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Adherence of *Streptococcus sanguis* to Conformationally Specific Determinants in Fibronectin

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The adherence of *Streptococcus sanguis* to specific receptors exposed or deposited at the site of endothelial damage may play an important role in the development of infective endocarditis. Adherence of the Challis strain of *S. sanguis* to gelatin (or collagen) and gelatin-binding components of plasma was examined with an enzyme-linked immunosorbent assay. *S. sanguis* adhered poorly to immobilized gelatin and to molecular or fibrillar collagen. However, in the presence of fresh human plasma, the adherence of *S. sanguis* to all three substrates increased as much as eightfold. Removal of gelatin-binding proteins eliminates the ability of plasma to enhance adherence of *S. sanguis* to the substrates. Addition of purified human plasma fibronectin (Fn) to the absorbed plasma restored the adherence-promoting ability in a dose-dependent manner. A similar dose-dependent increase in *S. sanguis* adherence was observed when increasing concentrations of Fn alone were added to the gelatin-coated assay wells. *S. sanguis* adherence to immobilized fibronectin could not be inhibited by preincubating either the bacteria or the gelatin-coated assay wells with Fn or by including excess soluble Fn in the assay mixture. Studies with peptides purified from trypsin digests of Fn indicated that the 160- to 180-kilodalton (kDa) fragments which retain both the gelatin-binding and the cell-binding regions of the intact molecule support adherence of *S. sanguis* to gelatin. The 160- to 180-kDa fragments inhibited the interaction of *S. sanguis* with immobilized Fn. In contrast, intact Fn and the 31-kDa amino-terminal fragment were unable to inhibit the adherence when used in equivalent or greater molar amounts. These in vitro results suggest that in the presence of whole plasma, *S. sanguis* binds to immobilized gelatin or collagen via Fn bound to the immobilized substrates. Our finding that adherence of *S. sanguis* to immobilized Fn can occur in the presence of large concentrations of Fn, whether in plasma or purified, indicates that a *S. sanguis*-binding domain is cryptic in the Fn molecule while in solution and is exposed by a conformational change when the Fn becomes bound to gelatin-coated plastic. The ability of peptide fragments of Fn to inhibit *S. sanguis* adherence is consistent with this hypothesis.

Streptococcus sanguis is a viridans streptococcal species that commonly colonizes the oral cavity and is usually considered avirulent. Nevertheless, this microorganism can cause infective endocarditis (IE). Intermittent bacteremia frequently occurs with this and other viridans streptococci, particularly after certain dental and medical manipulations, but bacteremia alone is not sufficient to initiate IE in normally healthy individuals (7, 10, 26). Certain individuals with underlying valvular heart disease are, however, at risk of developing IE from transient *S. sanguis* bacteremia (29). Current evidence suggests that a crucial step in the development of IE is the initial adherence of bacteria to one or more of the molecules present on damaged cardiac valvular surfaces (25). These surface components, which would not normally be exposed or deposited on healthy vascular tissues, could then act as receptors for circulating bacteria, such as *S. sanguis*. Since bacterial adherence to these receptors may lead to endocarditis, it would be of considerable importance to understand the molecular events that allow *S. sanguis* to colonize damaged cardiac valvular tissues.

Some tissue components that are likely to be present at sites of endocardial damage are the subepithelial collagenous matrix and certain plasma components that would become bound to the matrix once it is exposed (e.g., fibrin, platelets, fibronectin, etc.). One factor that must be considered when designing in vitro assays to study mechanisms of *S. sanguis* adherence to these tissue components is that the adhesin-receptor interaction takes place in the presence of blood. Hamill has suggested that Fn may play a role in IE by interacting with circulating bacteria (13). *S. sanguis* has been shown to bind Fn, but the role of this binding in the adherence of *S. sanguis* to tissues has not been studied (2, 34). Furthermore, there are large concentrations of soluble Fn in plasma, which should make it an unlikely adherence factor in this particular situation, since the soluble Fn should inhibit binding of *S. sanguis* to immobilized Fn.

We have begun to study the mechanisms of *S. sanguis* adherence by a relatively simple approach. Gelatin or collagen was deposited on assay wells, bacteria were combined with fresh plasma in these assay wells, and adherence was quantitated with an antiserum specific for *S. sanguis*. In this paper, we present data indicating that Fn may play a role in the adherence of *S. sanguis* to damaged endothelial tissues, since these bacteria bind to substrate-absorbed Fn even in the presence of high concentrations of soluble Fn. This suggests that *S. sanguis* adherence may be related to conformational changes that take place when Fn is immobilized.

MATERIALS AND METHODS

Bacteria. All streptococcal strains were grown in Todd-Hewitt broth for 18 h at 37°C. Bacteria were harvested by centrifugation, washed twice, and suspended in phosphate-buffered saline (PBS; 0.02 M PO_4, 0.15 M NaCl, pH 7.4).

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Bacterial suspensions were adjusted to indicated optical density values at 530 nm with a Coleman Junior II spectrophotometer.

Preparation of proteins. Human plasma fibronectin (Fn) was purified by affinity chromatography by the method of Engvall and Ruoslahti (9). Purified type I collagen from bovine skin was a gift from K. Hasty (Department of Anatomy and Neurobiology, University of Tennessee, Memphis). Gelatin was obtained commercially (Fisher Scientific Co., Fair Lawn, N.J.).

Preparation of plasma free of gelatin-binding components. Gelatin-binding proteins were removed from fresh human plasma by passage over a Sepharose column followed by three successive passages over a gelatin-Sepharose column. The absorbed plasma was dialyzed against PBS (pH 7.4), as was a control sample of unabsorbed plasma from the same batch. Each sample was then adjusted to the same optical density at 280 nm and used in adherence assays. No detectable levels of Fn were observed in the absorbed samples, while the unabsorbed samples were positive, as demonstrated by double-diffusion agar gel assays performed by the method of Munoz (22).

Preparation and purification of peptide fragments. Peptides of Fn were prepared and purified essentially by the method of Mosher and Proctor (21). Briefly, purified Fn (80 mg) was dialyzed against 0.05 M NaCl–0.01 M Tris (pH 7.4) and digested for 13 min at 37°C with trypsin (tolylsulfonyl phenylalanyl chloloromethyl ketone treated; Sigma Chemical Co., St. Louis, Mo.) at a concentration of 154 ng/mg of Fn. The digestion was terminated by the addition of soybean trypsin inhibitor (770 ng/mg of Fn). Tryptic digests were applied to a DEAE column equilibrated with 0.05 M NaCl–0.01 M Tris (pH 7.4) and then were eluted first with 0.01 M Tris (pH 7.4)–0.05 M NaCl and then with 0.4 M NaCl. Fractions from major peaks were pooled, dialyzed exhaustively against double-distilled water, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The protein content of the major peaks was measured by the method of Lowry et al. (20), and the fractions were snap frozen and stored at −70°C until needed.

Preparation of antisera and monoclonal antibodies. Antisera directed against *S. sanguis* were prepared by intracutaneous injection of New Zealand White rabbits in the back of the neck with an inoculum of 0.5 ml of 10^8 CFU of *S. sanguis* per ml in PBS emulsified in Freund complete adjuvant. Two subsequent booster injections were given intravenously at 2-week intervals with the same number of bacteria in sterile PBS (pH 7.4). Antisera were collected, assayed for antibody titer, and stored at 4°C until needed. Antiserum against group A streptococci (*Streptococcus pyogenes* type M5) was a gift from H. Courtney (Veterans Administration Medical Center, Memphis, Tenn.) and was prepared as described previously (5). Preparation and characterization of the monoclonal antibodies used here have been described in detail previously (14).

ELISA for adherence of *S. sanguis* to immobilized substrates. Gelatin or Fn was immobilized onto microdilution plates (F16, high binding, microwell module; Nunc, Roskilde, Denmark) by adding 200 μl of the protein solution (50 μg/ml in 0.05 M sodium carbonate buffer, pH 9.6) to each well and incubating the plates for 16 h at 4°C. The molecular collagen or collagen fibrils were immobilized in the same manner and with the same protein concentration, except the protein was in 0.2 M NaCl–0.01 M Tris hydrochloride (pH 7.4). Collagen fibrils were formed from molecular collagen which was warmed to 37°C before use. Plates were washed before use three times with PBS (all subsequent washes were the same). Bacteria were harvested by centrifugation for 10 min at 8,000 rpm in a Beckman JA20 rotor and suspended in PBS to an optical density of 0.9 at 530 nm. Bacterial suspensions were then mixed with equal volumes of plasma, PBS alone, or PBS containing Fn or Fn fragments and applied to the assay plates (100 μl per well). Microdilution plates were incubated at room temperature for 30 min with gentle circular shaking. Nonadherent bacteria were removed with several washes of PBS. Adherent bacteria were fixed to the plates with a 15-min incubation at 60°C. A 1:200 dilution of antisera against *S. sanguis* or *S. pyogenes* (in PBS with 5% bovine serum albumin) was added to each well and incubated for 1 h at 37°C. Plates were washed with PBS, and a 1:1,000 dilution (in PBS with 5% bovine serum albumin) of peroxidase-labeled goat anti-rabbit immunoglobulin G (Cooper Biomedical, Inc., Malvern, Pa.) was applied and incubated for 1 h at 37°C. *o*-Phenylenediamine was used as the chromophore, and the color was allowed to develop for 30 min, after which the A_{450} was measured with a MicroElisa Reader (Dynatech Laboratories, Alexandria, Va.).

In each experiment, six replicate sample wells were assayed and each experiment was performed at least twice. Initial studies showed that the absorbance values measured were directly related to the number of added bacteria. The absorbance increased proportionately when the concentration of bacterial suspensions increased from an optical density of 0.1 to 0.4 (530 nm) and then became saturated at higher bacterial concentrations. Bacteria from cultures grown for 7, 13, 16, and 20 h bound equally well to immobilized Fn. Adherence of the bacteria was optimal between pHs 6 and 7 and dropped off rapidly above or below this pH range. Adherence of the bacteria was not affected by Mg^{2+} or Ca^{2+} concentrations ranging from 1 to 50 mM. Under the assay conditions, bacterial adherence was maximal within 10 min. Most of the adherence assays reported in this study used gelatin instead of collagen due to the availability and cost of gelatin.

Statistical evaluation. Data were evaluated by the use of Student's *t* test for unpaired data to determine statistically significant differences.

RESULTS

Plasma-enhanced adherence of *S. sanguis* to gelatin. Gelatin, molecular collagen, and collagen fibrils were immobilized on microdilution wells, and *S. sanguis* binding was assayed by a modification of the typical enzyme-linked immunosorbent assay (8). The Challis strain of *S. sanguis* adhered poorly to these three immobilized extracellular proteins in the absence of plasma (Fig. 1). However, in the presence of plasma, the adherence of this strain increased as much as eightfold over the buffer control. These data suggest that components of plasma enhance the adherence of some strains of *S. sanguis* to immobilized gelatin, molecular collagen, and collagen fibrils.

When gelatin-binding components were removed from human plasma by several passages over gelatin-Sepharose affinity columns, the absorbed plasma no longer supported adherence of *S. sanguis* to gelatin-coated microdilution plates (Fig. 2). Plasma enhanced adherence of *S. sanguis* to gelatin. A principal gelatin-binding protein of plasma is Fn. Therefore, purified Fn was added back to plasma previously depleted of gelatin-binding proteins. Addition of increasing amounts of purified Fn restored the ability of absorbed plasma to enhance adherence of *S. sanguis* to immobilized

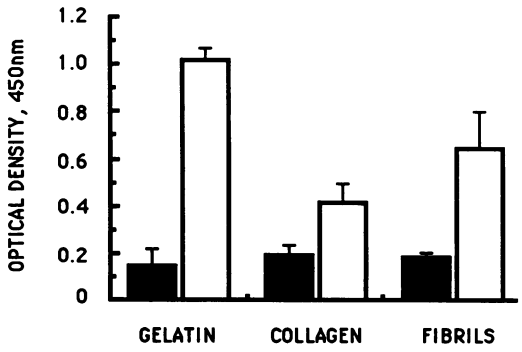

FIG. 1. Effect of plasma on *S. sanguis* adherence to immobilized substrates. Gelatin, collagen, and collagen fibrils were immobilized on microdilution wells at 50 μg/ml. Bacteria were allowed to adhere for 30 min at room temperature in the presence of PBS (black bars) or whole plasma (white bars). The vertical lines on each bar indicate the standard error of the mean. Experimental values were significantly different from the PBS control values ($n = 3$, $P < 0.05$).

gelatin in a dose-dependent manner (Fig. 3). While it was not possible with the highest concentration of Fn that we were able to test (one-third to one-half of the normal plasma concentration) to completely restore the adherence levels to that observed with whole plasma, it is clear from these results that Fn has a major effect on adherence.

If Fn is the major component of plasma that supports *S. sanguis* adherence to gelatin substrates, purified plasma Fn should promote adherence in the absence of other plasma components. To investigate this hypothesis, purified Fn in PBS (pH 7.4) was assayed for the ability to increase *S. sanguis* adherence to gelatin, collagen, and collagen fibrils. Similar increases to the adherence level observed with whole plasma were recorded (Fig. 4). In addition, when increasing amounts of Fn (1 to 100 μg/ml) in PBS were present during the adherence assay, a dose-dependent response, similar to that seen with the addition of Fn to absorbed plasma, was observed. Together, these results suggest that the major component of plasma which binds to gelatin, molecular collagen, and collagen fibrils and enhances the adherence of *S. sanguis* is Fn.

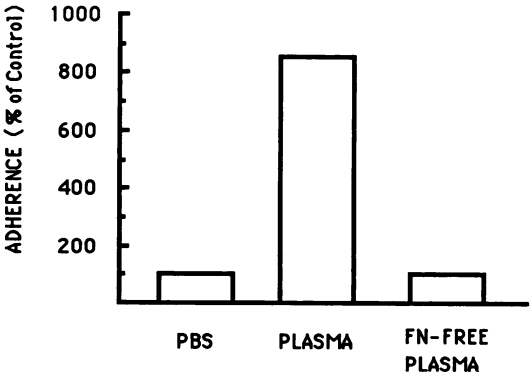

FIG. 2. Effect of gelatin-binding components of plasma on *S. sanguis* adherence to gelatin. Bacteria were allowed to adhere to gelatin-coated microdilution wells (50 μg/ml) in the presence of PBS, whole plasma, or plasma which had gelatin-binding components removed by three successive passes over a gelatin affinity column. In five replicate experiments ($n = 5$), the assay wells containing plasma were different from the wells containing PBS and the Fn-free plasma ($P < 0.001$), while the wells containing PBS and Fn-free plasma were not different from each other.

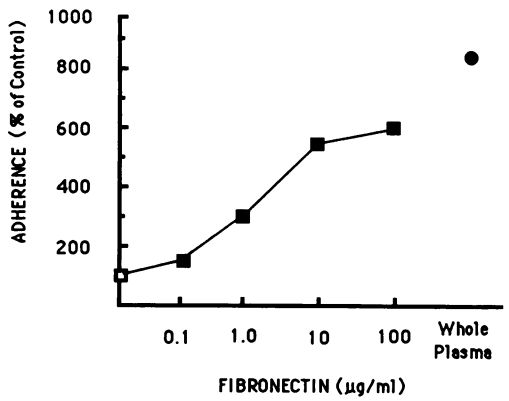

FIG. 3. Effect of purified Fn on *S. sanguis* adherence to gelatin-coated microdilution wells in the presence of absorbed plasma reconstituted with purified Fn. Adherence conditions were the same as for previous assays. Adherence was recorded in the presence of Fn-free plasma (□), reconstituted plasma (■), and whole plasma (●) ($n = 4$). Addition of 10 and 100 μg of Fn caused a significant increase ($P < 0.001$) in the adherence of *S. sanguis*.

Effects of preincubation of bacteria and gelatin substrate with fibronectin. In the adherence assays described above, the bacteria were added to the microdilution wells in the presence of plasma or Fn. To determine whether soluble Fn enhanced or inhibited the interaction of *S. sanguis* with gelatin, bacteria were preincubated with soluble Fn or PBS alone for 30 min. Unbound Fn was removed by washing three times with PBS prior to incubation. Preincubation of *S. sanguis* with Fn (adherence, 0.06 ± 0.007) did not increase adherence significantly over the gelatin-coated control wells (adherence, 0.03 ± 0.002). Conversely, if the gelatin-coated wells were preincubated with 50 μg of Fn per ml for 30 min and then washed three times with PBS prior to the addition of the bacteria, a sevenfold or better increase in the adherence of *S. sanguis* was observed (adherence, 0.03 ± 0.002 versus 0.41 ± 0.02 for PBS-treated bacteria on PBS- and fibronectin-treated wells, respectively, and 0.06 ± 0.007 versus 0.43 ± 0.03 for fibronectin-treated cells on PBS- and fibronectin-treated wells, respectively). There was not a

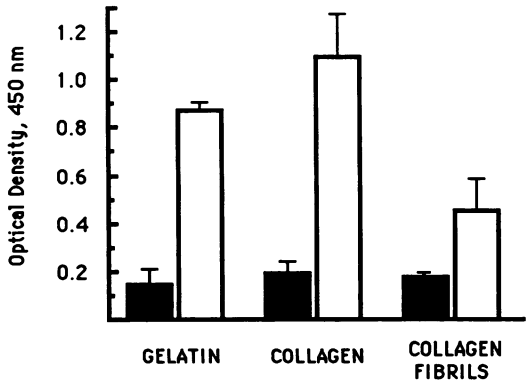

FIG. 4. Effect of purified Fn on adherence of *S. sanguis* to microdilution wells coated with gelatin, molecular collagen, or collagen fibrils. Bacteria were suspended in PBS (black bars) or PBS containing Fn (white bars) at a concentration of 100 μg/ml and were allowed to adhere for 30 min at room temperature. The vertical lines on each bar indicate the standard error of the mean. Experimental values were significantly different from the PBS control values ($n = 3$, $P < 0.005$).

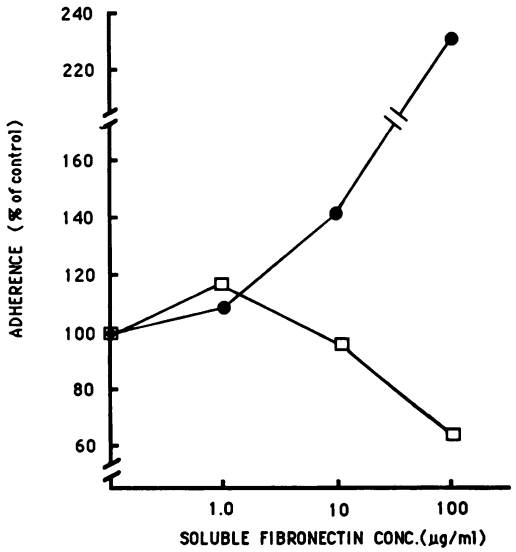

FIG. 5. Comparison of streptococcal adherence to immobilized Fn in the presence of soluble Fn. *S. sanguis* (●) or *S. pyogenes* (□) were washed and adjusted to an A_{530} of 0.40. The bacteria were mixed with increasing amounts of Fn (0.1 to 100 μg/ml) and added to gelatin-coated microdilution wells. In two replicate experiments (n = 6 in each), addition of 10 and 100 μg of Fn caused a significant increase in *S. sanguis* binding ($P < 0.001$) and a significant decrease in *S. pyogenes* binding ($P < 0.001$).

significant difference in adherence to Fn-pretreated gelatin assay wells between bacteria which were preincubated with Fn (adherence, 0.43 ± 0.03) and bacteria not preincubated with Fn (adherence, 0.41 ± 0.03). These data suggest that soluble Fn interacts primarily with the gelatin substrate, while *S. sanguis* adheres to Fn immobilized onto gelatin. If *S. sanguis* binds soluble Fn, this binding does not interfere with subsequent adherence of bacteria to immobilized Fn.

Comparison of streptococcal adherence to immobilized Fn. The data presented thus far indicate that *S. sanguis* interacts differently with soluble and immobilized Fn. In contrast, we have previously shown that *S. pyogenes* reacts identically with both soluble and immobilized Fn (4). To confirm these observations, the adherence of *S. sanguis* to immobilized Fn in the presence of increasing amounts of soluble Fn was compared to that of *S. pyogenes*. The adherence of *S. pyogenes* was inhibited in a concentration-dependent fashion, as would be predicted on the basis of our previous work (4). On the other hand, the adherence of *S. sanguis* was not inhibited by soluble Fn. Indeed, *S. sanguis* adherence to immobilized Fn increased to 230% of the control (Fig. 5). These data indicate that *S. sanguis* and *S. pyogenes* could interact with different regions of the Fn molecule. The inability of soluble Fn to inhibit the binding of *S. sanguis* to immobilized Fn in any of the tests we have performed suggests that a cryptic binding site(s) may become exposed when Fn becomes immobilized on plastic, gelatin, or collagen.

Effect of tryptic peptides on adherence of *S. sanguis* to gelatin. To determine which region of Fn might serve as a binding site(s) for *S. sanguis*, peptide fractions produced by limited trypsin digestion of Fn were used in the adherence assays. The fractions were separated on a DEAE column, and the fractions containing protein were characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis

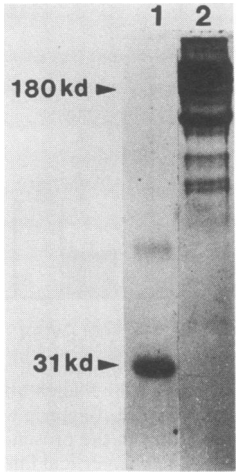

FIG. 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the peaks obtained from DEAE-Sepharose and heparin-agarose columns of a fibronectin tryptic digest. Peak I, shown in lane 1, is the amino-terminal fragment of Fn, and peak II, shown in lane 2, contains fragments that span the gelatin- and cell-binding regions of Fn (see text for details).

(Fig. 6) and identified by their reactivity with domain-specific polyclonal and monoclonal antibodies that have previously been described (14). Peak I contains the 31-kilodalton (kDa) amino-terminal fragment, as determined by reactivity with a polyclonal antibody (syn II) which is produced against a synthetic peptide from the amino terminus of the Fn molecule and which is specific for that fragment. Peak II contains 160- to 180-kDa fragments that span the gelatin-binding and cell-binding domains, on the basis of their reactivity with monoclonal antibodies D9b and IB10, respectively, which are specific for those domains (14). Western blot (immunoblot) analysis of these two major peaks also demonstrated that there was no detectable cross-contamination between the two peaks (data not shown). The 31-kDa fragment, which does not contain gelatin-binding sites, does not promote the adherence of *S. sanguis* to immobilized gelatin (Table 1). In contrast, the 160- to 180-kDa fragments do promote adherence, and the adherence is dose dependent. These data suggest that peak II contains an *S. sanguis* binding site. Although it is clear that peak I will not inhibit or support adherence in these assays, our results do not rule out the possibility that peak I could bind to *S. sanguis*.

To determine whether cryptic sites in the Fn molecule were exposed by trypsin digestion, fragments of Fn were

TABLE 1. Effect of tryptic peptides on adherence of *S. sanguis* to gelatin- or fibronectin-coated microdilution wells[a]

Fragment	Concn (μg/ml)	Adherence (% of PBS control)	
		Gelatin wells	Fn wells
31 kDa	50	80	90
	100	105	95
160 to 180 kDa	50	105	105
	100	820[b]	50[b]

[a] Peptides were purified as stated in Materials and Methods. Indicated concentrations of peptides were added to the bacterial suspension, which was then added to the wells for 30 min at room temperature; n = 5 experiments.
[b] $P < 0.001$ compared to PBS control; other results were not significantly different.

tested for their ability to inhibit *S. sanguis* binding to intact, immobilized Fn. When the 31-kDa and 160- to 180-kDa fragments were incubated with *S. sanguis* in the adherence assay, *S. sanguis* binding to immobilized Fn was not inhibited by the presence of the 31-kDa fragment at 50 or 100 μg/ml (Table 1). In contrast, the 160- to 180-kDa fragment inhibited the binding of the bacteria to immobilized Fn by 50% at 100 μg/ml. Thus, the 160- to 180-kDa fragments, which retain both the gelatin- and cell-binding regions, will support binding of *S. sanguis* to immobilized gelatin and will inhibit the binding of *S. sanguis* to intact, immobilized Fn.

DISCUSSION

Since Kuusela (17) initially described the binding of Fn to *Staphylococcus aureus* 10 years ago, a large number of other microorganisms have been shown to interact with this important glycoprotein (3, 23, 30, 32). It now appears that one of the primary functional consequences of the interaction of Fn with bacteria is in the initial adherence of the bacteria to host cells and tissues. Hamill has speculated in a recent review that Fn may play a role in infective endocarditis by acting as an adherence receptor on damaged cardiac valvular surfaces (13). At points of damage to the vascular endothelium, subcellular collagen is exposed, leading to platelet binding and activation and thrombus formation. It is known that Fn is one of the predominant components of these thrombi and is thus in a position to serve as a site for initial bacterial adherence. Indeed, *S. aureus* appears to bind much better to artificial thrombi formed in the presence of Fn than to thrombi made from Fn-deficient plasma (27). The question remains, however, whether Fn can in fact serve as a receptor for adherence of certain organisms to damaged heart valves, especially in view of the fact that there is a high concentration of soluble Fn in plasma.

S. sanguis is a normal inhabitant of the oral cavity, and *S. sanguis* bacteremia is a relatively common phenomenon. This microorganism is also one of the leading causes of infective endocarditis. In the present study, we have begun to investigate the mechanism of adherence of *S. sanguis* to extracellular macromolecules that may be exposed or deposited at points of endothelial cell damage. Our initial findings support and extend earlier reports of interactions between *S. sanguis* and Fn by demonstrating that Fn can serve as a receptor for adherence of the streptococci even in the presence of large amounts of soluble Fn (2, 34).

In this study, *S. sanguis* did not bind to immobilized gelatin, molecular type I collagen, or type I collagen fibrils, molecules that might also be expected to be exposed at points of damage to vascular endothelium. Exposure of gelatin- or collagen-coated plates to plasma, however, leads to adherence of the organisms. The plasma molecule that binds to the gelatin-coated plates and supports *S. sanguis* adherence is most likely Fn, since removal of Fn by gelatin-Sepharose chromatography eliminates the ability of plasma to stimulate adherence and since reconstitution of adsorbed plasma with purified Fn restores the adherence-stimulating effect. These were not unexpected findings, since some bacteria do not bind to gelatin or collagen and since a large number of bacteria are known to bind to substrate-adsorbed Fn. It was somewhat unexpected, however, to find that adherence of *S. sanguis* to substrate-adsorbed Fn was not inhibited by soluble Fn. This is, of course, a critical finding relative to the possibility that Fn may be an adherence receptor on damaged cardiac valves.

Although additional studies will be needed to determine how *S. sanguis* interacts differently with soluble versus substrate-adsorbed Fn, we believe that the following will be useful as an initial hypothesis. It is thought that Fn changes conformation and undergoes a surface activation when it becomes bound to certain other molecules or substrates, such as gelatin, collagen, or plastic (1). Williams et al. have demonstrated that plasma Fn exists in a folded globular configuration under physiological conditions and that it can unfold as the pH or ionic strength increases or as it interacts with certain other molecules (35). The same type of unfolding may also occur as Fn interacts with and becomes immobilized on a collagen matrix, and this unfolding could expose new sites which then support *S. sanguis* adherence. Vercellotti et al. have previously suggested this possibility in a study of the interaction of *Staphylococcus aureus* and several other bacteria, including *S. sanguis*, with extracellular matrix molecules (33). They found that *S. sanguis* was able to bind to a surface coated with Fn but did not aggregate in the presence of soluble Fn.

Such surface activation of Fn as it becomes immobilized has been suggested by several authors to explain certain other structural and functional differences that have been observed between soluble and insoluble Fn (1). For instance, plasminogen binds to insoluble Fn that has been immobilized on polystyrene surfaces but will not bind soluble Fn (24). Similar results have been demonstrated with hyaluronate, which binds only to immobilized Fn and not to soluble Fn (18). Another example is the observation that the soluble form of the 120-kDa chymotrypsin-generated cell-binding fragments of Fn did not contain glycosaminoglycan-binding sites (19). In contrast, if the same fragments were immobilized, glycosaminoglycan-binding sites were exposed, allowing the binding of dermatan sulfate proteoglycans. In addition, Grinnell and Feld demonstrated not only that anti-Fn antibodies react differently to immobilized Fn but also that the reactivity may depend upon the kind of surface used for immobilization (12).

Gibbons and Hay have described a somewhat similar situation for the adherence of *Actinomyces viscosus* LY3 to proline-rich proteins (PRPs) purified from human saliva (11). These investigators observed that *A. viscosus* could bind to PRP-coated hydroxyapatite in the presence of soluble PRPs. They suggested that as PRPs were immobilized on hydroxyapatite, regions that were hidden in the soluble form of the PRPs were exposed and able to interact with the microorganisms. These data support the principle that Fn and possibly other proteins contain additional binding sites which are not exposed in the soluble form but which are activated when the molecule is immobilized.

Results in the present study also suggest that the interactions of *S. sanguis* and *S. pyogenes* with Fn are distinct. *S. pyogenes* is undoubtedly the best-characterized streptococcal species with regard to interactions with Fn. Fatty acid chains of the lipoteichoic acid on the surface of the organism bind Fn via a fatty acid-binding site in the 27-kDa amino-terminal fragment of Fn (28). Soluble Fn binds to lipoteichoic acid and is a competitive inhibitor of the binding of group A streptococci to substrate-adsorbed Fn (4). In contrast, soluble Fn does not inhibit *S. sanguis* binding to substrate-adsorbed Fn.

In the experimental system used in this paper, proteolytic cleavage of Fn apparently mimics surface activation in terms of exposing a cryptic site in Fn for binding to *S. sanguis*. Brief trypsinization results in release of a small carboxy-terminal fragment that contains the disulfide bonds that join the Fn monomers, the 27-kDa amino-terminal domain, and the 160- to 180-kDa fragments that contain the gelatin-

binding and cell-binding domains (11, 19). Interestingly, the 31-kDa amino-terminal fragment that contains the primary binding sites for *S. pyogenes* and *S. aureus* does not affect adherence of *S. sanguis* to immobilized Fn. In addition, the 160- to 180-kDa fragments, but not the 31-kDa amino-terminal fragment, will support the binding of *S. sanguis* to gelatin. Thus, the interaction of *S. sanguis* and *S. pyogenes* with Fn would appear to occur by different mechanisms.

Other reports have demonstrated that functional domains can be removed from the intact molecule and still retain their activity (1, 17, 21). In addition, some of these studies were able to show that while intact Fn lacked or did not exhibit certain activities or features, proteolytically derived fragments were active. For example, a transformation-enhancing factor was not detected with the intact Fn molecule (6). However, plasmin- and cathepsin G-generated Fn fragments were highly active in promoting virus-induced morphological cell transformation (24). In another example, two heparin-binding fragments of Fn purified from cathepsin D or alpha-thrombin digests were specific, potent inhibitors of the growth of cultured bovine aortic endothelial cells, but intact Fn, even at high concentrations, was not able to inhibit growth (16). Our observations are consistent with this report in that the intact soluble Fn will not inhibit interactions with the immobilized molecule, whereas proteolytically derived fragments contain the binding domain for *S. sanguis* and will inhibit the interactions with the immobilized molecule. Although these fragments have been identified, more work will be needed to define the binding region for *S. sanguis* more precisely.

It has also been suggested that interactions of bacteria with platelets or laminin may play a role in IE by serving as substrates for the adherence and colonization of the host by pathogenic bacteria (15, 31). Nevertheless, adherence studies with these tissue components in which the bacteria and adherence receptors were incubated in the presence of plasma have not been performed. In this study, the data demonstrate a possible mechanism for the adherence of *S. sanguis* to immobilized Fn in the presence of plasma. It is possible, of course, that adherence of bacteria to damaged endothelial tissue may depend upon more than one mechanism, and various bacteria may also utilize different mechanisms to colonize damaged cardiac tissues. Production of well-characterized *S. sanguis* mutants which lack the ability to bind to specific molecules may allow one to dissect the role that adherence to one or more of the molecules present at the surface of damaged heart valves might actually play in the pathogenesis of IE.

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