INFECTION AND IMMUNITY, May 1985, p.318–323
0019-9567/85/050318-06$02.00/0

Vol. 48, No. 2

Binding of Fibronectin to Human Buccal Epithelial Cells Inhibits the Binding of Type 1 Fimbriated *Escherichia coli*

W. ANDREW SIMPSON,[1,2*] DAVID L. HASTY, [2] AND EDWIN H. BEACHEY[1,2]

Veterans Administration Medical Center[1] and University of Tennessee Center for the Health Sciences,[2] Memphis, Tennessee 38104

Received 1 August 1984/Accepted 30 January 1985

The interaction of purified human plasma fibronectin (FN) with human buccal epithelial cells was studied. Maximal binding of FN occurred at pH 5. The majority of the binding was specific and reversible. The binding of FN to buccal cells was saturable, reaching a maximum when 10^5 buccal cells were incubated with ~200 μg of radiolabeled protein per ml. The adherence of a type 1 fimbriated strain of *Escherichia coli* to buccal epithelial cells was inhibited by the addition of FN in a dose-related manner. Our results indicate that exogenous FN can bind to human buccal epithelial cells and block the attachment of a type 1 fimbriated strain of *E. coli*.

Fibronectin (FN) is a large glycoprotein (see references 4, 9, 14, 16, 21, 24, and 37 for reviews) which is found in the extracellular matrices of many tissues (13, 38) and in plasma (21), amniotic fluid (6), cerebrospinal fluid (18), and saliva (3, 27). Recent studies have shown that purified FN binds to a wide variety of bacteria, including *Staphylococcus aureus* (10, 11, 17, 20, 22–24, 32, 34), *Streptococcus pyogenes* (26, 28, 30, 31), *Streptococcus mutans* (3), and certain gram-negative species (33). The presence of FN on mammalian cell surfaces has been associated with the adherence of *Streptococcus pyogenes* to buccal mucosal cells (26, 27) and the adherence of *Staphylococcus aureus* to endothelial cells grown in tissue culture (34). Woods et al. suggested that the amount of FN on buccal mucosal cells was inversely related to the adherence of *Pseudomonas aeruginosa* to these cells (35, 36). Our results indicate that the buccal cell preparations used in in vitro adherence assays consist of a mixture of FN-positive and FN-negative cells and that the gram-negative species tested adhere primarily to FN-deficient cells (1). Since previous studies in our laboratories suggested that FN binds to buccal mucosal cells and increases the adherence of *Streptococcus pyogenes* cells (26), it was of interest to characterize the epithelial cell binding of this glycoprotein. We report evidence which indicates that FN binds to buccal mucosal cells at physiological hydrogen ion concentrations found in oral cavities. The ability to bind FN to these cells allowed us to test the hypothesis that there is an inverse relationship between the amount of FN on a cell surface and the ability of gram-negative bacteria to adhere. By using a type 1 fimbriated *Escherichia coli* strain, we showed that FN inhibited the attachment of these gram-negative bacteria in a dose-dependent manner.

MATERIALS AND METHODS

FN. FN was purified by affinity chromatography (25) and characterized as previously described (5, 26, 28). FN was iodinated by using a lactoperoxidase kit (New England Nuclear Corp., Boston, Mass.) and the directions supplied by the manufacturer. Radiolabeled FN was repurified on a gelatin-Sepharose column. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified protein indicated the presence of a characteristic 220,000-dalton doublet under reducing conditions.

E. coli. *E. coli* CSH50, which was used throughout these studies, expresses type 1 fimbriae and has been characterized previously in our laboratories (2). The adherence characteristics of this strain are typical of *E. coli* strains with mannose-sensitive adhesins. The bacteria were grown statically in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) for 48 h at 37°C. The cells were washed repeatedly with the appropriate buffer (see below) and collected by centrifugation as previously described (22). For some experiments, *E. coli* cells were washed and treated with 100 μg of FN per ml for 30 min at 37°C. After treatment, the bacteria were washed three additional times before they were added to the buccal epithelial cells.

Lipoteichoic acid. Lipoteichoic acid was extracted from *Streptococcus pyogenes* strain 1RP41 and chemically characterized as previously described (29).

Antibody. Antibody against human plasma FN was raised in New Zealand white rabbits and characterized as previously described (28). The antibody was affinity purified by using a column of FN immobilized on Sepharose beads in the method of March et al. (19). Rabbit antisera were diluted 1:4 with 0.05 M Tris-hydrochloride buffer (pH 7.4) containing 0.15 M NaCl and were applied to the FN column, which was equilibrated in the same buffer. The sample was cycled through the column twice, and the column was washed with 2 volumes of buffer and 2 volumes of the above-described buffer containing 0.5 M NaCl. The antibody was eluted with 0.2 M glycine hydrochloride buffer (pH 2.3) containing 0.5 M NaCl and immediately neutralized with Tris base. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated the presence of a single protein under nonreducing conditions and peptides characteristic of immunoglobulin G under reducing conditions. Control immunoglobulin G was purchased commercially (Miles Laboratories, Inc., Elkhart, Ind.) and was used without further purification.

FN binding studies. Each experiment in every study was run in duplicate, and each study was repeated at least twice. The reaction mixtures (total volume, 1 ml) contained either 1×10^5 or 5×10^4 buccal cells (see below), FN, and other test materials. Each mixture was contained in a 1.5-ml polypropylene test tube, which was rotated end over end for 1 h at 37°C. After incubation, the cells were pelleted by centrif-

* Corresponding author.

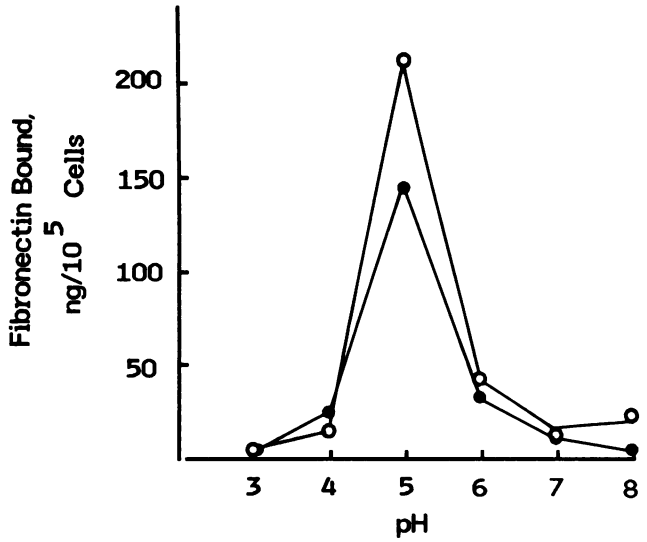

FIG. 1. Binding of FN to buccal epithelial cells as a function of pH. A l0-μg portion of ^{125}I-labeled FN was incubated with 5×10^4 buccal epithelial cells for 1 h at room temperature, and the amount of FN specifically bound was determined. The abscissa represents the amount of FN bound per 10^5 cells at each pH value tested. The results of two separate experiments performed on different days are shown.

ugation in a microcentrifuge. A sample of the supernatant was saved to determine the amount of unbound FN. The remainder of the supernatant was aspirated, and the cell pellet was suspended in fresh buffer. The centrifugation and washing steps were repeated twice, after which the cells were resuspended in the same buffer. A measured sample was transferred to a fresh tube for quantitation of the bound FN. Each experiment except the saturation curve and inhibition experiments was corrected for nonspecific binding by including an identical set of tubes which contained a 50-fold excess of unlabeled FN (8).

In addition to these controls, each experiment contained a set of tubes identical to the tubes described above, except that buffer was substituted for the buccal cell suspension. The amount of radioactivity collected from these control tubes was combined with the amount of ^{3}H-labeled FN bound by cells in the presence of a 50-fold excess of unlabeled FN and subtracted from the amount of radioactivity bound by the test cells to calculate specific binding. The radioactivity transferred from control tubes (without epithelial cells) never contained more than 1.2% of the counts bound by the cells. Up to 41% of the total binding was judged to be nonspecific by these criteria. The solubility of FN precluded the use of nonspecific binding controls at the higher concentrations of FN necessary to reach saturation.

The buffers used throughout the study contained 0.18 M NaCl, 0.1% bovine serum albumin, and either 0.01 M phosphate (pH 7 or above) or 0.01 M acetate (below pH 7). Initial studies were conducted in which the amount of FN remained constant and the specific activity of the radiolabel was varied from 140,000 to 2,000 cpm/μg. Under the conditions of this assay, the amount of FN bound did not vary, suggesting that the radiolabeling procedure did not perceptibly alter the binding characteristics of the FN molecule. Thus, the radiolabeled compound was diluted with unlabeled FN to a specific activity of ~30,000 cpm/μg for use in the binding assays. Compounds tested for their ability to inhibit the interaction of FN with buccal cells were preincubated with 1 μg of radiolabeled FN for 30 min prior to the addition of cells.

Additional control experiments indicated that the amount of FN bound when 10 μg of radiolabeled FN was incubated with varying numbers of epithelial cells (10^4 to 5×10^5 cells) increased in direct proportion to the number of epithelial cells added.

Saturation binding studies. Radiolabeled FN was diluted to different specific activities with unlabeled FN in pH 5 buffer and added to epithelial cells suspended in the same buffer. The binding experiments were then carried out as described above, except that the set of tubes containing an additional 50-fold excess of unlabeled FN was not included. Thus, the saturation binding experiments were corrected only by control tubes run with all components except the epithelial cells present.

Adherence tests. Epithelial cells were obtained from the buccal mucosa by gently scraping the mucosa with a sterile cotton swab. The cells were washed, mixed with the bacteria, separated from nonadherent bacteria by differential centrifugation, dried, and stained, and adherent bacteria were counted as previously described (1). The average number of adherent bacteria ranged between 6 and 12 per epithelial cell, and the background level (number of indigenous bacteria adherent to test cells) never exceeded 1 bacterium per cell. Adherence test mixtures contained approximately 5×10^8 bacteria per ml.

Epithelial cells were pretreated with FN for 1 h at 37°C in buffer at either pH 5 (maximal FN binding) or pH 6 (maximal *E. coli* adherence) (12). The cells were washed three times in the same buffer and resuspended, and the bacteria were added. Alternatively, FN was added to the epithelial cell suspension, and the bacteria were added immediately. No substantive pH effects were observed; thus, the data reported are the data that were generated at pH 6.

Chemicals. All chemicals used in our studies were of the highest quality and purity available.

Statistical methods. Differences in means were evaluated by the Student *t* test. Differences in frequency distribution were evaluated by using χ^2 analysis.

RESULTS

FN bound to buccal mucosa cells over a range of pH values normally encountered in oral cavities (Fig. 1). The

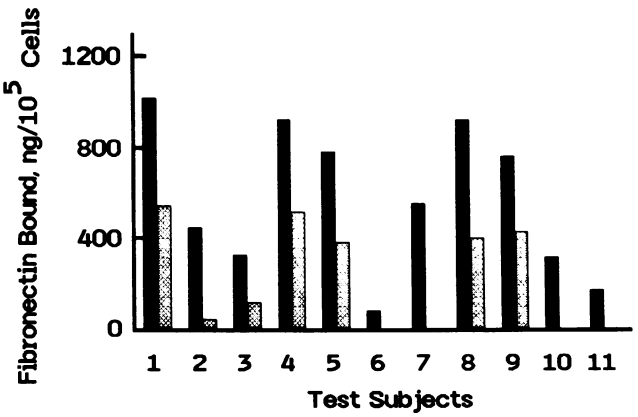

FIG. 2. Binding of FN to buccal cells isolated from 11 volunteers. The levels for both total (solid bars) and specific (cross-hatched bars) FN binding to cells isolated from different volunteers are shown.

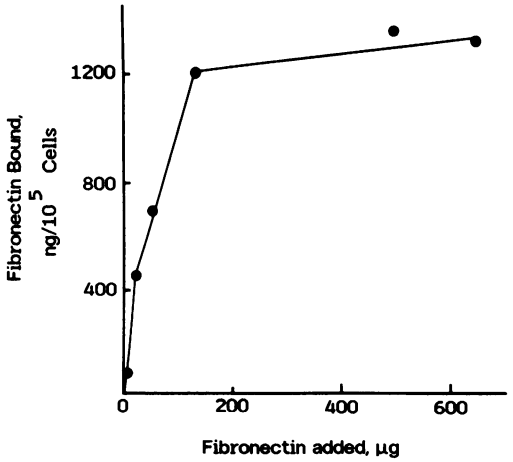

FIG. 3. Binding of radiolabeled FN to buccal cells at pH 5. Increasing amounts of radiolabeled FN (ordinate) were incubated with 10^4 buccal cells, and the amount of FN bound was determined (abscissa). Maximal binding occurred at a concentration of approximately 200 µg/ml.

binding of FN to isolated buccal cells was maximal at pH 5, and almost no binding was detected below pH 4 or above pH 7. In two experiments, the specific binding of FN to buccal cells at pH 5.0 varied from 140 to 210 ng/10^5 buccal cells (Fig. 1). Because of the experimental differences in binding, the abilities of cells isolated from individual volunteers to bind radiolabeled FN were compared. When cells were incubated with 10 µg of radiolabeled FN per ml, the total level of binding ranged from 81 to 1,004 ng/10^5 buccal cells. The level of specific binding ranged from 0 to 549 ng/10^5 buccal cells, with a specific binding average of 221 ng/10^5 cells (Fig. 2). By comparing the results shown in Fig. 1 and 2, we obtained an average specific binding value of 192 ± 41 ng/10^5 epithelial cells. To minimize the variability inherent in the assay, buccal cell pools containing cells from at least five individuals were used in each assay. A comparison of the levels of specific binding on different days suggested that the variations shown in Fig. 1 were typical; therefore, the remainder of the data are presented as averages of equivalent points from separate experiments.

Because FN binds to a large number of bacteria and because buccal cells isolated from some individuals have many adherent bacteria, as an additional control we compared the levels of binding of FN to buccal epithelial cells with varying levels of background (indigenous) bacteria. We found no relationship between the level of background bacteria and the amount of FN bound among the buccal cells from 11 different individuals.

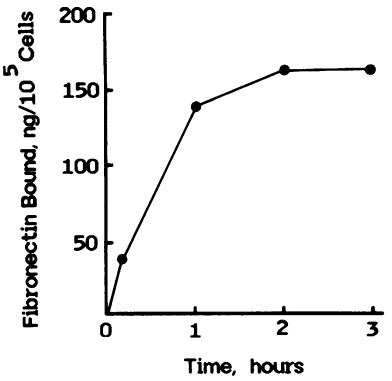

FIG. 4. Binding of FN to buccal cells as a function of time. Radiolabeled FN (10 µg/ml) was added to 5×10^4 buccal cells, and the amount of FN bound was determined as described in the text.

TABLE 1. Effects of various compounds on binding of FN to buccal cells

Test material[a]	Amt bound (cpm)	% Inhibition
Buffer	2,968	0
FN	805	73
Gelatin	2,528	15
Ovalbumin	2,178	26
Heparin	4,780	
Lipoteichoic acid	5,898	
Glucose	2,761	7
Mannose	2,731	8

[a] All test materials were added to the assay mixtures at a concentration of 100 µg/ml. The tests were performed twice in duplicate, and the results are the averages of these tests.

The binding of radiolabeled FN to buccal cells was saturable at pH 5 (Fig. 3). Because of the limited solubility of FN, saturation data are presented as levels of total binding. Maximal binding was obtained at an FN concentration of 200 µg/ml. The binding of radiolabeled FN reached a maximum after 2 h of incubation at 37°C; the level of binding reached 85% within 1 h (Fig. 4). The binding of radiolabeled FN was inhibited by unlabeled FN (73%) but was inhibited only slightly by gelatin and ovalbumin (15 and 26%, respectively) (Table 1). Both lipoteichoic acid and heparin increased the binding of FN to buccal cells, whereas mannose and glucose had no effect. FN-affinity-purified antibody inhibited the binding of human plasma FN to buccal cells in a dose-dependent manner (Fig. 5), suggesting that the blocking of specific antigenic epitopes can inhibit the interaction of FN and buccal cells. FN was bound to buccal cells in a reversible manner (Fig. 6). Approximately 50% of the FN bound could be eluted over a 4-h period when washed cells were suspended in 4 ml of buffer, whereas in the same time period the addition of excess unlabeled FN (500 µg/ml) displaced up to 70% of the bound FN (Fig. 6).

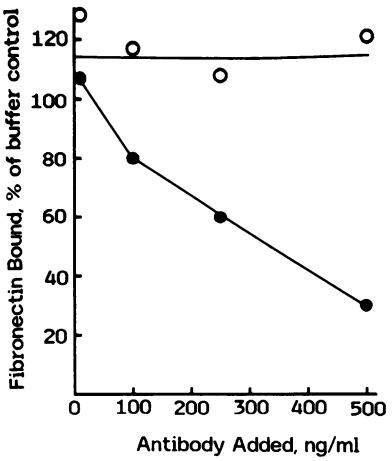

FIG. 5. Inhibition of FN binding to buccal cells by antibody. Affinity-purified anti-FN inhibited the binding of FN to buccal cells in a dose-dependent manner (●). Incubation of FN with a purified rabbit immunoglobulin G fraction had no effect (○).

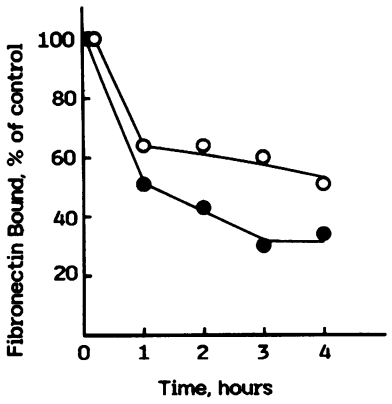

FIG. 6. Reversibility of FN binding to buccal cells. FN bound to buccal cells could be eluted by resuspending washed cells in 4 ml of buffer (○) or buffer containing 50 μg of unlabeled FN per ml (●).

Having established that FN binds to isolated buccal epithelial cells, we decided to determine the effect of the bound FN on the adherence of a gram-negative bacterium. The effect of FN on the adherence of gram-negative bacteria was of particular interest in view of previous clinical studies which showed an inverse relationship between buccal epithelial cell content of FN and the binding of *P. aeruginosa* (35). When buccal cells were preincubated with 100 μg of FN per ml and washed, the adherence of *E. coli* strain CSH50 was reduced from 12.7 ± 3.8 to 7.2 ± 2.1 bacteria per cell. When three separate experiments (five replicates per experiment) were compared, this difference was significant ($t = 6.46$; $P < 0.001$), suggesting that FN reduced the average adherence of *E. coli* to the buccal cells. This hypothesis was tested by analyzing the adherence distribution in the three experiments (Fig. 7). To establish the frequency distribution, the results from the three experiments were combined, and the numbers of epithelial cells binding 0, 1 to 10, 11 to 20, 21 to 30, 31 to 40, 41 to 50, and more than 50 bacteria were determined. When the number of buccal cells binding a given number of bacteria was compared in a 2 by 7 table by χ^2 analysis, the experimental group was significantly different from the control group ($\chi^2 = 120$; $P < 0.001$). Because the major differences observed appeared to be in the two extreme categories (0 bacteria and more than 50 bacteria), the data were recombined into a 2 by 5 table to minimize the effect of these differences. When the data were analyzed, significant differences were still found ($\chi^2 = 115$; $P < 0.001$). Thus, it appears that FN significantly inhibits the binding of *E. coli* strain CSH50 to buccal cells, and the principal effect of this molecule is to shift the distribution of epithelial cells by increasing the number of buccal cells that bind few (≤10) bacterial cells. This effect is more clearly illustrated in Fig. 8, in which the ratio of the number of buccal cells in an experimental group to the number of cells in the control group is plotted as a function of the adherent bacterial distribution. When the data are plotted in this manner, it is clear that the extreme groups were most affected by FN treatment. The number of epithelial cells without bacteria increased 10-fold after FN pretreatment, whereas the number of cells with more than 50 bacteria decreased 10-fold. In another set of experiments, pretreatment of buccal cells with FN inhibited the adherence of type 1 fimbriated *E. coli* strain CSH50 in a dose-dependent manner (Fig. 9). As little as 1 μg of FN per ml inhibited the adherence of *E. coli* by approximately 50%. When FN was included in the reaction mixture, a dose-dependent inhibition was also observed, and at the maximum concentration tested, the inhibitory effect of FN approached that of a 2.5% solution of α-methyl mannoside (65 versus 80%), the receptor analog for *E. coli* type 1 fimbriae (22). When the bacteria were pretreated with 100 μg of FN per ml and washed prior to introduction into the adherence test mixture, no inhibition was observed.

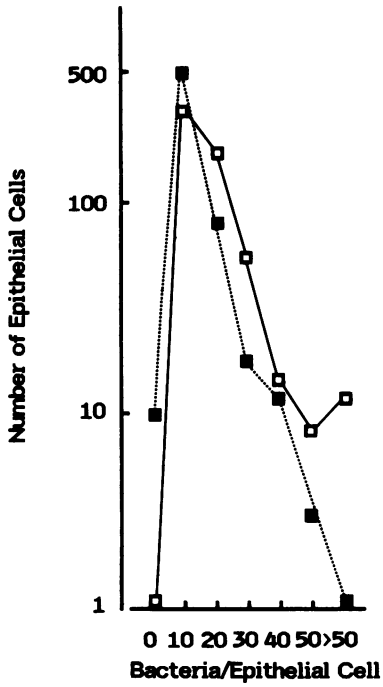

FIG. 7. Frequency distribution of the binding of *E. coli* to control (□) and FN-treated (■) epithelial cells. The numbers of epithelial cells binding 0, 1 to 10, 11 to 20, 21 to 30, 31 to 40, 41 to 50, and more than 50 bacteria were totaled for 15 separate experiments. Only the final number for each determination is shown for clarity.

DISCUSSION

Our data demonstrate that isolated buccal epithelial cells have the ability to bind exogenous FN. A large proportion of

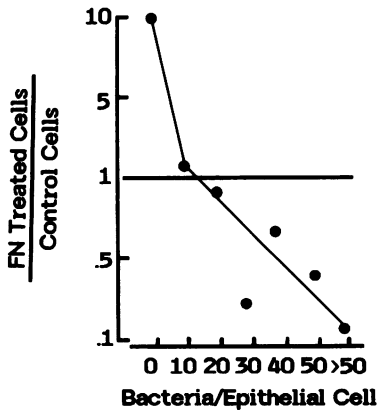

FIG. 8. Ratio of the number of cells in each interval in the experimental (FN-treated) and control groups. The data in Fig. 7 were used to calculate the ratio for each adherence interval.

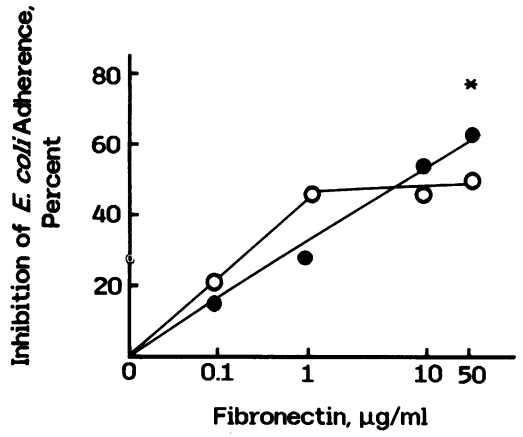

FIG. 9. Inhibition of adherence of *E. coli* to buccal cells by FN. Either buccal cells were pretreated and washed (○) or FN was included in the reaction mixture (●) during the adherence test at different concentrations of FN (ordinate). The results shown are the averages obtained from three separate experiments. αMM (2.5%) was always used as a control in the inhibition experiments, and the average level of inhibition obtained with this sugar is included for comparison (asterisk).

the FN binding in our assay appeared to be specific (60 to 70%), reversible, and saturable, suggesting a receptor-mediated reaction. However, a significant amount of the FN binding was also nonspecific and nonreversible. We have recently demonstrated that salivary FN or plasma FN added exogenously to saliva can bind to buccal epithelial cells in the presence of whole saliva (27a), suggesting that interactions similar to those observed in this study may also occur in vivo.

The ability of cells obtained from individual volunteers to bind FN varies. Variation occurred in both specific binding (the amount of binding that could be inhibited by a 50-fold excess of unlabeled FN) and total binding. All of the cells studied bound FN; however, cells from some individuals bound FN only non-specifically under the conditions of our assay system. Further investigation will be needed to determine the nature of the individual differences observed.

Although the addition of exogenous FN to buccal cells decreased adherence of type 1 fimbriated *E. coli*, the mechanism of the inhibition remains to be determined. Several studies (1, 35, 36) have suggested that gram-negative bacteria bind in greater numbers to buccal cells devoid of FN. In recent studies Vercellotti et al. (34) demonstrated that several gram-negative species tested do not bind to either a FN-collagen matrix or to the FN on endothelial cells. However, it has also been shown that FN binds to a number of gram-negative bacteria (33), and preliminary results in our laboratories indicate that *E. coli* strain CSH50 binds plasma FN (Hasty et al., unpublished data). Nevertheless, the binding of soluble FN to the surfaces of these organisms does not support their attachment to phagocytes (33), endothelial cells (34), epithelial cells (this study), or FN immobilized on latex beads (26). Thus, the physiological significance of the interaction of FN with certain gram-negative bacteria is unclear.

Gram-negative bacteria such as *E. coli*, *P. aeruginosa*, and *Klebsiella pneumoniae* are not part of the normal flora of oral cavities. However, certain patient populations often become colonized with gram-negative organisms (15), and the colonization appears to be accompanied by a loss of FN on the buccal cells and a concomitant increase in the number of gram-negative bacterial receptors (36). Previous attempts by Woods et al. (36) to prevent the adherence of *Pseudomonas* cells to epithelial cells with FN were unsuccessful. Our studies of the effect of pH suggest that this lack of success may have been due to the poor binding of FN to epithelial cells at pH 7.4, the conditions used by Woods and co-workers (36). The ability of FN to block the binding of type 1 fimbriated *E. coli* cells to buccal epithelial cells, both in an artificial buffer system and in the presence of whole saliva (27a), suggests that this glycoprotein may play an important role in the ecology of the bacterial populations in oral cavities.

ACKNOWLEDGMENTS

We thank Sandra Tsiu and Carolyn Sturdivant for excellent technical assistance and Pamela Swann and Johnnie Smith for excellent secretarial assistance.

This work was supported by funds from the Veterans Administration Research Service and by Public Health Service grants AI-10085 and AI-13550 from the National Institutes of Health.

LITERATURE CITED

1. **Abraham, S. N., E. H. Beachey, and W. A. Simpson.** 1983. Adherence of *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* to fibronectin-coated and uncoated epithelial cells. Infect. Immun. **41**:1261–1268.
2. **Abraham, S. N., D. L. Hasty, W. A. Simpson, and E. H. Beachey.** 1983. Anti-adhesive properties of a quanternary structure-specific hybridoma antibody against type 1 fimbriae of *Escherichia coli*. J. Exp. Med. **158**:1114–1128.
3. **Babu, J. P., W. A. Simpson, H. S. Courtney, and E. H. Beachey.** 1983. Interaction of human plasma fibronectin with cariogenic and non-cariogenic oral streptococci. Infect. Immun. **41**:162–168.
4. **Beachey, E. H., and W. A. Simpson.** 1982. The adherence of group A streptococci to oropharyngeal cells: the lipoteichoic acid adhesin and fibronectin receptor. Infection **10**:107–111.
5. **Courtney, H. S., W. A. Simpson, and E. H. Beachey.** 1983. Binding of streptococcal lipoteichoic acid to fatty acid binding sites on human plasma fibronectin. J. Bacteriol. **153**:763–770.
6. **Crouch, E., G. Balian, K. Holbrook, D. Duksin, and P. Bornstein.** 1978. Amniotic fluid fibronectin. Characterization and synthesis by cells in culture. J. Cell Biol. **78**:701–715.
7. **Culp, L. A., B. A. Murray, and B. J. Rollins.** 1979. Fibronectin and proteoglycans as determinants of cell substratum adhesion. J. Supramol. Struct. **11**:401–427.
8. **Dahlquist, F. W.** 1978. The meaning of Scatchard and Hill plots. Methods Enzymol. **48**:270–307.
9. **Doran, J. E.** 1983. A critical assessment of fibronectin's opsonic role for bacteria and microaggregates. Vox Sang. **45**:337–348.
10. **Doran, J. E., and R. H. Raynor.** 1981. Fibronectin binding to protein A-containing staphylococci. Infect. Immun. **33**:683–689.
11. **Esperson, F., and I. Clemmensen.** 1982. Isolation of a fibronectin-binding protein from *Staphylococcus aureus*. Infect. Immun. **37**:526–531.
12. **Harber, M. J., R. MacKenzie, and A. W. Asscher.** 1983. A rapid bioluminescence method for quantifying bacterial adhesion to polystyrene. J. Gen. Microbiol. **129**:621–632.
13. **Hedman, K., A. Vaheri, and J. Wartiovaara.** 1978. External fibronectin of cultured human fibroblasts is predominantly a matrix protein. J. Cell Biol. **76**:748–760.
14. **Hynes, R. O., and K. M. Yamada.** 1982. Fibronectins: multifunctional modular glycoproteins. J. Cell Biol. **95**:369–378.
15. **Johanson, W. G., A. K. Pierce, and J. P. Sanford.** 1969. Changing pharyngeal bacterial flora of hospitalized patients. Emergence of gram-negative bacilli. N. Engl. J. Med. **281**:1137–1140.
16. **Kleinman, H. K., R. J. Klebe, and G. R. Martin.** 1981. Role of collagenous matrices in the adhesion and growth of cells. J. Cell Biol. **88**:473-486.
17. **Kuusela, P.** 1978. Fibronectin binds to *Staphylococcus aureus*.

Nature (London) **276**:718–720.
18. **Kuusela, P., A. Vaheri, J. Palo, and E. Ruoslahti.** 1978. Demonstration of fibronectin in human cereobrospinal fluid. J. Lab. Clin. Med. **92**:595–601.
19. **March, S. C., I. Parikh, and P. Cuatrecasas.** 1974. A simplified method for cyanogen bromide activation of agarose for affinity chromatography. Anal. Biochem. **60**:149–152.
20. **Mosher, D. F., and R. A. Proctor.** 1980. Binding and factor $XIII_a$-mediated cross-linking of a 27-kilodalton fragment of fibronectin to *Staphylococcus aureus*. Science **209**:927–929.
21. **Mosseson, M. W., and D. L. Amrani.** 1980. The structure and biologic activities of plasma fibronectin. Blood **56**:145–158.
22. **Ofek, I., and E. H. Beachey.** 1978. Mannose binding and epithelial cell adherence of *Escherichia coli*. Infect. Immun. **22**:247–254.
23. **Proctor, R. A., R. J. Hamill, D. F. Mosher, J. A. Textor, and P. J. Olbrantz.** 1983. Effects of subinhibitory concentrations of antibiotics on *Staphylococcus aureus* interactions with fibronectin. J. Antimicrob. Chemother. **12**:85–95.
24. **Ruoslahti, E.** 1981. Fibronectin. J. Oral Pathol. **10**:3–13.
25. **Ruoslahti, E., H. Jalanko, D. E. Comings, A. M. Neville, and D. Raghaven.** 1981. Fibronectin from human germ-cell tumors resembles amniotic fluid fibronectin. Int. J. Cancer **27**:763–767.
26. **Simpson, W. A., and E. H. Beachey.** 1983. Adherence of group A streptococci to fibronectin on oral epithelial cells. Infect. Immun. **39**:275–279.
27. **Simpson, W. A., H. S. Courtney, and E. H. Beachey.** 1982. Fibronectin—a modulator of the oropharyngeal bacterial flora, p. 346–347. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.
27a. **Simpson, W. A., D. L. Hasty, and E. H. Beachey.** 1985. Inhibition of the adhesion of *Escherichia coli* to oral epithelial cells by fibronectin, p. 40–44. *In* S. E. Mergenhagen and B. Rosan (ed.), Molecular basis of oral microbial adhesion. American Society for Microbiology, Washington, D.C.
28. **Simpson, W. A., D. L. Hasty, J. M. Mason, and E. H. Beachey.** 1982. Fibronectin mediates the binding of streptococci to human polymorphonuclear leukocytes. Infect. Immun. **37**:805–810.
29. **Simpson, W. A., I. Ofek, and E. H. Beachey.** 1980. Binding of streptococcal lipoteichoic acid to the fatty acid binding sites on serum albumin. J. Biol. Chem. **255**:6092–6097.
30. **Speziale, P., M. Hook, L. M. Switalski, and T. Wadstrom.** 1984. Fibronectin binding a *Streptococcus pyogenes* strain. J. Bacteriol. **157**:420–427.
31. **Switalski, L. M., A. Ljungh, C. Ryden, K. Rubin, M. Hook, and T. Wadstrom.** 1982. Binding of fibronectin to the surface of group A, C and G streptococci isolated from human infections. Eur. J. Clin. Microbiol. **1**:381–387.
32. **Switalski, L. M., C. Ryden, K. Rubin, A. Ljungh, M. Hook, and T. Wadstrom.** 1983. Binding of fibronectin to *Staphylococcus* strains. Infect. Immun. **42**:628–633.
33. **van de Water, L., A. T. Destree, and R. O. Hynes.** 1983. Fibronectin binds to some bacteria but does not promote their uptake by phagocytic cells. Science **220**:201–204.
34. **Vercellotti, G. M., D. Lussenhop, P. K. Peterson, L. T. Furcht, J. B. McCarthy, H. S. Jacob, and C. F. Moldow.** 1984. Bacterial adherence to fibronectin and endothelial cells: a possible mechanism for bacterial tissue tropism. J. Lab. Clin. Med. **103**:34–43.
35. **Woods, D. E., D. C. Straus, W. G. Johanson, Jr., 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.
36. **Woods, D. E., D. C. Straus, W. G. Johanson, Jr., and J. B. Bass.** 1981. Role of salivary protease activity in adherence of gram-negative bacilli to mammalian buccal epithelial cells *in vivo*. J. Clin. Invest. **68**:1435–1440.
37. **Yamada, K. M.** 1983. Cell surface interactions with extracellular materials. Annu. Rev. Biochem. **52**:761–799.
38. **Zetter, B. R., T. E. Daniels, C. Quadra-White, and J. S. Greenspan.** 1979. LETS protein in normal and pathologic human oral epithelium. J. Dent. Res. **58**:484–488.