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# Adherence of *Staphylococcus aureus* to Squamous Epithelium: Role of Fibronectin and Teichoic Acid

**Raza Aly and Steve Levit**

*From the Departments of Dermatology and Microbiology, University of California, San Francisco, California*

For bacteria to colonize mucosal surfaces, they must be able to attach to epithelial cells. One of the most important factors in determining this attachment is bacterial adherence. The preferential adherence of a bacteria to a particular tissue influences the site of infection and the virulence of the organism. The glycoprotein fibronectin mediates adhesion of the bacteria to eukaryotic cells. Recent investigations have revealed that the precise locations of the binding sites for *Staphylococcus aureus* are close to the $NH_2$-terminal and at the COOH-terminal regions of the fibronectin molecule. Teichoic acids are major cell-wall components of staphylococci that have been found to mediate the capacity of the bacteria to adhere to epithelial cells. By use of biologic assays based on the specific adherence of *S. aureus* to nasal epithelium, it was determined that the binding site for fibronectin appears to be teichoic acid.

The process by which microorganisms bind to host epithelial cells should be considered the initiating event for many infectious illnesses. Under predisposing conditions the ability of bacteria to attach themselves to host tissue defines adherence. Thus adherence proportionately influences the capacity of a microorganism to colonize the mucosa [1, 2].

The current in vitro model, which utilizes isolated epithelial cells and bacteria, most closely resembles the in vivo situation. This model facilitates the study of specific bacterial and host factors independently.

Bacteria vary in their ability to attach to epithelial cells. *Staphylococcus aureus*, because it is the commonest cause of posttraumatic skin and soft tissue infections, has been the microorganism most often studied. It is not surprising that *S. aureus* seems to have a very high binding capacity for epithelial cells; this interaction will be discussed in greater detail below. However, many of the various genera of pathogens and resident microorganisms demonstrate a preference for certain cell types. *Streptococcus salivarius* and *Streptococcus sanguis*, resident organisms found abundantly on oral epithelial surfaces, display greatest affinity for these cells; in contrast, *Streptococcus mutans* prefers the surfaces of teeth. *S. mutans* manifests only feeble adherence to oral epithelial cells and thus only appears in relatively small numbers on these mucosal surfaces [1]. Furthermore, *Streptococcus pyogenes* strains that possess M protein attach more easily to human epithelial cells than do avirulent mutants lacking this component [3].

The pili of gonococci enable them to attach to epithelial cells, and studies utilizing a variety of host cells have established that fimbriated gonococci have significantly more adhesive ability than do nonfimbriated forms. It is not surprising that strains of *Neisseria gonorrhoeae* that adhere strongly to mucosal surfaces are more virulent than strains that do not attach as well [4, 5].

Microbial flora in the gastrointestinal tract selectively colonize mucosal epithelial cells along the gut. For example, lactobacilli harbor on the keratinizing stratified squamous epithelial cells of the nonsecreting portion of the stomach in normal mice [2], whereas yeast of the genus *Torulopsis* colonize columnar epithelium of the secreting portion of the stomach. *Torulopsis* appears to adapt and grow efficiently even in the stomach mucin.

Several important microbiologic activities can be related to the recognized ability of fibronectin to mediate substrate adhesion to both eukaryotic cells and bacteria. Fibronectin is a glycoprotein found in soluble form in many biologic liquids, e.g., plasma, and in insoluble form on the surface of fibroblasts [6–8]. Fibronectin has been reported to act as an opsonin, mediating the phagocytosis of gelatin-coated particles by macrophages [9]. More recently, the precise locations of the fibronectin-*Staphylococcus* binding sites were determined to be close to the $NH_2$-terminal and at the COOH-terminal regions of the fibronectin molecule [10, 11].

Please address requests for reprints to Dr. R. Aly, 1012 HSE, Department of Dermatology, University of California, San Francisco, California 94143.

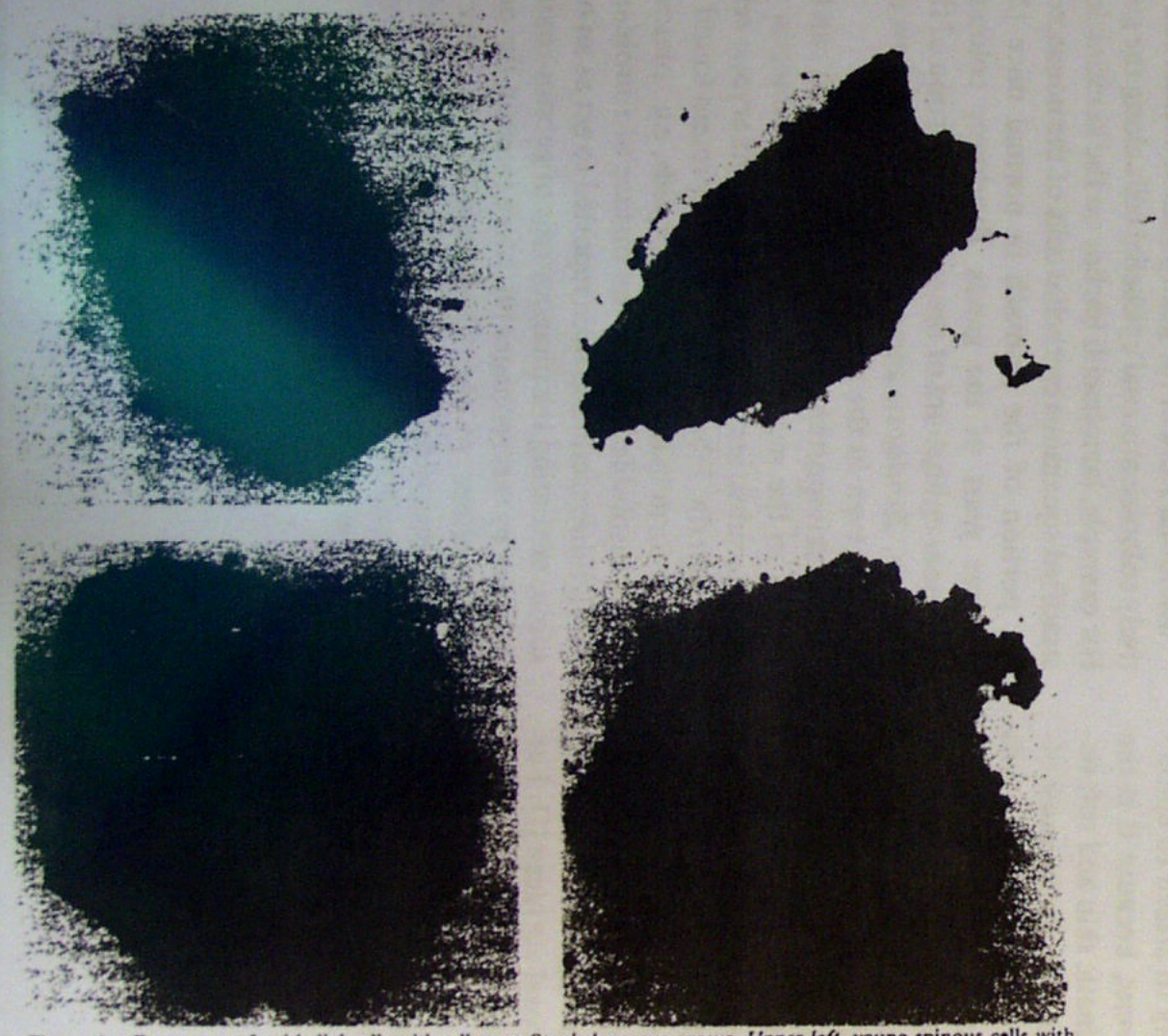

**Figure 1.** Four types of epithelial cells with adherent *Staphylococcus aureus*. *Upper left*, young spinous cells with deeply stained nucleus. *Upper right*, low-level granular cell. *Lower left*, high-level granular cell. *Lower right*, external keratinized cell.

Our objective in this review is to discuss the binding of microorganisms, particularly *S. aureus*, to mucosal epithelium. In particular, we will present recent findings documenting the role of fibronectin in this adherence process.

### Importance of the Keratinized Epithelial Cell

Keratinization is an extremely complex maturation process during which epithelial cells prepare to form the skin's protective barrier against a harsh external environment. As the cell matures through each of its successive strata, it changes shape, loses its nucleus, and becomes more granular. These changes are characterized physiologically and biochemically, in part, by massive sulfhydryl cross-linking of fibrillar proteins; dehydration; lipid degeneration; and a gradual alteration in the quantity and type of the cell's receptors, antigens, and other surface components. Under normal circumstances only fully keratinized cells in the highest layers of the epidermis are initially exposed to transient bacteria. For this reason we first focused our studies on bacterial adherence to mucosal and epidermal epithelial cells.



**Figure 2.** Adherence of *Staphylococcus aureus* to nasal epithelial cells as viewed by light microscopy.

The results of these studies show that adherence directly relates to the age of the epithelial cell and to the state of keratinization throughout the entire spectrum of maturation. We attempted to classify stratified epithelial cells into four common histologic categories on the basis of size, shape, condition or absence of the nucleus, and cytoplasmic consistency, i.e., degree of granulation (figure 1). Fully keratinized cells often had twice as many bacteria adhering to them as did upper-level granular cells, which in turn bound nearly twice as many bacteria as did the cells of lower layers. We could not account for this geometric progression of attachment on the basis of cell size alone. Furthermore, this relation held true for different bacterial species [12].

### Bacterial Adherence to Nasal Epithelial Cells from Adults

For certain bacteria, particularly *S. aureus*, the nose is a primary site of multiplication and dissemination. The clinical importance of this fact is illustrated by observations that depression of nasal staphylococci results in a rapid reduction of skin and aerial concentrations of this pathogen [13]. We developed an in vitro model to demonstrate the host-parasite relationship of bacterial adherence to nasal mucosal cells [14].

We collected nasal epithelial cells by gently scraping tissue from the anterior nares with a sterile wooden applicator. The cells were washed in phosphate buffer then incubated with various types of bacteria at 35°C for 90 min. After incubation, cells were washed free of unattached bacteria. Direct smears were prepared from this epithelial cell suspension and stained for 15 sec with crystal violet. The slides were examined by light microscopy, and the number of bacteria adhering to a cell was manually enumerated (figure 2).

We observed a selective ability of different bacteria to adhere to nasal mucosal cells. Significant adherence could be measured with *Pseudomonas aeruginosa*, *S. aureus*, *Staphylococcus epidermidis*, *S. pyogenes*, and diphtheroids. Less binding occurred with *S. mutans* and *Klebsiella pneumoniae* (table 1). Staphylococci, which constitute the major component of the flora of the anterior nares, possess a distinct advantage over *Streptococcus mitis* and *S. mutans*, which are the predominant components of the flora of the buccal mucosa and teeth, respectively. *S. pyogenes* and *P. aeruginosa* demonstrated good attachment despite the fact that these organisms are not noted in a healthy adult population. Other ecologic factors, both host and bacterial, that are active in vivo may have been altered in our in vitro experiments.

**Table 1.** Selective adherence of bacteria to nasal epithelial cells.

| Bacteria* | No. of bacteria/cell (mean ± SD) | Background counts/cell† (mean ± SD) |
|---|---|---|
| *Staphylococcus aureus* (20) | 53 ± 24 | 5 ± 2 |
| *Staphylococcus epidermidis* (5) | 58 ± 16 | 3 ± 2 |
| *Streptococcus pyogenes* (5) | 120 ± 85 | 3 ± 2 |
| *Streptococcus mitis* (5) | 10 ± 2 | 3 ± 2 |
| *Streptococcus mutans* (5) | 3 ± 1 | 3 ± 2 |
| *Streptococcus salivarius* (5) | 19 ± 9 | 3 ± 2 |
| Coryneforms (6) | 47 ± 41 | 2 ± 1 |
| *Klebsiella pneumoniae* (6) | 4 ± 3 | 2 ± 1 |
| *Pseudomonas aeruginosa* (6) | 153 ± 92 | 2 ± 1 |
| *Micrococcus leteus* (6) | 10 ± 2 | 2 ± 1 |

NOTE. Table is adapted from [15].

* The number in parentheses is the number of subjects from whom epithelial cells were taken.

† For determinations of background counts, the mucosal cells were treated with phosphate buffer rather than with bacteria.

The question of why some people become nasal carriers of *S. aureus* while others do not is often asked but has not yet been answered satisfactorily. Our results showed the adherence of *S. aureus* to be significantly greater ($P < .005$) for the carriers than for the noncarriers, i.e., 132 ± 82 bacteria per cell for carriers and 67 ± 70 bacteria per cell for noncarriers. This observation suggested that the greater affinity for bacterial adherence of mucosal cells from staphylococcal carriers might be a property of the mucosal cell or host environment rather than of the bacteria. Using this model we are currently investigating several bacterial and host factors to determine their relevance to staphylococcal carriage status.

### Bacterial Adherence to Nasal Mucosal Cells from Infants

The human fetus is bacteriologically sterile under normal conditions in utero, as evidenced by negative cultures obtained at the time of elective cesarian section [16]. However, by the end of the first two weeks of life, the infant has acquired most of the organisms found on corresponding areas of the adult. This is so despite the fact that the skin of the newborn at birth is physiologically different from that of the adult.

We also know that bacterial colonization of the human oral cavity is at a relatively low level during the first 24 hr after birth and increases rapidly thereafter [17, 18]. Other studies have demonstrated that the capacity of buccal epithelial cells to bind group A streptococci was minimal on postpartum days 1 and 2 yet reached adult levels by day 3 [19]. Most investigators attribute the reduced binding capacity of buccal epithelial cells to immature receptor sites or to other host factors.

We investigated the adherence of *S. aureus* to nasal epithelial cells obtained from infants on days 1–5 after birth. Our results demonstrated that the adherence of *S. aureus* to neonatal nasal epithelial cells is relatively depressed during the first four days of life but that it reaches adult levels on the fifth day. We compared the adherence of *S. aureus* to nasal mucosal cells from infants and adults (table 2). Some investigators have suggested that the reduced binding of buccal mucosal cells to groups A and B streptococci is due to diminished capacity of neonatal epithelial cells to bind lipoteichoic acid found on the surface of the streptococci [20]. We believe that teichoic acids, which are major cell-wall components of staphylococci, may play the same role for staphylococcal binding to neonatal and adult epithelial cells.

### Bacterial Adherence to Vulvar Epithelial Cells from Adults

In humans of both sexes, but particularly in females, the perineal region serves as a reservoir for *S. aureus* [21]. Because previous studies of bacterial adherence to the female genitalia were limited to the vagina, we attempted to characterize more satisfactorily this phenomenon for the entire vulvar region. Thus, we focused on the labia majora and the labia minora as well as on the vagina to demonstrate features of microbial adherence unique to the perineum. For this study we chose to examine the ability of seven different microorganisms to attach to vulvar epithelial cells: *S. aureus*, *Candida albicans*, *Escherichia coli*, *Acinetobacter calcoaceticus*, *S. pyogenes*, *P. aeruginosa*, and an α-hemolytic *Streptococcus*.

Seven healthy women who were not using oral contraceptives volunteered for this investigation. We harvested cells from the midlabium majus, labium minus, and vagina, as well as cells from the buccal and nasal mucosa for comparison. The cells were washed with phosphate buffer and then incubated with the test bacteria by previously described methods [14]. A micrometer eye-piece on a light microscope was used to measure the size of at least 20 epithelial cells. The test organisms adhering to the epithelial cells were counted, and the average density of adherence was computed. Two general cell types from the labium majus could be distinguished: medium-size cells with a smooth texture and even perimeter and larger cells with a rough consistency and irregular shape. Transitional cells also appeared commonly.

**Table 2.** Adherence of *Staphylococcus aureus* to nasal epithelial cells obtained from newborn infants of various ages.

| Mean age of infants ± SD (hr) | Percent adherence* |
|---|---|
| 24 ± 3 | 22 |
| 48 ± 4 | 25 |
| 72 ± 4 | 38 |
| 96 ± 3 | 35 |
| 120 ± 4 | 98 |

* Adherence of *S. aureus* to mucosal cells obtained from adults was considered to be 100%.

**Table 3.** Adherence of various microorganisms to labium majus cells.

| Microorganism | No. of microorganisms/cell (mean ± SD)* | |
|---|---|---|
| | Medium-size, smooth cells | Large, rough cells |
| *Staphylococcus aureus* | 47.28 ± 14.52 | 85.03 ± 22.35 |
| *Streptococcus pyogenes* | 23.20 ± 9.29 | 37.20 ± 8.36 |
| α-Hemolytic streptococci | 24.56 ± 14.13 | 28.89 ± 12.57 |
| *Escherichia coli* | 1.11 ± 1.36 | 1.14 ± 1.21 |
| *Pseudomonas aeruginosa* | 16.58 ± 7.41 | 28.17 ± 19.20 |
| *Acinetobacter calcoaceticus* | 8.21 ± 6.53 | 11.43 ± 6.61 |
| *Candida albicans* | 9.31 ± 4.87 | 24.54 ± 9.03 |

* In each case, 18–21 labium majus cells were examined.

Superior adherence of *S. aureus* to labium majus cells was obvious (table 3); however, the densities of *S. aureus* on labium minus and vaginal cells were lower than those of other organisms [22]. *S. pyogenes* and α-hemolytic streptococci attained intermediate levels of binding to labium majus cells. *C. albicans* and *A. calcoaceticus* manifested poor adherence. *E. coli* did not attach to any of the surfaces studied. Menstruation influenced attachment—highest counts of bacteria were reached between the third and fourth weeks of the menstrual cycle [22]. This temporal relation is reasonable when the diverse direct and indirect effects of hormones on cellular biology are considered.

### Bacterial Adherence to Skin and Nasal Cells from Patients with Atopic Dermatitis

Previous comparisons between normal skin and the skin of patients suffering from atopic dermatitis have revealed important differences in colonization by *S. aureus* [15, 23, 24]. *S. aureus* colonization is extremely high in patients with atopic dermatitis, and this organism can be readily isolated from affected skin, adjacent uninvolved areas, and the anterior nares. Although not to the same degree, high counts of *S. aureus* also are found in psoriatic plaques. Thus, we considered increased adherence as a possible contributory cause of colonization.

Although psoriasis is primarily a skin disorder of unknown origin, atopic dermatitis has been attributed to an aberrant, hyperactive immunologic condition. Thus, it may be merely one symptom of a systemic disorder that probably also affects the nasal mucosa and other regions of the respiratory tract. Patients diagnosed as having psoriasis or atopic dermatitis at a dermatology clinic volunteered to provide samples of epidermal skin from their forearms and specimens of nasal mucosal tissue; laboratory personnel without these maladies provided control samples. Cells collected with a surgical blade from the volar surface of the arm near the antecubital fossa and with a sterile wooden spatula from the anterior nares were mixed with *S. aureus*. Both types of harvested epithelial cells were placed on slides, air dried, heat fixed, stained, and examined by light microscopy.

Despite the profound alteration in microarchitecture, antigenicity, and metabolism of epidermal cells in psoriatic plaques, we found no enhanced adherence with the skin and nasal cells of such patients. There was no significant statistical difference between these cells and those of healthy individuals. Although atopic dermatitic cells appear more normal than those from psoriatic plaques and are characterized by eczematous hyperkeratinization and dryness, atopic cells from the skin and nose showed enhanced binding of *S. aureus*. While further investigation into this phenomenon will be important, we conclude that adherence appears to be a mechanism responsible for the carriage of *S. aureus* by patients with atopic dermatitis. Furthermore, the enhanced binding of the staphylococci implies an inherent cellular alteration unrelated to any immunologic abberation associated with the disease.

Our analyses of keratinization and dermatitic cells indicate the existence of two types of receptors for *S. aureus* on nasal cells. The first, which becomes more numerous with the progressive development of the granular cell, is unaffected by staphylococcal teichoic acid. The other, recognized with keratinized cells only, is secondary, supplemental, and blocked by teichoic acid [14]. Furthermore, patients with atopic dermatitis may carry *S. aureus* for at least four months, but their carriage seems to be of a different form than that of healthy carriers. Compared with the epithelial cells of healthy noncarriers, those of healthy carriers bind 100% more *S. aureus* [8]; atopic nasal cells support 30% more staphylococci than do control cells [15, 23].

### Role of Teichoic Acid in Staphylococcal Adherence

Teichoic acids are major cell-wall components of staphylococci. The teichoic acid of *S. aureus* contains polymers of ribitol connected by phosphate

diester linkages with side chains of D-alanine and *N*-acetylglucosamine. The phosphate groups and the amino groups of the alanine-ester residues of teichoic acid have a profound effect on cation binding. The molecule itself is composed of eight repeating units of ribitol phosphate [21, 25].

We investigated the role of teichoic acid in the binding of nasal epithelial cells to *S. aureus*. The method of Baddily et al. [26] was used to extract teichoic acid from *S. aureus*. Nasal epithelial cells were washed with PBS and preincubated for 30 min at 35°C with teichoic acid or with lipoteichoic acid from group A streptococci (each at a concentration of 1 mg/ml) [19, 25]. Control epithelial cells were treated with PBS only. After treating epithelial cells with $10^8$ CFU of bacteria/ml, we prepared smears by staining with crystal violet. Light microscopy was used to determine adherence. At the same time, we investigated the adherence of *S. aureus* to epithelial cells pretreated with a whole cell-wall preparation of *S. aureus*.

Epithelial cells treated with teichoic acid and lipoteichoic acid demonstrated 71% and 60% reductions of binding to *S. aureus*, respectively. Epithelial cells treated with whole cell-wall preparations manifested a 49% reduction in binding to *S. aureus*. However, only a minimal diminution (17%) [25] in the binding ability of group A streptococci was noted when epithelial cells were treated with teichoic acid.

These results suggest that lipoteichoic acid combines with an epithelial receptor site for both *S. aureus* and group A streptococci, thus reducing the binding of these organisms to the epithelial cells. Teichoic acid is specifically bound to the attachment sites for staphylococci but not to those for streptococci. As a result treatment of epithelial cells with teichoic acid did not significantly reduce streptococcal binding.

These results suggest that both lipoteichoic acid and teichoic acid can bind to nasal epithelial cells to inhibit adherence of *S. aureus* to the mucosa. The data also suggest that lipoteichoic acid on the surface of streptococci mediates the binding of this organism, while teichoic acid mediates the binding of staphylococci.

Electron microscopy of streptococci and various other gram-positive organisms has previously demonstrated the presence of polysaccharide fibers on the surface of these bacteria [1]. Mounting evidence suggests that these fibers are involved in the attachment of bacteria to epithelial cells.

Results of our investigations demonstrate the presence of similar polysaccharide material on the surface of *S. aureus*. To obtain electron micrographs on which this material is revealed, we stained cellular material with ruthenium red, which reacts strongly with acid polysaccharides and other polyanions of high charge density. Our results also suggest that the adherence of *S. aureus* to human nasal epithelial cells is mediated by these polysaccharide fibers. Certain gram-negative bacteria also have been observed to possess pili that impart adhesive qualities [5]. In view of their obvious clinical and ecologic significance, the surface components of bacteria involved in adherent interactions with host surfaces merit additional serious attention.

## Mechanisms of Adherence: The *Staphylococcus aureus* "Receptor" for Fibronectin

Over the past decade, one of the most enticing theories regarding possible mechanism of bacterial adherence to mucosal surfaces has been that of Costerton et al. [27]. Mammalian epithelial cells and bacterial cell-wall surfaces possess polysaccharide fibers with exquisite chemical specificity. Negatively charged bacteria can form polar bonds with host-cell polysaccharides by way of divalent positive ions in the host environment. Lectins, proteins with specific attractions for the bacterial and host-cell polysaccharides, can also form bridges between the two cells. These interactions are specific – bacteria whose fibers can bind neither to the host cell nor to suitable divalent ions in the system simply do not bind.

Bacterial infection begins with the specific adhesion of organisms to the host mucosal surfaces. Recent observations suggest that human fibronectin binds to *S. aureus* [10, 28–32]. Utilizing two variations of a unique biologic assay, we have elucidated evidence that teichoic acid is a staphylococcal receptor for fibronectin.

*Staphylococcus aureus* strain 502A was grown for 18 hr at 37°C in tryptic soy broth (Difco, Detroit, Mich.). After washing and resuspending the organisms in PBS, we incubated 1-ml suspensions of *S. aureus* with nasal cells for 90 min at 37°C. After unattached bacteria were washed away by vacuum filtration, the cell preparations were affixed to slides for staining with crystal violet. 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

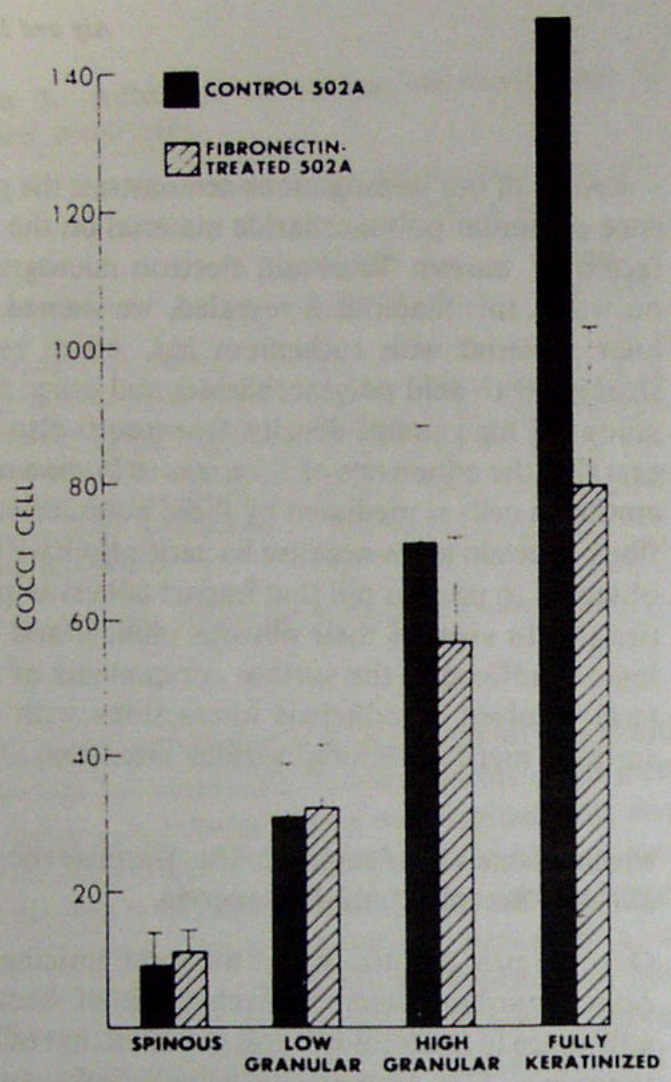


**Figure 3.** The ability of fibronectin-bound *Staphylococcus aureus* strain 502A to adhere to cells from various layers of nasal epithelium.

at 37°C for 90 min then washed in PBS. (Reduced fibronectin yielded a sharp single band after 5% SDS-PAGE.) For both preparations, testing for adherence proceeded as usual. A reduction in adherence by fibronectin in treated *S. aureus* would indicate the presence of the glycoprotein on the bacteria.

The binding of fibronectin to the staphylococci was immediately apparent when centrifugation of the suspension yielded a film against the culture tube instead of the expected pellet. Microscopic inspection showed that the bacteria were plentiful. As shown in figure 3, fibronectin partially blocked adherence of *S. aureus* to the keratinized nasal cells. The two trials of this experiment produced nearly identical results, with statistically significant differences in the effect of fibronectin on binding between the high-layer granular cells and the fully keratinized cells ($P < .005$ and $P < .001$, respectively). Younger cells were unaffected. This variation eliminates the possibility of a detrimental effect of increased clustering. We noted no change in adherence with the combination of untreated cocci and fibronectin-treated nasal cells.

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 protein A, *N*-acetyl-D-glucosamine, *N*-acetylmuramic acid, or ribitol teichoic acid, each at a concentration of 1 mg/ml, 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 bacteria were 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 cell-wall component, it then would be free to couple with the staphylococci and thereby affect adherence.

The pretreatment of fibronectin in this system with individual staphylococcal cell-wall components pro-

**Table 4.** Ability of teichoic acid and other cell-wall components to block the coupling of fibronectin to *Staphylococcus aureus*.

| Treatment of fibronectin | No. of *S. aureus* per nasal cell (mean ± SD)* | | |
|---|---|---|---|
| | Low-level granular cells | High-level granular cells | Fully keratinized cells |
| None (untreated *S. aureus*) | 21.2 ± 8.9 | 55.3 ± 14.4 | 115.1 ± 29.6 |
| *N*-acetylglucosamine | 24.1 ± 12.3 | 52.2 ± 17.5 | 70.7 ± 20.9† |
| *N*-acetylmuramic acid | 19.2 ± 9.4 | 56.4 ± 16.4 | 75.1 ± 22.7† |
| Protein A | 20.5 ± 7.5 | 46.7 ± 16.0 | 70.0 ± 13.4† |
| Teichoic acid | 22.7 ± 8.4 | 60.5 ± 15.3 | 116.2 ± 21.9† |

NOTE. *S. aureus* were treated with fibronectin plus the indicated cell-wall component. See text for details.

* In each case, 12–24 nasal cells were examined.

† $P < .001$ by *t* test.

duced the data presented in table 4. *N*-acetylglucosamine, *N*-acetylmuramic 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. The presence of antibody to teichoic acid or of other antibodies in the fibronectin preparation, could confuse the results, but it is likely that they were present only at trace levels.

Because our previous studies demonstrated that teichoic acid can block adherence of staphylococci to nasal cells, we regard teichoic acid as a staphylococcal adhesin for epithelial binding sites in this system. Thus, this knowledge permits another approach for testing the ability of teichoic acid to bind to fibronectin. We postulated that fibronectin, like an anti-antibody, would bind to teichoic acid and prevent the blockage of *S. aureus* binding sites on the nasal cells, thereby allowing uninhibited adherence of the staphylococci.

We first mixed 2 mg of teichoic acid/ml with 2 mg of fibronectin/ml for 60 min at 37°C. Washed nasal cells were added to this solution for an additional 90 min of incubation, allowing cellular binding of any free teichoic acid. After residual fibronectin and teichoic acid were washed away 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 staphylococci (table 5). The adherence scores of the test preparations were essentially the same as those of normal and fibronectin controls. Fibronectin had neutralized the blocking of adherence by teichoic acid.

To determine whether other cell-wall components could be tested in this manner, we first needed to learn whether any of these components could attach to nasal cells. Before adding the staphylococci in the basic adherence system, we mixed 1 mg of *N*-acetylmuramic 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 also tested mixtures of the components of ribitol teichoic acid—*N*-acetylglucosamine, ribose phosphate, and alanine—with an incubation period of 60 min. None of the cell-wall components tested could effectively interfere with adherence by attachment to nasal cells [33].

These results demonstrate that fibronectin binds to teichoic acid, a major structural component of the *S. aureus* cell wall; they do not confirm previous observations, which suggested that fibronectin binds to protein A, the other major cell-wall structural compound [33a]. We believe that our assays, although less sensitive, present a picture more relevant to the situation in vivo, since plasma fibronectin may serve as a receptor for microbial colonization and, in the case of macrophages, for host defense. Verbrug et al. [34] showed that purified, radiolabeled fibronectin not only bound to a similar degree to several laboratory strains and to the fresh clinical isolates of *S. aureus*, but also to *S. aureus* strains deficient in cell-wall teichoic acid. Also, purified peptidoglycan and teichoic acid isolated from *S. aureus* H failed to bind fibronectin, but crude cell-wall preparations of this strain containing protein constituents bound considerable amounts of fibronectin. The authors' explanation was that teichoic acid

**Table 5.** Failure of fibronectin-bound teichoic acid to block adherence of *Staphylococcus aureus* to nasal cells.

| Cell treatment | No. of *S. aureus*/cell (mean ± SD)* | | |
|---|---|---|---|
| | Low-level granular cells | High-level granular cells | Fully keratinized cells |
| None | 22.8 ± 8.9 | 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† |
| Fibronectin + teichoic acid | 28.8 ± 8.8 | 73.6 ± 11.6 | 146.0 ± 34.7 |

NOTE. See text for details of experiment.

* In each case, 12–21 nasal cells were examined.

† $P < .001$ by $t$ test.

and peptidoglycan together constitute a major part of the cell wall of *S. aureus* H; binding of fibronectin probably occurred to a minor (by weight) component of the staphylococcal cell wall that was present in isolated crude cell-wall preparations but was removed in subsequent purification procedures.

Because we observed that fibronectin-coated staphylococci were still able to adhere to nasal cells, although in diminished numbers, we feel that fibronectin is a secondary nasal cell binding site for the adhesin teichoic acid. However, recent investigations have elucidated evidence that fibronectin in fibrin thrombi promoted adherence of most strains of pathogenic *S. aureus*. This may prove important for understanding the pathogenesis of wound infections [35]. Fibronectin is vital in wound healing because of its adhesive property; it is ironic that this same property may also promote the adherence of staphylococci to the wound site.

The determination of any potential clinical significance for fibronectin's role in bacterial adherence requires further study. However, fibronectin, in both its insoluble form on the surface of many human cell types and its soluble form as part of the extracellular matrix, seems to affect attachment differently among various organisms. The environmental relation between fibronectin in its various forms and locations and the bacteria in question may assist certain pathogens in becoming established and thus initiate local infections while inhibiting others. Fibronectin and attachment may also determine which organisms are infectious under normal conditions in the host.

**References**


1. Gibbons RJ, Van Houte J. Bacterial adherence in oral microbial ecology. Annu Rev Microbiol 1975;**29**:19–44
2. Savage DC. Survival on mucosal epithelia, mucosal epithelia penetration and growth in tissue of pathogenic bacteria. In: Smith H, Pearch JH, eds. Microbial pathogenicity in man and animal. London: Cambridge University Press, 1972:25–8
3. Ellen RP, Gibbons RJ. M protein-associated adherence of *Streptococcus pyogenes* to epithelial surfaces: prerequisite for virulence. Infect Immun 1972;**5**:826–30
4. Swanson J. Studies on gonococcus infection. IV. Pili: their role in attachment of gonococci to tissue culture cells. J Exp Med 1973;**137**:571–89
5. Punsalang AP Jr, Sawyer WD. Role of pili in the virulence of *Neisseria gonorrhoeae*. Infect Immun 1973;**8**:255–63
6. Hynes RO. Cell surface proteins and malignant transformation. Biochim Biophys Acta 1976;**458**:71–107
7. Vaheri A, Ruoslahti E, Mosher DF, eds. Fibroblast surface protein. Ann NY Acad Sci 1978;**312**:1–456
8. Morrison PR, Edsall JT, Miller SG. Preparation and properties of serum and plasma proteins. XVIII. The separation of purified fibrinogen from fraction I of human plasma. Journal of the American Chemical Society 1948; **70**:3103–8
9. Villiger B, Kelley DG, Engleman W, Kuhn C III, McDonald JA. Human alveolar macrophage fibronectin: synthesis, secretion and ultrastructural localization during gelatin-coated latex particle binding. J Cell Biol 1981;**90**:711–8
10. Mosher DF, Proctor RA. Binding and factor XIIIa-mediated cross-linking of a 27-kilodalton fragment of fibronectin to *Staphylococcus aureus*. Science 1980;**209**:927–49
11. Kuusela P, Vartio M, Vuento TM, Myhre EB. Binding sites for streptococci and staphylococci in fibronectin. Infect Immun 1984;**45**:433–6
12. Bibel DJ, Aly R, Shinefield HR, Maibach HI, Strauss WG. Importance of the keratinized epithelial cell in bacterial adherence. J Invest Dermatol 1982;**79**:250–3
13. White A, Smith J. Nasal reservoir as the source of extranasal staphylococci. Antimicrob Agents Chemother 1963;**3**: 679–83
14. Aly R, Shinefield HI, Strauss WG, Maibach HI. Bacterial adherence to nasal mucosal cells. Infect Immun 1977;**17**: 546–99
15. Aly R, Maibach HI, Shinefield HR. Microbial flora of atopic dermatitis. Arch Dermatol 1977;**113**:780–2
16. Aly R, Shinefield HR, Maibach HI. Adherence of *Staphylococcus aureus* to infant and adult nasal mucosal cells. In: Maibach HI, Boisits EK, eds. Neonatal skin: structure and function. New York: Marcel Dekker, 1982:183–7
17. McCarthy C, Snyder ML, Parker RB. The indigenous oral flora of man, I. The newborn to the 1-year-old infant. Arch Oral Biol 1965;**10**:61–70
18. Torrey JC, Reese MK. Initial aerobic flora of newborn infants: selective tolerance of the upper respiratory tract for bacteria. Am J Dis Child 1945;**69**:208–14
19. Ofek I, Beachey EH, Eyal F, Morrison JC. Postnatal development of binding of streptococci and lipoteichoic acid by oral cells of humans. J Infect Dis 1977;**135**:267–74
20. Beachey EH. Binding of group A streptococci to human oral mucosal cells by lipoteichoic acid. Trans Assoc Am Physicians 1975;**88**:285–92
21. Aly R, Shinefield HR, Maibach HI. *Staphylococcus aureus* adherence to nasal epithelial cells: studies of some parameters. In: Aly R, Maibach HI, eds. Skin microbiology: relevance to clinical infection. New York: Springer-Verlag, 1981:171–9
22. Bibel DJ, Aly R, Lahti L, Shinefield HR, Maibach HI. Microbial adherence to vulvar epithelial cells. J Med Microbiol 1987;**23**:75–82
23. Leyden JJ, Marples RR, Kligman AM. *Staphylococcus aureus* in the lesions of atopic dermatitis. Br J Dermatol 1974;**90**:525–30
24. Marples RR, Heaton CL, Kligman AM. *Staphylococcus aureus* in psoriasis. Arch Dermatol 1973;**107**:568–70
25. 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 1980;**141**:463–5
26. Baddiley J, Buchanan JG, Rajbhandary UL, Sanderson AR.

Teichoic acid from the walls of *Staphylococcus aureus* H structure of the *N*-acetylglucosaminylribitol residues. Biochem J 1962;**82**:439–48

27. Costerton JW, Geesy GG, Cheng KJ. How bacteria stick. Sci Am 1978;**238**:86–95
28. Kuusela P. Fibronectin binds to *Staphylococcus aureus*. Nature 1978;**276**:718–20
29. Proctor RA, Mosher DF, Olbrantz PJ. Fibronectin binding to *Staphylococcus aureus*. J Biol Chem 1982;**257**:14788–94
30. Rydén C, Rubin K, Speziale P, Höök M, Lindberg M, Wadström T. Fibronectin receptors from *Staphylococcus aureus*. J Biol Chem 1983;**258**:3396–401
31. Espersen F, Clemmensen I. Isolation of a fibronectin-binding protein from *Staphylococcus aureus*. Infect Immun 1982; **37**:520–31
32. Proctor RA. The staphylococcal fibronectin receptor: evidence for its importance in invasive infections. Rev Infect Dis 1987;**9**(Suppl 4):S335–40
33. Bibel JD, Aly R, Shinefield HR, Maibach HI. The *Staphylococcus aureus* receptor for fibronectin. J Invest Dermatol 1983;**80**:494–6

33a. Doran JE, Raynor RH. Fibronectin binding to protein A–containing staphylococci. Infect Immun 1981;**33**:683–9

34. Verbrug HA, Peterson PK, Smith DE, Nguyen BT, Hoidel JR, Wilkinson BJ, Verhoef J, Furcht LT. Human fibronectin binding to staphylococcal surface protein and its relative inefficiency in promoting phagocytosis by human polymorphonuclear leukocytes, monocytes and alveolar macrophages. Infect Immun 1981;**33**:811–9
35. Toy PTCY, Lai L, Drake TA, Sande MA. Effect of fibronectin on adherence of *Staphylococcus aureus* to fibrin thrombin in vitro. Infect Immun 1985;**48**:83–6