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Microbial Adhesion to Fibronectin *in Vitro* Correlates with Production of Endocarditis in Rabbits (42205)

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Abstract. Microbial adhesion to the constituents of nonbacterial thrombotic endocarditis (NBTE) is an important early event in the pathogenesis of infective endocarditis. Fibronectin is a ubiquitous mammalian glycoprotein with diverse functions which binds to certain bacteria but not to others. In this study, we determined that fibronectin is present on the surface of NBTE (after catheter-induced aortic valve trauma) but not on normal rabbit cardiac valvular endothelium. The adhesion of various bacteria and yeasts to human fibronectin in tissue culture wells was then measured. Microorganisms with a high isolation frequency from endocarditis cases (*Staphylococcus aureus, Candida tropicalis, C. albicans, Streptococcus faecalis, S. sanguis*) bound significantly better ($P < 0.01$) to fibronectin *in vitro* than other organisms (*Escherichia coli, C. krusei, Pseudomonas aeruginosa*) rarely implicated in this disease. Microbial adhesion to fibronectin correlated closely with the propensity of each organism to produce endocarditis in rabbits (e.g., ID_{50}) with preexistent NBTE. A similar distribution was noted after binding of soluble radiolabeled fibronectin to bacteria in suspension. The results suggest that fibronectin, expressed on the surface of NBTE, may mediate microbial adhesion of circulating organisms to initiate colonization during the early pathogenesis of infective endocarditis.

Microbial adhesion to the constituents of nonbacterial thrombotic endocarditis (NBTE), composed primarily of fibrin and platelets, is a critical early event in the pathogenesis of infective endocarditis (1–3). Bacteria or yeast cells that adhere avidly to artificial fibrin–platelet matrices (simulating NBTE) or damaged cardiac valvular endothelium (expressing NBTE) *in vitro* produce endocarditis more readily in rabbits with NBTE following aortic valve trauma and are associated with >90% of infective endocarditis cases in humans (1–7). Various cell surface components including dextrans (5, 8, 9), lipoteichoic acid (10), or mannans (11) have been suggested as mediators of this important microbial–NBTE interaction but the NBTE receptor(s) promoting microbial adhesion remains unknown. Microbial adhesion to the components of NBTE *in vitro* is reduced by specific antibody (12, 13) and pretreatment of the organisms with subinhibitory concentrations of certain antibiotics (14, 15); both of these manipulations can prevent endocarditis in experimental animal models of this disease (12–15).

Fibronectin is a large (≅440 kDa) ubiquitous mammalian glycoprotein found in a soluble form in plasma, cerebrospinal fluid, and amniotic fluid and in a less soluble form in extracellular matrix and basement membranes (16–18). Fibronectin displays many interesting structural and functional properties and may play a role in cell binding, transformation, and movement, organization and cross-linking of connective tissue, regulation of the coagulation process, wound healing, opsonization of particulate matter (and perhaps microorganisms) in the bloodstream, promotion of bacterial or yeast adhesion to epithelial cells, and others (16–20). The fibrin–platelet matrix of NBTE could contain fibronectin, based on the following evidence: (1) fibronectin is required for cell adhesion to fibrin, a major constituent of NBTE (18, 19); (2) fibronectin forms a coat on fibrin clots within wounds, and promotes fibroblast adhesion (18, 19, 21); and (3) this protein is manufactured by endothelial cells and may be exposed after denudation and

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NBTE formation (22, 23). Thus, fibronectin may be present on the surface of NBTE and promote microbial adhesion from the circulating blood in the early pathogenesis of infective endocarditis.

Also, fibronectin binds avidly to certain bacterial species but not to others. In general, binding is greatest with various gram-positive cocci (18, 20, 24–34), the major pathogens of infective endocarditis. Fibronectin binds poorly, if at all, to gram-negative bacteria, as a group rarely implicated in bacterial endocarditis. In fact, loss of cell surface fibronectin actually promotes bacterial adhesion of gram-negative organisms to oropharyngeal epithelial cells (35–37). These studies have examined fibronectin binding to the bacterial cells or bacterial adhesion to epithelial cells *in vitro* but have not examined bacterial adhesion to purified fibronectin directly. Nevertheless, the bacterial spectrum with high fibronectin binding includes the major pathogens of infective endocarditis and organisms with low affinity for this glycoprotein rarely cause this disease. In addition, yeasts commonly isolated from endocarditis patients (e.g., *Candida albicans*) bind to purified plasma fibronectin more avidly *in vitro* than yeasts that rarely cause endocarditis, e.g., *C. krusei* (37A, 38). Thus, fibronectin may be present in NBTE *in vivo*, binds to certain microorganisms with a high isolation frequency in endocarditis, and may promote the early adhesion events to NBTE. The role of fibronectin in the pathogenesis of infective endocarditis remains unknown, however.

The objectives of this study were (1) to determine if fibronectin is present on or within NBTE after catheter-induced aortic valve trauma in rabbits; (2) to examine the adhesion of various microorganisms to fibronectin *in vitro* under strictly standardized conditions; and (3) to correlate microbial adhesion to fibronectin *in vitro* with the propensity of the organism to produce endocarditis in rabbits.

Materials and Methods. *Assay for detection of fibronectin in NBTE.* The aortic valve endothelium was examined from normal rabbits and from animals with catheter-induced trauma (with NBTE) for the presence of fibronectin by fluorescent-microscopy techniques. Normal, 2- to 3-kg New Zealand White rabbits ($n = 4$) were compared with rabbits ($n = 6$) following 30 min of catheterization across the aortic valve via carotid artery insertion (PE-90; Intramedic; Becton, Dickinson, and Co., Parsippany, N.J.). This period of valvular trauma uniformly results in loss of endothelial cells and the formation of microscopic fibrin–platelet deposits, e.g., NBTE (39, 40). After catheter removal, the animals were rapidly sacrificed and the aortic valves were removed aseptically. The valves were pinned to cork and frozen rapidly in isopentane. After sectioning on a cryomicrotome, the sections were put on microslides (Bellco Glassware), air-dried, and fixed for 10 min in absolute ethyl alcohol. The slides were washed three times with PBS and covered with anti-human fibronectin prepared in goats (Bethesda Research Lab., 1:10 dilution) and incubated in a wet chamber at room temperature for 30 min. After washing with PBS (3×, 5 min per wash), the specimen was covered with fluorescent (FITC) conjugated anti-goat IgG prepared in rabbits (Miles-Yeda, 1:20 dilution, 10 min, room temperature). The slides were washed in PBS, air-dried, covered with buffered glycerin (9:1 ratio) and examined in a fluorescent microscope. All specimens were read blind and the fluorescence graded from 0 to 4.

Microbial strains. All bacterial and yeast strains were clinical isolates from blood cultures, the majority from patients with infective endocarditis. The following strains, all from endocarditis cases, were originally isolated at the Univ. of Virginia: *Staphylococcus aureus, Streptococcus sanguis* (5, 12, 15), *S. faecalis,* and *Pseudomonas aeruginosa.* The *Escherichia coli* isolate, K_1 antigen positive, was isolated from blood and CSF of a neonate and kindly provided by Dr. George McCracken (Dallas). The *S. pyogenes* (M type 5) strain was kindly provided by Dr. Edwin Beachey (Memphis). The *S. epidermidis* strain was isolated from an adult with prosthetic valve endocarditis and provided by Dr. Gordon Archer (Richmond, Va.). The *C. albicans* strain (clone 4) has been described previously (7, 11, 13). *C. tropicalis* was a clinical isolate from an addict with endocarditis at the Georgetown Univ. Hospital; the *C. krusei* isolate was kindly provided by Dr. R. D. King.

Isolation of fibronectin. Fibronectin was obtained from human plasma, after centrifugation of whole blood at 2400*g* for 10 min, by

the method of Hooper *et al.* (41). The plasma was passed through a cyanogen-activated Sepharose 4B column (Pharmacia, bed volume = 18 ml) to which gelatin had been conjugated (5 mg gelatin/ml of gel, Fisher Chemical Co.) for 24 hr at 4°C. After extensive washing of the column with PBS (3 bed volumes), the fibronectin was eluted from the gelatin with 8 *M* urea. The urea-soluble proteins were rechromatographed on Sepharose, dialyzed against 10 m*M* CAPS buffer (pH 11.0, containing 150 m*M* NaCl and 1 m*M* $CaCl_2$), and stored at −70°C. Protein was quantitated by the procedure of Lowry *et al.* (42). The yield is 600 mg from 10 units of plasma by OD_{280}, $E^{1\%}_{280} = 13$. The material yields a single line of identity in Oucterlony gels against rabbit or goat monospecific antibody to human fibronectin (Bethesda Research Laboratories, Gaithersburg, Md.).

Assay of adhesion to fibronectin. All strains used in this study were grown for 18 hr in appropriate media prior to use. No significant differences in adhesion to fibronectin were noted for all strains when grown for 6–8 hr (log phase) vs 18 hr (stationary phase) organisms. The bacteria, excluding streptococci, were grown in trypticase soy broth (Difco). The streptococci were grown in brain–heart infusion broth (BHIB, Difco) supplemented with 5% defibrinated sheep's blood. All bacteria were grown at 37°C. All strains of yeast were grown for 18 hr at 30°C in phytone–peptone broth (BBL Microbiology Systems, Cockeysville, Md.) 10 g/liter supplemented with 1 mg/ml glucose at 125 rpm. The cells were harvested by centrifugation (3000*g* × 15 min), washed three times in PBS containing 1 m*M* $CaCl_2$, and standardized to 1×10^8 cells/ml in a hemacytometer, and verified by OD readings in a Gilford spectrophotometer and serial 10-fold dilutions in appropriate agar pour plates. In all studies, except for some involving *Candida* species, adhesion to fibronectin was quantitated by a direct CFU assay of adherent organisms. In other experiments with *Candida* the results were verified with radiolabeled yeasts. The cells (1×10^8/ml in 10 ml PBS plus 1 m*M* $CaCl_2$) were pulsed with [^{3}H]leucine (20 μl, 50 μCi/m*M*, New England Nuclear) for 1 hr at 37°C, repeatedly washed, and standardized to 1×10^7 cells/ml in PBS containing 1 m*M* $CaCl_2$.

The fibronectin was coated in wells of tissue culture dishes (Costar, 24 wells, 16 mm). The fibronectin concentration used was 70–90 μg protein/well in a volume of 0.1 ml; the wells were allowed to dry at room temperature and were used fresh on the day of preparation. When the fibronectin-coated wells were dried, 3×10^4 CFU of bacterial or yeast cell suspension (total volume = 0.15 ml) in PBS plus 1 m*M* $CaCl_2$ was added. In some experiments, $CaCl_2$ was omitted from the assay buffer. The tissue culture dishes were incubated at 37°C for 30 min in a New Brunswick Gyrotory Shaker at 120 rpm of gentle agitation. The fibronectin-coated wells were then washed five times with PBS and the pooled washes (nonadherent bacteria or yeasts) were serially diluted and quantitated after 24 hr of incubation at 37°C in appropriate agar pour plates. Adherent bacteria or yeast cells were removed by trypsinization of the fibronectin [100 μg trypsin (Sigma) per well, 10 min at 37°C] and the wells washed five times with PBS. Trypsinized bacteria or yeast cells and washes were pooled and CFU were quantitated as described above. In experiments with ^{3}H-radiolabeled *Candida,* the respective samples were collected on GF/A filters (Whatman) and the inoculum used was 2.5×10^6 CFU/well. The yeast cells were digested for 1 hr in tissue solubilizer (NCS Amersham); liquid scintillation fluid (5 ml) was added, and radioactivity was determined. Zero-time controls were performed in all experiments by adding bacteria or yeast cell suspensions to fibronectin-coated dishes incubated on ice and treated immediately as described above. The adhesion of each species to fibronectin was expressed as a percentage by the following formula: adhesion % = adherent (trypsinized) CFU or cpm/initial inoculum CFU or cpm. The percentage adhesion was validated only if percentage recovery was >95% (e.g., adherent + nonadherent washes/initial inoculum ≥ 0.95). The actual value for all experiments included in this study was 0.977.

Inhibition of binding by fibronectin. The above experiments were repeated for each strain except the bacteria were suspended in PBS plus 250 μg/ml purified fibronectin (similar to concentrations present in blood) and 1 m*M* $CaCl_2$ instead of PBS alone. The adhesion results ($n \geq 8$, each strain) are expressed as percentage of control.

[^3H] fibronectin binding to bacteria or yeasts) values. Each strain employed in the above experiments was grown at 37°C in BHIB at 200 rpm for 18–20 hr, centrifuged, and washed three times in PBS and standardized to 1×10^9 cells/ml. The cells were pulsed with ^{3}Hfibronectin (prepared for RAC by Amersham), 20 μl at 34 μCi/m*M*, and incubated at 37°C for 1 hr. After washing three times in PBS, the cells were resuspended in 1 ml PBS. A 100-μl spot was applied to filter paper (GF/A, Whatman) and radioactivity was determined.

Production of endocarditis. The relative propensity for each strain employed in the *in vitro* studies to produce endocarditis *in vivo* was investigated with a rabbit model. The infectious dose for 50% of the animals (ID_{50}) was determined for each strain. Endocarditis was produced in 2- to 3-kg New Zealand white rabbits by modifications of techniques described previously (5, 7, 12, 13, 15, 39). A polyethylene catheter (Intramedic PE-90; Becton, Dickinson, and Co.) was introduced through the right carotid artery and threaded across the aortic valve into the left ventricle. The catheter was left in place for 1 hr; this period of trauma uniformly elicits fibrin–platelet deposition (e.g., NBTE) on the valvular endothelium (13, 39). All inoculations were performed intravenously after a 1-cm catheter withdrawal; the catheter was removed 10 min later and the wound was closed. All inocula were prepared from 18 hr growth in appropriate broth, washed 2× in PBS after centrifugation, and verified by serial 10-fold dilutions and subsequent CFU enumeration in appropriate agar pour plates. Groups of 6–10 animals were injected with the following inocula (CFU in 1.0 ml, washed in PBS): 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8. All inocula were not included for each organism due to initial results, thus the experimental groups included 30–48 animals. Quantitative blood cultures (1.0 ml) and temperature recordings were performed 24 hr after inoculation. The presence (or absence) of endocarditis was confirmed 5 days later when the animals were sacrificed, the aortic valve area and vegetations were removed by wide excision, homogenized in PBS, and quantitatively titered onto duplicate agar pour plates of appropriate media, as described previously (5, 7, 12, 13, 15). The ID_{50} of the rabbits was calculated for each organism (based on the number of vegetations with positive cultures in the groups at each inoculum concentration), and statistical analysis performed after simplification of the dose–response curves by probit analysis, as previously described (7, 12, 13).

Results. *Detection of fibronectin in NBTE.* Fibronectin was not visualized by immunofluorescence on the nontraumatized rabbit aortic valve endothelial surface exposed to circulating blood. In contrast, fibronectin was detected readily on the surface of NBTE following 30 min of catheter-induced aortic valve trauma. The fluorescence was always graded as 3 to 4+ by several different observers in an analysis of blinded samples. Thus, fibronectin is present within NBTE (and exposed on the surface) but not on the luminal surface of normal rabbit aortic valve endothelium. In addition, fibronectin was not present on the endothelial surface of samples obtained from other intracardiac areas in rabbits including the mitral valve, ventricular septum, and free wall.

Adhesion of bacteria and yeasts to fibronectin in vitro. Because *S. aureus* and *C. albicans* were noted to adhere avidly to fibronectin (38; see below), these organisms were used to standardize the assay procedure. Adhesion of these organisms to fibronectin was equivalent when incubated at 30 or 37°C but was only 47–49% of control values when the experiments were conducted at 4°C. All subsequent studies were performed at 37°C. Adhesion at zero time on ice was <3% for both organisms (data not shown). As noted above, >95% of the organisms in the initial inoculum were recovered in the nonadherent washes and trypsinized adherent pools. Adhesion was maximal at 30 min and exceeded values obtained after 5, 10, and 15 min of incubation (data not shown; 38); further prolongation of the experiments to 60 or 90 min did not increase adhesion.

Initially, we compared the adhesion of *S. aureus* and *C. albicans* to fibronectin and bovine serum albumin. Adhesion to fibronectin increased as the fibronectin concentration per well increased; maximal values, e.g., 35% for *C. albicans,* were obtained at a concentration of 70 μg protein/well. Further increases in fibronectin concentration (e.g., 100, 300 μg/well) did not increase adhesion but fibronectin concentrations of 0, 0.01, 1, 10, and 30 μg/well resulted in mean values of only 3.3–7.6%

adhesion compared to 70 μg/well for *C. albicans*. A fibronectin concentration of 70 μg/well was chosen for all subsequent studies. Adhesion of *S. aureus* and *C. albicans* to bovine serum albumin was always <1.0–2.45% at concentrations of 70, 100, and 300 μg/well, much less than values obtained for adhesion to fibronectin. In addition, adhesion to commercially prepared human fibronectin (Bethesda Research Laboratories) at 100 μg/well was 43.7 ± 2.1% for *C. albicans*, similar to the mean values (37.3 ± 3.3%) obtained for adhesion to fibronectin prepared in our laboratory.

Adhesion to fibronectin was greater with viable cells; the adhesion of heat-killed *C. albicans* was only 35% of control values. Calcium (1 m*M*) promoted adhesion but was not essential; adhesion was only 51% of control when calcium was omitted from the assay buffer. All subsequent experiments employed viable organisms and 1 m*M* $CaCl_2$ in the system. The adhesion of *C. albicans* and *S. aureus* to fibronectin (70 μg/well) was equivalent when results were tabulated by viable CFU or radiolabeled cpm end points. All values reported here are derived from the assay of viable organisms, using CFU adherent/CFU inoculum in analysis.

The adhesion of various bacteria and yeasts to human plasma fibronectin *in vitro* is shown in Table I. The mean adhesion of *S. aureus* was the highest of all organisms tested, in accordance with other studies of fibronectin-binding to this organism. The mean adhesion of two *C. albicans* strains and *C. tropicalis* to fibronectin was 25–33%, much greater than values obtained for *C. krusei*. The adhesion of *C. pseudotropicalis* and *S. cerevisiae* to fibronectin under identical conditions was 0, e.g., no organisms were recovered in the trypsinized layer. The mean adhesion of the streptococci to fibronectin was intermediate (≅5–10%) and sixfold greater than the values observed with gram-negative aerobic bacilli and *S. epidermidis*. Thus, three groups of organisms were delineated: those with mean adhesion of >25%, an intermediate group of streptococci, and those that essentially did not adhere (gram-negative bacilli, *S. epidermidis*, and nonpathogenic yeasts). The mean adhesion of the streptococci, *Candida* sp., and *S. aureus* were significantly ($P < 0.01$) greater than values obtained in this latter group. In general, organisms that commonly cause infective endocarditis in humans bind to fibronectin significantly better than organisms rarely implicated in endocarditis.

TABLE I. ADHESION OF VARIOUS BACTERIA AND YEASTS TO HUMAN PLASMA FIBRONECTIN *in Vitro*

Organism (No. experiments)	Mean ± SD (% adhesion)	Adhesion suspended in 250 μg/ml fibronectin mean ± SD (% of control)
S. aureus (14)	56.2 ± 8.1	20.2 ± 4.9
C. tropicalis (18)	33.6 ± 1.9	96.4 ± 3.8
C. albicans (C-4) (30)	28.6 ± 2.0	105.8 ± 6.8
C. albicans (4918) (12)	25.9 ± 1.4	91.5 ± 4.9
S. sanguis (16)	9.1 ± 1.8	10.1 ± 3.4
S. faecalis (15)	5.6 ± 1.9	5.8 ± 4.0
S. pyogenes (9)	5.5 ± 1.8	16.3 ± 2.6
S. epidermidis (10)	1.4 ± 0.8	88.7 ± 12.5
P. aeruginosa (13)	1.4 ± 0.6	94.6 ± 12.1
E. coli (19)	0.9 ± 0.5	111.5 ± 6.5
C. krusei (18)	0.7 ± 0.8	79.3 ± 4.1

Adhesion of all strains to fibronection was altered in the presence of exogenous purified fibronectin (250 μg/ml) (Table I). Marked decreases in adhesion to fibronectin (e.g., 5–10% of control values) were noted for the staphylococci and streptococci in the presence of fibronectin, but the overall rank order of adhesion was preserved. In contrast, exogenous fibronectin had essentially no influence ($P > 0.05$) an adhesion of the gram-negative aerobic bacilli or *Candida* sp. to fibronectin *in vitro*.

Production of endocarditis in vivo. In general, there was a strong correlation between binding (adhesion) to fibronectin *in vitro* and the ability of the organism to produce endocarditis *in vivo* in rabbits with preexistent NBTE (Table II). The ID_{50} for endocarditis production in rabbits was $<10^6$ CFU for streptococci, staphylococci, and pathogenic *Candida* sp. but was 10^7 CFU or greater for gram-negative aerobic bacilli and *C. krusei*. Again, the organisms most commonly found in endocarditis cases in humans displayed the lowest ID_{50}s for endocarditis production in rabbits. Two exceptions in rank order for ID_{50} occurred when compared to the relative adhesion to fibronectin. The ID_{50} for *S. faecalis*

TABLE II. CORRELATION OF BACTERIAL OR YEAST ADHESION TO FIBRONECTIN *in Vitro* WITH ABILITY TO PRODUCE ENDOCARDITIS *in Vivo*

Strain	No. animals	Mean ± SD ID_{50}[a] for endocarditis production in rabbits
S. aureus	36	$10^{3.61\pm0.25}$
C. tropicalis	40	$10^{4.17\pm0.18}$
C. albicans (C-4)	47	$10^{4.86\pm0.26}$
S. faecalis	31	$10^{5.21\pm0.10}$
S. sanguis	48	$10^{5.78\pm0.24}$
S. pyogenes	22	$10^{6.67\pm0.32}$
E. coli	30	$10^{6.93\pm0.40}$
C. krusei	42	$10^{7.27\pm0.31}$
S. epidermidis	34	$10^{7.54\pm0.82}$
P. aeruginosa	33	$10^{8.31\pm0.25}$

[a] ID_{50} = Infectious dose for 50% of animals.

was lower than that obtained with *S. sanguis* (although adhesion to fibronectin was similar) and the ID_{50} for *P. aeruginosa* was the highest observed, despite mean percentage adhesion to fibronectin exceeding *E. coli* and *C. krusei* (Table II). All of these strains were serum resistant when analyzed for lysis in normal rabbit serum after incubation of 5×10^5 CFU for 2 hr at 37°C.

Binding of [^{3}H]fibronectin to organisms in suspension. Except for *C. albicans,* there was a direct correlation between microorganism adhesion to fibronectin in tissue culture wells (Table I) and binding of radiolabeled fibronectin to cells in suspension (Table III). The results (Table III) indicate the means of three experiments. Fibronectin bound avidly to staphylococci and streptococci but not to gram-negative bacilli or *C. albicans.* The poor binding of fibronectin to *Candida* yeast cells in suspension is surprising given the results in Table I; this discrepancy remains unexplained. However, the results are consistent with the lack of inhibition of exogenous fibronectin (250 μg/ml) on *Candida* sp. adhesion to fibronectin *in vitro* (Table I).

Discussion. This study demonstrates the following: (1) fibronectin is present on and within NBTE *in vivo* but not on the luminal surface of normal rabbit valvular endothelium; (2) bacterial or yeast adhesion to human plasma fibronectin *in vitro* is variable (Tables I and II), and adhesion is highest for strains commonly implicated in endocarditis; (3) bacterial or yeast adhesion to fibronectin *in vitro* correlates highly with the propensity of the organism to produce endocarditis in rabbits with preexistent NBTE (Table II); and (4) the binding of soluble [^{3}H]fibronectin to bacterial or yeast cells in suspension is also variable (Table III) and correlates well with the results summarized under 2 and 3 above, except for *C. albicans.* This last discrepancy has been noted by others. For example, mammalian cells in suspension bind less exogenous fibronectin than do the same cells in tissue culture monolayers (43). In addition, nutritionally variant streptococci (NVS) are an important cause of endocarditis, bind to fibronectin substrate *in vitro,* but do not demonstrate binding of soluble I^{125}-fibronectin in suspension (≅6–8 ng/10^9 cells; Dr. Ivo van de Ryn, personal communication). Nevertheless, the results of this study suggest that fibronectin, as a component of NBTE, may facilitate adhesion (e.g., act as a receptor) of circulating microorganisms to initiate colonization of NBTE in the early pathogenesis of infective endocarditis.

Since NBTE is a thrombus *in situ,* the presence of fibronectin in this lesion is not unexpected. About 3–4% of the normal blood clot consists of fibronectin (16–19, 44), and NBTE is similar in structure (e.g., fibrin plus platelets) to a clot. Fibronectin has a fibrin cross-linking domain and is covalently cross-linked to fibrin by factor XIIIa (17, 18). The source of fibronection in NBTE may be the plasma or platelets, since the platelet release reaction results in extrusion of fibronectin from α-granules (45). Fibronectin, through fibrin–factor XIIIa interactions, also provides the best substratum for fibroblast adhesion to the developing clot, an important factor in wound healing (18, 21). Staphylococcal adhesion to fibronectin within the wound coagulum may be an important early event in the initiation of wound infection

TABLE III. BINDING OF HUMAN PLASMA FIBRONECTIN TO MICROORGANISMS IN SUSPENSION

Strain	Mean ± SD [^{3}H]fibronectin bound (ng/10^9 cells)
S. aureus	1605 ± 216
S. sanguis	452 ± 80
S. faecalis	535 ± 76
C. albicans	22 ± 10
P. aeruginosa	23 ± 5
E. coli	10 ± 6

(26, 27, 30, 31, 34). As shown in the present experiments, these concepts may also be directly relevant to the initiation of infective endocarditis.

Since the vascular endothelium is the major source of circulating plasma fibronectin (18, 22, 23), the lack of this glycoprotein on the luminal surface of rabbit aortic valve (and other intracardiac) endothelial cells by immunofluorescence microscopy may appear unusual. Endothelial cells in tissue culture manufacture fibronectin, but the protein is expressed on the antiluminal surface to secure anchoring to the underlying basal lamina (46). Plasma fibronectin produced by the endothelium may be rapidly removed by the circulating blood. The lack of luminal surface fibronectin may actually serve as a protective mechanism for the vascular endothelium from microbial adhesion and the initiation of infection. Fibronectin expression after endothelial damage, e.g., NBTE formation, may conversely favor microbial adhesion with the development of infective endocarditis if valvular tissue is involved. These processes may be relevant to the pathogenesis of metastatic foci of infection elsewhere in the vasculature, but this hypothesis remains untested.

In most (if not all) cases, the adhesion of circulating microorganisms to the components of NBTE is a critical early event in the pathogenesis of infective endocarditis (1). The normal valvular endothelium is very resistant to colonization; it is nearly impossible to induce infective endocarditis in rabbits (and other experimental animals) unless NBTE formation precedes inoculation. NBTE thus serves as a nidus for colonization by microorganisms during a subsequent bacteremia. Infective endocarditis is a dynamic process and microorganisms may be constantly adhering to and being shed from NBTE during the constant low-grade bacteremia characteristic of this disorder. The present study suggests that fibronectin may mediate microorganism–NBTE interactions during these complex events.

Despite the relatively frequent occurrence of bacteremia or fungemia due to a myriad of microorganisms, the vast majority of cases of infective endocarditis are caused by a small number of species. Staphylococci and streptococci are still isolated from approximately 80% of patients with infective endocarditis involving native valves (47, 48). Furthermore, there is a heirarchy within each genus defining the relative propensity for a given organism to cause endocarditis in humans. For example, *C. tropicalis* or *C. albicans* are recovered from virtually all cases of endocarditis due to *Candida* sp.; *C. krusei* and others are very rarely involved (49). The relative propensity for endocarditis production correlates closely with the ability of the organism to produce endocarditis in rabbits with preexistent NBTE, as shown in Table II. The infectious dose for 50% of the animals (ID_{50}) were all $\leq 10^{5.78}$ for common etiologic agents of endocarditis (e.g., *S. aureus, C. tropicalis, C. albicans, S. faecalis,* and *S. sanguis*) but were much higher ($\geq 10^7$ CFU) for gram-negative aerobic bacilli, *S. pyogenes, S. epidermidis,* and *C. krusei.* This distribution closely parallels isolation frequencies from infective endocarditis of humans (47, 48). Furthermore, the distribution closely correlates with the relative adhesion of each microorganism to fibronectin *in vitro,* suggesting that adhesion to fibronectin within NBTE may influence, or determine, the striking proclivity of gram-positive cocci to produce endocarditis in experimental animal models and in humans. The precise cell surface characteristic(s) mediating this adhesion to fibronectin are unknown but extracellular dextrans (5, 8, 9), teichoic acid (10), mannan (11), and protein A have all been suggested. Further study is warranted in this important area.

Although the present studies suggest that fibronectin may mediate microorganism adhesion to NBTE *in vivo,* it is doubtful that fibronectin serves as the sole receptor in all cases. Other components of NBTE, e.g., platelets, are also known to bind bacteria (50). Fibrin or other constituents of the extracellular matrix forming NBTE may also be responsible. Furthermore, multiple bacterial binding sites to fibronectin may exist and tissue (not plasma) fibronectin may also play a role, especially on damaged endothelium. These concepts are under current investigation. In addition, fibronectin binds to some bacteria that are uncommon pathogens in infective endocarditis including groups A, C, and G streptococci and various coagulase-negative staphylococci (25, 28, 29, 32–34). This discrepancy between lack of endocarditis production and avid binding

to fibronectin *in vitro* remains unexplained but many other factors relating to host defense mechanisms or reversible affinity may be operative. Nevertheless, the experiments reported here suggest that microorganism adhesion to fibronectin within NBTE may mediate a crucial early step in the pathogenesis of infective endocarditis. In addition, the ID_{50} for *S. epidermidis* and *S. pyogenes* in this rabbit model is much greater than those noted for *S. aureus* and viridans streptococci, despite the known adhesion of *S. pyogenes* to fibronectin. Similar conclusions were reached recently by others employing a similar *in vitro* adhesion assay (51).

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