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Role of Fibronectin in Attachment of *Streptococcus pyogenes* and *Escherichia coli* to Human Cell Lines and Isolated Oral Epithelial Cells

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We studied the binding of cells of *Streptococcus pyogenes* and mannose-sensitive *Escherichia coli* to human fibroblast cell lines and isolated buccal epithelial cells in relation to the cell-associated endogenous or exogenous fibronectin of the host cells. The degree of bacterial binding to cell lines correlated directly with the content of endogenous fibronectin on the surface of the cultured cells, although the correlation was better with *S. pyogenes* than with *E. coli*. The addition of exogenous plasma fibronectin to the cell lines or oral epithelial cells enhanced binding of *S. pyogenes* but suppressed binding of mannose-sensitive *E. coli*. These findings are consistent with the notion that exogenously acquired fibronectin on the surface of host cells modulates bacterial adherence by providing attachment sites for certain pathogens, such as *S. pyogenes*, and by blocking receptors for others, such as mannose-sensitive *E. coli*.

Fibronectin is a high-molecular-weight glycoprotein present in an insoluble form on the surfaces of many animal cells and in a soluble form in plasma (14, 15, 33). It possesses binding sites for a number of bacteria (2, 7, 18, 30), especially streptococci and staphylococci (2, 8, 10, 16, 19–22, 24, 26, 28, 29). More specifically, plasma fibronectin possesses binding sites for the lipid portion of streptococcal lipoteichoic acid (6, 22). Since lipoteichoic acid resides on streptococcal surfaces, it has been suggested that fibronectin on buccal epithelial cells serves as a receptor for the binding of this upper respiratory pathogen (4, 22). However, fibronectin may also inhibit bacterial adherence since its removal from the cells results in increased binding of *Pseudomonas aeruginosa* (31, 32). Moreover, Abraham et al. (1) have shown that buccal epithelial cells, rich in fibronectin, bind more streptococci and fewer mannose-sensitive (MS) *Escherichia coli* as compared with fibronectin-poor cells, whereas the latter cells bind more *E. coli* and fewer streptococci. It should be noted that the source of fibronectin on buccal cells has not been established, but it is likely to originate from saliva (2, 23). The relationship between the salivary fibronectin and other forms of fibronectin is not known.

In the present study, we examined the adherence of *Streptococcus pyogenes* and of MS *E. coli* to several lines of human tissue culture cells and to human oral cells in relation to their fibronectin content. For comparison, the effect of exogenously added fibronectin on the adherence of the same bacteria was also investigated.

The human tissue culture cell lines employed were: GM11, 8-week-old human skin embryo fibroblasts; HEF299, fibroblastlike cells derived from embryonic lung tissue; and SKF-IU and 302, adult skin fibroblasts. The cells were grown in minimal essential medium containing 10% fetal calf serum supplemented with 100 μg of streptomycin and penicillin per ml. Buccal epithelial cells were obtained from healthy laboratory personnel and were used in adherence tests as previously described (1).

S. pyogenes M type 5 and *E. coli* 346, a type 1 fimbriated MS strain, were grown in brain heart infusion broth (Difco Laboratories) at 37°C for 24 and 48 h, respectively. The microorganisms were harvested and washed twice with 0.02 M phosphate–0.15 M NaCl (pH 7.4) (PBS) and suspended in this buffer to obtain a reading of 150 Klett units (approximately 5×10^8 bacterial cells per ml). The adherence of *S. pyogenes* and *E. coli* to tissue culture cell lines and the amount of the cell-associated fibronectin were evaluated by an enzyme-linked immunosorbent assay (ELISA). The cells were grown to confluence in Falcon 96-well polystyrene plates (BD Labware) over a 3 to 4-day period. The cellular monolayers were washed three times with warm (37°C) PBS, fixed with 0.25% glutaraldehyde for 10 min at 4°C, and then treated with 0.2 M glycine and 0.1% bovine serum albumin for 30 min at ambient temperature. In preliminary experiments employing nonfixed cell monolayers, the magnitude of bacterial adherence was the same as that to glutaraldehyde-fixed cells. Glutaraldehyde fixation was necessary to retain the confluent monolayers intact throughout the multiple washes required for quantitation by ELISA (see below). To evaluate the magnitude of bacterial attachment, the wells were washed with PBS; 100 μl of bacterial suspension containing 10 mg of hemoglobin (Sigma Chemical Co.) per ml to prevent nonspecific binding was added and incubated for 30 min at 37°C. The monolayers were then washed five times with PBS, followed by addition of 100 μl of anti-*S. pyogenes* (3) or anti-*E. coli* immune serum; the antisera were added at dilutions of 1:5,000, and 1:1,000 in PBS, respectively. After 30 min of incubation at ambient temperature, the cell monolayers were washed five times with a solution consisting of 0.9% NaCl, 0.05% Tween 20, and 0.02% NaN^3 and incubated with 100 μl of a 1:10,000 dilution of affinity-purified goat anti-rabbit immunoglobulin G conjugated to

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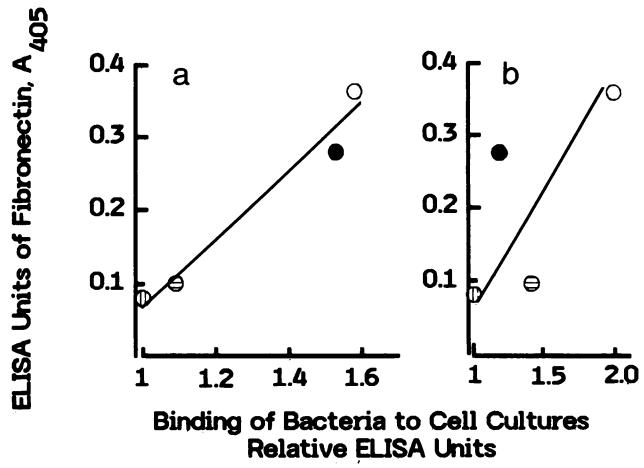

FIG. 1. Correlation between fibronectin content and bacterial adherence to tissue culture cell lines 302 (◍), HEF (⊖), GM11 (●), and IV (○). One relative ELISA unit is equal to A_{405} readings of 0.108 and 0.176 for *S. pyogenes* (a) and *E. coli* (b), respectively.

alkaline phosphatase for 40 min. After washing five times, 100 μl of *p*-nitrophenylphosphate (Sigma) was added as a substrate and incubated at 37°C. After 20 min, the absorbance at 405 nm (A_{405}) was recorded in an MR580 MicroElisa Autoreader (Dynatech Instruments Co.). Mannose-specific adherence of *E. coli* was determined by recording the difference in A_{405} between cells exposed to bacterial suspensions supplemented with 2.5% methyl α-D-mannoside, an inhibitory sugar, or with methyl α-D-glucoside, a noninhibitory sugar. Nonspecific binding of *E. coli* (i.e., in the presence of methyl α-D-mannoside) was 20 to 40% of the total *E. coli* bound, suggesting that hydrophobic and other forces contribute to the overall adherence of the bacteria to the cells.

To evaluate the fibronectin content of the cells, the same ELISA procedure was followed, except that PBS instead of the bacterial suspension and antifibronectin serum instead of the antibacterial serum were added. In some experiments, cells were coated with plasma fibronectin by adding 100 μl of a 200-μg/ml solution of fibronectin and incubating for 30 min at room temperature; the binding of plasma fibronectin to cells in culture has been reported previously (17). The cells were then washed with PBS and immediately assayed for bacterial adherence as described above. Each test was carried out in triplicate. As a control, normal rabbit serum was substituted for the specific rabbit antisera. Fibronectin was prepared from human plasma by affinity chromatography on a gelatin-Sepharose column (9). The eluted protein was judged to be pure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lipoteichoic acid was purified by methods described previously (25). Antibacterial sera were raised in rabbits as previously described (3).

A direct relationship was found between the level of cell-associated fibronectin and the amount of *S. pyogenes* that adhered to the four cell lines examined (Fig. 1). Moreover, coating of the cells with plasma fibronectin resulted in a marked increase in streptococcal adherence, which was proportional to the increase in the amount of plasma fibronectin on the cells (Table 1). With MS *E. coli*, different results were obtained. In this case too, there seemed to be a direct relationship between the level of cell-associated fibronectin and the amount of bacteria bound (Fig. 1), although the correlation was not as good as that with *S. pyogenes*. In

TABLE 1. Effect of plasma fibronectin on adherence of *S. pyogenes* and *E. coli* to human fibroblast cell lines

Cell lines treated with 100 μg of fibronectin per ml	Fibronectin content (% increase ± SD)	Bacterial adherence (% change[a] ± SD)	
		S. pyogenes	*E. coli*
HEF	163 ± 10	121 ± 10	−28 ± 10
GM11	65 ± 6	45 ± 6	42 ± 6
IV	70 ± 8	56 ± 5	−4.4 ± 0.7

[a] Percent change was calculated from ELISA values as follows: (A_{405} of Fn treated − A_{405} of not treated)/(A_{405} of untreated cells) × 100. Changes in adherence and increase in fibronectin content were significantly different as compared with untreated cells at the level of $P \leq 0.01$ for fibronectin and *S. pyogenes* and at the level of $P \leq 0.05$ for *E. coli*, except for the change in adherence of *E. coli* to the type IV cell line, which was not significant.

contradistinction, coating the cells with plasma fibronectin caused a significant decrease in *E. coli* attachment to two of the cell lines and no change in attachment to the third line (Table 1). To establish whether plasma fibronectin would block adherence of MS *E. coli* to human buccal epithelial cells, we coated the cells with increasing amounts of fibronectin shortly after they were scraped from the cheeks of human volunteers. It was found that the added fibronectin inhibited the adhesion of the *E. coli* to the buccal epithelial cells in a dose-response fashion (Fig. 2).

The molecular mechanism of the adherence of *S. pyogenes* to epithelial cells has been under intensive investigation during the last decade (4). The data with *S. pyogenes* are consistent with the notion that plasma or cellular fibronectin serves as a receptor for the binding of this organism, both to primary and cultured cells.

The relationship between fibronectin and mannose-specific *E. coli* adherence is, at first glance, less clear. Whereas an increase in cell-associated fibronectin led to an increase in the adherence of these bacteria, coating the cells with plasma fibronectin caused a decrease in *E. coli* adherence with two of the cell lines examined, as with the isolated human oral epithelial cells. The discrepancy in the effect on *E. coli* adherence between exogenously acquired and cell-associated fibronectin may be explained by the assumption that there are structural differences between the two types of

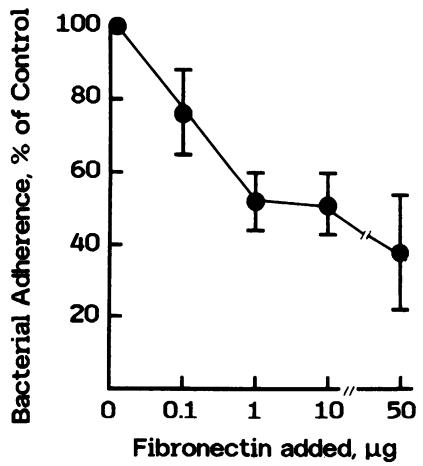

FIG. 2. Dose-response of the inhibition of the adherence of *E. coli* to oral epithelial cells by fibronectin. Vertical bars indicate standard deviations of the mean.

fibronectin, especially with respect to their oligosaccharide chains. For example, Chou-Rong Zhu et al. (5) very recently noted such differences between plasma fibronectin and placental fibronectin, the latter being probably identical with the cell associated one. Moreover, these authors also reported that some of the glycopeptides obtained from the placental fibronectin bound to concanavalin A, a lectin whose sugar specificity overlaps that of the mannose-specific *E. coli* (11–13). This supports earlier work of Olden et al. (20) who showed that cellular fibronectin isolated from chicken fibroblasts binds concanavalin A. Thus, although cellular fibronectin may provide binding sites for both *E. coli* and *S. pyogenes*, the soluble form (e.g., salivary fibronectin) will bind only the latter organism.

Our results are consistent with the notion (22, 23) that exogenously acquired fibronectin on the surface of host cells modulates bacterial adherence by providing attachment sites for certain pathogens (e.g., streptococci) and by blocking receptors for others (e.g., MS *E. coli*), thereby affecting colonization of various bacterial species on mucosal surfaces. Since oral epithelial cells in vivo most probably acquire their fibronectin from saliva (2, 23), the variations reported in the binding of MS *E. coli* to such cells (27) may be ascribed to differences in the quantities of bound salivary fibronectin as suggested by Abraham et al. (1). Our proposal also would account for the clinical observations where increases in gram-negative infections of the upper respiratory tract are associated with decreased levels of cell-associated fibronectin in the oral cavities of patients in the hospital (31, 32).

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