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Enhanced Levels of Attachment of Fibronectin-Primed *Treponema pallidum* to Extracellular Matrix

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Freshly extracted *Treponema pallidum* organisms treated with exogenous human fibronectin (Fn) (Fn-primed treponemes) showed a 6- to 15-fold increased level of attachment to Fn-coated cover slips and to extracellular matrix (ECM) when compared with unprimed treponemes. Treponemes primed with collagen or laminin showed no similar enhanced binding to immobilized Fn or ECM. Preexposure of immobilized Fn and ECM to anti-Fn serum but not to anti-collagen or anti-laminin serum prevented treponemal adherence. Also, the presence of proteoglycanlike molecules such as dextran sulfate or heparan sulfate inhibited Fn-primed treponemal attachment to Fn or ECM. In contrast Fn-primed treponemes did not exhibit elevated levels of attachment to eucaryotic cell monolayers. To understand the increased tropism of Fn-primed *T. pallidum* organisms for Fn and ECM-like surfaces, we radiolabeled freshly extracted treponemes with [^{35}S]methionine and examined them for the presence of surface immunoreactive Fn. Magnetic protAspheres and glass beads coated with monospecific anti-Fn serum bound only 20 to 30% of radiolabeled treponemes. Nonadherent treponemes failed to bind to gelatin-agarose, further confirming the absence of surface Fn or Fn-like material. Fn-free organisms, however, did attach to Fn-coated cover slips and to cell monolayers like treponemes of the original population. Incubation of Fn-free treponemes with human Fn resulted in almost total binding of organisms to anti-Fn antibody on glass beads and also produced increased attachment to Fn-coated cover slips and ECM. These results suggest that enhanced interactions between *T. pallidum* and the host are dependent on the presence of Fn on syphilis spirochetes and the specific location and orientation of Fn in vivo.

The syphilis spirochete, *Treponema pallidum*, parasitizes host cell surfaces (cytadherence) in a specific, high-affinity, and energy-requiring manner (5, 6, 11, 14). Treponemal adherence appears to involve association with fibronectin (Fn) on eucaryotic cell membranes mediated by the important arginine-glycine-aspartic acid tripeptide of the cell-binding domain of Fn (12, 19, 22, 23). Little information is available, however, concerning the attachment of treponemes to Fn or other components making up the extracellular matrix (ECM) that exists between cells and tissues (13, 15, 17, 18). It is, therefore, essential to characterize and compare *T. pallidum* interactions with Fn found in ECM-type materials and on host cell surfaces to understand the in situ tropism (18) of *T. pallidum*.

In this study we demonstrated significantly enhanced binding of Fn-primed *T. pallidum* to Fn immobilized on glass cover slips and deposited in ECM (13) but not to host cells in monolayer culture. The data also indicated that associations between Fn on treponemes and Fn in ECM are mediated by proteoglycanlike molecules. We discuss our results in terms of relevance to dissemination of syphilis infection in the host.

MATERIALS AND METHODS

Bacteria. *T. pallidum* organisms were harvested in a serum-free extraction medium with reducing agents (1) from infected rabbit testicular tissue as previously described (1, 7, 8). Treponemes were clarified of host cellular debris by centrifugation twice at 500 × *g* for 10 min followed by centrifugation at 650 × *g* for 20 min on a 0.8% Methacel (Dow Chemical Co., Midland, Mich.)-50% Hypaque (Winthrop Laboratories, Div. Sterling Drug Inc., New York, N.Y.) gradient. Supernatant containing 1×10^8 to 2×10^8 spirochetes per ml was used for all assays.

Radiolabeling of *T. pallidum*. Ten milliliters consisting of approximately 2×10^9 motile treponemes was radiolabeled with 50 to 100 μCi of [^{35}S]methionine (specific activity, 1,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) per ml for 3 h at 34°C in an air atmosphere (2, 3, 6). For both cytadherence assays and attachment to Fn or ECM on glass cover slips, ^{35}S-labeled treponemes were first centrifuged at 17,000 × *g* (2) and suspended in extraction medium without radioisotope.

Preparation of antisera. Rabbits were immunized with an initial injection of 300 μg of purified human plasma Fn emulsified in Freund complete adjuvant (19). Booster injections of 300 μg in Freund incomplete adjuvant were given three additional times at 10-day intervals. Rabbits were bled 14 days after the last booster inoculation. Anti-Fn antibodies were measured by enzyme-linked immunosorbent assay with Fn-coated microtiter wells (4). Antiserum to type I rat collagen was also prepared by this method. Antiserum to laminin was obtained from Bethesda Research Laboratories, Inc., Gaithersburg, Md. Pooled normal rabbit sera (NRS) served as controls. Serum immunoglobulin G (IgG) fractions were purified by protein A-agarose (Sigma Chemical Co., St. Louis, Mo.) affinity chromatography.

***T. pallidum* attachment to protein-coated cover slips and eucaryotic cells in monolayer culture.** Glass cover slips (9 by 35 mm) were coated with individual protein preparations of human plasma Fn (19), laminin (Bethesda Research Laboratories), type I collagen, or bovine serum albumin as previously described (19, 22). Collagen was solubilized in 0.1 M acetic acid, and other proteins were dissolved in phosphate-buffered saline (PBS). A 50-μl volume of each of the respective protein solutions (1 mg/ml) was spread evenly over the entire glass surface and allowed to air dry at room temper-

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ature (RT) (19). Indirect immunofluorescence demonstrated a uniform protein layer on glass surfaces (19, 22).

Human tumor (HT1080) cells (American Type Culture Collection, Rockville, Md.) were maintained in Dulbecco minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum (KC Biologicals, Lenexa, Kans.). Chinese hamster ovary (CHO) cells (American Type Culture Collection) were cultured in Ham F-12 nutrient medium (GIBCO) containing 10% fetal calf serum. Cell monolayers used for cytadherence experiments were grown on 9- by 35-mm glass cover slips in Leighton tubes as recently detailed (19, 22).

One milliliter of medium containing unlabeled or ^{35}S-labeled treponemes was added to Leighton tubes containing ECM, protein-coated cover slips, or cell monolayers and incubated at 34°C for 2 h (22). For some experiments, treponemes obtained from the same extraction were also pretreated with increasing amounts of Fn, collagen, laminin, dextran sulfate, heparan sulfate, dextran, or a heptapeptide containing the arginine-glycine-aspartic acid sequence (20, 23) at RT for 30 min prior to incubation with test cover slips.

Attachment of *T. pallidum* to ECM. Bovine corneal endothelial cells used for generating ECM were isolated from eyes of freshly killed cows as described by Gospodarowicz (13). Briefly, corneas of two eyes were removed and washed sequentially with 95% ethanol and PBS. Cells were then gently scraped from the corneas with a sterile scalpel blade and released into a 35-mm^2 tissue culture dish containing Dulbecco minimal essential medium–10% fetal calf serum supplemented with 50 μg of gentamicin and 0.25 μg of amphotericin B per ml. After incubation at 37°C in a humidified CO_2 atmosphere for 5 days, 25 ng of brain fibroblast growth factor (Collaborative Research, Inc., Waltham, Mass.) per ml was added to the medium. Cells at 80 to 90% confluency were expanded into 25-cm^2 flasks after dissociation of cell monolayers with 0.25% trypsin in 0.2% EDTA–PBS.

A 1-ml suspension of 5×10^5 to 6×10^5 endothelial cells derived from confluent monolayers in 75-cm^2 flasks was added to individual 24-well plates containing ethanol-washed, 12-mm diameter round glass cover slips. Plates were incubated at 37°C for 3 weeks to allow deposition of the ECM (13), and medium was changed every 4 days. Cells on glass coverslips were treated with 1.0 ml of 20 mM NH_4OH at RT for 30 min. This treatment removed the cells without disturbing intact ECM attached to the glass cover slips (13, 17). Wells were then washed twice with distilled H_2O and once with 70% ethanol. ECM preparations on glass cover slips were stored at −20°C. When needed, cover slips in 24-well plates were washed twice with PBS at RT before addition of treponemes. Cover slips were incubated, washed, and counted for radioactivity as described above. In some experiments, ECM was treated with 1:50 dilutions of antiserum to Fn, laminin, or collagen for 1 h at 34°C prior to addition of treponemes.

The presence of Fn, collagen, and laminin in ECM was confirmed by two methods. First, a colorimetric enzyme-linked immunosorbent assay (4, 25) was performed on ECM-coated cover slips by using specific antisera to respective ECM components. The assay was accomplished as described previously (25), except that the reaction was conducted in 24-well plates with 300-μl reaction volumes. After incubation with substrate, 200 μl from each well was transferred to a 96-well plate for evaluation at 405 nm. Additionally, ECM material associated with cover slips was solubilized overnight in 1 ml of 2 M NaOH at 37°C (16) and subsequently electrophoresed and transferred to nitrocellulose (24). Nitrocellulose blots were probed with antiserum to Fn, laminin, or collagen, and reactive bands were visualized with goat anti-rabbit IgG horseradish peroxidase conjugate (ImmunoBlot Assay Kit; Bio-Rad Laboratories, Richmond, Calif.). Purified Fn, laminin, and collagen type I were used as standards.

Isolation of Fn-free *T. pallidum* organisms. For each experiment, 40 μl of magnetic protAspheres (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) washed twice with PBS–0.1% Tween 80 was mixed with 40 μg of purified IgG fraction from rabbit anti-human Fn or NRS. After incubation for 1 h at RT, protAspheres were again washed six times with PBS–0.1% Tween 80. Approximately 10^6 radiolabeled treponemes in 0.5 ml of PBS–0.1% Tween 80 were added to pelleted protAspheres. After 2 h of gentle mixing on a Tekpro Dimensional Rotator at RT, protAspheres were separated with a magnet placed on the side of the tube for 5 min. The supernatant containing nonadherent treponemes was then readsorbed with an additional preparation of IgG-treated protAspheres. Treponemes adherent to the anti-Fn antibody-coated protAspheres were combined. Quantitation of adherent and nonadherent populations of *T. pallidum* organisms was established by scintillation spectroscopy.

Because of limitations in recovery of treponemes from experiments with protAspheres, we attempted isolation of Fn-free organisms by affinity chromatography. Glass beads (1-mm diameter) were placed in siliconized 9-in. (1 in. = 2.54 cm) Pasteur pipettes and treated with a solution of 3% polycarbonate (Plastics Supply Co., San Antonio, Tex.) in methylene chloride. After drying, the beads were incubated with 1.0 mg of the IgG fraction of anti-human Fn serum or NRS in 15 mM carbonate buffer, pH 9.6. After overnight incubation at 4°C, the columns were washed with 10 volumes of serum-free treponemal extraction medium. A 2-ml volume of ^{35}S-labeled or unlabeled motile treponemes was added to the glass bead column, and nonadherent treponemes were eluted with PBS. Fn-free spirochetes were then tested for their ability to attach to cover slips coated with various proteins and gelatin-agarose. Cytadherence assays were performed with HT1080 and CHO cells as described above (21). For studies involving gelatin-agarose, a 1.0-ml suspension containing 7×10^7 nonadherent, ^{35}S-labeled treponemes was mixed with 1.0 ml of packed gelatin-agarose washed in serum-free extraction medium. After incubation at RT for 20 min, nonadherent organisms were incubated with new gelatin-agarose. The extent of treponemal attachment to gelatin-agarose, protein-coated cover slips, and host cells was determined by counting radioactivity or by enumerating treponemes (22). Results were compared with freshly extracted treponemes adjusted to the same density and handled similarly. Spirochete motility remained ≧80% throughout these experiments.

RESULTS

Enhanced attachment of Fn-primed *T. pallidum* to Fn on glass surfaces. Figure 1 (panel A) is a typical dark-field micrograph showing the attachment of freshly extracted *T. pallidum* to Fn-coated glass cover slips (19, 22). Interestingly, when organisms were first incubated (primed) with increasing amounts of Fn (up to 25 μg) prior to addition to Fn-coated cover slips, greater numbers of organisms attached to Fn on cover slips (Fig. 1b). We therefore attempted to quantitate the extent of enhanced attachment of ^{35}S-labeled treponemes resulting from preincubation of spirochetes with Fn. Table 1 shows a correlation between

numbers of treponemes bound to immobilized Fn and amounts of added Fn. The presence of 50 μg of Fn resulted in a 16-fold higher level of treponemal binding to Fn-coated cover slips. No agglutination of Fn-primed *T. pallidum* organisms was observed under these conditions. Priming of organisms with laminin or collagen in similar experiments failed to augment *T. pallidum* attachment to Fn (data not shown). Also, no treponemal binding was observed when Fn-primed organisms were incubated with bovine serum albumin-coated or uncoated cover slips, indicating that specific Fn-Fn-type interactions were mediating the enhanced parasite recognition of Fn on glass cover slips.

Fn priming and association of treponemes with ECM. We next examined attachment of treponemes to bovine corneal endothelial cell ECM. This attachment appeared to be mediated by Fn, since pretreatment of ECM with anti-Fn antibody inhibited attachment up to 75% of positive control values (Table 2). Anti-collagen and anti-laminin antibodies which extensively bound to ECM based on immunofluorescence, enzyme-linked immunosorbent assay, and immunoblot data (see Materials and Methods) were ineffective in preventing attachment. Furthermore, priming of parasites with Fn resulted in a sixfold or greater overall increase in levels of parasite attachment to ECM (Table 3). These data show the ability of syphilis spirochetes to interact with Fn or ECM and also illustrate the ability to achieve greater numbers of parasites bound to ECM when treponemes were first primed with Fn.

TABLE 1. Representative experiment showing the effect of exogenous Fn on *T. pallidum* attachment to Fn-coated cover slips[a]

Amt of Fn added (μg)	Recovered cpm[b]	Fold increase
0.00	11,000 ± 482	1.0
0.05	18,556 ± 1,138	1.7
0.50	29,908 ± 1,188	2.7
5.00	47,600 ± 2,266	4.3
50.00	174,600 ± 2,663	15.9

[a] Proteins were added to a 1.0-ml suspension of 7×10^7 ^{35}S-labeled treponemes and incubated for 30 min at 34°C prior to addition to cover slips. After incubation for 2 h at 34°C, cover slips were removed, washed, and measured for radioactivity.

[b] Data represent the mean ± standard deviation of three determinations.

Inhibitory effect of Fn on *T. pallidum* cytadherence. It was important to determine whether Fn-primed treponemes demonstrated elevated levels of host cell parasitism. Table 4 shows that Fn-primed organisms did not exhibit enhanced cytadherence. No effect was also observed for levels of Fn between 10 and 100 μg, where increased treponemal attachment to ECM was evident (Table 3), and in fact, inhibition of *T. pallidum* attachment to host cells was observed when treponemes were preincubated with ≧500μg/ml concentrations of Fn.

Inhibition of enhanced attachment of Fn-primed treponemes to Fn-coated surfaces. Aggregation of Fn molecules is mediated by proteoglycans such as heparan sulfate (9). We therefore tested whether proteoglycans might modulate reactions between treponemal surface Fn and immobilized Fn (Tables 1 and 3). We found that 15% sulfated dextran sulfate and heparan sulfate significantly reduced attachment of Fn-primed treponemes to Fn-coated cover slips (Table 5). In contrast, dextran, poorly sulfated (1%) dextran sulfate, and a synthetic heptapeptide containing the arginine-glycine-

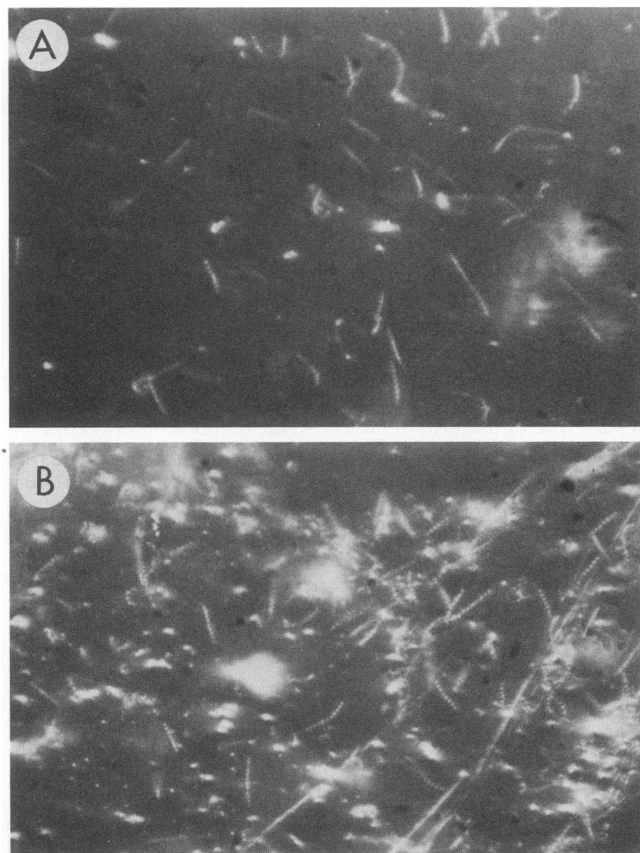

FIG. 1. Effect of exogenous Fn on *T. pallidum* attachment to Fn-coated cover slips. Dark-field microscopy of representative fields showing treponemal attachment to Fn-coated cover slips in the absence of exogenous Fn (A) and after preincubation of treponemes with 25 μg of Fn (B).

TABLE 2. *T. pallidum* attachment to ECM[a]

Expt no.	Antibody used for pretreatment of ECM[b]	Recovered cpm[c]	% of control
1	NRS	8,034 ± 107	100
	Anti-laminin	7,780 ± 243	97
	Anti-collagen	7,870 ± 154	98
	Anti-Fn	2,358 ± 141	29
2	NRS	10,232 ± 269	100
	Anti-laminin	9,560 ± 201	94
	Anti-collagen	9,832 ± 193	96
	Anti-Fn	2,560 ± 214	25
3	NRS	9,838 ± 177	100
	Anti-laminin	9,402 ± 153	96
	Anti-collagen	9,716 ± 165	99
	Anti-Fn	3,212 ± 159	33

[a] A 1.0-ml suspension of 7×10^7 ^{35}S-labeled treponemes was added to each ECM-coated cover slip. The remainder of the procedure was as described in Table 1, footnote a.

[b] A 1:50 dilution of the indicated antiserum in PBS was incubated on ECM-coated cover slips for 1 h at 34°C before addition of treponemes. After 2 h of incubation at 34°C, cover slips were removed, washed, and measured for radioactivity. Controls were pretreated with PBS in the absence of antibody.

[c] Data represent the mean ± the standard deviation of three determinations.

TABLE 3. Attachment of Fn-primed *T. pallidum* to ECM[a]

Amt of Fn added (μg)[b]	Recovered cpm[c]	Fold increase
0	2,583 ± 83	1.0
10	2,673 ± 241	1.0
25	7,563 ± 1,250	3.0
50	9,800 ± 648	3.8
100	15,527 ± 936	6.0

[a] A 1.0-ml suspension of 7×10^7 ^{35}S-labeled treponemes was added to each ECM-coated cover slip. The remainder of the procedure was as described in Table 1, footnote a.

[b] The indicated amount of Fn was incubated with treponemes for 30 min at 34°C prior to their addition to cover slips. After 2 h of incubation at 34°C, cover slips were removed, washed, and measured for radioactivity.

[c] Data represent the mean ± the standard deviation of six determinations of a representative experiment.

aspartic acid sequence (20, 23, 26) showed little or no inhibition of spirochete binding to Fn. In addition, 15% sulfated dextran sulfate (500 μg/ml) inhibited attachment of *T. pallidum* to ECM by up to 80%. These results suggest a role for glycosaminoglycans in the attachment of Fn-primed treponemes to Fn-coated surfaces.

Differentiation of freshly extracted *T. pallidum* organisms by anti-Fn antibody. Because of the dramatic effect of Fn bound to *T. pallidum* on treponemal adherence to Fn-containing surfaces, we screened freshly extracted treponemes for differences in surface Fn or Fn-like material. Radiolabeled treponemes were incubated with magnetic protAspheres coated with anti-Fn IgG antibody, and numbers of bound versus nonadherent organisms were determined. In three separate experiments, only ≦30% of freshly extracted organisms possessed cross-reactive Fn-like material (data not shown). These results suggest that freshly harvested organisms can be differentiated into subpopulations based on the presence or absence of surface Fn or Fn-like molecules.

To isolate greater numbers of treponemal subpopulations for further studies, we performed chromatography of freshly extracted organisms on glass beads coated with anti-Fn IgG antibody. The amount of immunoglobulin bound to glass beads was ~0.8 mg as measured by protein determination of column eluate. Consistent with the data from protAsphere experiments, only approximately 20 to 30% of radiolabeled treponemes was bound by anti-Fn antibody. In contrast, only 3% of freshly extracted organisms from a duplicate preparation bound nonspecifically to control glass beads coated with NRS IgG. Importantly, incubation of washed treponemes with 25 μg of Fn prior to chromatography resulted in almost total binding of radiolabeled organisms to Fn-coated glass beads.

Glass bead-purified, nonadherent (Fn-free), ^{35}S-labeled treponemes were then incubated at RT with gelatin-agarose beads to confirm the absence of Fn from this *T. pallidum* subpopulation. Greater than 98% of radioactivity remained unassociated with gelatin. As expected, Fn-free treponemes recognized Fn on cover slips in a manner similar to that of parasites from the original extraction. Priming of Fn-free treponemes with Fn also yielded results analagous to data presented earlier (Tables 1 and 2). These data demonstrate that freshly harvested treponemes without surface Fn or Fn-like material are capable of binding Fn in vitro.

TABLE 4. Effect of Fn priming on *T. pallidum* cytadherence[a]

Cell type	Amt of Fn added (μg/ml)	Recovered cpm	% of control
HT1080	0	14,448 ± 146	100
	250	13,638 ± 456	94
	500	12,830 ± 604	89
	1,000	9,200 ± 157	64
	1,500	6,747 ± 377	47
CHO	0	13,099 ± 8	100
	250	12,237 ± 138	93
	500	10,822 ± 83	83
	1,000	6,089 ± 156	59
	1,500	6,089 ± 176	46

[a] Fn at the indicated levels was added to a 1-ml volume containing 7×10^7 ^{35}S-labeled treponemes for 30 min at RT prior to addition to cell monolayers. After incubation for 2 h at 34°C, the extent of cytadherence was determined by measurement of radioactivity. Data represent the mean ± the standard deviation of three determinations in a representative experiment.

DISCUSSION

We have previously demonstrated the ability of freshly extracted treponemes to adhere to Fn on cell surfaces or immobilized on glass cover slips (19). Furthermore, acquisition by *T. pallidum* of human Fn was recently shown to be mediated by three treponemal outer envelope proteins (19) in a specific receptor-ligand fashion (22). Because syphilis spirochetes in vivo are exposed to Fn present in plasma and other body fluids (16–18, 26), we wanted to investigate the parasitic properties of this pathogenic microorganism under

TABLE 5. Inhibition by dextran sulfate and heparan sulfate of the enhanced attachment of Fn-primed *T. pallidum* to Fn-coated glass cover slips[a]

Pretreatment and coincubation (amt added, μg)	Recovered cpm	% of control
15%[b] Dextran sulfate		
0	79,623 ± 331	100
100	77,027 ± 270	97
250	69,423 ± 567	81
500	48,303 ± 360	61
1,000	36,181 ± 368	45
Heparan sulfate		
0	79,686 ± 304	100
250	68,110 ± 219	85
500	51,655 ± 535	65
1%[b] Dextran sulfate		
0	77,299 ± 314	100
250	77,225 ± 204	100
500	76,815 ± 420	99
Dextran		
0	76,913 ± 131	100
500	76,699 ± 193	100
1,000	76,118 ± 128	99
GRGDSPC[c]		
0	79,801 ± 138	100
500	78,603 ± 657	98

[a] Proteins were added to make a 1.0-ml solution containing 7×10^7 Fn-primed ^{35}S-treponemes. Solutions were incubated for 30 min at 34°C before addition to cover slips. After incubation for 2 h at 34°C, cover slips were removed, washed, and measured for radioactivity. Data represent the mean ± standard deviation for three determinations in a representative experiment.

[b] 15% and 1% refer to the extent of sulfated dextran sulfate.

[c] Gly-Arg-Gly-Asp-Ser-Pro-Cys (23).

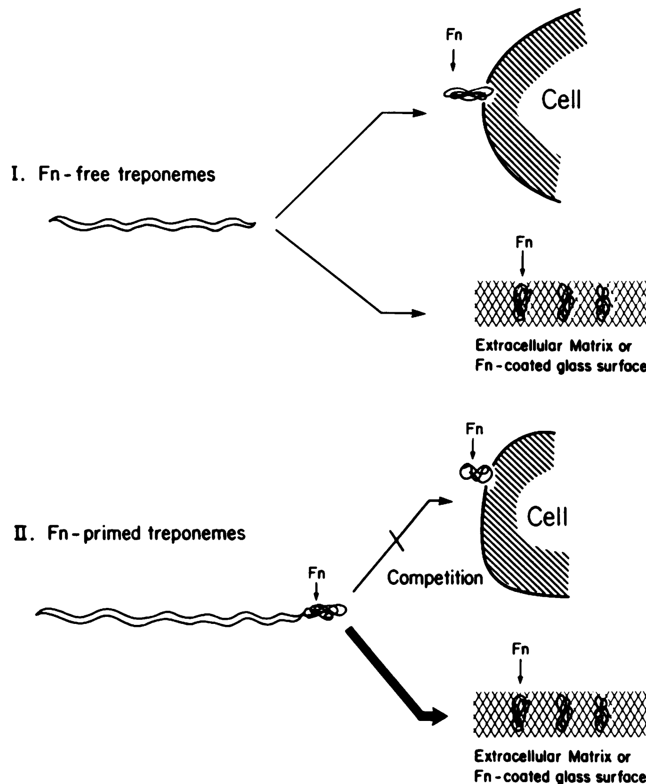

FIG. 2. Schematic showing the tropism of Fn-primed *T. pallidum* organisms to ECM versus host cell surfaces. (I) Attachment of Fn-free organisms to either Fn on host cells or Fn on ECM or glass cover slips. (II) Increased capacity of treponemes with bound Fn (primed) to attach to ECM compared with host cell surfaces. At levels of external Fn $\geq$ 500 μg/ml (Table 4), competition in treponemal attachment to cells was observed, as indicated by the slashed line.

physiologic conditions relevant to the in vivo environment. In this report we describe our observation that Fn-primed spirochetes demonstrate dramatically enhanced ability to bind not only to Fn on glass cover slips (Table 1) but also to a more physiologic in situ model, the ECM (Table 3).

The ability of Fn priming to achieve a manyfold enhancement in the extent of treponemal attachment to immobilized Fn (Tables 1 and 2) is noteworthy (Fig. 2). The inhibition of treponemal binding by prior treatment of Fn cover slips or ECM with only anti-Fn antibody (Table 2) indicates that unique Fn-Fn interactions occur between Fn on treponemes and Fn on coated surfaces. Priming of *T. pallidum* with other ECM molecules like collagen and laminin was ineffective in promoting similar treponeme-ECM associations, confirming the established specificities in *T. pallidum*-Fn events (19, 22). The data are especially meaningful because laminin and collagen bind to *T. pallidum* at 38 and 31%, respectively, of the binding seen at saturating levels of Fn (unpublished data). The absence of elevated cytadherence levels following similar coincubation of eucaryotic cell monolayers with Fn-primed treponemes may also be significant. It is possible that the paradoxical inhibition of treponemal adherence to cell surfaces concomitant with increased *T. pallidum* attachment to ECM (Fig. 2) demonstrates the capacity of *T. pallidum* to target tissues preferentially during infection.

The observation that dextran sulfate and heparan sulfate inhibit attachment of Fn-primed parasites to Fn on cover slips as well as ECM (Table 4) is in agreement with the possible important role of glycosaminoglycans during syphilis (10). Since the important arginine-glycine-aspartic acid (RGD) tripeptide of the Fn cell-binding domain (22, 23) and poorly sulfated dextran show no inhibitory properties, the data indicate a specificity for Fn interactions with charged or sulfated heparinlike molecules. Consistent with our data are reports which illustrate a role for glycosaminoglycans in Fn-mediated cell attachment and Fn-Fn aggregation (9, 17). Clearly, additional work is necessary to identify more precisely the functional glycosaminoglycan components and the Fn domains (15, 17, 20, 26) which are responsible for the observations in this report.

Finally, differences in the presence or absence of Fn or an Fn-like material on treponemal surfaces were observed among freshly extracted spirochetes. Magnetic prot-Aspheres and glass beads coated with anti-Fn IgG bound 20 to 30% of *T. pallidum* organisms. The remaining (70 to 80%) treponemes were nonreactive with anti-Fn antibody and did not bind to gelatin-agarose, yet these parasites readily acquire host Fn (22). The enhanced attachment of Fn-free *T. pallidum* organisms to ECM in the presence of exogenous Fn also indicates acquisition of Fn by these spirochetes. Finally, all freshly harvested organisms pretreated with Fn bound to immobilized anti-Fn antibody, indicating that treponemes were homogeneous with respect to receptor-mediated binding of human Fn (19, 22).

We are hopeful that our work will ultimately help to explain the disseminated nature of syphilis infection and the tropism of *T. pallidum* for distinct tissue sites. We recognize the need for further experimentation to understand at a molecular level the nature of cell and tissue parasitism by the syphilis spirochete mediated by Fn and other host molecules.

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