0022-202X/83/8006-0494$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 80:494–496, 1983

Vol. 80, No. 6
Printed in U.S.A.

The *Staphylococcus aureus* Receptor for Fibronectin

DEBRA JAN BIBEL, PH.D., RAZA ALY, PH.D., HENRY R. SHINEFIELD, M.D., AND HOWARD I. MAIBACH, M.D.

Department of Dermatology, University of California, San Francisco, and Department of Pediatrics, Kaiser/Permanente Medical Center, San Francisco, California, U.S.A.

The *Staphylococcus aureus* binding site for human fibronectin was determined by unique biologic assays based upon the specific adherence of the bacterium to nasal epithelial cells. Fibronectin treatment of *S. aureus* caused a reduction in adherence tallies on high granular and fully keratinized cells compared to controls ($p < 0.05$; $p < 0.001$). Spinous and low granular cells showed no significant differences. The cell wall materials N-acetyl-D-glucosamine, N-acetyl muramic acid, and protein A were unable to inhibit the coupling of fibronectin to *S. aureus*. Only ribitol teichoic acid had this property. Furthermore, fibronectin could neutralize the adherence-blocking ability of teichoic acid, which affects keratinized cells. Thus, teichoic acid seems to be a receptor for fibronectin.

The ability of human fibronectin to bind to *Staphylococcus aureus* has been recently reported [1,2]. Fibronectin is a 200,000-dalton glycoprotein found in the plasma and on the surface of connective tissue, basement membranes, and certain epithelial cells [3–5]. Studies have suggested that among other functions it may serve as a cell receptor in bacterial adherence and as an opsonin [3]. The coupling with *S. aureus* is specific, since only trace amounts of fibronectin were found on *Mycobacterium butyricum* and *Escherichia coli* [1,3]. Furthermore, *Pseudomonas aeruginosa* is also unaffected, for an increase in its attachment to buccal cells has been correlated with the loss of fibronectin from the cell surface [5].

The molecular identity of the binding site on *S. aureus* is still unresolved, but protein A appears to be the leading candidate [2]. In this report, we demonstrate through a unique biologic assay that teichoic acid is a staphylococcal receptor for fibronectin.

MATERIALS AND METHODS

Bacterium

S. aureus stain 502A was grown 18 h at 37°C in Tryptic soy broth (*DIFCO*). Prior to testing, the culture was centrifuged, and the pellet was washed twice and resuspended in 0.067 M phosphate-buffered saline (PBS), pH 6.8. The test concentration was about 10^8 colony-forming units/ml.

Nasal Cells

Healthy volunteers not carrying *S. aureus* in the nose obtained their own anterior mucosal cells from both nares with a wooden spatula fashioned from a tongue depressor [6]. The specimens, each having been dispersed in 1 ml of PBS, were pooled and vacuum washed with 30 ml of PBS onto a membrane (10-μm pore size, Millipore Corp., Bedford, Massachusetts). A vortex mixer dislodged the cells into 1–3 ml of buffer.

Adherence Testing

The standard procedure of testing for adherence has been presented in detail [6,7]. In brief, we combined 1-ml suspensions of *S. aureus* and nasal cells for 90 min of incubation at 37°C. After washing unattached bacteria away by vacuum filtration, the cell preparation was affixed to slides for staining with crystal violet. Nasal cells were differentiated by layer of origin [6], and the adhering bacteria on each were tallied.

Fibronectin and Staphylococcal Wall Components

To determine the suitability of this adherence system as an assay for fibronectin binding, *S. aureus* and nasal cells were each mixed with 1 mg of human plasma fibronectin (Lot 81-1271, Collaborative Research Corporation, Waltham, Massachusetts) at 37°C for 90 min and then washed in PBS. (Reduced fibronectin yielded a sharp single peak after 5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis.) For both preparations, testing for adherence proceeded as usual. A reduction in adherence by fibronectin-treated *S. aureus* would indicate the presence of the glycoprotein on the bacterium.

We next examined the ability of fibronectin to couple with the basic constituents of the staphylococcal cell wall. Equal volumes of fibronectin (0.5 mg/ml) were mixed with 1 mg/ml of protein A (Sigma Chemical Co., St. Louis, Missouri), N-acetyl-D-glucosamine, N-acetyl muramic acid, or ribitol teichoic acid [8] in PBS. After incubation at 37°C for 60 min, each solution was added in parallel to a tube containing a twice-washed and centrifuged pellet of a 1-ml 18-hr culture of *S. aureus*. The bacterium was then resuspended and incubated for 90 min at 37°C. Next, the preparations were centrifuged and washed twice with PBS. We mixed each suspension of treated staphylococci with nasal cells to test for adherence and compared the result with that obtained using untreated bacteria. In this situation, if fibronectin failed to combine with a tested wall component, it then would be free to couple with the staphylococcus and thereby affect adherence.

Our previous studies [6,8] had shown that teichoic acid can block adherence of *S. aureus* to nasal cells, and hence it is regarded as a staphylococcal adhesin for epithelial receptors in this system. This knowledge permitted another approach for testing the ability of teichoic acid to link with fibronectin. We first mixed 2 mg/ml of teichoic acid with 2 mg/ml of fibronectin for 60 min at 37°C. Washed nasal cells were added to the above solution for an additional 90 min of incubation, allowing cellular binding of any free teichoic acid. After washing away residual fibronectin and teichoic acid with PBS, the nasal cells were incubated with *S. aureus* for 90 min at 37°C. Controls included untreated cells, cells subjected to fibronectin only, and cells suspended with teichoic acid only. Basic to the experimental design was that teichoic acid, if fibronectin did not bind it, would have been available to attach to the nasal cells and thereby prevent adherence of the staphylococcus.

To determine whether the other wall components could also be tested in this manner, we first needed to learn whether any could attach to nasal cells. Before adding the staphylococcus in the basic adherence system, we mixed 1 mg of N-acetyl muramic acid, N-acetyl-D-glucosamine, protein A, D-adonitol (ribitol), ribose, or D-ribose-1-phosphate with the nasal cells for 30 min at 37°C. Testing was repeated, and protein A was examined an additional time. We furthermore tested, with an incubation period of 60 min, the mixed components of ribitol teichoic acid: acetyl glucosamine, ribose-phosphate, and alanine.

RESULTS

The binding of fibronectin to the staphylococcus was immediately apparent when centrifugation of the suspension yielded not the expected pellet but rather a film against the culture tube. Microscopic inspection showed that many of the bacteria were in aggregates only a little larger than usual, but diplococci were also plentiful. As recorded in Fig 1, fibronectin partially

Manuscript received July 26, 1982; accepted for publication November 19, 1982.
Supported in part by the Community Service Program of Kaiser Foundation Hospitals and the Permanente Medical Group.
Reprint requests to: Dr. Raza Aly, Department of Dermatology, University of California, San Francisco, California 94143.
Abbreviations:
PBS: phosphate-buffered saline

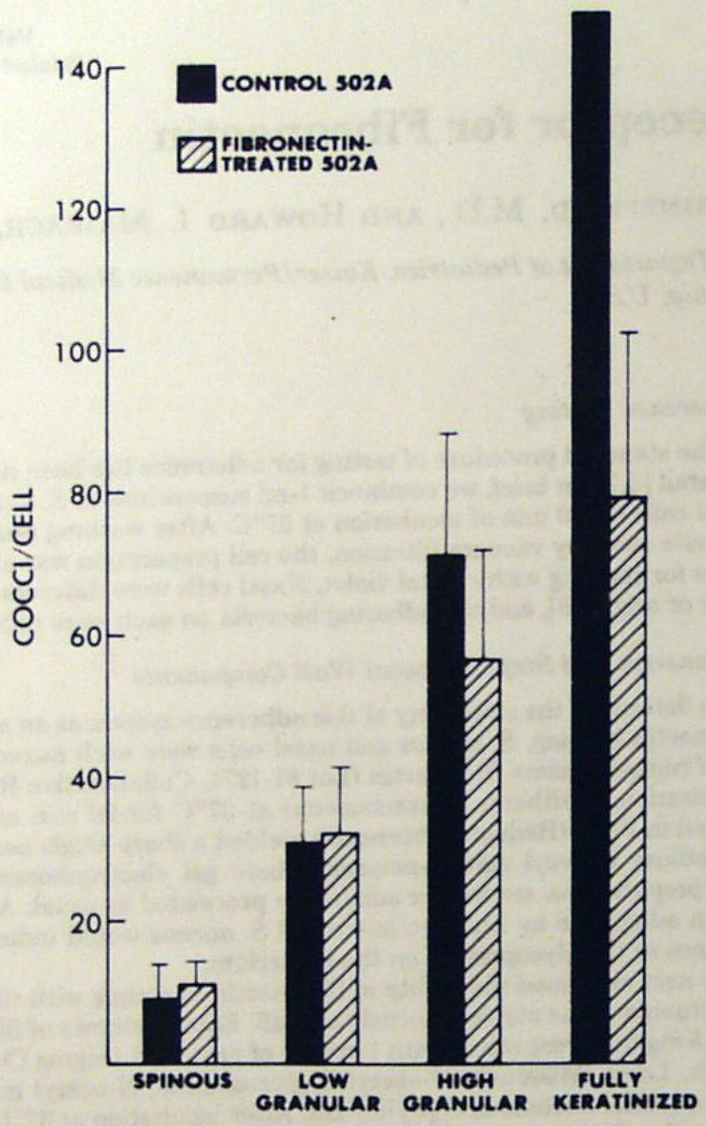

FIG 1. The ability of fibronectin-bound *S. aureus* to adhere to nasal cells from various layers.

blocked adherence of *S. aureus* to the keratinized nasal cells. The two trials of this experiment produced nearly identical results with statistically significant differences between tallies on high-layer granular cells and fully keratinized cells ($p < 0.005$; $p < 0.001$). Younger cells were unaffected. This variation eliminates the possibility of a detrimental effect of increased clustering. We noted no change in adherence counts with the combination of untreated cocci and fibronectin-treated nasal cells.

The pretreatment of fibronectin in this system with individual staphylococcal wall components produced the data of Table I. Acetyl glucosamine, acetyl muramic acid, and protein A were unable to prevent the binding of fibronectin to *S. aureus*. Only teichoic acid could couple with fibronectin and thus permit the unaltered bacterium to attach to the fully keratinized nasal cells at normal levels. Any presence of antiteichoic acid or other antibodies in the fibronectin preparation, which could confuse the results, was likely to exist only at trace levels.

The involvement of keratinized cells resembled our previous tests of staphylococcal teichoic acid as an adhesin to cell receptors [6], where prior incubation of nasal cells with the wall material prevented the attachment of subsequently added *S. aureus*. We adapted this assay to confirm the union of fibronectin and teichoic acid. Table II displays the results of one typical trial. The adherence scores of the test preparation were essentially the same as normal and fibronectin controls. Like an anti-antibody, fibronectin had neutralized adherence-blocking teichoic acid. This procedure could not be used for the other cell wall materials, since none could effectively interfere with adherence by attaching to nasal cells (Table III).

TABLE I. *Ability of teichoic acid to block the coupling of fibronectin to S. aureus*

Fibronectin treatment	Adherent cocci/nasal cell[a]		
	Low granular	High granular	Fully keratinized
Control (untreated cocci)	21.2 ± 8.9[b]	55.3 ± 14.4	115.1 ± 29.6
Acetyl glucosamine	24.1 ± 12.3	52.2 ± 17.5	70.7 ± 20.9[c]
Acetyl muramic acid	19.2 ± 9.4	56.4 ± 16.4	75.1 ± 22.7[c]
Protein A	20.5 ± 7.5	46.7 ± 16.0	70.0 ± 13.4[c]
Teichoic acid	22.7 ± 8.4	60.5 ± 15.3	116.2 ± 21.9

[a] *S. aureus* treated with fibronectin plus wall component.
[b] Mean ± SD of 12–24 cells.
[c] $p < 0.001$ by *t*-test.

TABLE II. *Failure of fibronectin-bound teichoic acid to block adherence of S. aureus to nasal cells*

Cell treatment	Adherent cocci/cell type		
	Low granular	High granular	Fully keratinized
None	22.8 ± 8.9[a]	70.1 ± 21.6	144.8 ± 32.1
Fibronectin only	30.5 ± 7.8	69.2 ± 14.2	144.6 ± 38.6
Teichoic acid only	18.3 ± 8.6	60.6 ± 12.6	100.2 ± 23.9[b]
Fibronectin + teichoic acid	28.8 ± 8.8	73.6 ± 11.6	146.0 ± 34.7

[a] Mean ± SD of 12–21 cells.
[b] $p < 0.001$ by *t*-test.

TABLE III. *Effect of staphylococcal cell wall components on S. aureus adherence to nasal cells*

Test agent	Percent of control/cell type		
	Low granular	High granular	Fully keratinized
Adonitol	67	107	82
Ribose	92	94	91
Ribose-phosphate	97	102	99
Acetyl-glucosamine	116	86	108
Protein A	110	98	88
Ribitol teichoic acid [6]	100	100	62[a]
Ribose-phosphate, alanine, acetyl glucosamine	74	85	100
Acetyl-muramic acid	104	89	109

[a] $p < 0.005$ by *t*-test.

DISCUSSION

The cell wall of *S. aureus* consists essentially of protein A and ribitol teichoic acid on a peptidoglycan frame. By two variations of a unique indirect biologic assay, we have demonstrated that fibronectin couples with the teichoic acid. Our data, which eliminates acetyl glucosamine, acetyl muramic acid, and protein A as fibronectin receptors, confirm the work of Kuusela [1]. Her studies involved the inhibition of binding of radiolabeled fibronectin to *S. aureus*. However, protein A was implicated recently by Doran and Raynor [2]. Using similarly tagged fibronectin and protein A-normal and -deficient strains, they found binding quantitatively correlated with the protein A content of the staphylococci. Protein A-deficient strains did not bind fibronectin. Their inhibition studies with protein A produced only a 50% reduction in coupling, leaving room for another wall component of protein A-positive strains. Yet another investigative team, using protein A-deficient and protein A-excessive mutants, found no correlation of protein A to fibronectin binding*. Further studies are needed to explain and untangle the conflicting results.

* Wadström T, Jonsson P, Switalski LM, Rubin K, Ryden C, Höök M, Lindberg M: Decreased binding of fibronectin to *Staphylococcus aureus* mutants of low virulence (abstr). XIII International Congress of Microbiology, Boston, Massachusetts, 1982.

Our assays, although less sensitive than the above direct chemical tests, are the first to examine teichoic acid. They also present a more in vivo-related picture, since plasma fibronectin may serve as a receptor for microbial colonization and, in the instance of macrophages, for host defense [3,9]. As we observed, fibronectin-coated *S. aureus* was still able to adhere to nasal cells, although in fewer number on the environmentally important, most external, fully keratinized cells. This information, with the knowledge that the teichoic acid blockage of nasal cells produces the same effect, suggests that fibronectin is a secondary nasal cell receptor for the *S. aureus* adhesin teichoic acid. However, our preliminary immunofluorescent and proteolytic enzyme studies (unpublished data) could not detect the glycoprotein. Perhaps its nonantigenic receptive portion [1,10,11] or a different surface molecule that acts like fibronectin may be found.

The authors thank Charlene Bayles for her helpful advice.

REFERENCES

1. Kuusela P: Fibronectin binds to *Staphylococcus aureus*. Nature 276:718–720, 1978
2. Doran JE, Raynor RH: Fibronectin binding to protein A-containing staphylococci. Infect Immun 33:683–689, 1981
3. Mosher DF, Furcht LT: Fibronectin: review of its structure and possible functions. J Invest Dermatol 77:175–180, 1981
4. Pearlstein E, Gold LI, Garcia-Pardo A: Fibronectin: a review of its structure and biological activity. Mol Cell Biochem 29:103–128, 1980
5. Woods DE, Straus DC, Johanson WB Jr, Bass JA: Role of fibronectin in the prevention of adherence of *Pseudomonas aeruginosa* to buccal cells. J Infect Dis 143:784–790, 1981
6. Bibel DJ, Aly R, Shinefield HR, Maibach HI, Strauss WG: Importance of the keratinized epithelial cell in bacterial adherence. J Invest Dermatol 79:250–253, 1982
7. Aly R, Shinefield HR, Strauss WG, Maibach HI: Bacterial adherence to nasal mucosal cells. Infect Immun 17:546–549, 1977
8. Aly R, Shinefield HR, Litz C, Maibach HI: Role of teichoic acid in the binding of *Staphylococcus aureus* to nasal epithelial cells. J Infect Dis 141:463–465, 1980
9. Johnannson S, Rubin K, Höök M, Ahlgren T, Seljelid R: In vitro biosynthesis of cold-insoluble globulin (fibronectin) by mouse peritoneal macrophages. FEBS Lett 105:313, 1979
10. Ruoslahti E, Hayman EG, Engvall E, Cothran WC, Butler WT: Alignment of biologically active domains in the fibronectin molecule. J Biol Chem 256:7277–7281, 1981
11. Mosher DF, Proctor RA: Binding and factor XIIIa mediated cross-linking of a 27 kilodalton fragment of fibronectin to *Staphylococcus aureus*. Science 209:927–929, 1980