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Role of *Staphylococcus aureus* Coagulase and Clumping Factor in Pathogenesis of Experimental Endocarditis

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The pathogenic role of staphylococcal coagulase and clumping factor was investigated in the rat model of endocarditis. The coagulase-producing and clumping factor-producing parent strain *Staphylococcus aureus* Newman and a series of mutants defective in either coagulase, clumping factor, or both were tested for their ability (i) to attach in vitro to either rat fibrinogen or platelet-fibrin clots and (ii) to produce endocarditis in rats with catheter-induced aortic vegetations. In vitro, the clumping factor-defective mutants were up to 100 times less able than the wild type strain to attach to fibrinogen and also significantly less adherent than the parents to platelet-fibrin clots. Coagulase-defective mutants, in contrast, were not altered in their in vitro adherence phenotype. The rate of in vivo infection was inoculum dependent. Clumping factor-defective mutants produced ca. 50% less endocarditis than the parent organisms when injected at inoculum sizes infecting, respectively, 40 and 80% (ID_{40} and ID_{80}, respectively) of rats with the wild-type strain. This was a trend at the ID_{40} but was statistically significant at the ID_{80} ($P < 0.05$). Coagulase-defective bacteria were not affected in their infectivity. Complementation of a clumping factor-defective mutant with a copy of the wild-type clumping factor gene restored both its in vitro adherence and its in vivo infectivity. These results show that clumping factor plays a specific role in the pathogenesis of *S. aureus* endocarditis. Nevertheless, the rate of endocarditis with clumping factor-defective mutants increased with larger inocula, indicating the contribution of additional pathogenic determinants in the infective process.

Bacteria which cause infectious endocarditis have the capacity to adhere to damaged cardiac tissue and to platelet-fibrin clots in vitro (2, 11, 14, 26). Therefore, a likely mechanism for the initiation of endocarditis is the direct attachment of circulating bacteria to damaged valves (1, 2). Several structures in the bacterial envelope have been implicated in ligand-receptor interactions with cardiac vegetations. These have been reviewed recently (2) and include polysaccharides, lipoteichoic acids, proteins in streptococci and enterococci (10, 17, 21, 22, 27, 29), and surface proteins and, possibly, polysaccharides in staphylococci (6, 9, 19, 30).

Staphylococcus aureus has at least three surface determinants which might mediate adherence to damaged valves and promote endocarditis, namely, (i) coagulase, which is mainly secreted in the medium (5, 24), (ii) clumping factor (or fibrinogen-binding protein) (8, 23, 24), and (iii) fibronectin-binding protein. Recent studies have addressed the roles of these structures in pathogenesis. Cheung et al. showed that mutants inactivated in either the *sar* locus (9) or the *sar* and *agr* loci (6), both of which regulate the expression of several surface proteins, including coagulase, clumping factor, and fibronectin-binding protein, had a decreased capacity to induce experimental endocarditis in rabbits. In addition, staphylococcal and streptococcal mutants defective in fibronectin-binding protein were impeded in their ability to produce experimental endocarditis in rats when compared with that of the parental strains (19, 21). On the other hand, Baddour et al. (3) observed no decrease in the infectivity of coagulase-defective mutants of *S. aureus*, suggesting that coagulase alone is not involved in the infective process. These important studies shed new light on the role of adhesins in the pathogenesis of endocarditis. However, they did not determine the respective significance of coagulase, clumping factor, and fibronectin-binding protein in the infective process. Solving this question for *S. aureus* requires comparison of the infectivities of staphylococcal mutants specifically inactivated in each of the genes concerned, both singly and in combination.

Recently, the staphylococcal coagulase (*coa*) and clumping factor (*clfA*) genes have been characterized (23, 28), and site-specific mutants defective in these two determinants have been generated (23, 24). This allowed further investigation of the capacity of these components to promote experimental endocarditis. In the experiments described here, *S. aureus* mutants defective in either the coagulase gene or the clumping factor gene alone or in both determinants were tested for their ability to adhere to fibrinogen or platelet-fibrin clots in vitro and to induce experimental endocarditis in vivo. To confirm the specific role of clumping factor, we also tested in parallel a clumping factor-negative mutant complemented with a copy of the cloned *clfA* gene.

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TABLE 1. Bacterial strains used in the study

S. aureus strain	Relevant genotype	Relevant phenotype	Source or reference
Newman	*coa*$^+$ *clfA*$^+$	High level of clumping factor	13
DU5854	Δ*coa*::Tc^r	Coagulase defective	24
DU5852	*clfA1*::Tn*917*	Clumping factor defective	23
DU5858	Δ*coa*::Tc^r *clfA1*::Tn*917*	Coagulase and clumping factor defective	12
DU5896	*clfA1*::Tn*917 geh*::pCF16	Clumping factor positive, lipase negative	23

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MATERIALS AND METHODS

Bacterial strains and growth conditions. Strain Newman which produces both coagulase and clumping factor (13, 24) and site-specific mutants lacking these factors are listed in Table 1. Strain DU5854 is a coagulase-defective mutant with a deletion in the *coa* gene substituted by a fragment encoding tetracycline resistance (Δ*coa*::Tc^r). The mutation was isolated in strain RN4220 by allelic replacement and transduced into strain Newman (24). The clumping factor-defective mutant DU5852 was isolated by transposon Tn*917* mutagenesis of strain Newman (23), forming *clfA1*::Tn*917*. The *coa clfA* double mutant of Newman (DU5858) was constructed by transducing the *clfA1* mutation into DU5854, selecting for Tc^r and Em^r. The *clfA1* mutation was complemented by use of a single-copy integration vector, pCL83, carrying the cloned wild-type *clfA* gene. The integrant was isolated in RN4220 and transduced into Newman *clfA1*::Tn*917*, selecting for Tc^r (DU5896) (20, 23).

Unless otherwise stated, all of the organisms were grown at 37°C either on solid medium (tryptic soy agar; Difco Laboratories, Detroit, Mich.) or in aerated liquid cultures (tryptic soy broth; Difco) in a rotating incubator at 120 rpm. The mutant strains were grown in antibiotic-containing medium appropriately supplemented with either 2 μg of tetracycline per ml or 5 μg of erythromycin per ml. The strains grew at similar rates in liquid cultures, as determined by parallel increases in optical densities at 620 nm. Frozen stocks of bacteria were kept at −70°C in tryptic soy broth supplemented with 10% glycerol.

Antibiotics and reagents. Erythromycin, tetracycline, and rat fibrinogen were purchased from Sigma Chemical Co. (St. Louis, Mo.). Rat fibrinogen was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis to test for purity. Three bands were visualized, as expected for human fibrinogen. Thrombin was obtained from Diagnotec (Liestal, Switzerland).

In vitro attachment of bacterial strains to rat fibrinogen. The attachment of the *S. aureus* strains was measured by a previously described adhesion assay using polymethylmethacrylate (PMMA) coverslips coated with purified rat fibrinogen (23, 24, 32). Two-sided coverslips were coated for 60 min at 37°C with increasing concentrations (from 0.5 to 2 μg/ml) of rat fibrinogen. At the end of the protein coating phase, the coverslips were rinsed in phosphate-buffered saline (PBS) (23, 24, 32, 33). The adhesion characteristics of the strains were evaluated by incubating the fibrinogen-coated PMMA coverslips for 60 min at 37°C with 4×10^6 CFU of washed log-phase cells labelled by growth in Mueller-Hinton broth containing [^{3}H]thymidine (23, 24, 32, 33). The adhesion medium was PBS with 1 mM Ca^{2+} and 0.5 mM Mg^{2+}, supplemented with 5 mg of serum albumin per ml to prevent nonspecific adhesion of *S. aureus* (23, 24, 32, 33). In certain experiments, bacteria were sonicated briefly to disrupt possible clumps. This procedure did not essentially affect the results. Coverslips were then rinsed, and the number of attached bacteria was estimated from radioactive counts. Albumin-coated coverslips were used as controls (23, 24, 32, 33).

Attachment of *S. aureus* to protein-coated PMMA coverslips was scored as the number of adherent CFU $\times 10^3/cm^2$. The statistical significance of the different attachment properties of parent and mutant strains was evaluated by one-way analysis of variance (ANOVA), and pairwise differences between the means of groups were determined by the *t* test with the Bonferroni correction. Data were considered significant when P was <0.05 by use of two-tailed significance levels.

In vitro attachment to rat platelet-fibrin clots. Adherence to rat platelet-fibrin clots was measured by slight modifications of a previously described method (26). First, blood freshly drawn from a pool of donor Wistar rats (weight, 300 to 400 g) was treated with 10% (vol/vol) chloride-citrate buffer (100 mM; pH 4.8) to prevent coagulation before centrifugation at low speed at room temperature for 5 to 15 min to pellet the erythrocytes and leukocytes. The conditions were set to keep the platelet counts between 15×10^9 and 50×10^9/liter in the cleared plasma. Platelet-fibrin clots were produced by pouring 0.5-ml volumes of plasma into a series of 30-mm-diameter culture plates (Costar Corp., Cambridge, Mass.) and then adding 100 μl of a 500-U/ml NIH bovine thrombin solution and 100 μl of a 0.2 mM $CaCl_2$ solution. The components were mixed gently by hand and allowed to coagulate at room temperature. The clots were then dehydrated overnight at 37°C and kept at 4°C before being used. To determine bacterial adherence, 2-ml volumes of 50 mM sodium phosphate buffer (pH 7) containing 5×10^3 to 1×10^4 CFU/ml were added to the wells (prewarmed at 37°C), and the plates were agitated for 3 min at 120 rpm on a rotating incubator. There was no difference in levels of clumping of the various test strains as assessed by phase-contrast microscopy. The fluid was gently decanted, and the clots were washed twice for 5 min with 2 ml of the same buffer to remove nonadherent bacteria. The clots were then overlaid with 3 ml of tryptic soy agar, which was allowed to solidify before incubation at 37°C. The number of adherent bacteria giving rise to colonies was determined after 24 h of incubation and expressed as the percentage of the number of bacteria in the original inoculum (termed the adherence ratio). Statistical differences between groups were analyzed as described above.

Experimental endocarditis in rats. Sterile aortic vegetations were produced in female Wistar rats (weight, 200 g) by a previously described method (16). In brief, a polyethylene catheter (Guerbet Biomédical, Louvres, France) was inserted via the right carotid artery through the aortic valve. The catheter was secured with a silk ligature and left in place for the remainder of the experiment. Twenty-four hours after catheterization, groups of rats were inoculated through a tail vein with 0.5 ml of saline containing different numbers of wild-type strain Newman cells prepared from a culture in the exponential phase of growth. These titration experiments allowed the determination of the minimum inoculum of this organism required to infect 40 to 50% (40% infectious dose [ID_{40}]), 80 to 90% (ID_{80}), or more ($4 \times ID_{80}$ to $5 \times ID_{80}$) of the animals. The infectivity of the mutants was then compared with that of the parent strain by injecting the same number of cells into groups of 5 to 10 animals. The rats were sacrificed 12 h later, the aortic valves and vegetations were excised, weighed, and homogenized in 1 ml of saline, and dilutions were plated for colony counts. Mutant bacteria were plated on both antibiotic-containing and antibiotic-free agars to ascertain the stability of the markers. Bacterial densities in the vegetations were expressed as $\log_{10}$ CFU per gram of tissue; ≥ 2 $\log_{10}$ CFU/g of vegetation could be detected. Rats with sterile valve cultures were considered uninfected. Quantitative cultures of the spleen and qualitative blood cultures were performed in parallel.

Statistical differences between the frequencies of valvular infections were evaluated by the χ^2 test with Yates correction. The Bonferroni correction was used for multiple group comparisons. Differences between mean bacterial densities in infected tissues were analyzed by one-way ANOVA, and pairwise differences between the means of groups were determined by the *t* test with the Bonferroni correction. Differences between groups were considered significant when P was ≤ 0.05 by use of two-tailed significance levels.

RESULTS

Adherence of *S. aureus* strains in vitro. Figure 1 shows that both the ClfA-defective single mutant and the ClfA- and Coa-defective double mutant were completely defective in attachment to surface-bound rat fibrinogen compared with the

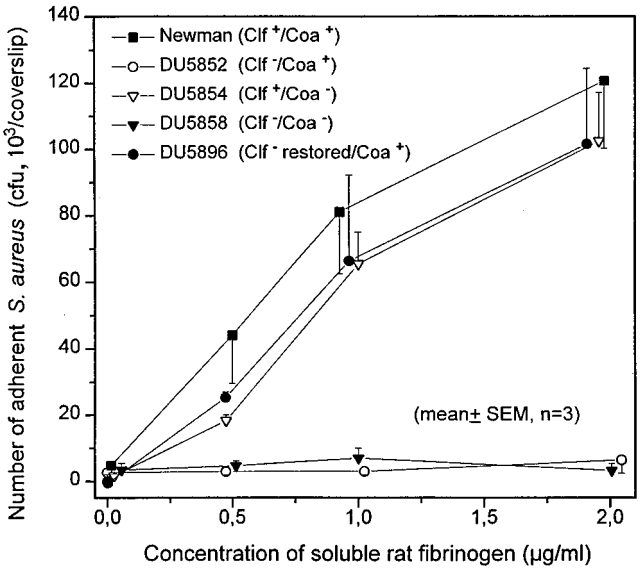

FIG. 1. Attachment properties of parental strain Newman of *S. aureus* and its coagulase- and/or clumping factor-defective mutants to PMMA coverslips coated in vitro with increasing concentrations of purified rat fibrinogen.

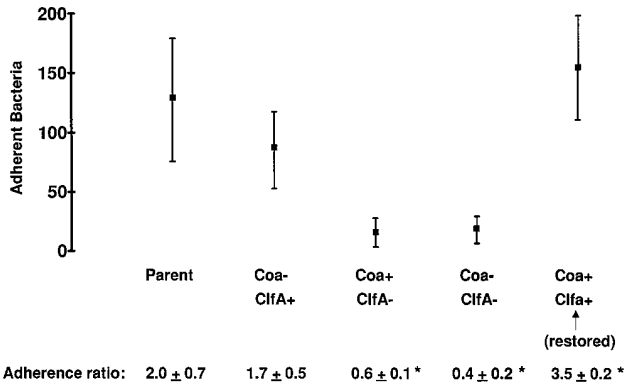

FIG. 2. In vitro adherence of the various test organisms (described in Table 1) to platelet-fibrin clots. Two milliliters of sodium phosphate buffer (50 mM; pH 7) containing 5×10^3 to 1×10^4 CFU/ml were added to the clots, and the mixture was agitated for 3 min at 120 rpm. The clots were rinsed extensively and overlaid with nutrient agar. Adherent CFUs were counted after 24 h of incubation at 37°C. The figure shows the mean ± standard deviation of 12 to 24 determinations for each of the test organisms. The bottom of the figure shows the Coa and ClfA phenotypes of the organisms and the adherence ratio after correction for the individual inoculum sizes. The asterisks indicate statistically significant differences ($P \leq 0.05$) between groups as determined by one-way ANOVA and pairwise comparisons using the *t* test with the Bonferroni correction.

wild-type strain Newman ($P < 0.01$). In contrast, both the Coa-defective single mutant and the ClfA-defective mutant restored with the chromosomally integrated *clfA* gene showed no significant decrease in attachment to surface-bound fibrinogen compared with that of the parental strain Newman. Bacterial attachment of parental and mutant strains of *S. aureus* promoted by rat fibrinogen was equivalent to that of human fibrinogen (data not shown). These data confirm previous findings that clumping factor, rather than coagulase, was involved in primary bacterial attachment to fibrinogen in the present static assay (23, 24, 33) as well as in a recently described dynamic assay involving shearing flow force to determine in vitro adherence (12).

Since cardiac vegetations contain numerous proteins and platelet factors (15) not present in the purified fibrinogen assay described above, we repeated the adherence experiments with a more realistic model of platelet-fibrin clots from platelet-containing rat plasma. Figure 2 shows that the profile of adherence in this assay was similar to that observed on fibrinogen-coated coverslips. The *coa*::Tc^r mutation affected neither the adherence of the wild-type strain Newman nor the adherence of the *coa clfA* double mutant, indicating that the production of coagulase did not contribute to the attachment to platelet-fibrin clots in vitro. The only notable difference from the fibrinogen assay was that the *clfA1* mutation decreased adherence by ca. four- to sixfold on platelet-fibrin clots as compared with ca. 100-fold on pure fibrinogen (Fig. 1). This might result from the contribution of bacterial adhesins in platelet-fibrin clots which are not present in purified fibrinogen. Note that the adherence ratio of the restored mutant was slightly greater than that of the parent. Although statistically significant ($P < 0.05$), the biological meaning of this small difference is questionable.

Results of experimental endocarditis. Figure 3 shows the titration of the infectivity of the various organisms. It can be seen that the rate of valvular infection increased with the size of the inoculum. With small inocula, the ClfA-defective mutants tended to produce approximately 50% less endocarditis than either of the ClfA-producing organisms, including the parent strain *S. aureus* Newman, the coagulase-negative (but clumping-factor-positive) strain DU5854, and the reconstituted mutant complemented with an extra copy of the *clfA* gene. This trend was already observed with the smaller inoculum, which infected ca. 40% of animals challenged with the control strain Newman (Fig. 3, left panels). The difference remained proportionally similar and was statistically significant ($P < 0.05$) when the size of the inoculum was increased to infect 80 to 90% of the rats with the ClfA-producing parent

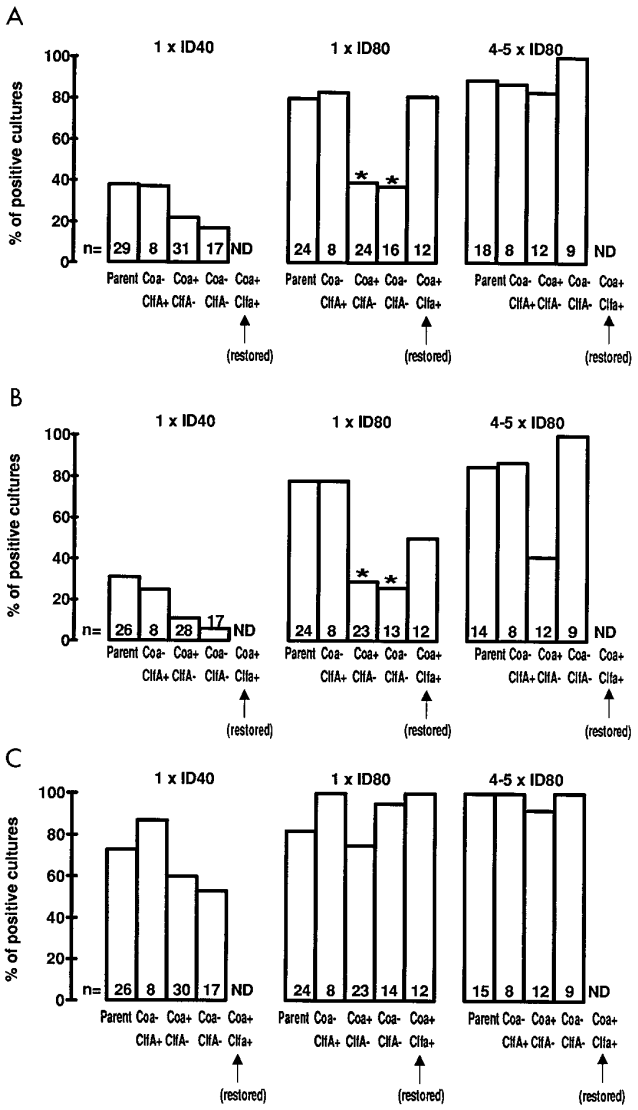

FIG. 3. Infectivity titration of the various test organisms (described in Table 1) in the rat model of experimental endocarditis. The figure shows a compilation of 12 separate experiments. The rats were challenged with bacterial inocula of gradually increasing sizes producing rates of endocarditis of ca. 40 and 80% with the parent strain *S. aureus* Newman (ID_{40} and ID_{80}) as well as with an inoculum four to five times larger than the ID_{80}. The columns indicate the percentage of positive vegetations (A), blood cultures (B), and spleen cultures (C). The number of animals per group (*n*) and the Coa and ClfA phenotypes are indicated at the bottom of the columns. ND, not determined. The asterisks on the columns indicate statistically significant differences compared with the parent strain Newman, as determined by the χ^2 test with the Yates correction. After correction for multiple group comparison (Bonferroni), the difference between the parent and the single ClfA-defective mutant (Coa^+ $ClfA^-$ phenotype) remained significant ($P = 0.03$), whereas the difference between the parent and the Coa^- $ClfA^-$ double mutant became marginally significant ($P = 0.08$).

TABLE 2. Bacterial densities in vegetations and spleens of rats which developed experimental endocarditis after challenge with inoculum sizes equal to the ID_{80} of the parent strain Newman

Infection site	Bacterial density (mean ± SD) in infected tissues[a]				
	Newman (Coa^+ $ClfA^+$)[b]	DU5854 (Coa^- $ClfA^+$)	DU5852 (Coa^+ $ClfA^-$)	DU5858 (Coa^- $ClfA^-$)	DU5896[c] (Coa^+ $ClfA^+$)
Vegetations	6.45 ± 1.47 (19)	6.10 ± 1.5 (7)	6.50 ± 1.6 (9)	7.01 ± 0.8 (6)	7.91 ± 1.22 (10)
Spleens	3.73 ± 0.69 (19)	3.71 ± 0.79 (7)	3.77 ± 0.68 (8)[d]	3.10 ± 0.90 (6)	3.85 ± 0.69 (10)

[a] Bacterial density is expressed as $\log_{10}$ CFU per gram of tissue. The numbers in parentheses represent the numbers of animals. Differences between values were not statistically different ($P > 0.05$) as analyzed by one-way ANOVA.
[b] Phenotypes are shown in parentheses after strain designations.
[c] *clfA1*::Tn*917* mutant complemented with a copy of the cloned *clfA* gene.
[d] One rat had no spleen culture.

(Fig. 3, middle panels). However, the difference leveled off as the inoculum size was further increased another four- to fivefold (Fig. 3, right panels). This indicated that clumping factor was not the sole determinant in endocarditis pathogenesis and that additional elements were also involved in the infectious process.

In spite of this fact, however, clumping factor clearly played a pathogenic role since inactivation of its genetic determinant impeded the ability of the bacteria to infect damaged valves. This was demonstrated by both the overall profile of inoculum titration in Fig. 3 and the fact that the infectivity of the defective mutant could be restored by complementation with a copy of the wild-type *clfA* gene (Fig. 3A, middle panel). Deletion of the coagulase gene, on the other hand, did not decrease the ability of bacteria to infect cardiac vegetations, whether the mutation was carried alone or in combination with clumping factor inactivation. The latter results were in accordance with recent observations by Baddour et al. (3), who also found that coagulase inactivation had no effect on experimental endocarditis.

Figure 3 also shows the pattern of positive blood and spleen cultures at the time of sacrifice, i.e., 12 h after inoculation. Blood cultures were positive in virtually all of the animals with infected vegetations, whereas they were sterile in rats with uninfected valves. Positive blood culture is a classic in bacterial endocarditis, where circulating microorganisms are constantly released from infected vegetations. In contrast, spleen cultures were positive in most of the cases, indicating that this organ had been successfully colonized by all of the test organisms.

Specific alteration in the infectivity of ClfA-defective mutants. The lower infectivity of the ClfA-defective mutants might have resulted from either of two alterations which might operate alone or in combination. First, the ClfA-defective mutants might be altered in their ability to colonize the damaged valves during the transient bacteremia following intravenous inoculation. Second, they might have a decreased ability to multiply in the infected vegetations after having colonized the valves. To determine which of these two possibilities was responsible for the decreased infectivity of the ClfA-defective mutants, the numbers of viable bacteria in the vegetations and the spleens of the animals at the time of sacrifice were determined. Table 2 shows that, at the ID_{80} of the parent strain Newman, the few animals which developed endocarditis with the ClfA-defective mutants had vegetations and spleens with bacterial densities equivalent to those of animals infected with $ClfA^+$ bacteria ($P > 0.05$). These infections were not due to $ClfA^+$ revertants because the bacteria recovered from the vegetations retained the Ery^r marker. Therefore, growth of ClfA-defective bacteria in these two sites was not impaired once they had colonized the valves. This suggests that the ClfA-defective mutants were most likely hampered in an early step in the pathogenesis of endocarditis, probably at the stage of colonization of the damaged valves.

DISCUSSION

The results presented in this paper highlight the respective roles of coagulase and clumping factor in the pathogenesis of experimental staphylococcal endocarditis. While coagulase has been suspected to facilitate infection via its procoagulant and fibrinogen-binding activity (5), it did not appear to promote either adherence to platelet-fibrin clots in vitro or experimental endocarditis in rats in the present experiments. These results support recent observations with a similar rat model of experimental endocarditis and Coa-defective staphylococcal mutants (3). Clumping factor, on the other hand, clearly affected both adherence and valvular infection, as demonstrated by experiments with the ClfA-defective mutants (and the ClfA-reconstituted mutant).

There is a theoretical rationale for a pathogenic role for coagulase in the model. By triggering coagulation, vegetation-adherent bacteria might promote additional deposits of platelets and fibrin on top of the infection nidus and thus become protected from further mechanical detachment and/or cellular host defense mechanisms. Support of this hypothesis was provided by Herzberg et al. (17), who showed that streptococcal mutants affected in the production of surface proteins mediating platelet aggregation (Agg-negative phenotype) were less virulent and produced smaller vegetations than Agg^+ parent strains in the rabbit model of endocarditis. Thus, it was somewhat unexpected that in the present experiments, staphylococcal coagulase did not play a role in the pathogenesis of endocarditis. However, the staphylococcal and streptococcal investigations outlined above may not be directly comparable. Indeed, not only did they test different organisms which expressed quite different procoagulant activities (i.e., fibrinogen polymerization for staphylococci versus platelet aggregation for streptococci), but they also used different study designs. By killing the animals soon after inoculation (12 h postchallenge in the present experiments), the researchers in the staphylococcal experiments investigated whether coagulase could affect the early establishment of valvular infection. They did not, however, determine the impact of coagulase on more-advanced disease. In the streptococcal investigation, on the other hand, the animals were killed between 1 and 4 days after inoculation and provided a glimpse at a later stage of infection. Thus, while the available experimental data clearly demonstrated that the staphylococcal coagulase did not affect the initiation of experimental endocarditis, they did not rule out a role of coagulase in the later infection. Further investigations will be necessary to clarify this issue.

Clumping factor is different from coagulase because it is

attached to the bacterial surface (23, 24) and mediates direct adherence to fibrinogen and fibrin, which are present in large amounts in the vegetation. Clumping factor also mediates platelet aggregation (4), which is another major factor in the constitution of cardiac vegetations. Alteration of clumping factor decreased both bacterial adherence to rat fibrinogen and platelet-fibrin clots in vitro as well as the ability of bacteria to infect damaged valves in vivo. In addition, the adherence and infectivity of ClfA-defective mutants could be restored by complementation of these organisms with the wild-type *clfA* gene, providing further support for a specific role of clumping factor in the staphylococcal pathogenicity. The lower infectivity of the mutants was not due to a decreased bacterial fitness in vivo, as ClfA-defective mutants grew well in cardiac vegetations of those animals which became infected with the defective strains. Therefore, ClfA-defective mutants were not altered in their potential to maintain an established infection but rather in their capacity to infect sterile vegetations.

An additional argument for the specific role of clumping factor in valve infection was that the spleens were equally colonized by parents and ClfA-defective mutants. After inoculation, circulating bacteria are trapped in the spleen, where they are eventually phagocytized. Lower spleen bacterial densities after challenge with ClfA-defective mutants would have suggested faster clearance of the mutants than of the parent strain by host defense mechanisms and introduced a bias in the experimental system. Thus, a likely explanation for the lower infectivity of the defective mutants is that they were specifically impeded in their ability to colonize and infect damaged valves but not in their overall resistance to cellular host defenses. Whether this involves primarily adherence to fibrinogen, adherence and triggering of platelet aggregation, or both remains to be determined.

The probability of valvular infection depends on both the magnitude of postchallenge bacteremia and the ligand-receptor interactions between bacterial surface components and constituents of damaged valves. This was exemplified in the last series of experiments, showing that inoculation of greater numbers of ClfA-defective mutants resulted in an increased rate of infection. This was also demonstrated by experiments using mutants and double mutants in the *sar* and *agr* global regulatory loci (6, 9). The *sar* and *agr* mutants are affected in the expression of numerous secreted and surface-bound proteins, including hemolysins, coagulase, clumping factor, and fibrinogen-binding proteins (7, 18). The *sar* mutants were altered in their ability both to adhere to damaged valves and to induce experimental endocarditis (9). In addition, when both *sar* and *agr* were inactivated, the infectivity of the double mutants was further reduced (6), suggesting that the *sar* and *agr* mutations had an additive, if not synergistic, effect on pathogenesis. This supports the hypothesis that several staphylococcal surface structures are involved in the initiation of endocarditis and cooperate to promote infection.

Unraveling the molecular mechanisms of staphylococcal pathogenesis carries the hope of generating new antistaphylococcal prophylactic and/or therapeutic alternatives. Not only are there multiple surface determinants to consider, but the strategies to block these components and their beneficial effect for the patient have yet to be defined. As in the experimental model, colonization of endovascular lesions or prostheses in humans is a yes-or-no phenomenon arising during transient low-grade bacteremia, which often occurs spontaneously. Alteration of staphylococcal adherence in vivo could possibly be achieved by a variety of means, including vaccination against specific bacterial determinants or devising prosthetic material with antiadherent properties. Indeed, the adherence of certain staphylococci and group B streptococci to fibrinogen or fibronectin can vary with the physicochemical state of these molecules, depending on whether they are in soluble form or attached to specific supports (31, 33). Since foreign material becomes coated rapidly with fibrin-fibrinogen, fibronectin, and other plasma proteins soon after implantation, bacterial adherence might be modulated by designing new surfaces which would force these adherent molecules to expose nonadherent domains once attached to prosthetic material.

In summary, the present experiments demonstrate that clumping factor has a specific role in the pathogenesis of staphylococcal endocarditis. Moreover, they underline the involvement of additional bacterial surface determinants in the infectious process, as recently suggested by studies with the pleiotropic *sar* and *agr* mutants (6, 9). The prospect of obtaining single and multiple mutants in each of these pathogenic factors will help further define the most appropriate bacterial target(s) for the future development of new antistaphylococcal agents.

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