INFECTION AND IMMUNITY, Aug. 1982, p. 805–810
0019-9567/82/080805-06$02.00/0

Vol. 37, No. 2

Fibronectin-Mediated Binding of Group A Streptococci to Human Polymorphonuclear Leukocytes

W. ANDREW SIMPSON,[1,2,3] D. L. HASTY,[4] J. M. MASON,[3] AND E. H. BEACHEY[1,2,5]*

Veterans Administration Medical Center,[1] and Departments of Medicine,[2] Anatomy,[4] Pathology,[3] and Microbiology,[5] University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38104

Received 11 December 1981/Accepted 29 April 1982

Human plasma was shown to promote the attachment of an avirulent strain of *Streptococcus pyogenes* to human polymorphonuclear leukocytes. Removal of the fibronectin by absorption of the plasma with agarose-immobilized gelatin abolished the attachment-promoting activity. The activity of the absorbed plasma was restored with purified human serum fibronectin. The fibronectin was found to promote the attachment of three M protein-negative, but not of three M protein-positive, strains of streptococci. Radiolabeled fibronectin was shown to bind in various degrees to all strains of *S. pyogenes* tested. Thus, in the absence of streptococcal surface M protein, fibronectin promotes the association of group A streptococci with human polymorphonuclear leukocytes.

Serum fibronectin acts as a nonimmune opsonin against a variety of foreign particles, including denatured collagen (5), gelatin-coated colloidal carbon (10), and gelatin-coated latex beads (2). Whether fibronectin plays a role in the opsonization and clearance of bacteria remains to be established. Nevertheless, certain clinical evidence suggests that serum fibronectin may play a protective role against serious bacterial infections in patients who have suffered extensive burns, undergone major surgery, or suffered other forms of serious trauma (11). It has been shown, for example, that the administration of serum cryoprecipitates which are rich in fibronectin markedly reduced the incidence and duration of bacterial septicemia in victims of trauma. In vitro studies have shown that purified serum fibronectin binds to and agglutinates cells of *Staphylococcus aureus* (8) but not *Escherichia coli*, and preliminary studies suggest that fibronectin promotes the ingestion of *S. aureus* cells by phagocytic cells (R. A. Proctor, E. Pendergrost, and D. F. Mosher, Clin. Res. 27:650A, 1979).

In the present study we investigated the ability of human serum fibronectin to promote the association of group A streptococci (*Streptococcus pyogenes*) with polymorphonuclear leukocytes (PMNs) in vitro. We present data to show that although fibronectin was able to bind to both M protein-positive (M+) and M protein-negative (M−) strains of *S. pyogenes*, it promoted the association with PMNs only of the M− strains.

MATERIALS AND METHODS

Bacterial strains. Seven strains of group A streptococci were kindly provided by Rebecca Lancefield, The Rockefeller University, New York, and the type 1av and type 1V strains were kindly supplied by Roger Cole, National Institutes of Health, Bethesda, Md. Types 5, 19, and 24 group A streptococci as well as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are clinical isolates stored as stock strains in our laboratory. The Cowan I strain of *S. aureus* was obtained originally from Paul Quie of the University of Minnesota. All bacteria were grown in Todd-Hewitt broth for 16 h (stationary phase) and washed twice in ice-cold 0.02 M phosphate–0.15 M NaCl, pH 7.4 (PBS). The organisms used for the PMN binding studies were suspended in PBS and adjusted to an absorbancy of 0.7 at 530 nm ($\simeq 10^9$ bacteria per ml) in a Coleman Junior II spectrophotometer. Bacterial counts were performed with a Petroff-Hausser chamber or by quantitative colony counts on blood agar pour plates on all strains before and after treatment with fibronectin. No decrease in the number or viability of the organisms was noted.

Purification of fibronectin. All reagents used were of analytical grade and were obtained from commercial suppliers unless otherwise noted. Fibronectin was isolated from pooled human plasma salvaged from outdated blood which was obtained from the blood bank of the city of Memphis hospitals. Benzamidine (5 mM) and sodium azide (0.02%) were added to the plasma which was then centrifuged at 20,000 × *g* for 1 h to remove insoluble material. Fibronectin was purified by affinity chromatography on columns of gelatin and arginine covalently linked to cyanogen bromide-activated agarose beads (gelatin-agarose and arginine-agarose) according to the method of Vuento and Vaheri (13). To remove any agarose-binding proteins, 250 ml of plasma was filtered through a column (5 by 11 cm) of agarose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) equilibrated with 0.05 M Tris at pH 7.4 supplemented with 5 mM benzamidine and 0.02% sodium azide (Tris buffer). The eluted plasma was then applied to a column (1.5 by 20 cm) of gelatin-agarose which was washed first with 4 column volumes of Tris buffer followed by 4 column volumes of 1

M NaCl and then 2 column volumes of 0.2 M arginine in the same buffer. Finally, absorbed fibronectin was eluted with 1 M arginine, dialyzed extensively against Tris buffer, centrifuged at 10,000 × *g* for 10 min, and reapplied to a column (1.5 by 10 cm) of agarose-arginine. After washing with 3 column volumes of Tris buffer, the final product was eluted with 0.1 M NaCl in Tris buffer. Purity of the fibronectin preparations was monitored by electrophoresis in 5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate. Before electrophoresis, the samples were boiled for 2 min in 1% sodium dodecyl sulfate–5% β-mercaptoethanol. The purified preparation migrated as a closely spaced Coomassie blue-staining doublet consistent with an approximate molecular weight of 220,000 (8). No other Coomassie blue-staining band was detected.

Preparation of blood PMNs. Heparinized (10 U/ml) venous blood from healthy donors was sedimented with 6% dextran (Sigma Chemical Co., St. Louis, Mo.) for 30 min. The leukocyte-rich supernatant was layered over Ficoll-Hypaque (Pharmacia Fine Chemicals, Inc.) and centrifuged for 25 min at 800 × *g* (1). The pellet containing PMNs and erythrocytes was resuspended in an erythrocyte-lysing solution (0.15 M NH_4Cl, 0.01 M $KHCO_3$, 0.0001 M EDTA [pH 7.3]) and incubated for 5 min at 4°C. The PMNs were then washed two times with cold PBS and finally resuspended in PBS to the desired concentration. The purity of the PMN preparations was 91 ± 4.6% as determined by morphological and staining characteristics. PMN monolayers were prepared by incubating 50 μl of the PMN suspension (10^6/ml) on glass cover slips contained in a petri dish for 5 min at room temperature. Nonadherent cells were removed by washing the cover slips three times with ice-cold PBS. The cells were covered with 2 ml of ice-cold PBS and kept at 4°C until ready for use. Monolayers were used within 10 min of preparation.

Immunocytochemical procedures. The antiserum used to localize fibronectin was prepared in rabbits by immunization with purified human plasma fibronectin. New Zealand white rabbits were injected intracutaneously in multiple sites on the back of the neck with a 100-μg dose of fibronectin emulsified in complete Freund adjuvant. The rabbits were boosted 3 weeks later with a 100-μg dose of fibronectin emulsified in incomplete Freund adjuvant injected subcutaneously, and the serum was obtained at 2-week intervals thereafter. Immunoelectrophoresis and double immunodiffusion assays in agar gels with the immune serum against human plasma resulted in a single precipitin line, indicating that the antibody was directed against a single plasma protein.

Electron microscopy. Bacteria exposed to various concentrations of purified fibronectin under the conditions described above for the PMN association test were fixed at room temperature in 0.1% glutaraldehyde–4% paraformaldehyde buffered with 0.1 M sodium cacodylate (pH 7.3). After 1 h they were transferred to a solution containing buffered paraformaldehyde only and incubated at 4°C for approximately 18 h and then washed extensively with PBS containing 0.1 M glycine to block free aldehyde groups remaining bound to the organisms. The fixed bacteria were incubated with a 1:400 dilution of antiserum or preimmune serum overnight at 4°C, washed thoroughly with PBS, stained with a 1:1000 dilution of peroxidase-conjugated goat anti-rabbit immunoglobulin G (Cappel Laboratories, Cochranville, Pa.) for 1 h at ambient temperature, and thoroughly washed with PBS followed by 0.05 M Tris buffer (pH 7.2). The samples then were reacted with a freshly prepared solution of 0.05% 3,3'-diaminobenzidine hydrochloride (Electron Microscopy Sciences, Ft. Washington, Pa.)–0.0025% H_2O_2 for 10 to 12 min at 0°C, washed with Tris buffer, postfixed in 2.5% glutaraldehyde for 30 min and in 2% OsO_4 for 1 h, dehydrated, and embedded in Spurr plastic. Sections with and without lead citrate stain were examined with an AEI transmission electron microscope.

PMN association tests. A 1-ml amount of the bacterial suspension and 100 μl of PBS, with or without the test substance, were added to each monolayer (total volume, 1.1 ml); the monolayers were shaken at the lowest speed on a Fisher Clinical Rotator (60 rpm) at ambient temperature for 15 min. Unbound bacteria were removed by washing the monolayer three times with 2 ml of ice-cold PBS. The cells were fixed with 95% ethanol and stained for 6 min with Giemsa blood stain (Harleco, Philadelphia, Pa.), and the number of associated streptococci per PMN was counted. The results are expressed either as the number of streptococci bound per 100 PMNs or as a ratio of the number of bacteria bound in the presence of fibronectin to the number of bacteria bound in PBS alone. When binding assays were performed with streptococci pretreated with fibronectin, 20 μg of fibronectin was added to 1 ml of PBS containing streptococci adjusted to an absorbancy of 0.2 at 530 nm. Concentrations of fibronectin above 20 μg/ml caused significant agglutination of the streptococci and were not studied. The sample was incubated for 1 h at 37°C, washed twice with ice-cold PBS, and finally resuspended in sufficient PBS to give an absorbancy of 0.2 at 530 nm. One milliliter of pretreated and washed streptococci was added to each monolayer, and the association assay was performed as described above. All experiments were repeated at least three times, and representative results are reported.

Binding studies. Purified fibronectin was labeled with [^{3}H]formaldehyde (specific activity, 100 mCi/mmol; New England Nuclear Corp., Boston, Mass.) according to the procedure of Grinnel (4). Initial studies indicated that labeled fibronectin could not be distinguished from native fibronectin in the PMN adherence assay. The organisms used for the binding studies were washed and resuspended in ice-cold PBS at a concentration of 10^9 bacteria per ml. A 0.5-ml amount of the bacterial suspension was mixed with 5 μg of [^{3}H]formaldehyde-labeled fibronectin (≃5000 cpm/μg) in a total volume of 1 ml and incubated for 1 h at 37°C. All organisms were centrifuged at 12,000 × *g* for 30 s in a microcentrifuge (Eppendorf) and washed twice with PBS. The washed pellet was resuspended in 1 ml of distilled water, and the amount of bound fibronectin was determined with a liquid scintillation counter (Hewlett-Packard, Palo Alto, Calif.). Preliminary binding studies with types 1av and 5 streptococci indicated that the binding of fibronectin increased linearly with increasing numbers of streptococci, reached saturation with increasing concentrations of fibronectin, reached maximal binding within 30 min at 37°C, and could be inhibited by inclusion of unlabeled fibronectin in the assay. Quench controls consisted of a known amount of [^{3}H]formaldehyde-labeled fibro-

TABLE 1. Fibronectin-mediated association of type 1av streptococci with human PMNs

Streptococci suspended in:	Total protein (mg)[a]	Fibronectin (μg)[b]	No. of streptococci adherent per 100 PMNs
Fresh plasma	2.5	20	>1,000
Control plasma[c]	2.5	20	21
Fibronectin-depleted plasma	2.5	<1	2
Fibronectin-reconstituted plasma	2.5	20	14
PBS	0	0	2

[a] Protein was estimated by the method of Lowry.
[b] Fibronectin was estimated by unidimensional electrophoresis in antibody-containing gels.
[c] Control plasma was obtained from outdated blood and passed over three columns of agarose.

nectin plus bacteria and water equivalent to the amounts used in each experiment. The bacteria were inspected and counted microscopically before and after fibronectin treatment to exclude artifacts caused by aggregation or loss of bacteria.

RESULTS

Effect of fibronectin on association of streptococci with PMNs. Depletion of fibronectin reduced the ability of plasma to promote the attachment of type 1av streptococci to PMNs to levels observed when buffer alone was added to the assay (Table 1). Reconstitution of the depleted plasma with purified human fibronectin restored the ability of the depleted plasma to promote binding of streptococci to 63% of the level of control plasma, indicating that fibronectin may play a role in promoting the adherence of type 1av streptococci to human PMNs. As expected, fresh plasma was the most effective promoter of PMN-streptococcus association (Table 1).

Several strains of M− and M+ streptococci were tested for their ability to bind to PMNs in the presence of purified fibronectin or PBS. M− strains (types 1av, D58X, and 1RP41) showed increased binding to PMNs ($\bar{x}$ = 313%; range, 200 to 400%) in the presence of fibronectin whereas M+ strains showed no increased binding compared with PBS controls ($\bar{x}$ = 87%; range, 75 to 98%).

When type 1av streptococci were pretreated with fibronectin (20 μg/ml) in PBS for 1 h at 4°C and washed three times before incubation with the PMN monolayer, nearly a 22-fold increase in binding was observed (Table 2). Pretreatment of the M protein-rich type 24 strain with fibronectin produced only a slight (2.5) increase in association.

TABLE 2. Effect of pretreatment of streptococci with fibronectin on association with PMNs

Streptococcal type	Treatment	Streptococci per 100 PMNs
1av	PBS	0.8
	Fibronectin[a]	17.5
24	PBS	2.2
	Fibronectin[a]	5.5

[a] The concentration of fibronectin was 20 μg/ml.

Electron microscopy. When streptococci were incubated with plasma or purified fibronectin, the bound fibronectin was identified on the surface of the organisms by immunocytochemical techniques. The typical globular reaction product of horseradish peroxidase was observed on the surface of the organisms exposed to immune rabbit serum (Fig. 1a). Figures 1a and b are photomicrographs made from unstained sections. Both type 1av (not shown) and type 24 (shown in Fig. 1) showed similar patterns of staining after incubation with fibronectin and horseradish peroxidase-labeled antiserum.

The heavy staining observed on the cytoplasmic membrane (Fig. 1a) may be an artifact due to diffusion of the reaction product and absorption to the membrane. Similar artifacts have been reported by several other investigators (3).

Binding studies with radiolabeled fibronectin. Because fibronectin was demonstrated by electron microscopy on the surface of both M+ and M− streptococci, we performed binding assays to determine whether the differences observed in the PMN association assays were related to differences in the amounts of fibronectin bound by the various strains. Initial binding studies, with 5 μg of [^{3}H]formaldehyde-labeled fibronectin and 10^9 type 1av streptococci, indicated that maximum binding occurred within 30 min at room temperature. These assay conditions were used to determine the relative amount of fibronectin binding to each strain studied (Table 3). All of the gram-positive organisms tested bound radiolabeled fibronectin, whereas the two gram-negative organisms did not.

An M− strain (1RP41) and an M+ strain (type 1V) bound the highest amounts of fibronectin; type 1av (M−) and type 24 (M+) bound the least amounts of fibronectin in this assay. Thus, the presence or absence of M protein on the surface

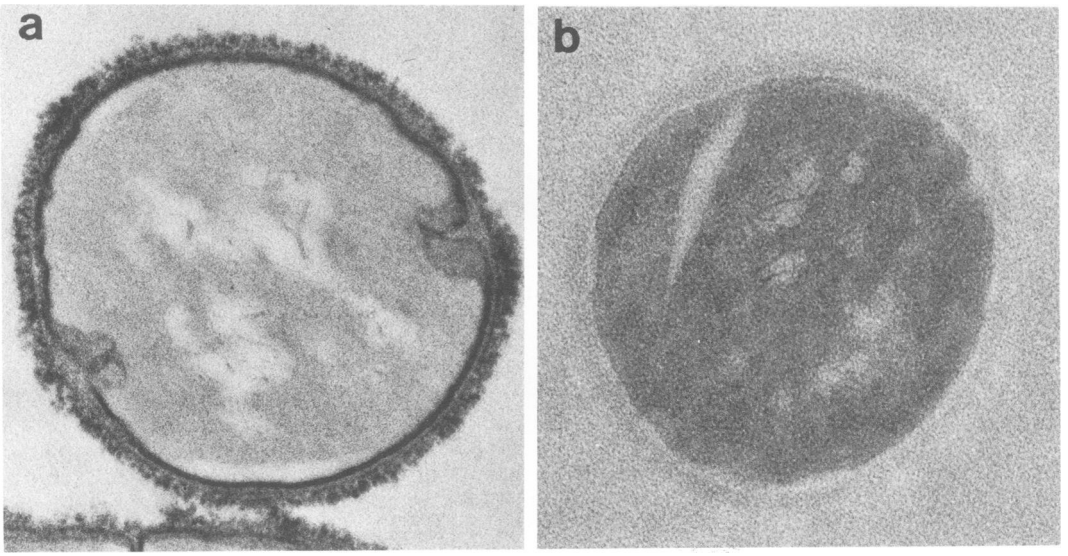

FIG. 1. Electron micrographs of ultrathin sections of group A streptococci (type 24) treated with fibronectin and (a) rabbit anti-human fibronectin or (b) normal rabbit serum. Both were then treated with horseradish peroxidase-labeled goat anti-rabbit immunoglobulin G. The sections were photographed without further staining to emphasize the peroxidase reaction product deposited on (a).

of the bacteria did not appear to influence the amount of fibronectin bound.

DISCUSSION

Our studies indicate that the removal of gelatin-binding proteins from plasma by exhaustive absorption decreased the ability of the plasma to promote the binding of streptococci to PMNs. Moreover, most of the original activity of the plasma could be restored by adding purified fibronectin to the depleted plasma.

The results are in agreement with a recent study which indicated that fibronectin is necessary for the optimal serum-mediated phagocytosis of *S. aureus* by human and rat neutrophils (M. E. Lanser and T. M. Saba, Circ. Shock **8**:209, 1981), although more recently, Verbrugh et al. (12) concluded that fibronectin did not promote phagocytosis of bacteria by human neutrophils, monocytes, or macrophages. It must be stressed, however, that Verbrugh et al. (12) were careful to preserve complement activity and that the conditions of their assay were designed to allow phagocytosis of the bacteria by the three populations of phagocytic cells studied. In our studies, plasma salvaged from outdated blood and passed over three chromatography columns at ambient temperature was used. Indeed, when fresh plasma was added to our test system, the association of bacteria with the PMN monolayers increased severalfold and may have easily hidden the differences in association observed. It should be emphasized that we do not mean to imply that fibronectin plays a major role in the opsonization of group A streptococci. However, since our data indicate that fibronectin promotes the association of M− streptococci with PMNs, it is possible that this serum glycoprotein works in concert with other serum components for the efficient opsonization of invading gram-positive bacteria.

Perhaps the most interesting observation presented in this work is the demonstration that fibronectin binds in significant amounts to some strains of *S. pyogenes* that bear M protein on their surface without effectively promoting their attachment to PMNs. It is not surprising that

TABLE 3. Binding of radiolabeled fibronectin to bacteria

Bacteria	Strain	^{3}H-labeled fibronectin bound (ng) per 10^9 bacteria[a]
S. pyogenes	D58X	781 ± 89
	Type 1av	63 ± 5
	1RP41	1,089 ± 4.2
	Type 1V	922 ± 13
	Type 5 (Manfredo)	461 ± 61
	Type 19	510 ± 32
	Type 24 (Vaughn)	169 ± 35
S. aureus	Cowan I	1,079 ± 5.8
P. aeruginosa	Clinical isolate	10 ± 3
K. pneumoniae	Clinical isolate	7 ± 2

[a] The values are recorded as the mean of triplicate assays ± one standard deviation.

organisms expressing M protein were unaffected by purified fibronectin because these virulent streptococci are characterized by their innate ability to grow in whole human blood (7). Indeed, the only effective opsonin known for these bacteria is antibody against the M protein molecule in conjunction with classical complement components (7). It has been shown previously (5a) that the C3 component of complement also binds to the surface of M+ streptococci in the absence of M antibodies in quantities that are sufficient to opsonize M− strains without promoting phagocytosis of the former. Thus, at least two proteins which are opsonic for M− streptococci bind avidly to M+ strains without overcoming the antiphagocytic effects of the M protein. The mechanism of the antiphagocytic action of M protein remains unclear. It is apparent, however, that its presence does not interfere with the binding of fibronectin. The binding studies presented here were designed only to ascertain relative binding of fibronectin. Nothing is known about either the affinity or the number of binding sites for fibronectin on the strains studied, nor is it known whether the binding sites for purified fibronectin on M+ and M− streptococci are the same. However, the data clearly demonstrate that binding of fibronectin to bacteria is not sufficient to increase phagocytic recognition of the bacteria. A similar disparity recently has been reported in preliminary form for another gram-positive organism, *S. aureus* (J. E. Doran, R. H. Raynor, A. C. Reese, and H. T. Edmondson, Fed. Proc. **40**:3063, 1981). They reported that fibronectin promoted phagocytosis only of *S. aureus* strains lacking protein A, yet the binding of fibronectin to the protein A-bearing Cowan I strain of *S. aureus* has been extensively characterized (9).

Our studies show that fibronectin does not bind to gram-negative organisms which is in agreement with preliminary studies of *E. coli* (6) and *P. aeruginosa* (J. Sadoff, S. Futrosky, S. Peyser, U. Haynes, and H. Colins, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, B16, p.17). These investigators, furthermore, were unable to show a clear-cut relationship between fibronectin levels and either phagocytosis of, or infections produced by, *P. aeruginosa* in an experimental rat model of burn injury. Thus, the accumulated data suggest that serum fibronectin may be important only in defense against certain strains of gram-positive bacteria.

Fibronectin may play an important role in the ecology of the indigenous flora of the upper respiratory tract. Woods et al. have recently shown that *P. aeruginosa* adhere in high numbers to oral epithelial cells from patients with cystic fibrosis (14) and seriously ill patients in intensive care units (15) and that this increased adherence is correlated with a greatly reduced level of fibronectin on the epithelial cell surfaces. We have recently found that group A streptococci adhere only to populations of oral epithelial cells exhibiting fibronectin on their surfaces and that this adherence can be blocked by the addition of purified fibronectin (Simpson and Beachey, manuscript in preparation), suggesting that fibronectin may be an important modulator of bacterial adherence and colonization as well as a nonimmune modulator of the phagocytic defenses of the host.

ACKNOWLEDGMENTS

These studies were supported by program-directed research funds from the U.S. Veterans Administration and by Public Health Service research grants AI-10085 and AI-13550 from the National Institutes of Health. E.H.B. is the recipient of a Medical Investigatorship Award from the U.S. Veterans Administration, and D.L.H. and W.A.S. are the recipients of Public Health Service Young Investigatorship Awards (DE-05062 and DE-05773, respectively) from the National Institutes of Health.

We thank J. B. Dale for his helpful comments and discussions, Ella Pinals, Harry Courtney, Loretta Hatmaker, Ellen Looney, and Nancy Foster for technical assistance, and Johnnie Smith for secretarial assistance.

LITERATURE CITED

1. **Courtney, H., I. Ofek, W. A. Simpson, and E. H. Beachey.** 1981. Characterization of lipoteichoic acid binding to polymorphonuclear leukocytes of human blood. Infect. Immun. **32**:625–631.
2. **Doran, J. E., A. R. Mansberger, H. T. Edmondson, and A. C. Reese.** 1981. Cold insoluble globulin and heparin interactions in phagocytosis by macrophage monolayers: lack of heparin requirement. Res. J. Reticuloendothel. Soc. **29**:275–283.
3. **Goldfischer, S.** 1980. Effusion antifact: a chronic problem in DAB cytochemistry. J. Histochem. Cytochem. **28**:1360–1361.
4. **Grinnel, F.** 1980. Fibroblast receptor for cell-substratum adhesion: studies on the interaction of baby hamster kidney cells with latex beads coated by cold insoluble globulin (plasma fibronectin). J. Cell Biol. **86**: 104–112.
5. **Hopper, K. E., B. C. Adelmann, G. Gentner, and S. Gay.** 1976. Recognition by guinea-pig peritoneal exudate cells of conformationally different states of the collagen molecule. Immunology **30**:249–259.

5a. **Jadss-Weiss, J., Y. Kim, and P. P. Cleary.** 1982. Restricted deposition of C3 on M^+ group A streptococci: correlation with resistance to phagocytosis. J. Immunol. **128**:1897–1902.

6. **Kuusela, P.** 1978. Fibronectin binds to *Staphylococcus aureus*. Nature (London) **276**:718–720.
7. **Lancefield, R. C.** 1962. Current knowledge of type-specific M antigens of group A streptococci. J. Immunol. **89**:207–313.
8. **Mosher, D. F.** 1980. Fibronectin, p. 111–151. *In* T. H. Spate (ed.), Progress in hemostasis and thrombosis-5. The Grune Co., New York.
9. **Mosher, D. F., and R. A. Proctor.** 1980. Binding and factor $X111_a$-mediated cross-linking of a 27-kilodalton fragment of fibronectin to *Staphylococcus aureus*. Science **209**:927–929.
10. **Pardy, B. J., R. C. Spencer, and H. A. F. Dudley.** 1977. Hepatic reticuloendothelial protection against bacteremia in experimental hemorrhagic shock. Surgery **81**:193–197.
11. **Saba, T. M., F. A. Blumerstock, W. A. Scovill, and H. Bernard.** 1978. Cryoprecipitate reversal of opsonic α_2-

surface binding glycoprotein deficiency in septic surgical and trauma patients. Science **201**:622–624.

12. **Verbrugh, H. A., P. K. Peterson, D. E. Smith, B. T. Nguyen, J. R. Hoidal, B. J. Wilkinson, J. Verhoef, and L. T. Furcht.** 1981. Human fibronectin binding to staphylococcal surface protein and its relative inefficiency in promoting phagocytosis by human polymorphonuclear leukocytes, monocytes, and alveolar macrophages. Infect. Immun. **33**:811–819.
13. **Vuento, M., and A. Vaheri.** 1979. Purification of fibronectin from human plasma by affinity chromatography under nondenaturing conditions. Biochem. J. **183**:331–337.
14. **Woods, D. E., J. A. Bass, W. G. Johanson, Jr., and D. C. Straus.** 1980. Role of adherence in the pathogenesis of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. Infect. Immun. **30**:694–699.
15. **Woods, D. E., D. C. Straus, W. G. Johanson, and J. A. Bass.** 1981. Role of fibronectin in the prevention of adherence of *Pseudomonas aeruginosa* to buccal cells. J. Infect. Dis. **143**:784–790.