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FimA, a Major Virulence Factor Associated with *Streptococcus parasanguis* Endocarditis

DANA BURNETTE-CURLEY,[1] VIRGINIA WELLS,[2] HELEN VISCOUNT,[1] CINDY L. MUNRO,[3] J. CHRISTOPHER FENNO,[4]† P. FIVES-TAYLOR,[4] AND FRANCIS L. MACRINA[1]*

Department of Microbiology and Immunology,[1] Department of Medicine,[2] and Department of Adult Health Nursing,[3] Medical College of Virginia-Virginia Commonwealth University, Richmond, Virginia 23298, and Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, Vermont 05405[4]

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Adherence of microorganisms to damaged heart tissue is a crucial event in the pathogenesis of infective endocarditis. In the present study, we investigated the role of the FimA protein as a potential virulence factor associated with *Streptococcus parasanguis* endocarditis. FimA is a 36-kDa surface protein that is a recognized adhesin in the oral cavity where it mediates adherence to the salivary pellicle. An insertion mutant and a deletion mutant of *S. parasanguis* were employed in the rat model of endocarditis to determine the relevance of FimA in endocarditis pathogenesis. Catheterized rats were infected with either the *fimA* deletion mutant VT929, the *fimA* insertion mutant VT930, or the isogenic, wild-type *S. parasanguis* FW213. Rats inoculated with FW213 developed endocarditis more frequently (50.9%) than animals inoculated with either the deletion mutant (2.7%) or the insertion mutant (7.6%) ($P < 0.001$). A series of in vitro assays were performed to explore the mechanism(s) by which FimA enhanced the infectivity of *S. parasanguis*. FimA did not inhibit the uptake or the subsequent killing of *S. parasanguis* by phagocytic granulocytes. Similarly, FimA did not play a role in the adherence to or the aggregation of platelets. Significant differences were noted between FW213 and VT929 ($P < 0.05$) and FW213 and VT930 ($P < 0.001$) in their abilities to bind to fibrin monolayers. The mean percent adherence of FW213 to fibrin monolayers (2.1%) was greater than those of VT929 (0.5%) and VT930 (0.12%). Taken together, these results indicate that FimA is a major virulence determinant associated with *S. parasanguis* endocarditis and further suggest that its role is associated with initial colonization of damaged heart tissue.

Viridans streptococci are the leading cause of native valve endocarditis in nonimmunocompromised individuals, accounting for approximately 60% of infections (2, 25). Infective endocarditis results from a complex interaction among vascular endothelium, blood components, circulating bacteria, and host defense mechanisms (16). Adherence of bacteria to previously damaged heart tissue is a crucial event in the pathogenesis of infective endocarditis. Fibrin and platelets are deposited at the site of endothelial cell trauma, forming a sterile vegetation. During bacteremia, this sterile vegetation provides a site for bacterial adherence and colonization. Adherence is followed by bacterial division, deposition of additional fibrin, and aggregation of platelets. The deposition of additional host proteins and cells after bacterial adherence leads to the formation of a larger vegetation and may serve to hide the organisms from circulating immune cells. The formation of a larger, mature vegetation can lead to mechanical dysfunction of cardiac valves and to the release of septic thrombi.

Several host cell factors and bacterial components have been identified as virulence determinants of endocarditis. Subendothelial matrix proteins such as collagen, fibronectin, and laminin have been shown to serve as receptors for streptococci (15, 20, 22, 23, 26). Additionally, fibrin and platelets deposited at the damaged site have been shown to play a role in streptococcal adhesion to vegetations (11, 12, 21). It is thought that interactions between two or more of these vegetation-associated factors may create binding sites for microorganisms. Exopolysaccharides produced by microorganisms also have been shown to enhance adherence of bacteria to vegetations. Using allelic exchange mutagenesis, Munro and Macrina (17) demonstrated that a *Streptococcus mutans* mutant (V1996) which was deficient in the synthesis of all exopolysaccharides, had decreased infectivity in the rat model of endocarditis compared with the wild-type strain (V403). Furthermore, this polymer-deficient mutant was killed more effectively by phagocytic granulocytes, and its adherence to fibrin monolayers was significantly decreased.

A group of streptococcal genes encoding related proteins that function as oral adhesins has been identified; these adhesins include FimA from *Streptococcus parasanguis* (5), SsaB from *Streptococcus sanguis* (8), ScaA from *Streptococcus gordonii* (13), and PsaA from *Streptococcus pneumoniae* (19). It is possible that this group of streptococcal adhesins also plays a role in endocarditis by promoting adherence to vegetations. Normal oral flora can gain entry into the bloodstream and establish infections in individuals with cardiac disease or preexisting valvular damage. The involvement of a protein related to these oral adhesins in endocarditis has been suggested in the case of *Enterococcus faecalis*. The product of a gene designated *efaA* that encodes a protein with 55 to 60% homology to the streptococcal oral adhesins has been identified in *E. faecalis* (14). EfaA has been hypothesized to be an adhesin in endocarditis, and it appears to be an immunodominant antigen in patients with endocarditis.

* Corresponding author. Mailing address: Department of Microbiology and Immunology, Virginia Commonwealth University, Box 980678 MCV Station, Richmond, VA 23298-0678. Phone: (804) 828-9728. Fax: (804) 828-9946. Electronic mail address: MACRINA@GEMS.VCU.EDU.

† Present address: Department of Microbiology, University of British Columbia, Vancouver, BC Canada.

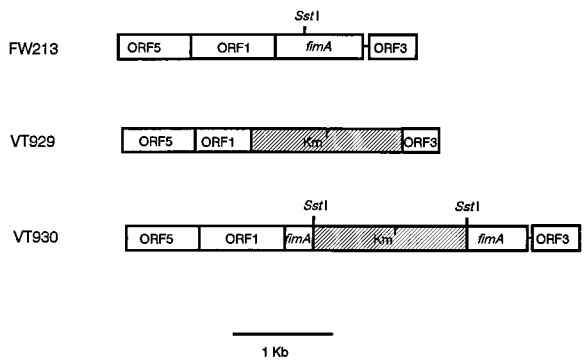

FIG. 1. Construction of the FW213 allelic exchange mutants VT929 and VT930. ORF5 and ORF1 protein products share homology with ATP-binding membrane transport proteins. ORF3 encodes a protein having identity with a protein located downstream of *ssaB*, a *fimA* homolog. A kanamycin resistance gene cassette was used to construct both mutants. VT929 is a *fimA* deletion mutant; in addition, the carboxyl terminus of ORF1 and the amino terminus of ORF3 are deleted in VT929. VT930 was constructed by inserting a kanamycin antibiotic resistance gene cassette in the *Sst*I site of *fimA*. Both ORF5 and ORF1 are intact in VT930.

In the present study, we investigated the role of FimA as a potential virulence factor in a rodent endocarditis model. *fimA* encodes a 36-kDa fimbrial adhesin of *S. parasanguis* FW213 (5–7). This gene is highly conserved in all four genetic groups of sanguis streptococci. Studies have shown that this fimbrial adhesin is localized at the tips of fimbriae (7) and functions in adherence of the sanguis streptococci to saliva-coated hydroxyapatite (18). Insertion and deletion mutants of *fimA*, VT930 (6) and VT929 (7), respectively, were tested in the rat model along with the wild-type strain, FW213. FimA was shown to be an important virulence determinant associated with endocarditis. Several in vitro assays were performed to determine the mechanism by which FimA promotes infectivity in endocarditis. Our data suggested that FimA functions in adherence of bacteria to fibrin associated with vegetations.

MATERIALS AND METHODS

Bacterial strains and media. Wild-type *S. parasanguis* FW213 and its isogenic *fimA* mutants, VT929 and VT930, have been described previously (6, 7). The identities of wild-type and mutant strains were indistinguishable by Facklam's biochemical testing methods (4) and restriction endonuclease-cleaved genomic fragment patterns. VT929 is a *fimA* deletion mutant, and VT930 is a *fimA* insertion mutant. The *aphA-3* kanamycin resistance gene cassette (24) was used to construct both mutants, as illustrated in Fig. 1. Use of this Km^r marker prevents effects on the expression of upstream or downstream gene sequences. The presence of a kanamycin antibiotic resistance gene in VT929 and VT930 was confirmed by growth on solid medium containing 100 μg of kanamycin per ml. Streptococcal strains were grown in brain heart infusion (BHI) broth or Todd Hewitt broth (Difco Laboratories, Detroit, Mich.) anaerobically (10% CO_2, 10% H_2, 80% N_2) at 37°C. Glucose was added to medium to a final concentration of 0.35%. Agar (Difco) was added to a final concentration of 1.5% in the preparation of solid medium. Under these growth conditions, VT929 and VT930 grew at the same rate as FW213.

Rat model of endocarditis. The rat model of endocarditis, as described by Munro and Macrina (17), was employed in this study. Approval for animal use was obtained from the Virginia Commonwealth University Institutional Animal Care and Use Committee (protocol no. 9410-2082) prior to initiation of experiments. Male Sprague-Dawley rats (Harlan, Indianapolis, Ind.) were challenged with a 10^7 bacteria 24 to 48 h after catheterization. The significance of differences between the numbers of streptococcus-infected vegetations obtained from animals challenged with wild-type or mutant strains was calculated by Fisher's exact test.

Radiolabeling of bacteria. Overnight cultures of bacteria grown in BHI were diluted 1:10 in fresh BHI supplemented with 50 μCi of [methyl-^{3}H]thymidine (NEN Research Products, Boston, Mass.; 20 Ci/mmol) per ml. Cultures were grown anaerobically at 37°C to an optical density at 660 nm of ~0.600. No differences in growth rates between the two strains in BHI or BHI and tritiated thymidine were noted. Bacteria were suspended in Hanks' balanced salt solution (HBSS) (Sigma, St. Louis, Mo.) at 10^8 cells per ml and were used in phagocytosis assays. An appropriately diluted sample was inoculated onto BHI agar to confirm bacterial concentrations.

Phagocytosis assay. Phagocytosis assays were performed as previously described (17). Briefly, venous blood was obtained before each assay from healthy adult volunteers. Informed consent was obtained from all donors. One hundred microliters of labeled bacterial cells (10^8/ml) was incubated with 900 μl of granulocytes (5.5×10^6/ml) in Hanks' balanced salt solution (HBSS). Cocultures were incubated in 5% CO_2 at 37°C for 1 h. After the 1 h of incubation, 24-well plates containing the cocultures were centrifuged at $500 \times g$ for 10 min to pellet granulocytes and associated bacteria. Supernatant fluids were removed. Three 50-μl samples from each sample well were spotted onto filter disks. The remaining supernatant fluid from each well was serially diluted and plated onto BHI agar. Granulocyte pellets in each well were lysed in 1 ml of ice-cold distilled H_2O. Three 50-μl samples of the suspended cell pellet from each well were spotted onto filter disks. The remaining pellet fluid from each well was serially diluted and plated onto BHI agar. Acid-insoluble material present on filter disks was precipitated with two 5-min washes of ice-cold 5% trichloroacetic acid followed by two 5-min washes in ice-cold ethanol. Disks were air dried, and their radioactivity was determined by liquid scintillation counting. The percentage of bacteria associated with the granulocytes at the conclusion of the assay was calculated by the following equation: (mean cpm of cell pellet fluid/mean cpm of cell pellet fluid + mean cpm of supernatant fluid) × 100%. To examine bacterial sensitivity to killing, a comparison was made between the mean number of live bacteria recovered from the cell pellet fluid and the number predicted to be alive on the basis of counts per minute of the cell pellet. The predicted number of bacteria in the cell pellet was calculated by the following equation: (number of CFU recovered from supernatant/mean cpm of supernatant) × (mean cpm of cell pellet fluid). The Wilcoxon rank sum test was performed to determine significant differences in wild-type and mutant strain phagocytosis by granulocytes.

Fibrin binding assay. Fibrin binding assays were performed as previously described (17) to determine differences in binding between FW213 and VT929 and FW213 and VT930. Briefly, fibrin monolayers were prepared by the addition of 0.25 ml of human thrombin (25 U/ml) (Sigma) to 1 ml of human fibrinogen (1 mg/ml) (Sigma). Monolayers then were washed with phosphate-buffered saline (PBS). Overnight bacterial cultures were diluted 1:10 in fresh BHI containing 0.35% glucose and were grown anaerobically to an optical density at 660 nm of ~0.600. Cells were washed twice with PBS and resuspended at a concentration of 10^8 cells per ml. One milliter of cells was added to the monolayer for 30 min at 37°C with gentle agitation. After the 30-min incubation, the nonadherent cells were removed from the monolayer and the monolayer was washed four times with PBS to remove remaining nonadherent bacteria. The monolayer was then dissolved by the addition of 0.5 ml of a 2.5% trypsin solution, and the resulting fluid was sonicated and plated onto BHI. The plates were incubated anaerobically at 37°C for 48 h. The percent adherence was calculated as (number of CFU recovered/number of cells introduced onto fibrin plate) × 100%. Statistical analysis was performed by utilizing Student's *t* test.

Platelet aggregation and adhesion assays. Outdated human platelet-rich plasma (PRP), containing the anticoagulant acid citrate-glucose, was supplied by the Medical College of Virginia Hospital's Blood Bank (Richmond, Va.) and was used in platelet adhesion assays. Platelets were centrifuged at $2{,}000 \times g$ for 30 min at 4°C. The platelets were washed three times in 0.05 M Tris-hydrochloride–0.1 M NaCl–0.02 M EDTA buffer (Tris-NaCl-EDTA buffer [pH 7.25]). The washed platelets (WP) were then reconstituted in a known volume of Tris-NaCl-EDTA buffer to an A_{620} of ~2.0.

For platelet aggregation assays, fresh PRP was prepared. Venous blood was obtained before each assay from donors who were free of infection and medication for at least 2 weeks before the study. Informed consent was obtained from all donors. Blood was drawn into Vacutainer tubes (Becton Dickinson) containing 3.8% buffered citrate solution and was centrifuged at $400 \times g$ for 20 min. PRP was removed, and the remaining blood was centrifuged at $2{,}000 \times g$ for 10 min to obtain platelet-poor plasma (PPP). A platelet count was performed on the PRP with a Coulter counter. The count was adjusted to 3×10^8 cells per ml with PPP.

Bacteria were harvested by centrifugation from late-stationary-phase growth (18 h) in Todd-Hewitt or BHI broth. The cells were washed four times in cold Tris-NaCl-EDTA buffer. After washing, bacteria were dispersed by sonication, suspended to an A_{620} of ~0.500, centrifuged, and suspended to one-fourth the volume (A_{620} of ~2.0) in HBSS or Tris-NaCl-EDTA buffer.

A method adapted from Herzberg et al. (9) was employed to determine bacterial adhesion to platelets. In this assay, 105 μl each of standard WP and bacterial suspensions was mixed in V wells of microtiter plates (Dynatech Laboratories, Inc., Chantilly, Va.), and the plates were incubated at 37°C for 30 min. Suspensions of 210 μl of WP or bacteria were placed in separate wells and incubated for 30 min at 37°C. These served as controls for interbacterial or platelet adhesion. WP-bacterium interactions were terminated by centrifugation of the microwell plates at $35 \times g$ for 5 min at 4°C to separate platelets with bound bacteria from noninteracting cells. Supernatants (200 μl) from each well were diluted 1:5 with Tris-NaCl-EDTA buffer, and the A_{620} was recorded. The percentage adhesion was calculated by the following formula: 100% × {1 − [mix-

TABLE 1. Endocarditis cases in rats inoculated with *S. parasanguis* FW213, VT929, or VT930[a]

Strain[b]	Cases[c]	Total[d]	Percent infected
FW213	26	51	50.9
VT929	1	37	2.7
VT930	1	13	7.6

[a] Initially, catheterized rats were infected with FW213 or VT929. Cumulative data from four experiments are shown. Subsequently, catheterized rats were infected with FW213 or VT930 to determine whether VT930 infection frequencies were similar to those of VT929.
[b] Animals were inoculated with 10^7 CFU.
[c] Number of animals with streptococci recovered from vegetations.
[d] Surviving animals with proper catheter placement at necropsy.

ture A_{620}/(bacterial A_{620} + WP A_{620}) ÷ 2]}. All experiments were performed in triplicate on at least two occasions.

The method described by Herzberg et al. (9) was used to determine the ability of bacteria to aggregate platelets. Fresh PRP obtained from a single donor was used in each experiment. After establishment of a baseline with 450 μl of PRP in a recording aggregometer (Chrono-Log Corp., Havertown, Pa.), 50 μl of bacterial suspension was added to the cuvette to give a 1:1 bacterium-to-platelet ratio. Light transmission was recorded for 40 min or until platelet aggregation was completed. A positive aggregation response was defined as an increase in light transmittance of 50% or greater through the platelet-bacterial suspension. Chrono-Par Collagen (Chrono-Log Corp.) was used as a positive control for platelet aggregation. HBSS was used as a negative control.

RESULTS

Infectivity in the rat model of endocarditis. The ability of the *fimA* deletion and insertion mutants, VT929 and VT930, to produce endocarditis was compared with that of the wild-type strain FW213 in the rat model. Twenty-four hours after catheterization, the rats were inoculated with the bacterial strains. Forty-eight hours after inoculation, the animals were sacrificed and their hearts were removed and visually inspected for correct catheter placement and the presence or absence of vegetations. Vegetations were observed in every case in which catheter placement was correct. Only animals with correct catheter placement were included in the data analysis. The numbers of animals that developed infective endocarditis, which were determined by positive vegetation cultures, were significantly different by comparison of the wild-type and mutant inoculated animals. As illustrated in Table 1, 50.9% of the animals infected with FW213 developed endocarditis. Only 2.7% of the animals infected with the deletion mutant VT929 and 7.6% of the animals infected with the insertion mutant VT930 developed endocarditis. By Fisher's exact test, the differences between the FW213 and VT929 and FW213 and VT930 infection frequencies were significant ($P < 0.001$).

Phagocytosis assays. The *fimA* deletion mutant VT929 was employed in subsequent in vitro assays to determine the mechanism by which FimA promoted infectivity in endocarditis. Phagocytosis assays were employed to determine whether FimA may function to inhibit phagocytosis and subsequent killing of bacteria by granulocytes, since these cells are a first line of defense for clearance of bacteria. Each experiment used blood from a single human donor. As illustrated in Fig. 2A, the percentages of bacteria associated with the granulocytes at the conclusion of the assay were similar for wild-type FW213 and mutant VT929. In four experiments, the mean percentage of FW213 associated with granulocytes was 87.3% ± 3.7%, and the mean percentage of VT929 associated with granulocytes was 88.3% ± 4.8%. The numbers of bacteria which could be recovered alive following association with granulocytes were similar for both FW213 and VT929. Data from four representative experiments are presented in Fig. 2B. The mean number

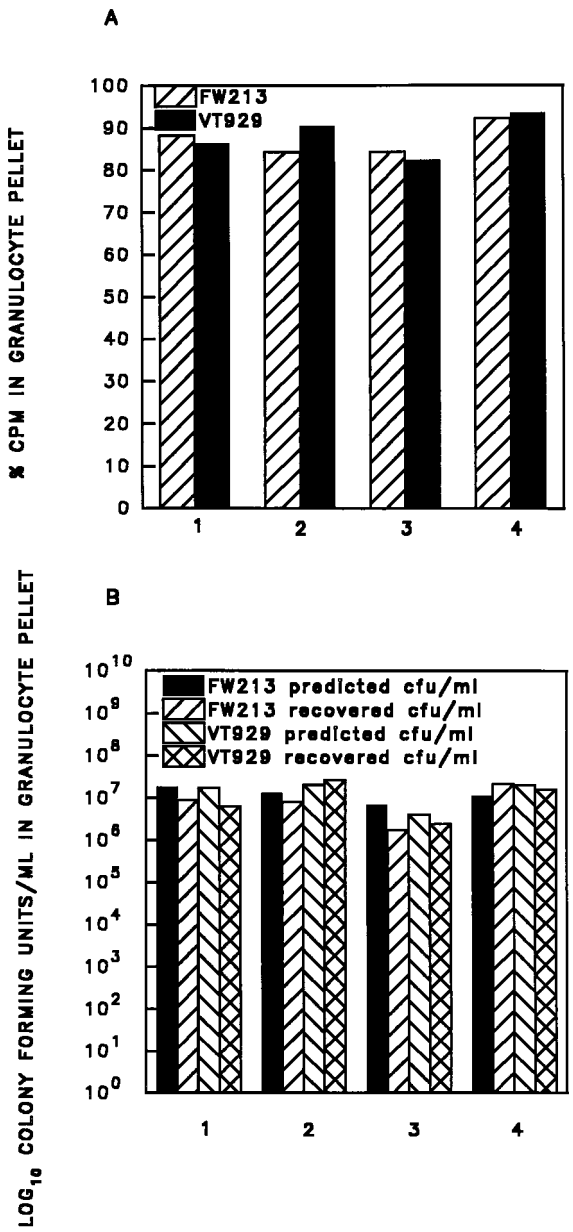

FIG. 2. Interaction of *S. parasanguis* and human granulocytes in vitro. (A) Percentages of *S. parasanguis* FW213 and VT929 associated with granulocytes in phagocytosis assay. Data from four representative experiments performed in triplicate are shown. (B) *S. parasanguis* sensitivity to killing by granulocytes. The number of bacteria in the granulocyte pellet that were viable at the conclusion of the assay and the number of bacteria predicted to be present in the pellet were determined as described in Materials and Methods. Data from four representative experiments performed in triplicate are shown.

of FW213 recovered alive from cell pellets was 6.87 ± 0.45 $\log_{10}$ CFU ml^{-}; the predicted mean number of FW213 in cell pellets was 7.085 ± 0.17 $\log_{10}$ CFU ml^{-}. The mean number of VT929 recovered alive from cell pellets was 6.96 ± 0.46 $\log_{10}$ CFU ml^{-}; the predicted mean number of VT929 in cell pellets was 7.12 ± 0.34 $\log_{10}$ CFU ml^{-}. These data showed that there were no differences between wild-type and mutant *S. parasanguis* in regard to bacterial sensitivity to killing.

Comparison of adherence of FW213 and VT929 to fibrin. Fibrin and platelets within the sterile vegetation on damaged

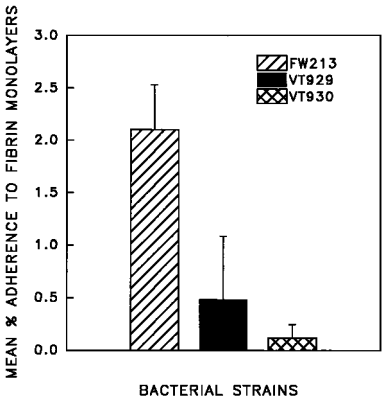

FIG. 3. Percent adherence of *S. parasanguis* FW213, VT929, and VT930 to fibrin monolayers. Bacteria were incubated on fibrin-coated disposable petri dishes (60 by 15 mm) for 30 min at 37°C. Bars show mean percent adherence of *S. parasanguis* to fibrin monolayers ± standard deviations. The adherence of VT929 was significantly different from the mean percent adherence obtained with wild-type FW213 at a level of $P < 0.05$. Similarly, the adherence of VT930 was significantly different from the mean percent adherence obtained with wild-type FW213 at a level of $P < 0.001$.

cardiac surfaces are thought to provide sites for bacterial adherence, a crucial event in the pathogenesis of infective endocarditis. Accordingly, we tested the role of FimA in adherence to fibrin. Fibrin monolayers were prepared, and the percentage of bacterial adherence to the monolayers was determined. The results from three replicate experiments, each performed in triplicate, are presented in Fig. 3. The mean percent adherence of the mutant strain, VT929 (0.5% ± 0.6%), was significantly different from that of the wild-type strain, FW213 (2.1% ± 0.43%) ($P < 0.05$; Student's *t* test). Similarly, the mean percent adherence of the insertion mutant, VT930 (0.12% ± 0.13%), was significantly different from that of the wild type, FW213 ($P < 0.001$). The difference between the adherence of VT930 to fibrin and the adherence of VT929 to fibrin was not statistically significant.

Platelet adherence and aggregation assays. Certain strains of *S. sanguis* adhere to platelets and cause them to aggregate (9). Thus, platelet adhesion and aggregation may serve as potential virulence factors of endocarditis, causing the formation of larger vegetations and hiding organisms from phagocytic immune cells. To determine whether *S. parasanguis*-mediated platelet adhesion and aggregation played a role in virulence, in vitro assays were performed. As illustrated in Fig. 4, there were no significant differences between the mean percent adhesion of wild-type FW213 (72.5% ± 1.5%) and the mean percent adhesion of the mutant VT929 (72.3% ± 2.6%) to platelets. Additionally, neither FW213 nor VT929 aggregated platelets obtained from several individual donors (data not shown). Platelets were monitored for aggregation by FW213 or VT929 grown in either Todd-Hewitt or BHI broth. However, no aggregation was observed, even after 40 min of incubation. Platelets aggregated after a 4-min incubation with collagen.

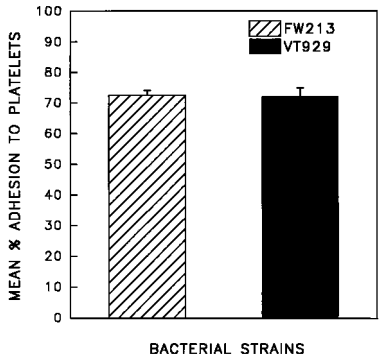

FIG. 4. Platelet adhesion of *S. parasanguis* FW213 and VT929. Platelets and bacteria were incubated at 37°C for 30 min in microtiter plates, as described in Materials and Methods. PRP was obtained from multiple donors and tested individually. Bars show mean percent adhesion of *S. parasanguis* to platelets ± standard deviations.

DISCUSSION

The production of endovascular infection requires four sequential events: (i) disruption of the normal valve surface, (ii) deposition of host cell proteins at the altered site, forming a sterile vegetation, (iii) adherence of blood-borne microorganisms to the traumatized surface, and (iv) formation of a larger, mature vegetation. Adherence of microorganisms to preexisting endocardial lesions is paramount to establishing an infection. We have used the rat model of endocarditis to identify adherence factors which serve as potential virulence determinants of streptococcal endocarditis. The results of the present study demonstrate that FimA, a surface protein of *S. parasanguis*, is an important virulence determinant of endocarditis.

Forty to fifty percent of endocarditis cases are attributed to infections with viridans streptococci (2). Dental work has been shown to precede approximately one-third of streptococcal viridans endocarditis cases (2). *S. sanguis*, *S. mitis*, *S. milleri*, *S. mutans*, and *S. salivarius* are all normal inhabitants of the oral cavity and are responsible for a majority of endocarditis cases (2, 25). Following dental manipulations or oral infections, it is possible for the streptococci to enter the bloodstream and colonize damaged heart valves, leading to the development of endocarditis. Interestingly, a number of oral adhesins have been identified in streptococcal species, leading to the hypothesis that one of these oral adhesins, FimA, may play a role in the adherence of *S. parasanguis* to damaged valvular surfaces in infective endocarditis.

The use of allelic exchange mutants differing from wild-type strains in the production of one gene product provides a powerful tool for determining the role of a specific gene product in infectivity. In the present study, a *fimA* deletion mutant, VT929, was used to infect catheterized rats. Animals inoculated with VT929 had a greatly decreased incidence of infected vegetations than did animals inoculated with FW213. Construction of VT929 involved deletion of *fimA* and substitution of a kanamycin antibiotic resistance gene cassette in its place. Recent studies have identified additional sequences located upstream and downstream of *fimA* that also were deleted in the construction of VT929 (7). As illustrated in Fig. 1, two open reading frames (ORFs), ORF5 and ORF1, are located upstream of *fimA*, and one ORF, ORF3, is located downstream of *fimA*. The carboxyl terminus of ORF1 and the amino terminus of ORF3 are deleted in VT929. The protein products of ORF5 and ORF1 share homology with members of a superfamily of ATP-binding cassette membrane transport proteins. ORF3 encodes a protein having identity with a protein encoded downstream of *ssaB*, a *fimA* homolog. To ensure that *fimA* and not ORF1 and ORF3 functions as a virulence factor associated with endocarditis, we infected catheterized rats with the insertion mutant VT930. VT930 was constructed by inserting a kanamycin antibiotic resistance gene cassette in the *Sst*I site of *fimA*. ORF1 and ORF5 are intact in this mutant, and

Fenno et al. (7) have demonstrated that expression of *fimA* is not linked to expression of ORF3. Infection of catheterized rats with VT930 resulted in a decreased incidence of infected vegetations upon comparison with infection with FW213. Therefore, we concluded from these experiments that the absence of *fimA* expression in both VT929 and VT930 accounts for the decreased level of infectivity associated with these bacterial strains in the rat model of endocarditis.

To determine the role of FimA in endocarditis, we performed several in vitro assays. Granulocytes are very important in the clearance of bacteria; thus, we employed a phagocytic assay to determine whether FimA may inhibit phagocytosis and subsequent killing of *S. parasanguis*. No differences were noted between the ability of granulocytes to phagocytose wild-type FW213 and mutant VT929. Eighty to ninety percent of bacteria were associated with the granulocyte pellet. Additionally, no differences were noted between FW213 and VT929 in their sensitivities to killing by granulocytes.

Because FimA functions as an adhesin in the oral cavity, we employed multiple in vitro assays to determine whether FimA may mediate adhesion of *S. parasanguis* to fibrin present in vegetations formed on damaged cardiac valves. Significant differences in the binding of FW213 (2.1%) and VT929 (0.5%) ($P < 0.05$) and FW213 (2.1%) and VT930 (0.12%) ($P < 0.001$) to fibrin monolayers were detected. Fibronectin, a high-molecular-weight matrix protein important in cellular adherence, was included in the fibrin monolayer in order to evaluate its effect on the adherence of FW213. Addition of fibronectin (50 to 200 μg) failed to increase adhesion to fibrin monolayers (data not shown).

Experiments also were performed to assess the abilities of FW213 and VT929 to adhere to and to aggregate platelets. Bacterial adhesion and aggregation of platelets into the vegetative lesion in infective endocarditis may bring about the formation of larger vegetations and, therefore, a more severe clinical course of endocarditis. This has been shown in the rabbit model of endocarditis (21). Bacterial aggregation of platelets also may serve to hide organisms from host immune cells such as granulocytes. By the platelet adhesion assay, there were no significant differences between the binding of FW213 (72.5%) and VT929 (72.3%) to platelets. By aggregometry, neither FW213 nor VT929 aggregated platelets (data not shown), which is a result that was in agreement with that of Douglas et al. (3). In contrast to these results, Herzberg et al. (10) have shown that FW213 aggregates platelets in 12 min. In the present study, we monitored aggregation up to 40 min, with no positive results. Platelets may play an additional role in the pathogenesis of infective endocarditis. Recent investigations have identified platelet microbicidal proteins (PMPs) (30) that are released from the α-granules of platelets upon thrombin stimulation. These PMPs are lethal in vitro to bacterial and fungal pathogens that cause endovascular infection (28–30). Bacterial resistance to these PMPs correlates with the ability of viridans streptococcal strains to adhere to platelets and to facilitate the development of endocarditis (1, 31). Therefore, resistance to PMPs may promote bacterial adherence to vegetations and subsequent colonization. Moreover, staphylococcal and viridans streptococcal strains isolated from patients with infective endocarditis are less susceptible to PMPs than those obtained from patients without endocarditis (27). FimA, an adhesin located at the tips of *S. parasanguis* fimbriae, may protect this bacterium from the bactericidal activities of PMPs. Studies are in progress to address this hypothesis.

In conclusion, the data obtained in the present study demonstrate that FimA is a major virulence factor associated with *S. parasanguis* endocarditis. Mutants deficient in the production of FimA are significantly decreased in their ability to bring about endocarditis in the rat model as measured by culture-positive vegetations. The exact mechanism by which FimA functions as a virulence factor of endocarditis has yet to be determined. Our data strongly suggest that FimA plays a role in adherence to fibrin deposits associated with damaged cardiac valves. Because FimA appears to be a major virulence determinant associated with *S. parasanguis* endocarditis, future studies should be performed to determine whether immunization with FimA offers protection against infection with FW213. Additionally, adhesins homologous to FimA should be studied as potential virulence determinants of infective endocarditis. Further characterization of the regulation and the role of FimA and its homologs in infective endocarditis will further our understanding of streptococcal pathogenesis in endovascular infection and may provide insight for the development of improved mechanisms for prophylaxis or therapy.

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