INFECTION AND IMMUNITY, May 1996, p. 1876–1878
0019-9567/96/$04.00+0
Copyright © 1996, American Society for Microbiology
Vol. 64, No. 5

Reconsideration of the Role of Fibronectin Binding in Endocarditis Caused by *Staphylococcus aureus*

J.-I. FLOCK,[1,2]* S. A. HIENZ,[1,2] A. HEIMDAHL,[2] AND T. SCHENNINGS[2]

Department of Microbiology, Immunology, Pathology, and Infectious Diseases[1] and Department of Oral Surgery,[2] Karolinska Institutet, Huddinge University Hospital, S-141 86 Huddinge, Sweden

Received 26 October 1995/Returned for modification 15 December 1995/Accepted 9 February 1996

The adherence characteristics in vivo and virulence of two isogenic strains of *Staphylococcus aureus* differing in fibronectin binding were compared in a rat model of catheter-induced infective endocarditis. No differences were found between the two strains. The results strongly point to the multifactorial nature of bacterial adherence to damaged heart valves and suggest that other binding functions can compensate for the lack of fibronectin binding in *S. aureus*.

Infective endocarditis is strongly correlated with the preexistence of nonbacterial thrombotic endocarditis, indicated by the presence of sterile vegetations on the heart valves. Vegetations are usually found on the left side of the heart affecting the mitral and aortic valves and consist of platelets and fibrin adherent to the subendothelial matrix as a result of endothelial cell damage (23). Since circulating fibronectin, vitronectin, plasmin, and other plasma proteins can bind to fibrinogen, the composition of vegetations is very complex.

Bacteremia in patients with valvular vegetations can easily lead to infective endocarditis (IE), and once attached to the vegetations, the bacteria will propagate and stimulate enlargement of the vegetations (4, 18).

Main causative agents of IE are staphylococci. *Staphylococcus aureus* has been reported to specifically bind to fibronectin (5, 11, 20), fibrinogen (1, 14), collagen (2, 17), elastin (16), laminin (22), prothrombin (1), vitronectin (3, 13), and thrombospondin (9). The identification of a fibronectin-binding protein on the surface of *S. aureus* and the presence of fibronectin in the sterile vegetations and on the subendothelial matrix led to the reasonable assumption that fibronectin binding is a major factor contributing to IE caused by *S. aureus* (7). This assumption was supported by a study by Kuypers and Proctor (12) in which an *S. aureus* strain with reduced fibronectin-binding capability showed reduced adherence to valvular vegetations. The degrees of virulence of two isogenic strains of *S. aureus*, one carrying insertional inactivations of the genes encoding fibronectin-binding proteins, were compared in a rat model of catheter-induced IE.

S. aureus 8325-4 has two closely linked genes for fibronectin

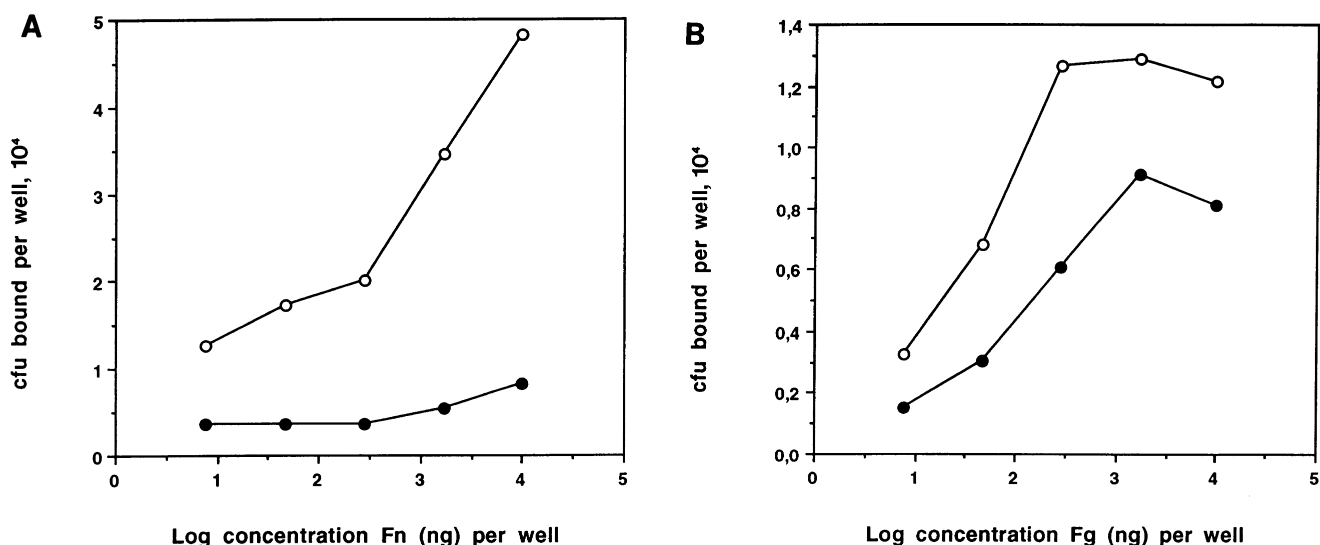

FIG. 1. Bacterial binding to fibronectin (A) and fibrinogen (B). Microtiter plates were coated with the indicated concentrations of fibronectin or fibrinogen, 5×10^6 CFU of radiolabelled *S. aureus* 8325-4 (○) or DU 5883 (●) was added, and the number of bound CFU was estimated.

* Corresponding author. Mailing address: Department of Microbiology, Immunology, Pathology and Infectious Diseases, Section for Oral Microbiology, Karolinska Institutet, Huddinge University Hospital F88, S-141 86 Huddinge, Sweden. Phone: 46 8 746 5032. Fax: 46 8 711 3918. Electronic mail address: jifl@bimd01.hs.sll.se.

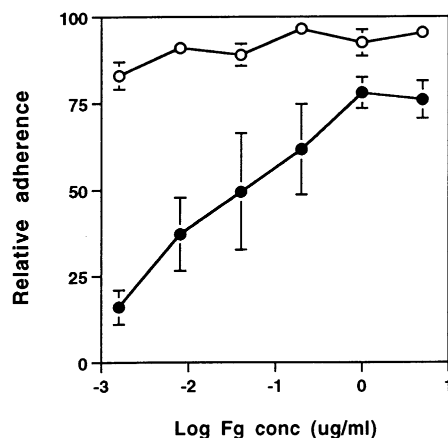

FIG. 2. Bacterial binding to fibronectin and fibrinogen mixed. Fibronectin (10 μg/ml) mixed with fibrinogen at the indicated concentrations was used to coat microtiter plates, 10^8 CFU of *S. aureus* 8325-4 (○) or DU 5883 (●) was added, and the relative adherence was determined with a microplate reader (A_{405}). The means and standard errors (error bars) of triplicate determinations are shown.

binding, *fnbA* and *fnbB* (10), that have been inactivated by insertion mutagenesis (6). Both genes are expressed and both contribute to the ability of 8325-4 to adhere to solid-phase fibronectin (6). The *fnbA fnbB* double mutant, strain DU 5883, was defective in adherence to fibronectin but not to fibrinogen (Fig. 1). Bacteria were radiolabelled and grown in Luria-Bertani medium containing 20 μCi of [^{3}H]thymidine (Amersham, Amersham, United Kingdom) per ml for 5 h. The specific activity was 80 to 110 CFU/cpm. Microtiter wells (Costar, Cambridge, Mass.) were coated with fibronectin (Sigma Chemical Co., St. Louis, Mo.) or fibrinogen (Kabi, Stockholm, Sweden) at room temperature overnight and blocked with 2% bovine serum albumin (ICN Biomedicals Ltd., High Wycombe, United Kingdom) for 1 h. Bacteria (5×10^6 CFU) in phosphate-buffered saline with 0.05% Tween 20 were added to each well and incubated for 2 h at 37°C. The wells were washed, and the numbers of adherent bacteria were estimated by scintillation counting. Different concentrations of bacteria yielded equivalent results.

The two strains were also tested for their adherence to a mixture of fibronectin and fibrinogen. The same steps were followed as described above for the adhesion assay. However, nonradiolabelled bacteria were used and the amount of adherent microorganisms was determined in a microplate reader (A_{405}). This assay gave results comparable to those obtained by the method described above. Briefly, bacteria were allowed to adhere to microtiter wells that had been previously coated with a constant amount of fibronectin (10 μg/ml) mixed with a dilution of fibrinogen, ranging from 0.002 to 5 μg/ml. Nonadherent bacteria were washed away, and the amount of light absorbance of the remaining bacteria was determined with a microplate reader. This method yielded a satisfactory signal-to-background ratio. With increasing fibrinogen concentration, the difference between the two strains became smaller, and at a fibrinogen concentration of ≈10% of the fibronectin concentration, mutant strain DU 5883 reached the same level of adherence as 8325-4, implying that fibrinogen is more efficient than fibronectin in stimulating adherence of 8325-4 (Fig. 2). We assume this is also the case in an in vivo setting.

The adherence of 8325-4 and DU 5883 to traumatized aortic and nondamaged pulmonary heart valves was studied. Rats were catheterized via the right carotid artery as described previously (8, 19). Twenty-four hours after catheterization, the catheters were removed or left in place, and the animals were challenged intravenously with inocula of gradually increasing sizes.

The adherence capability of hematogenously spread bacteria to traumatized heart valves was determined after 1 h. Secondly, we determined the rate of infection and bacterial counts after 24 h, which is a measure of the ability of the bacteria to adhere, propagate, and escape host defense mechanisms. To exclude a possible difference in the clearance rates of strains 8325-4 and DU 5883, two rats were injected intravenously with a 1:1 mixture of 5×10^7 CFU of each strain. Blood cultures were then taken every 10 min for 1 h from the right carotid artery. No difference in the clearance rates of 8325-4 and DU 5883 was detected.

The adherence ratio, defined as the number of bound bacteria per number of injected bacteria expressed as log CFU values, was found to be the same for the two strains on the aortic valve ($P = 0.3$ at a challenge dose of 1.2×10^8 CFU; $P = 0.1$ at 5×10^6 CFU) and was independent of the number of bacteria injected (Table 1). As expected, the adhesion ratio was much lower for the pulmonary valve than for the aortic valve, and no difference in the adhesion capabilities of the two strains was found ($P = 0.07$) (Table 1). In addition, the adhesion ratios were not influenced by the presence of the catheter prior to intravenous challenge (Table 1). Thus, it seems that neither deposition of fibronectin on the catheter nor a disturbed blood flow through the valves influenced the adherence of the microorganisms.

Our results, indicating that there is no difference between the mutant and its parent strain, are inconsistent with the findings by Kuypers and Proctor (12), who used a transposon-mutagenized strain with reduced adherence to fibronectin (21) in a similar experiment. Deducing the adherence ratios from the data in Kuypers and Proctor's study and comparing it with the ratios from our study can only be weak at best. The adherence ratio of the fibronectin-binding strain of Kuypers and Proctor was −1.87, or 1.3%. However, in our study, we generally found adherence ratios at the aortic valves that ranged

TABLE 1. Number of bacteria recovered from aortic and pulmonary valves of rats at 1 h postchallenge with different *S. aureus* strains

S. aureus strain	Challenge dose (log CFU)	Mean log CFU recovered ± SE (adherence ratio)			
		Aortic valve		Pulmonary valve	
		Catheter removed	Catheter in place	Catheter removed	Catheter in place
DU 5883	6.70	1.90 ± 0.17 (−4.80) (*n* = 16)			
8325-4	6.70	2.26 ± 0.10 (−4.44) (*n* = 12)			
DU 5883	8.08	3.34 ± 0.08 (−4.74) (*n* = 13)	3.43 ± 0.11 (−4.65) (*n* = 9)	1.84 ± 0.14 (−6.24) (*n* = 13)	1.91 ± 0.19 (−6.17) (*n* = 9)
8325-4	8.08	3.11 ± 0.20 (−4.97) (*n* = 14)	3.22 ± 0.15 (−4.86) (*n* = 10)	1.42 ± 0.18 (−6.66) (*n* = 14)	1.80 ± 0.12 (−6.28) (*n* = 10)

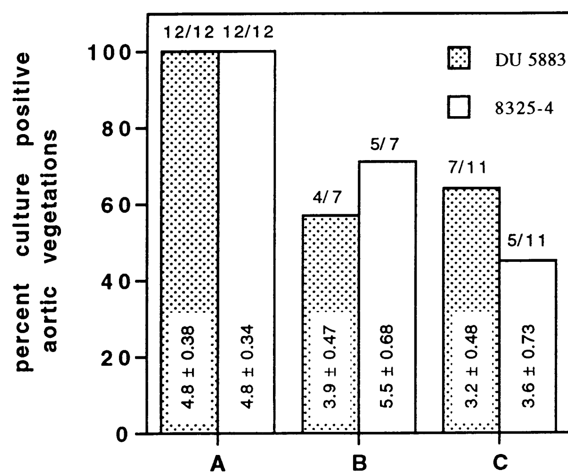

FIG. 3. Rate of infection as a function of inoculum size. The percentage of animals with endocarditis, defined as culture-positive vegetations, was plotted for each inoculum size: 3×10^6 (A), 1×10^5 (B), and 4×10^4 CFU (C). The numbers above the bars indicate the number of infected animals per the total number of animals in a specific group. The numbers within the bars are mean log CFUs and standard errors of culture-positive vegetations.

between −4.97 and −3.74 for DU 8553, 8325-4 (Table 1), and several other strains (data not shown).

In another experiment, inoculum sizes of 4×10^4, 1×10^5, and 3×10^6 CFU of both strains were used. The catheters were removed before inoculation, and the animals were sacrificed 24 h postchallenge. The percentages of animals with endocarditis, defined by culture-positive vegetations, were determined. The rates of infection were similar for both strains in all experiments (Fig. 3). The mean values of log CFU of the positive vegetations, shown in Fig. 3, did not differ significantly between the strains ($P > 0.12$).

In conclusion, fibronectin binding alone is not enough to promote adhesion of *S. aureus* 8325-4 to heart valve vegetations. This finding indicates that bacterial adherence is a multifactorial event and that other binding functions, such as binding to fibrinogen, vitronectin, or thrombospondin, might compensate for the lack of fibronectin binding. Elimination of the clumping factor responsible for adherence to fibrinogen was recently shown to reduce virulence in IE (15).

This work was supported by a grant from the Swedish Medical Research Council (B95 16X 09092-06B) and the Einar Belvén Foundation.

Ingegerd Löfving-Arvholm and Carin Coster are gratefully acknowledged for skillful technical assistance.

REFERENCES

1. **Bodén, M., and J.-I. Flock.** 1992. Evidence for three different fibrinogen-binding proteins with unique properties from *Staphylococcus aureus* strain Newman. Microb. Pathog. **12:**289–298.
2. **Buxton, T. B., J. P. Rissing, J. A. Horner, K. M. Plowman, D. F. Scott, T. J. Sprinkle, and G. K. Best.** 1990. Binding of a *Staphylococcus aureus* bone pathogen to type I collagen. Microb. Pathog. **8:**441–448.
3. **Chhatwal, G. S., K. T. Preissner, G. Muller-Berghaus, and H. Blobel.** 1987. Specific binding of the human S (vitronectin) protein to streptococci, *Staphylococcus aureus*, and *Escherichia coli*. Infect. Immun. **55:**1878–1883.
4. **Durack, D. T., and P. B. Beeson.** 1972. Experimental bacterial endocarditis. I. Colonization of a sterile vegetation. Br. J. Exp. Pathol. **53:**44–49.
5. **Flock, J.-I., G. Fröman, K. Jonsson, B. Guss, C. Signäs, B. Nilsson, G. Raucci, M. Höök, T. Wadström, and M. Lindberg.** 1987. Cloning and expression of the gene for a fibronectin-binding protein from *Staphylococcus aureus*. EMBO J. **6:**2351–2357.
6. **Greene, C., D. McDevitt, P. Francois, P. Vaudaux, D. P. Lew, and T. Foster.** 1995. Adhesion properties of mutants of *Staphylococcus aureus* defective in fibronectin binding proteins and studies on the expression of *fnb* genes. Mol. Microbiol. **17:**1143–1152.
7. **Hamill, R.** 1987. Role of fibronectin in infective endocarditis. Rev. Infect. Dis. **9:**360–371.
8. **Héraïef, E., M. P. Glauser, and F. R. Freedman.** 1982. Natural history of aortic valve endocarditis in rats. Infect. Immun. **37:**127–131.
9. **Herrmann, M., S. J. Suchard, L. A. Boxer, F. A. Waldvogel, and P. D. Lew.** 1991. Thrombospondin binds to *Staphylococcus aureus* and promotes staphylococcal adherence to surfaces. Infect. Immun. **59:**279–288.
10. **Jönsson, K., C. Signäs, H.-P. Muller, and M. Lindberg.** 1991. Two different genes encode fibronectin binding proteins in *Staphylococcus aureus*: the complete nucleotide sequence and characterization of the second gene. Eur. J. Biochem. **202:**1041–1048.
11. **Kuusela, P.** 1978. Fibronectin binds to *Staphylococcus aureus*. Nature (London) **276:**718–720.
12. **Kuypers, J., and R. Proctor.** 1989. Reduced adherence to traumatized rat heart valves by a low fibronectin binding mutant of *Staphylococcus aureus*. Infect. Immun. **57:**2306–2312.
13. **Liang, O., J.-I. Flock, and T. Wadström.** 1994. Evidence that the heparin-binding consensus sequence of vitronectin is recognized by *Staphylococcus aureus*. J. Biochem. **116:**457–463.
14. **McDevitt, D., P. Vaudaux, and T. J. Foster.** 1992. Genetic evidence that bound coagulase of *Staphylococcus aureus* is not clumping factor. Infect. Immun. **60:**1514–1523.
15. **Moreillon, P., J. Entenza, P. Francioli, D. McDevitt, T. Foster, P. Francois, and P. Vaudaux.** 1995. Role of *Staphylococcus aureus* coagulase and clumping factor in pathogenesis of experimental endocarditis. Infect. Immun. **63:** 4738–4743.
16. **Park, P. W., D. D. Roberts, L. E. Grosso, W. C. Parks, J. Rosenbloom, W. R. Abrams, and R. P. Mecham.** 1991. Binding of elastin to *Staphylococcus aureus*. J. Biol. Chem. **266:**23399–23406.
17. **Patti, J. M., J. Boles, and M. Höök.** 1993. Identification and biochemical characterization of the ligand binding domain of the collagen adhesin from *Staphylococcus aureus*. Biochemistry **32:**11428–11435.
18. **Scheld, W. M., R. W. Strunk, G. Balian, and R. A. Calderone.** 1985. Microbial adhesion to fibronectin in vitro correlates with production of endocarditis in rabbits. Proc. Soc. Exp. Biol. Med. **180:**474–482.
19. **Schennings, T., A. Heimdahl, K. Coster, and J.-I. Flock.** 1993. Immunization with fibronectin binding protein from *Staphylococcus aureus* protects against experimental endocarditis in rats. Microb. Pathog. **15:**227–236.
20. **Signäs, C., G. Raucci, K. Jönsson, P.-E. Lindgren, G. M. Anantharamaiah, M. Höök, and M. Lindberg.** 1989. Nucleotide sequence of the gene for a fibronectin-binding protein from *Staphylococcus aureus* and its use in the synthesis of biologically active peptides. Proc. Natl. Acad. Sci. USA **86:** 699–703.
21. **Vaudaux, P., P. Francois, R. Proctor, D. McDevitt, T. J. Foster, R. M. Albrecht, D. P. Lew, H. Wabers, and S. L. Cooper.** 1995. Use of adhesion-defective mutants of *Staphylococcus aureus* to define the role of specific plasma proteins in promoting bacterial adhesion to canine arteriovenous shunts. Infect. Immun. **63:**585–590.
22. **Vercelotti, G. M., P. McCarthy, P. Lindholm, P. K. Peterson, H. S. Jacob, and L. T. Furcht.** 1985. Extracellular matrix proteins (fibronectin, laminin, and type IV collagen) bind and aggregate bacteria. Am. J. Pathol. **120:**13–21.
23. **Vlodavsky, I., A. Eldor, E. HyAm, R. Atzmon, and Z. Fuks.** 1982. Platelet interaction with the extracellular matrix produced by cultured endothelial cells: a model to study the thrombogenicity of isolated subendothelial basal lamina. Thromb. Res. **28:**179–191.

Editor: V. A. Fischetti