

## Use of Adhesion-Defective Mutants of *Staphylococcus aureus* To Define the Role of Specific Plasma Proteins in Promoting Bacterial Adhesion to Canine Arteriovenous Shunts

PIERRE E. VAUDAUX,<sup>1\*</sup> PATRICE FRANÇOIS,<sup>1,2</sup> RICHARD A. PROCTOR,<sup>3</sup> DAMIEN McDEVITT,<sup>4</sup>  
TIMOTHY J. FOSTER,<sup>4</sup> RALPH M. ALBRECHT,<sup>5</sup> DANIEL P. LEW,<sup>1</sup> HUGH WABERS,<sup>6</sup>  
AND STUART L. COOPER<sup>6</sup>

Division of Infectious Diseases, University Hospital,<sup>1</sup> and Group of Applied Physics, University of Geneva,<sup>2</sup> Geneva, Switzerland; Departments of Medical Microbiology and Immunology,<sup>3</sup> Animal Health and Biomedical Sciences,<sup>5</sup> and Chemical Engineering,<sup>6</sup> University of Wisconsin, Madison, Wisconsin; and Microbiology Department, Trinity College, Dublin, Ireland<sup>4</sup>

Received 10 August 1994/Returned for modification 14 September 1994/Accepted 30 November 1994

**We used an ex vivo canine arteriovenous shunt model, previously developed to study plasma protein adsorption and thrombogenesis on polymeric biomaterials, to define the role of host proteins in promoting adhesion of *Staphylococcus aureus*. Either polyethylene or polyvinyl chloride tubings were exposed to canine blood for 5, 15, or 60 min at a flow rate of 300 ml/min and then were flushed in phosphate-buffered saline (PBS), cut into 1.5-cm segments, and stored at  $-70^{\circ}\text{C}$ . After thawing, each segment was preincubated in 0.5% albumin in PBS to prevent nonspecific staphylococcal attachment to surfaces that were not exposed to blood. Each segment was then incubated with  $4 \times 10^6$  CFU of [ $^3\text{H}$ ]thymidine-labelled *S. aureus* per ml for 60 min at  $37^{\circ}\text{C}$  in an in vitro adhesion assay. Two site-specific mutants of *S. aureus* were tested: one specifically defective in adhesion to fibrinogen (Fg<sub>Ad</sub>-def). Compared with their respective parental strains, the Fg<sub>Ad</sub>-def, but not the Fn<sub>Ad</sub>-def, mutant of *S. aureus* showed a strong (>80%) decrease in attachment to ex vivo tubings. The adhesion of each strain of *S. aureus* onto polyethylene was consistently more than twofold higher than the adhesion onto polyvinyl chloride segments exposed to flowing blood for 5 or 15 min, but adhesion became similar to that on polyvinyl chloride after 60 min of exposure. In conclusion, the specific adhesion-defective mutants of *S. aureus* suggested that fibrinogen was the most active adhesion-promoting protein in a short-term blood-material interaction. The experimental approach described in this study should prove useful for screening materials thought to be resistant to protein-mediated staphylococcal adhesion and colonization.**

*Staphylococcus aureus* is a major pathogenic organism involved in colonization of orthopedic implants or cardiovascular devices and is responsible for life-threatening metastatic infections (20). Blood-contacting materials such as vascular grafts, catheters, kidney dialyzers, and cardiac assist devices trigger multiple and complex events at their interfaces, involving adsorption of numerous plasma proteins and the deposition and activation of platelets. Colonization of cardiovascular biomaterials is thought to occur on plasma proteins and/or platelets (10, 43) that have been adsorbed on the artificial surfaces shortly after their implantation. Several in vitro studies have shown that *S. aureus* attachment to polymeric surfaces is strongly promoted by selectively adsorbed plasma or extracellular matrix proteins such as fibrinogen (2, 10, 12, 21), fibronectin (12, 34, 35, 38–42), collagen (13, 26, 27, 42), vitronectin (3, 18, 28), laminin (12, 19, 42), and thrombospondin (11). The clinical relevance of these in vitro observations was documented by clinical studies (35, 36) showing the in vivo contribution of fibronectin and fibrin or fibrinogen to *S. aureus* attachment to catheters inserted in hospitalized patients.

Progress has been made in elucidating bacterial cell wall-associated surface components that promote specific interactions with individual host proteins. These studies allowed characterization of the genes for the fibrinogen-binding protein

(clumping factor [21]), a collagen adhesin (26, 27), and two distinct but related fibronectin-binding proteins (7, 14). Also, site-specific mutants of *S. aureus* specifically defective in adhesion to a single host protein such as fibrinogen (21) or fibronectin (15) have been isolated.

While clinical studies using inserted catheters harvested from patients were instrumental in showing the in vivo contribution of fibronectin (36) and fibrinogen or fibrin (35) to staphylococcal adhesion, they were not designed to characterize the dynamic events of sequential plasma protein adsorption and their specific contribution to *S. aureus* adhesion onto polymer tubing. Previous reports have demonstrated that plasma protein and platelet coating of biomaterials is a dynamic process involving selective adsorption and then release of various components (5, 6, 17, 23, 24). To define the temporal relationship between plasma protein adsorption and bacterial adhesion, we selected an animal model of an arteriovenous shunt previously developed to study thrombogenesis on polymeric biomaterials (4, 5, 17). The ability to make sequential measurements over time in this in vivo model combined with the use of adhesion-defective bacterial mutants allowed identification of fibrinogen or fibrin as the most active plasma component promoting *S. aureus* attachment to the experimental shunt tubing in a brief exposure to whole, non-anticoagulant-treated blood.

(The results of this study were presented in part at the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, 1991 [37].)

\* Corresponding author. Mailing address: Division of Infectious Diseases, University Hospital, CH 1211 Geneva 14, Switzerland. Phone: (4122) 37 29 826. Fax: (4122) 37 29 830.

## MATERIALS AND METHODS

**Canine ex vivo arteriovenous shunt model.** The canine ex vivo femoral arteriovenous series shunt model allowing continuous quantitative monitoring of thromboembolization with non-anticoagulant-treated blood under the influence of shear flow (23–25) has been previously described (4, 5, 8, 17). This method also allows the simultaneous measurement of platelet and plasma protein deposition on different polymer surfaces. Either polyvinyl chloride (PVC; 0.125-in. [0.318-cm] internal diameter; Tygon, Norton Plastics, Akron, Ohio) or polyethylene (PE; 0.125-in. [0.318-cm] internal diameter; Intramedic, Parsippany, N.J.) polymer tubing was exposed to canine blood for 5, 15, or 60 min at a flow rate of 300 ml/min. The tubing was then flushed with 0.1 M phosphate-buffered saline (PBS) (pH 7.4), cut into 1.5-cm segments, and stored at  $-70^{\circ}\text{C}$ .

**Scanning electron microscopy.** To determine luminal surface morphology of the polymer tubings after 5, 15, and 60 min of exposure to canine blood, duplicate segments were serially dehydrated in ethanol-water conditions, dried by the critical point method, mounted, and sputter coated with gold for examination in a Jeol-JSM 35C scanning electron microscope with an accelerating voltage of 15 kV (25).

**In vitro attachment properties of bacterial strains.** The following strains of *S. aureus* were tested. (i) Strain Newman and its  $\text{Fg}_{\text{Ad}}\text{-def}$  mutant strain DU5852, which is defective in the production of the fibrinogen-binding protein (clumping factor) were tested. Strain DU5852 is the transposon Tn917 insertion mutant *clfA1* of strain Newman within the *clfA* gene encoding the fibrinogen adhesin, whose molecular characterization and functional properties have recently been described in detail (21). (ii) Strain 8325-4 and its  $\text{Fg}_{\text{Ad}}\text{-def}$  mutant of 8325-4 (DU5880), which carries the same transposon insertion in the *clfA* gene as DU5852 (21), were tested. An additional derivative strain of 8325-4, harboring multicopy plasmid pCF4 bearing the wild-type *clfA* gene enhancing adhesion to surface-bound fibrinogen as previously described (21), was used as a specific indicator of in vivo fibrinogen-promoted adhesion. (iii) Strain 879R4S and a Tn918 insertion mutant (879R4S/1536), which exhibits defective in vitro adhesion to fibronectin ( $\text{Fn}_{\text{Ad}}\text{-def}$  mutant) and reduced in vivo attachment to traumatized rat heart valves (15), were tested. Recent unpublished studies (9) indicate that the transposon insertion in strain 879R4S/1536 occurred between the promoter and coding sequence of the single fibronectin-binding gene carried out by this particular strain. (iv) Strain Cowan I, which adheres strongly to surface-bound fibronectin and surface-bound fibrinogen in a fashion similar to that of the bacteremic isolates (36), was used as an internal control of the bacterial adhesion system.

Canine fibronectin was purified from canine plasma as previously described (16, 33). Canine fibrinogen was purchased from Sigma (St. Louis, Mo.), and contaminating fibronectin was removed by gelatin adsorption (16, 33).

The attachment properties of adhesion-defective mutant and parental strains of *S. aureus* were measured by using a previously described adhesion assay with polymethylmethacrylate (PMMA) coverslips coated with purified proteins (21, 22, 36, 39). Two-sided coverslips, prepared as previously described (39), were coated with increasing amounts of immobilized fibrinogen by incubation for 60 min at  $37^{\circ}\text{C}$  with increasing concentrations (from 0.25 to 2  $\mu\text{g}/\text{ml}$ ) of purified canine fibrinogen in solution. At the end of the protein coating phase, coverslips were rinsed in PBS as previously described (21, 22). It was ascertained that the PMMA surfaces were coated in a dose-dependent manner with amounts of canine fibrinogen ranging from 32 to 248  $\text{ng}/\text{cm}^2$  (41 to 317 ng per coverslip) when fibrinogen, radiolabelled by reductive methylation with sodium boro [ $^3\text{H}$ ]hydride (36), was used.

To optimize adsorption of purified canine fibronectin from concentrations below 1  $\mu\text{g}/\text{ml}$ , PMMA coverslips were precoated with gelatin (1  $\text{mg}/\text{ml}$ ) as previously described (36, 39). After a rinsing in PBS, gelatin-coated PMMA coverslips were incubated for 60 min at  $37^{\circ}\text{C}$  with low concentrations (ranging from 0.125 to 1  $\mu\text{g}/\text{ml}$ ) of fibronectin, followed by rinsing in PBS (36, 39). These conditions allowed a linear coating of PMMA surfaces with  $^3\text{H}$ -labelled fibronectin ranging from 15 to 132  $\text{ng}/\text{cm}^2$  (22 to 190 ng per coverslip).

The adhesion characteristics of the different parental and mutant strains of *S. aureus* were evaluated by incubating the fibrinogen- or fibronectin-gelatin-coated PMMA coverslips with  $4 \times 10^6$  CFU of washed log-phase cultures, metabolically radiolabelled with [ $^3\text{H}$ ]thymidine in Mueller-Hinton broth as previously described (39). Each protein-coated PMMA coverslip was incubated with the suspension of radiolabelled bacteria for 60 min at  $37^{\circ}\text{C}$  as previously described (22, 39). The adhesion medium was PBS with 1 mM  $\text{Ca}^{2+}$  and 0.5 mM  $\text{Mg}^{2+}$ , supplemented with 5 mg of human serum albumin per ml, which prevented nonspecific adhesion of *S. aureus* (21, 22, 36, 39). Coverslips were then rinsed, and the number of attached bacteria was estimated from radioactive counts (39). Albumin-coated and gelatin-coated PMMA coverslips were used as controls of adhesion to fibrinogen- and fibronectin-coated surfaces, respectively (21, 22, 39).

Attachment of *S. aureus* to protein-coated PMMA coverslips was scored as the number of adherent CFU ( $10^3/\text{cm}^2$ ).

**Bacterial adhesion to ex vivo polymer tubing.** The conditions for assaying adhesion of *S. aureus* onto ex vivo polymer tubing segments were essentially similar to those described above for protein-coated PMMA coverslips. Frozen segments of blood-exposed PVC or PE tubing were thawed just before the assay and preincubated for 60 min at  $20^{\circ}\text{C}$  with 0.5% human albumin in PBS to prevent nonspecific attachment to the external, blood-unexposed surface.

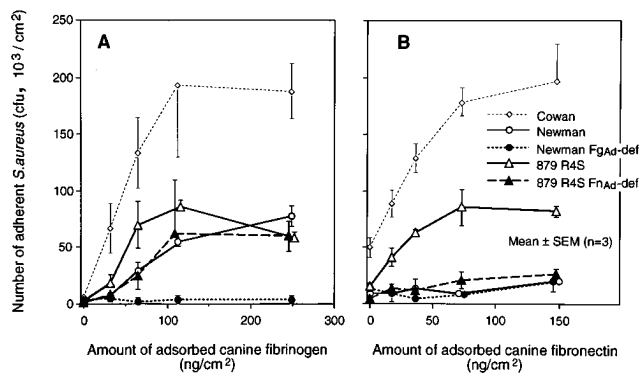


FIG. 1. Attachment properties of adhesion-defective mutant and parental strains of *S. aureus* tested on PMMA coverslips coated in vitro with either purified canine fibrinogen (A) or fibronectin (B). SEM, standard error of the mean.

Duplicate segments for each time point of blood exposure were then tested for promotion of bacterial adhesion by incubation with suspensions of each strain of *S. aureus* for 60 min at  $37^{\circ}\text{C}$ . The concentration ( $4 \times 10^6$  CFU/ml) of radiolabelled bacteria and the conditions for the adhesion assay were identical to those of the assay performed with PMMA coverslips. Attachment of *S. aureus* to each tubing segment was scored as the number of adherent CFU ( $10^3/\text{cm}^2$ ), to facilitate the comparison with attachment to protein-coated PMMA coverslips.

**Bacterial adhesion to in vitro fibrinogen-coated polymer tubing.** When the specific contribution of fibrinogen to *S. aureus* attachment to polymer tubing was studied, 1.5-cm-long segments of native PVC or PE, coated in vitro for 60 min at  $37^{\circ}\text{C}$  with concentrations of canine fibrinogen ranging from 1 to 16  $\mu\text{g}/\text{ml}$ , were incubated in the bacterial adhesion assay as described above. These concentrations of canine fibrinogen were selected from dose-response curves of canine fibrinogen immobilized on either type of polymer tubing, whose amounts were determined with  $^3\text{H}$ -labelled canine fibrinogen as described above.

## RESULTS

**Selective attachment of parent and mutant strains of *S. aureus*.** Figure 1A shows that promotion of bacterial attachment by immobilized canine fibrinogen is optimal at a concentration of 100  $\text{ng}/\text{cm}^2$ , both for the reference strain Cowan and for the parental strains Newman and 879R4S. The  $\text{Fg}_{\text{Ad}}\text{-def}$  (clumping factor defective) strain DU5852 (21) of Newman, but not the  $\text{Fn}_{\text{Ad}}\text{-def}$  mutant strain (879R4S/1536) of 879R4S (15), showed a selective defect in attachment to canine fibrinogen. Figure 1B shows that immobilized canine fibronectin promoted attachment of strains 879R4S and Cowan to the same extent as fibrinogen, but adhesion of strain Newman and its  $\text{Fg}_{\text{Ad}}\text{-def}$  mutant was not promoted. The  $\text{Fn}_{\text{Ad}}\text{-def}$  mutant strain of *S. aureus* 879R4S showed a selective decrease in attachment to surface-bound fibronectin.

Control experiments confirmed that bacterial attachment of parental and mutant strains of *S. aureus* promoted by canine fibrinogen or fibronectin was equivalent to that of the same proteins of human origin (data not shown).

**Bacterial attachment to ex vivo polymer tubings.** The promotion of *S. aureus* adhesion by segments of ex vivo polymer tubing exposed to canine blood for either 5, 15, or 60 min is shown in Fig. 2. After 5 min of blood exposure, adhesions of all parental and mutant strains to either PVC or PE tubing segments had already reached their maximum values. Significant differences were found between PVC and PE tubing segments exposed for either 5 or 15 min. Whereas adhesion of Newman, 879R4S, and Cowan strains to PVC tubing segments remained constant throughout the 60-min period of blood exposure (Fig. 2A), each of these strains showed an approximately twofold increase in attachment to PE compared with PVC exposed for 5 or 15 min (Fig. 2B). However, at 60 min, adhesion of strains

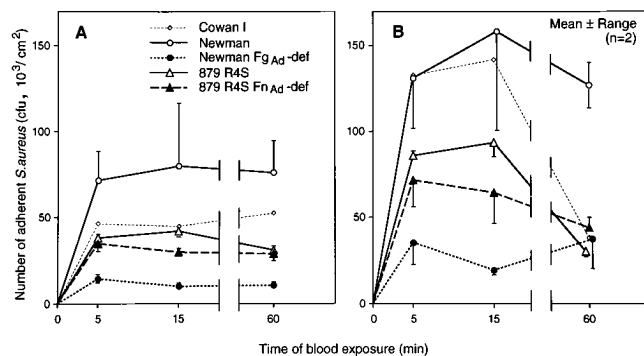


FIG. 2. Attachment of adhesion-defective mutant and parental strains of *S. aureus* to segments of ex vivo PVC (A) and PE (B) tubing exposed to canine blood for 5, 15, or 60 min.

Cowan, 879R4S, and, to a lesser extent, Newman onto ex vivo PE tubing segments decreased to levels almost equivalent to those found on PVC (Fig. 2B). On both types of polymer tubing, the Fg<sub>Ad</sub>-def mutant of strain Newman, but not the Fn<sub>Ad</sub>-def mutant of strain 879R4S, showed a strong (>80%) reduction in attachment compared with the parental strain. The selective reduction in the attachment of an Fg<sub>Ad</sub>-def mutant of *S. aureus* to both types of ex vivo polymer tubing identified fibrinogen or its derivative fibrin as the most active plasma component promoting *S. aureus* attachment to the canine shunt tubing.

To document further the contribution of fibrinogen to *S. aureus* attachment in the canine shunt model, we incubated ex vivo polymer tubing segments with a derivative of *S. aureus* 8325-4, which contains a multicopy plasmid leading to overexpression of clumping factor, whose molecular and functional properties have been previously described in detail (21). Figure 3 demonstrates that attachment of the plasmid-containing strain 8325-4 (pCF4) to both PVC and PE polymer tubing segments exposed to canine blood was markedly higher than that of the plasmid-free parental strain. These differences in the adhesion of the plasmid-enriched and nonenriched strains reflected the higher affinity of the former strain for fibrinogen immobilized on polymer surfaces as previously described in detail (21). As also expected from previous studies (21), the Fg<sub>Ad</sub>-def mutant strain DU5880 of 8325-4, which contains the same *clfA1* mutation as strain Newman, showed a strong defect in attachment to each type of polymer tubing exposed to canine blood (Fig. 3).

**Luminal surface morphology.** Figure 4 shows scanning electron micrographs of either PVC or PE tubing after a 15-min exposure to canine blood. Significant differences were found between the two types of polymer tubing. Whereas a large number of platelets were adherent to the surface of PVC, they did not show significant spreading on this surface (Fig. 4A). In contrast, extensive spreading of the adherent platelets occurred on the surface of PE tubing (Fig. 4B). The differences in platelet morphology on PE versus PVC tubing were also observed after 5 and 60 min of blood exposure (data not shown).

**Contribution of fibrinogen to the material-dependent differences in *S. aureus* attachment.** To study the specific contribution of fibrinogen to the transient increase in bacterial adhesion promoted by PE compared with PVC on blood-exposed shunt tubing, further in vitro experiments were performed. The most relevant concentrations of immobilized fibrinogen used for studying the promotion of bacterial adhesion to either type

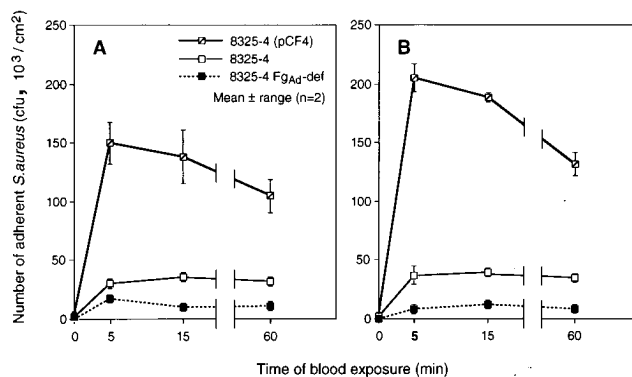


FIG. 3. Attachment of parental and mutant strains of *S. aureus* 8325-4 expressing different levels of affinity for fibrinogen onto ex vivo PVC (A) and PE (B) tubing exposed for 5, 15, or 60 min to canine blood.

of polymer tubing were selected from adsorption isotherms of <sup>3</sup>H-labelled canine fibrinogen to each type of polymer tubing shown in Fig. 5A. Protein adsorption was dose dependent from 1 to 16 μg of soluble canine fibrinogen per ml to both types of polymer tubing, followed by a plateau at higher protein concentrations. The plateau phases of fibrinogen adsorption on both PVC and PE tubings (Fig. 5A) likely reflected monolayer amounts of fibrinogen on these surfaces. Of note, the saturating amounts of radiolabelled fibrinogen on PVC were somewhat higher on PVC than on PE tubing, as shown in Fig. 5A. In contrast to this, *S. aureus* adhesion promoted by canine fibrinogen immobilized as a monolayer on PE (Fig. 5C) was higher than that on PVC tubing (Fig. 5B). The increases in adhesion were 65 and 45% for the parental strains Newman and 879R4S, respectively. As expected, the Fg<sub>Ad</sub>-def mutant of Newman showed a completely defective attachment to either type of in vitro fibrinogen-coated polymer tubing. Because we could exclude the possibility that the increased adhesion of parental strains Newman and 879R4S of *S. aureus* to fibrinogen-coated PE compared with PVC tubing reflected a higher amount of protein on the former surface, the most likely explanation was that the monolayer amounts of canine fibrinogen expressed a higher specific activity for promoting *S. aureus* attachment when the protein molecules were adsorbed onto PE than onto PVC. Such a difference in the specific activity of the protein immobilized on either type of polymer surface might contribute, at least in part, to the higher bacterial attachment to blood-exposed PE compared with PVC tubing in the ex vivo shunt model.

## DISCUSSION

Thrombogenesis is the most frequent complication of blood-contacting polymeric implants and devices. A relationship between catheter-related thrombogenesis and infection has been proposed at both the subclinical microscopic level (29, 35, 36) and the clinical level, as recently demonstrated with central venous catheters used in cancer patients (32). Despite their importance in the clinical situation, central venous catheters are not suitable for time course studies of thrombogenic events. Therefore, most experimental studies designed to evaluate the thrombogenic potential of various materials used for blood-contacting applications were performed in short-term ex vivo systems, including various types of arteriovenous shunt models (see review in reference 6). The major advantage of such ex vivo systems is they allow evaluation in a dynamic way of how the composition and structure of the polymer surface

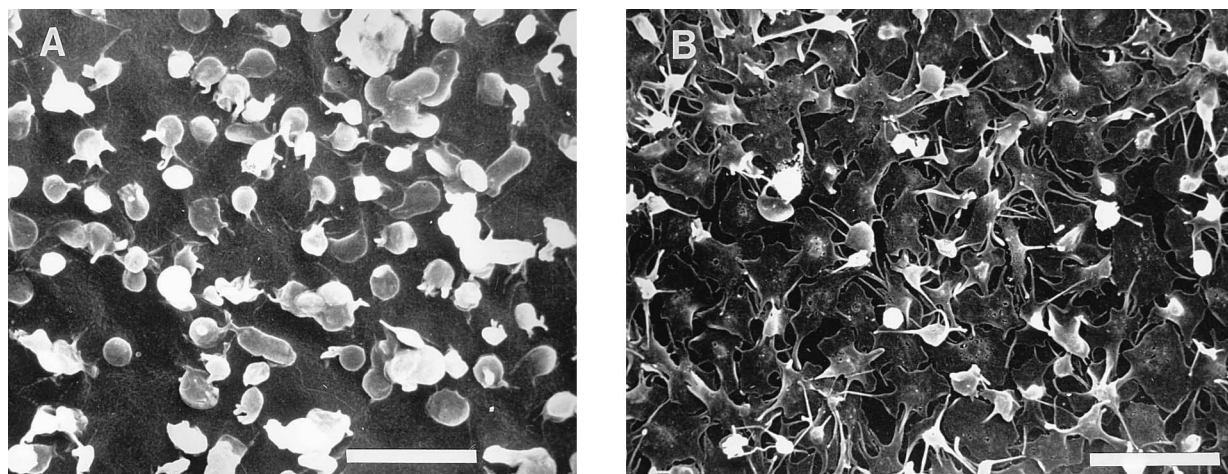


FIG. 4. Scanning electron micrographs showing platelets adherent to the luminal surface of polymer tubing exposed to canine blood for 15 min. (A) Nonspread platelets on PVC. (B) Extensively spread platelets on PE.

can influence thrombus and embolus formation within the first hours of blood-material interaction (6). In the canine acute ex vivo femoral arteriovenous shunt model, an early peak of platelet deposition, activation, and aggregation was shown to occur on a wide range of thrombogenic surfaces after 15 to 20 min of blood contact (17, 31). More detailed studies demonstrated that the thrombogenicity of a surface was determined by the composition of the initial protein layer rather than the total concentration of protein on the polymer surfaces (25). The most important protein component involved in thrombogenesis was shown to be fibrinogen (6, 17, 23–25), which can directly interact with the platelet receptors GPIIb/IIIa and contribute to platelet activation (30). Thus, the initial 60-min period of blood contact with the two different polymer surfaces tested in this model was considered appropriate for evaluating the short-term impact of plasma protein adsorption on *S. aureus* attachment.

In blood, which contains hundreds of proteins, the competitive and sequential adsorption of proteins is very complicated (31). In a preliminary analysis of ex vivo polymer tubing-associated proteins performed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), we found a large number of silver-stained protein components extracted from PVC or PE tubing segments at either 5, 15, or 60 min (unpublished data). Identification of major tubing-associated protein components will require the use of immunoblots with specific antibodies to a large panel of plasma and platelet components. In view of the large number of proteins bound to the tubing segments and because several of them might potentially interact with *S. aureus* and promote bacterial adhesion to artificial surfaces (2, 3, 10–13, 18, 19, 21, 26, 27, 34, 35, 38–42), identification of fibrinogen as the most active adhesion-promoting component would have been very difficult without the use of specific adhesion-defective mutants of *S. aureus* which lack attachment to either fibrinogen, fibrin, or fibronectin. Further studies and a greater range of experimental conditions and materials are required to precisely evaluate the contribution of fibronectin and additional plasma or platelet components, such as vitronectin (1, 18), thrombospondin (11), or collagen (26, 27), to *S. aureus* adhesion in the ex vivo system. This will require the development of further bacterial mutants exhibiting complete specific defects in adhesion to each of these proteins.

The significant transient increase in bacterial adhesion pro-

moted by PE compared with PVC blood-exposed tubing may reflect the higher intrinsic acute thrombogenicity of PE over PVC tubing, which has been documented in previous studies (see review in reference 6). Of note, the peak of *S. aureus* adhesion to blood-exposed PE tubing occurs at the same time points (5 and 15 min) as that of platelet deposition and activation (6, 17). Previous studies of fibrinogen and platelet deposition with radiolabeling techniques (6, 17) showed marginally different profiles on PE compared with PVC blood-exposed tubings. In contrast, ex vivo PE but not PVC tubing promoted major changes in platelet morphology which consisted of extensive spreading and activation of the PE-deposited platelets after 5 and 15 min of blood exposure. To evaluate the specific contribution of fibrinogen to the transient increase in bacterial attachment to ex vivo PE compared with PVC, comparative *S. aureus* adhesion to each material coated in vitro with purified fibrinogen was recorded. The results of these experiments showed that despite the lower in vitro affinity of PE versus PVC for fibrinogen leading to slightly lower protein surface coatings, fibrinogen immobilized as a monolayer on PE was significantly more active in promoting attachment of *S. aureus* than when on PVC. Therefore, these data seem to indicate that PE tubing not only activates deposited platelets but also activates fibrinogen for promoting bacterial attachment. It is possible that immobilized fibrinogen molecules undergo confor-

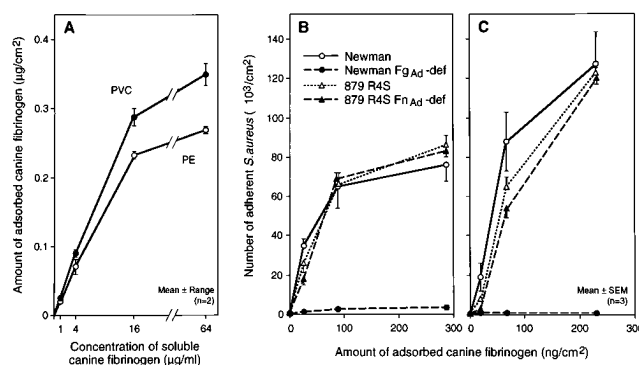


FIG. 5. In vitro adsorption (A) of purified canine fibrinogen and promotion of the adhesion of parental and mutant strains of *S. aureus* to PVC (B) or PE (C) protein-coated tubing segments. SEM, standard error of the mean.

mational changes which promote both platelet activation and *S. aureus* adhesion after adsorption on PE tubing. Further support for a link between the activation of deposited platelets and fibrinogen-mediated adhesion of *S. aureus* is suggested by a recent in vitro study using purified human platelets adsorbed onto PMMA coverslips in the presence of plasma (10). In this simplified in vitro system, platelets which spread on the surface bound more *S. aureus* than nonspread platelets and the interaction of fibrin or fibrinogen with thrombin-activated surface-bound platelets greatly increased *S. aureus* adhesion to the polymer surfaces.

The findings of this study with a short-term model of ex vivo blood-material interaction differ in several respects from those of a previous clinical study (36) which analyzed promotion of *S. aureus* adhesion by plasma proteins adsorbed onto central venous catheters used in patients for several days or weeks. In the latter study, chronically inserted catheters were found to be coated with a restricted number of plasma protein components, with fibrinogen and fibrin, plasminogen, and fibronectin being the predominant components identified by SDS-PAGE and immunoblots (41). In addition, central venous catheters showed a selective proteolytic inactivation of fibrin or fibrinogen over fibronectin which occurred during long-term insertion and led to a significant decrease in *S. aureus* adhesion (36, 41). Under the latter conditions, fibronectin and fibronectin proteolytic fragments seemed to be the most active protein component or components promoting *S. aureus* attachment to chronically implanted catheters (41). The very different results recorded with human intravenous catheters and the canine ex vivo system might be explained by significant differences in the time frame, material composition, clinical use, and hemodynamic conditions of both types of blood-contacting devices.

In conclusion, the canine arteriovenous shunt combined with the use of adhesion-defective mutants of *S. aureus* is a useful model for identification of the most active components promoting bacterial adhesion and colonization among plasma proteins adsorbed on various types of blood-contacting polymers. This study illustrates the role of fibrinogen or fibrin as a very active component promoting in vivo *S. aureus* adhesion in a short-term blood-material interaction, as opposed to fibronectin and fibronectin proteolytic fragments, which play a more important role in chronically implanted catheters (36). Finally, the experimental approach described in this study should prove useful for screening materials that might reduce protein-mediated staphylococcal adhesion and colonization.

#### ACKNOWLEDGMENTS

This work was supported by grants 32-30161.90 and 5002-35180 from the Swiss National Foundation and project grant 033403 from the Wellcome Trust, London, United Kingdom. This work was also supported by the National Institutes of Health (HL-29586) and by the graduate and Medical School Department of Medicine Research Funds. P. François' fellowship is supported in part by Ohmeda, Swindon, United Kingdom. S.L.C. thanks the National Science Foundation and National Institutes of Health for funding through the joint NSF-NIH grant RFA BCS-91-20660.

We thank Manuela Bento for technical assistance.

#### REFERENCES

- Bale, M. D., L. A. Wohlfahrt, D. F. Mosher, B. Tomasini, and R. C. Sutton. 1989. Identification of vitronectin as a major plasma protein adsorbed on polymer surfaces of different copolymer composition. *Blood* **74**:2698-2706.
- Cheung, A. L., and V. A. Fischetti. 1990. The role of fibrinogen in staphylococcal adherence to catheters in vitro. *J. Infect. Dis.* **161**:1177-1186.
- Chhatwal, G. S., G. Preissner, G. Müller-Berghaus, and H. Blobel. 1987. Specific binding of the human S protein (vitronectin) to streptococci, *Staphylococcus aureus*, and *Escherichia coli*. *Infect. Immun.* **55**:1878-1883.
- Collins, W. E., D. F. Mosher, A. R. Diwan, K. D. Murthy, S. R. Simmons, R. M. Albrecht, and S. L. Cooper. 1987. Ex vivo platelet deposition on fibronectin-adsorbed surfaces. *Scanning Microsc.* **1**:1669-1676.
- Collins, W. E., D. F. Mosher, B. R. Tomasini, and S. L. Cooper. 1987. A preliminary comparison of the thrombogenic activity of vitronectin and other RGD-containing proteins when bound to surfaces. *Ann. N. Y. Acad. Sci.* **516**:291-299.
- Cooper, S. L., D. J. Fabrizio, and T. G. Grasel. 1987. Methods of assessment of thrombosis ex vivo. *Ann. N. Y. Acad. Sci.* **516**:572-585.
- Flock, J. L., G. Froman, K. Jonsson, B. Guss, C. Signas, B. Nilsson, G. Raucci, M. Höök, T. Wadström, and M. Lindberg. 1987. Cloning and expression of the gene for a fibronectin-binding protein from *Staphylococcus aureus*. *EMBO J.* **6**:2351-2357.
- Grasel, T. G., and S. L. Cooper. 1989. Properties and biological interactions of polyurethane anionomers: effect of sulfonate incorporation. *J. Biomed. Mater. Res.* **23**:311-338.
- Greene, C., D. McDevitt, R. A. Proctor, P. Vaudaux, and T. J. Foster. Unpublished data.
- Hermann, M., Q. J. Lai, R. M. Albrecht, D. F. Mosher, and R. A. Proctor. 1993. Adhesion of *Staphylococcus aureus* to surface-bound platelets: role of fibrinogen/fibrin and platelet integrins. *J. Infect. Dis.* **167**:312-322.
- Herrmann, M., S. J. Suchard, L. A. Boxer, F. A. Waldvogel, and P. D. Lew. 1991. Thrombospondin binds to *Staphylococcus aureus* and promotes staphylococcal adherence to surfaces. *Infect. Immun.* **59**:279-288.
- Hermann, M., P. Vaudaux, D. Pittet, R. Auckenthaler, D. P. Lew, F. Schumacher-Perdreau, G. Peters, and F. A. Waldvogel. 1988. Fibronectin, fibrinogen and laminin act as mediators of adherence of clinical staphylococcal isolates to foreign material. *J. Infect. Dis.* **158**:693-701.
- Holderbaum, D., T. Spech, L. A. Ehrhart, T. Keys, and G. S. Hall. 1987. Collagen binding in clinical isolates of *Staphylococcus aureus*. *J. Clin. Microbiol.* **25**:2258-2261.
- Jonsson, K., C. Signas, H. P. Muller, and M. Lindberg. 1991. Two different genes encode fibronectin binding proteins in *Staphylococcus aureus*. The complete nucleotide sequence and characterization of the second gene. *Eur. J. Biochem.* **202**:1041-1048.
- Kuyppers, J. M., and R. A. Proctor. 1989. Reduced adherence to traumatized rat heart valves by a fibronectin low-fibronectin-binding mutant of *Staphylococcus aureus*. *Infect. Immun.* **57**:2306-2312.
- Lambrecht, L. K., B. R. Young, R. E. Stafford, K. Park, R. M. Albrecht, D. F. Mosher, and S. L. Cooper. 1986. The influence of preadsorbed canine von Willebrand factor, fibronectin and fibrinogen on ex vivo artificial surface-induced thrombosis. *Thromb. Res.* **41**:99-117.
- Lelah, M. D., L. K. Lambrecht, and S. L. Cooper. 1984. A canine ex vivo series shunt for evaluating thrombus deposition on polymer surfaces. *J. Biomed. Mater. Res.* **18**:475-496.
- Liang, O. D., M. Maccarana, J.-I. Flock, M. Paulsson, K. T. Preissner, and T. Wadström. 1993. Multiple interactions between human vitronectin and *Staphylococcus aureus*. *Biochim. Biophys. Acta* **1225**:57-63.
- Lopez, J. D., M. Dos Reis, and R. R. Bretani. 1985. Presence of laminin receptors in *Staphylococcus aureus*. *Science* **229**:275-277.
- Maki, D. G. 1982. Infections associated with intravascular lines, p. 309-363. In J. S. Remington and M. N. Swartz (ed.), *Current clinical topics in infectious diseases*. McGraw Hill Book Co., New York.
- McDevitt, D., P. Francois, P. Vaudaux, and T. J. Foster. 1994. Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. *Mol. Microbiol.* **11**:237-248.
- McDevitt, D., P. Vaudaux, and T. J. Foster. 1992. Genetic evidence that bound coagulase of *Staphylococcus aureus* is not clumping factor. *Infect. Immun.* **60**:1514-1523.
- Park, K., and S. L. Cooper. 1985. Importance of composition of the initial protein layer and platelet spreading in acute surface-induced thrombosis. *Trans. Am. Soc. Artif. Intern. Organs* **31**:483-488.
- Park, K., S. J. Gerndt, and S. L. Cooper. 1986. The effect of fibrinogen sialic acid residues on ex vivo platelet deposition on biomaterials. *Thromb. Res.* **43**:293-302.
- Park, K., D. F. Mosher, and S. L. Cooper. 1986. Acute surface-induced thrombosis in the canine ex vivo model: importance of protein composition of the initial monolayer and platelet activation. *J. Biomed. Mater. Res.* **20**:589-612.
- Patti, J. M., T. Bremell, D. Krajewska-Pietrasik, A. Abdelnour, A. Tarkowski, C. Rydén, and M. Höök. 1994. The *Staphylococcus aureus* collagen adhesin is a virulence determinant in experimental septic arthritis. *Infect. Immun.* **62**:152-161.
- Patti, J. M., H. Jonsson, B. Guss, L. M. Switalski, K. Wiberg, M. Lindberg, and M. Höök. 1992. Molecular characterization and expression of a gene encoding *Staphylococcus aureus* collagen adhesin. *J. Biol. Chem.* **267**:4766-4772.
- Paulsson, M., and T. Wadström. 1990. Vitronectin and type-I collagen binding by *Staphylococcus aureus* and coagulase-negative staphylococci. *FEMS Microbiol. Immunol.* **2**:55-62.
- Peters, W. R., W. H. Bush, R. D. McIntyre, and L. D. Hill. 1973. The development of fibrin sheath on indwelling venous catheters. *Surg. Gynecol. Obstet.* **137**:43-47.

30. **Phillips, D. R., L. A. Fitzgerald, I. F. Charo, and L. V. Parise.** 1987. The platelet membrane glycoprotein IIb/IIIa complex. Structure, function, and relationship to adhesive protein receptors in nucleated cells. *Ann. N. Y. Acad. Sci.* **509**:177–187.
31. **Pitt, W. G., B. R. Young, K. Park, and S. L. Cooper.** 1988. Plasma protein adsorption: in vitro and ex vivo observations. *Makromol. Chem. Macromol. Symp.* **17**:453–465. (Abstract.)
32. **Raad, I. I., M. Luna, S.-A. M. Khalil, J. W. Costerton, C. Lam, and G. P. Bodey.** 1994. The relationship between the thrombotic and infectious complications of central venous catheters. *JAMA* **271**:1014–1016.
33. **Ruoslahti, E., E. G. Hayman, M. Pierschbacher, and E. Engvall.** 1982. Fibronectin: purification, immunochemical properties, and biological activities. *Methods Enzymol.* **82**:803–831.
34. **Toy, P. T. C. Y., L.-W. Lai, T. A. Drake, and M. A. Sande.** 1985. Effect of fibronectin on adherence of *Staphylococcus aureus* to fibrin thrombi in vitro. *Infect. Immun.* **48**:83–86.
35. **Vaudaux, P., D. Pittet, A. Haerberli, E. Huggler, U. E. Nydegger, D. P. Lew, and F. A. Waldvogel.** 1989. Host factors selectively increase staphylococcal adherence on inserted catheters: a role for fibronectin and fibrinogen/fibrin. *J. Infect. Dis.* **160**:865–875.
36. **Vaudaux, P., D. Pittet, A. Haerberli, P. G. Lerch, J. J. Morgenthaler, R. A. Proctor, F. A. Waldvogel, and D. P. Lew.** 1993. Fibronectin is more active than fibrin or fibrinogen in promoting *Staphylococcus aureus* adherence to inserted intravascular catheters. *J. Infect. Dis.* **167**:633–641.
37. **Vaudaux, P., R. A. Proctor, D. McDevitt, T. Foster, D. P. Lew, H. Wabers, and S. Cooper.** 1991. Use of adherence-defective mutants of *Staphylococcus aureus* (SA) to identify adherence-promoting plasma proteins deposited from perfusing blood in a canine shunt model, abstr. 1068, p. 277. In Program and Abstracts of the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
38. **Vaudaux, P., R. Suzuki, F. A. Waldvogel, J. J. Morgenthaler, and U. E. Nydegger.** 1984. Foreign body infection: role of fibronectin as a ligand for the adherence of *Staphylococcus aureus*. *J. Infect. Dis.* **150**:546–553.
39. **Vaudaux, P., F. A. Waldvogel, J. J. Morgenthaler, and U. E. Nydegger.** 1984. Adsorption of fibronectin onto polymethylmethacrylate and promotion of *Staphylococcus aureus* adherence. *Infect. Immun.* **45**:768–774.
40. **Vaudaux, P., H. Yasuda, M. I. Velazco, E. Huggler, I. Ratti, F. A. Waldvogel, D. P. Lew, and R. A. Proctor.** 1990. Role of host and bacterial factors in modulating staphylococcal adhesion to implanted polymer surfaces. *J. Biomater. Appl.* **5**:134–153.
41. **Vaudaux, P. E., D. P. Lew, and F. A. Waldvogel.** 1994. Host factors predisposing to and influencing therapy of foreign body infections, p. 1–29. In A. L. Bisno and F. A. Waldvogel (ed.), *Infections associated with indwelling medical devices*, 2nd ed. American Society for Microbiology, Washington, D.C.
42. **Vercellotti, G. M., J. B. McCarthy, P. Lindholm, P. K. Peterson, H. S. Jacob, and L. T. Furcht.** 1985. Extracellular matrix proteins (fibronectin, laminin, and type IV collagen) bind and aggregate bacteria. *Am. J. Pathol.* **120**:13–31.
43. **Wang, L., J. M. Anderson, and R. E. Marchant.** 1993. *Staphylococcus epidermidis* adhesion to hydrophobic biomedical polymer is mediated by platelets. *J. Infect. Dis.* **167**:329–336.