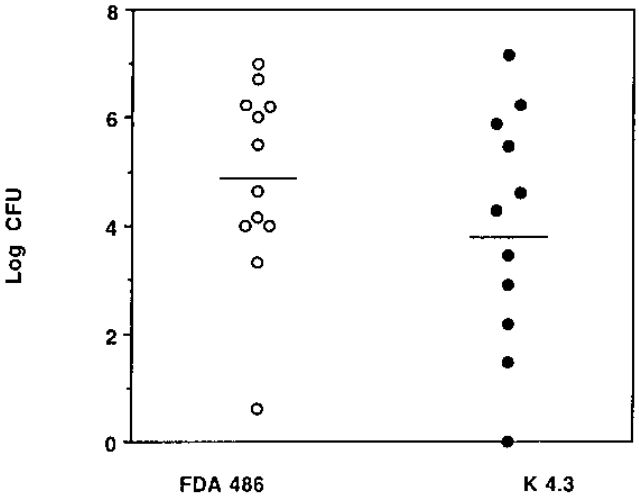

FIG. 5. Log number of CFU recovered from aortic valves with vegetations at 24 h postchallenge from animals infected with FDA486 and strain K4.3.

flanking *fib* was obtained. This flanking region is not included in the plasmid transferring *fib*::Gm^r into the recipient strain; thus, the linkage between Gm^r and this region is the result of recombination within *fib*.

The phenotypic alteration in K4.3 resulting from *fib*::Gm^r insertion, i.e., loss of the 19-kDa fibrinogen-binding protein, was shown by ligand affinity blotting using fibrinogen followed by antifibrinogen antibodies. Reactivity against fibrinogen-binding coagulases (60 and 87 kDa) demonstrates that the expression of these proteins has not been affected by insertional inactivation of the *fib* gene. We thus conclude that *fib* is the only gene encoding Fib. Earlier data (5) indicate that the region responsible for the fibrinogen binding is located downstream of the *Xba*I site, excluding the possibility of a truncated Fib-Gm^r fusion protein retaining fibrinogen-binding ability.

Adherence of *S. aureus* to immobilized fibrinogen and clumping in the presence of fibrinogen are typical characteristics of this species. We therefore tested adherence to fibrinogen and found that the mutant strain had no change in properties of adherence to fibrinogen. Fib has no typical cell wall anchoring region (18) or an LPXTG motif (28, 29), typical of cell surface association. Although a minor portion of Fib might be cell surface associated during a transient secretion process, the contribution of Fib to adherence of *S. aureus* to fibrinogen is probably minimal. Likewise, adherence to fibronectin is not affected by the loss of Fib. Neither strain FDA486 nor K4.3 can bind to collagen.

The allele replacement of *fib*::Gm^r for *fib* does not seem to affect general regulatory mechanisms in the recipient strain, since both alpha-toxin production and coagulase activity, properties regulated by *agr* (15), are unaltered. Furthermore, the abilities of the two isogenic strains to propagate on traumatized aortic valves, to escape host defense systems in endocarditis, and to adhere to vegetations are not affected by loss of Fib, since the two strains resulted in similar amounts of bacteria recovered from heart valves. Therefore, strain K4.3 does not seem to be generally crippled compared with FDA486 in an in vivo situation.

In this study, we compared the virulence of the two strains in a wound infection model based on a deep subcutaneous incision, with no foreign body present and with a moderate infectious dose applied locally at the time of injury. The same amount of bacteria given subcutaneously without injury did not lead to infection. The model mimics a natural wound infection as far as possible. The significantly ($P < 0.01$) reduced capacity of mutant K4.3 to induce severe wound infection indicates that extracellular Fib plays an important role in wound infection and reduces the rate of recovery. The different levels of severity in the groups are reflected by the different rates of weight loss ($P = 0.004$ at 4 days postinfection).

The importance of Fib is implied also by the high incidence of this protein; we have found that 105 isolates of *S. aureus* from various sources (endocarditis, wound infection, septicemia, and nasal isolates) all had the *fib* gene, as determined by PCR (7a). However, the finding that a lack of Fib in *S. aureus* experimental endocarditis does not reduce the propagation of the bacteria indicates that the role of Fib is different in different types of infections. The mechanism of action of Fib can only be speculated, but since a major component of blood clots in wounds is fibrin threads, it is conceivable that any disturbance to the wound healing process inflicted by an extracellular fibrinogen-binding protein is beneficial for the bacteria and delays the healing process.

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