

FIBRONECTIN MEDIATES *TREPONEMA PALLIDUM*  
CYTADHERENCE THROUGH RECOGNITION OF  
FIBRONECTIN CELL-BINDING DOMAIN

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Numerous studies (1–5) suggest that an early event in the pathogenesis of syphilis is *Treponema pallidum* adherence to host cells (cytadherence), mediated by tip-like structures on virulent spirochetes. Biochemical-molecular studies have identified three treponemal outer envelope proteins as putative ligands responsible for surface parasitism (3, 6). These proteins are also highly immunogenic, as demonstrated by radioimmunoassays using sera from syphilitic humans and experimentally infected animals (3, 15).

The observation that host fibronectin avidly binds to freshly extracted *T. pallidum* prompted further evaluation of the biological-biochemical properties conferred upon virulent treponemes by this host protein (6). Fibronectin appears to be important to *T. pallidum* cytadherence, because antifibronectin antibody prevents attachment of the syphilis spirochetes to host cells and to fibronectin-coated glass surfaces (6). In contrast, the avirulent Reiter treponeme fails to bind fibronectin and other plasma proteins, reinforcing the idea that host protein binding may be an attribute of virulent spirochetes. These observations also support the view that specific receptor-ligand events were responsible for the *T. pallidum*—host cell interaction.

This study was conducted to demonstrate the specificity of fibronectin as the mediator of *T. pallidum* cytadherence. In addition, since fibronectin is a dimeric polypeptide with defined structural and functional properties (7–10), we attempted to identify the responsible fibronectin functional domain(s) involved in the treponemal parasitism of host cells.

### Materials and Methods

**Bacteria.** Virulent *T. pallidum* (Nichols) were preserved in 10% dimethyl sulfoxide in liquid nitrogen (11) before intratesticular inoculation into New Zealand White rabbits, as previously described (12–14). Treponemes were harvested from minced tissue at peak orchitis in an extraction medium containing salts, glucose, and reducing agents (12). Treponeme suspensions were clarified of host cell contaminants by two centrifugations at 500 *g* for 10 min each before centrifugation on a 0.8 % Methacel (Dow Chemical Co., Midland, MI)–50% Hypaque (Winthrop Laboratories, New York) gradient at 650 *g* for

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20 min. The supernatant containing  $\sim 1-3 \times 10^8$  treponemes/ml was used for all assays unless stated otherwise.

**Radiolabeling of *T. pallidum*.**  $\sim 2 \times 10^9$  motile treponemes in 10 ml of extraction medium were radiolabeled with 1 mCi of [ $^{35}$ S]methionine (sp act, 1,000 Ci/mmol; Amersham Corp., Arlington Heights, IL) for 3 h at 34°C under aerobic conditions (1, 3, 15). For attachment assays with cultured mammalian cells, labeled treponemes were centrifuged at 17,000 g and resuspended in extraction medium (1).

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)<sup>1</sup> and Immunoblotting (IB) of Fibronectin Fragments.** SDS-PAGE and IB of fibronectin domains were performed as described previously (1, 3, 6, 15). Briefly, samples were loaded onto wells of 3% stacking and 7.5% separating acrylamide gels (1, 3, 15). Electrophoresis was performed on a 16 cm slab gel apparatus (Bio-Rad Laboratories, Richmond, CA) at 30 mA per gel. Electrophoretic protein transfer to nitrocellulose was performed by a modification of the method of Towbin et al. (16) using a Transblot cell (Bio-Rad Laboratories). IB was executed at 200 mA for 18 h in a solution containing 25 mM Tris base, 192 mM glycine, and 20% methanol, pH 8.3. After protein transfer, nitrocellulose was treated with antibody and  $^{125}$ I-protein A as described (6).

**Purification of Human Plasma Fibronectin.** Fibronectin was purified as detailed earlier (6). Samples containing purified fibronectin were pooled, dialyzed against a solution of 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) (Sigma Chemical Co., St. Louis, MO), 1 mM CaCl<sub>2</sub>, and 150 mM NaCl, pH 11.0, and frozen at -20°C until required (17). Before use, fibronectin was dialyzed against phosphate-buffered saline (PBS) and adjusted to a concentration of 1 mg/ml.

**Proteolytic Digestion of Purified Human Plasma Fibronectin.** The cell- and heparin-binding domains of purified human plasma fibronectin were isolated as described by Hayashi and Yamada (17). A schematic presentation of the procedure is shown in Fig. 1. Briefly, purified fibronectin in CAPS buffer was diluted to 3 mg/ml in 50 mM Tris, 30 mM NaCl, and 1 mM CaCl<sub>2</sub>, and warmed to 30°C. Trypsin (TPCK-treated; Sigma Chemical Co.) was added to 0.2% (wt/wt) and digestion was allowed to occur for 30 min at 30°C, when the reaction was quenched with phenylmethylsulfonyl fluoride. The digest was dialyzed into 10 mM Tris-HCl, pH 7.0, and applied to a 1.5 × 30 cm column of DEAE-cellulose. Nonadherent material represented N-terminal, heparin-binding domain fragments (Fig. 1A) (17) and was frozen at -70°C until required. Bound fragments were eluted with 500 mM NaCl in 10 mM Tris-HCl buffer, followed by dialysis in 10 mM Tris-HCl containing 1 mM MgCl<sub>2</sub>. The dialyzed sample was chromatographed on a 1.5 × 30 cm gelatin-agarose (Sigma Chemical Co.) column, and pooled nonadherent material adjusted to 2 mM EDTA before chromatography on a 1.5 × 30 cm heparin agarose (Bio-Rad Laboratories) column. Adherent protein contained cell-binding domain (see Fig. 1B), which was eluted with 130 mM NaCl and 10 mM Tris-HCl, pH 7.0. Fractions were evaluated by SDS-PAGE, and those enriched for the 68,000 (68 K) mol wt cell-binding domain were pooled and frozen until use.

The gelatin-binding domain was isolated after digestion of human plasma fibronectin with 1% thermolysin (Sigma Chemical Co.) (18, 19) for 30 min at 30°C. The thermolysin-treated fibronectin solution was adjusted to 50 mM EDTA before chromatography on a 1.5 × 30 cm gelatin-agarose column. Adherent polypeptides were eluted with 4 M urea in 10 mM Tris-HCl, pH 7.0 and the protein precipitated with solid ammonium sulfate to 70% saturation. After dialysis against 10 mM Tris-HCl, the gelatin-binding domain (see Fig. 1C) was analyzed by SDS-PAGE and stored at -70°C.

**Acquisition of Fibronectin- and Cell-binding Domain by *T. pallidum*.**  $\sim 5 \times 10^6$  live treponemes, washed twice with PBS, were pelleted in siliconized microfuge tubes by centrifugation at 17,500 g. Treponemes were resuspended to 1.0 ml in PBS containing increasing amounts of  $^{125}$ I-cell-binding domain (sp act,  $6.23 \times 10^5$  cpm/ $\mu$ g) or  $^{125}$ I-

<sup>1</sup> Abbreviations used in this paper: BSA, bovine serum albumin; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; CHO, Chinese hamster ovary cells; DMEM, Dulbecco's minimal essential medium; IB, immunoblotting; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

fibronectin (sp act,  $6.6 \times 10^4$  cpm/ $\mu$ g). After incubation at 23°C for 20 min, treponemes were washed twice with PBS and pellets monitored for radioactivity. Competition experiments were performed using radiolabeled and unlabeled preparations of fibronectin or purified fibronectin fragments.

*Attachment of T. pallidum to Protein-coated Coverslips and Cell Culture Monolayers.* Glass coverslips (9 × 35 mm) were incubated with 50  $\mu$ l of 1 mg/ml solutions of fibronectin, fibronectin fragments, or bovine serum albumin (BSA) in PBS. The solution was spread evenly on the glass surface and allowed to dry at room temperature (6). Indirect immunofluorescence microscopy was used to determine the uniformity of the protein layer on the glass surface (6). Human tumor (HT1080) cells (American Type Culture Collection [ATCC], Rockville, MD) were maintained in Dulbecco's minimal essential medium (DMEM) (Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum (KC Biological, Inc., Lenexa, KS). Chinese hamster ovary (CHO) cells (ATCC) were cultured in Ham's F-12 nutrient medium containing 10% fetal calf serum. Presence of laminin, fibronectin, and collagen on cell surfaces was confirmed using indirect immunofluorescence according to the method of Sun and Green (20). All cells were grown at 37°C in a humidified air atmosphere of 5% CO<sub>2</sub>. Cells were seeded at a density of  $5 \times 10^4$  cells into Leighton tubes containing a single 9 × 35 mm glass coverslip. Cultures were incubated overnight at 37°C. One-ml suspensions of freshly harvested unlabeled or <sup>35</sup>S-labeled treponemes were added to Leighton tubes containing protein-coated coverslips or cell monolayers, and the tubes were incubated at 34°C for 2 h. After incubation, the coverslips were removed and washed three times with PBS before microscopic observation or determination of radioactivity (6).

In some experiments, protein-coated coverslips and cells were incubated at room temperature for 30 min with antisera or monoclonal antibody diluted in DMEM, followed by washing with PBS. Cell surface parasitism was quantitated by counting the number of treponemes attached to 20 individual cells of typical morphology (6) or by placing the rinsed coverslips in scintillation vials containing cocktail and measuring vials for radioactivity in a Beckman scintillation counter. Microscopy was performed using darkfield optics on a Leitz Ortholux II.

*Monoclonal Antibody Reagents and Preparation of Antisera.* Monoclonal antibody 7.1E, which recognizes the fibronectin cell-binding domain (21), was kindly donated by Dr. Robert Klebe (UTHSCSA, San Antonio, TX). Monoclonal antibodies directed against the heparin-binding domain (type I) and toward the gelatin-binding domain (type III) were purchased from Calbiochem-Behring Corp. (San Diego, CA).

Antisera against fibronectin and type I collagen (also gifts from Dr. Robert Klebe, UTHSCSA) were prepared by immunizing rabbits with an initial injection of 300  $\mu$ g of purified human plasma fibronectin or collagen emulsified in Freund's complete adjuvant. Booster injections of 300  $\mu$ g of the respective preparation in Freund's incomplete adjuvant were given three times at 10-d intervals. The presence of antibodies was assessed by enzyme-linked immunosorbent assay using protein-coated microtiter wells. No contaminating fibronectin was detected in the collagen preparation used for immunization.

## Results

*Selective Recognition of Cell Surface Fibronectin by T. pallidum.* Culture monolayers of CHO cells, which synthesize fibronectin (22), and HT1080 cells, which synthesize fibronectin, laminin, and collagen (23), were used to determine the binding specificity of *T. pallidum* with fibronectin and other extracellular matrix molecules. Table I, summarizing two representative experiments, shows that pretreatment of cell monolayers with antifibronectin serum diminished adherence of radiolabeled treponemes to both cell types by 70%. Incubation of cells with high-titered antisera against laminin or type I collagen was without effect. As expected, preincubation of HT1080 cells with a mixture of antifibronectin, antilaminin, and anticollagen sera produced no greater inhibition of attachment

TABLE I  
*Specificity of Fibronectin in Attachment of <sup>35</sup>S-labeled T. pallidum to HT1080 and CHO Cells*

Experi- ment	Treatment*	Recovered cpm (percent of control) <sup>‡</sup>	
		HT1080 cells	CHO cells
1	DMEM	19,665 ± 219 (100.0)	15,019 ± 315 (100.0)
	Normal rabbit serum	19,421 ± 253 (98.8)	14,833 ± 232 (98.8)
	Antilaminin	18,261 ± 269 (92.9)	13,981 ± 171 (93.1)
	Anticollagen type I	19,007 ± 224 (96.7)	NT <sup>‡</sup>
	Antifibronectin	6,752 ± 168 (34.3)	4,179 ± 218 (27.8)
	Antifibronectin, antilaminin	6,313 ± 198 (32.1)	NT
2	DMEM	18,864 ± 213 (100.0)	14,610 ± 181 (100.0)
	Normal rabbit serum	18,935 ± 225 (100.3)	NT
	Antilaminin	17,822 ± 317 (94.4)	14,377 ± 195 (98.4)
	Anticollagen type I	18,669 ± 224 (98.9)	14,462 ± 186 (99.0)
	Antifibronectin	7,001 ± 241 (37.1)	4,228 ± 176 (28.9)
	Antifibronectin, anticollagen, antilaminin	6,833 ± 186 (36.2)	NT

\* Cells were treated with 1.0 ml of antiserum in DMEM at the following concentrations: antifibronectin, 1:5; antilaminin, 1:5; anticollagen, 1:10.

<sup>‡</sup> On darkfield examination, ~100% of cells showed attached treponemes. A 1 ml suspension of  $7 \times 10^7$  treponemes, representing 475,000 cpm, was added to each Leighton tube containing control or antiserum-treated cells.

<sup>‡</sup> Not tested.

than antifibronectin serum alone. These data support the idea that fibronectin is the principal mediator of *T. pallidum* cytoadherence.

*Monoclonal Antibody to Cell-binding Fragment Inhibits T. pallidum-Fibronectin Associations.* The specific nature of *T. pallidum* binding to cell surface fibronectin prompted us to further examine whether a functional domain of the fibronectin polypeptide (24) could be identified in the adherence event (5, 6). Because fibronectin-coated glass surfaces also promote tip-mediated *T. pallidum* attachment similar to that seen with host cells (6), we used this assay to determine whether monoclonal antibodies against fibronectin functional domains inhibited *T. pallidum* binding. As seen in Table II, the monoclonal antibody against the cell-binding fragment (21) inhibited *T. pallidum* attachment to the fibronectin surface to the same degree as antifibronectin serum. Antibody to the heparin-binding protein was less effective, and monoclonal antibody to the gelatin-binding protein fraction showed little inhibition.

*Acquisition by T. pallidum of Fibronectin and Fibronectin Cell-binding Domain.* To better assess the *T. pallidum*-fibronectin interaction, we performed proteolytic fragmentation of fibronectin and isolated the three functional domains (17-19) (Fig. 1). Fig. 2 shows the Coomassie-stained protein profiles of heparin-binding (Fig. 2A), gelatin-binding (B), and cell-binding (C) fractions of human plasma fibronectin; molecular weights for the respective fragments (arrows) are in agreement with reported values (17, 18).

We next tested the ability of these protein fragments to inhibit fibronectin association with *T. pallidum*. Fig. 3 demonstrates the saturation binding of fibronectin to *T. pallidum* organisms. The inset indicates that the unlabeled cell-

TABLE II  
Effect of Antifibronectin Antibodies on Attachment of *T. pallidum* to Protein-coated Coverslips

Antibody designation	Antibody reaction*	Protein on coverslip	Recovered cpm (percent of control)‡
—	—	Bovine serum albumin	2,450 ± 373 (8.3)
—	—	Fibronectin	29,597 ± 1,517 (100.0)
Antifibronectin serum	Fibronectin	Fibronectin	6,117 ± 663 (20.7)
mAb <sup>§</sup> 7.1E	Cell-binding domain	Fibronectin	8,312 ± 1,855 (28.0)
mAb type I	Heparin-binding domain	Fibronectin	12,328 ± 1,657 (41.6)
mAb type III	Gelatin-binding domain	Fibronectin	23,350 ± 3,493 (78.9)

\* Human plasma fibronectin domain recognition as reported in literature and/or as determined in Western blots.

‡ A 1.0 ml suspension of  $7 \times 10^7$  treponemes, representing 811,000 cpm, was added to each coverslip and incubated at 34 °C for 2 h. Data represent mean ± standard deviation for three determinations.

§ Monoclonal antibody.

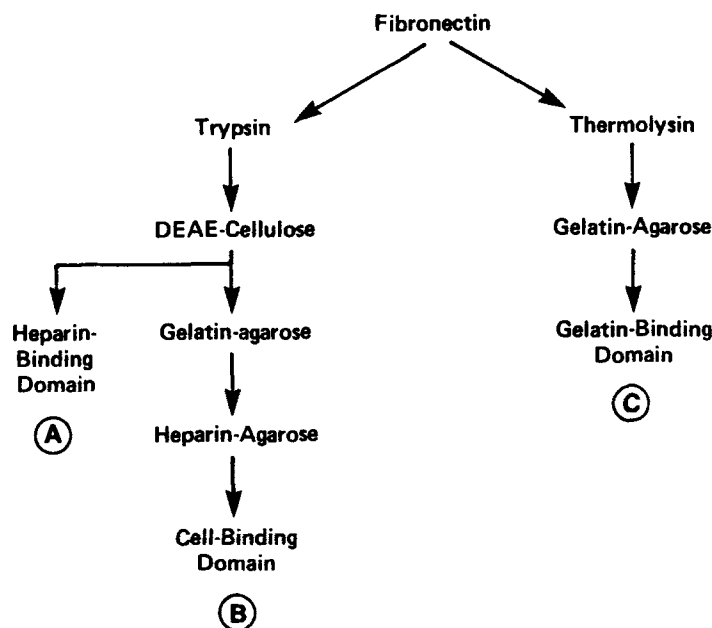


FIGURE 1. Purification scheme for fibronectin fragments. See Materials and Methods and references 17–19 for details.

binding protein fraction competes best with saturation levels (250  $\mu$ g) of iodinated fibronectin, for binding to *T. pallidum*. Unlabeled fibronectin was equally effective in competing with iodinated fibronectin (data not shown). These observations are consistent with experiments performed earlier (Table II), implicating the cell-binding domain as mediator of the specific *T. pallidum*–fibronectin interaction.

Next, the acquisition of cell-binding domain by live, motile treponemes was assessed. Fig. 4 shows the Coomassie-stained protein profile (lane A1) and

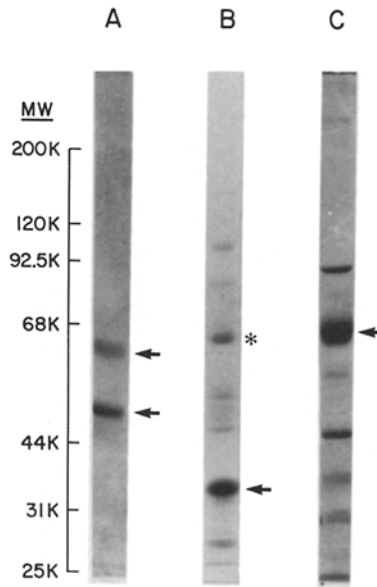


FIGURE 2. Representative Coomassie Brilliant Blue profile of partially purified fibronectin functional domains. (A) The gel pattern of the gelatin-binding fraction; (B) the heparin-binding domain fraction; and (C) the protein profile of the cell-binding domain fraction. Respective domains are indicated by arrows. Asterisk denotes presence of cell-binding domain in heparin-binding fraction. Molecular weight estimates for the gelatin-, heparin-, and cell-binding domains are 48, 35, and 68 K, respectively.

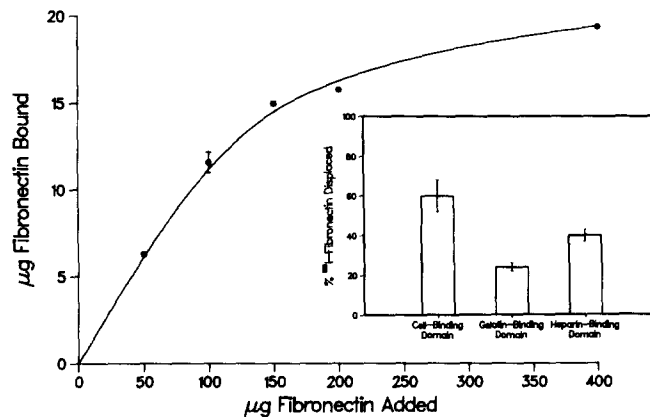


FIGURE 3. Concentration-dependent binding of <sup>125</sup>I-fibronectin by *T. pallidum*. Increasing amounts of fibronectin were incubated for 20 min at room temperature with  $7 \times 10^6$  live treponemes. Inset shows competition of <sup>125</sup>I-fibronectin (250 µg) by 2.5-fold excess of preparations of unlabeled fibronectin domains shown in Fig. 2.

corresponding autoradiogram (A2) of the partially purified, iodinated, cell-binding domain preparation used in binding experiments. Immunoblotting of the unlabeled fraction (A1) with monoclonal antibody 7.1E, which demonstrated significantly inhibited *T. pallidum* cytoadsorption (Table I), detected the same

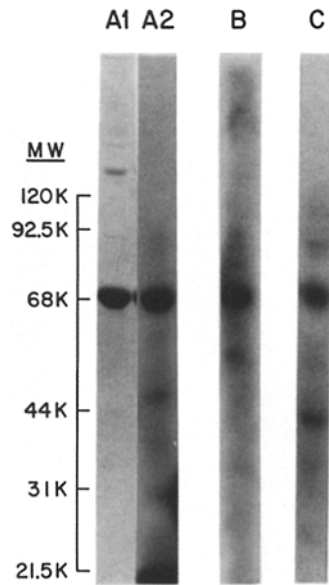


FIGURE 4. Protein profiles and immunodetection of cell-binding domain of fibronectin. (A1) Coomassie-stained protein profile of the enriched fraction. (A2) Autoradiographic profile of the iodinated preparation. (B) Immunoblot of A2 probed with monoclonal antibody to cell-binding domain. (C) Acquisition of cell-binding domain fraction by live *T. pallidum*.

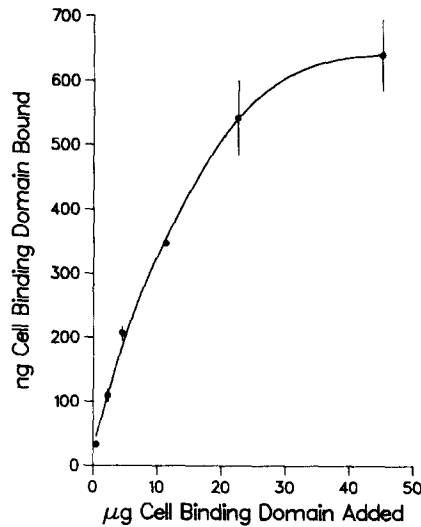


FIGURE 5. Saturation binding kinetics of *T. pallidum* acquisition of cell-binding domain. Increasing amounts of cell-binding domain were incubated with  $7 \times 10^6$  live treponemes for 20 min at room temperature.

major 68 K mol wt polypeptide (B). Fig. 4C indicates the binding by *T. pallidum* of the major iodinated 68 K mol wt cell-binding domain protein.

The specificity and kinetics of acquisition of cell-binding domain by *T. pallidum* of the cell-binding domain were further evaluated. As seen in Fig. 5, saturation binding was achieved

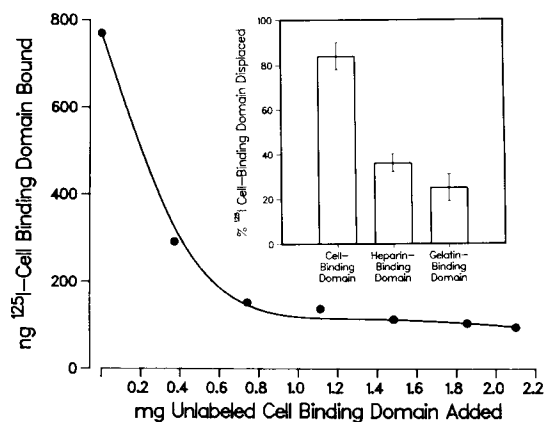


FIGURE 6. Competition of <sup>125</sup>I-cell-binding domain on live *T. pallidum* with unlabeled cell-binding fraction. The inset shows competition of iodinated cell-binding domain with other unlabeled fragments, as seen in Fig. 2. In this experiment, a 1 ml final volume of PBS containing  $7 \times 10^6$  treponemes was incubated at room temperature for 20 min with 48  $\mu$ g of <sup>125</sup>I-cell-binding domain and ~1,200  $\mu$ g respective unlabeled domain. Radioactivity was measured in a Beckman gamma counter, after washing of treponemes as discussed in Materials and Methods.

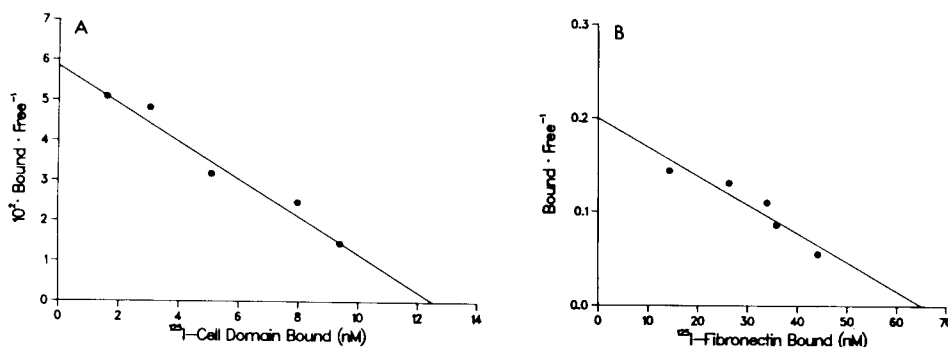


FIGURE 7. Scatchard plot analyses of saturation kinetics of <sup>125</sup>I-fibronectin (A) and <sup>125</sup>I-cell-binding domain (B) binding to *T. pallidum*.

after incubation of *T. pallidum* with increasing concentrations of iodinated ligand for 20 min at room temperature. We also observed efficient competition of radiolabeled cell-binding domain with a duplicate preparation of unlabeled ligand (Fig. 6). ~85% of the <sup>125</sup>I-cell-binding domain fraction could be competed with unlabeled cell-binding domain. Additionally, Fig. 6 (inset) demonstrates the inability of the heparin-binding domain and gelatin-binding domain to effectively compete with iodinated cell-binding domain acquisition.

A comparative Scatchard analysis of the data for both fibronectin and cell-binding domain acquisition was then performed (Fig. 7). We obtained evidence for a single class of treponemal surface molecules (receptors) that interact with fibronectin as well as cell-binding domain, and calculated apparent  $K_d$  of  $1.48 \times 10^{-7}$  M and  $2.1 \times 10^{-7}$  M for fibronectin and cell-binding domain, respectively. The average number of treponemal receptors that recognize fibronectin and



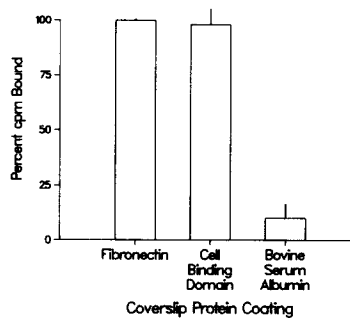


FIGURE 8. Attachment of  $^{35}\text{S}$ -labeled *T. pallidum* to substrates immobilized on  $9 \times 35$  mm Leighton tube glass coverslips as described in Materials and Methods.

cell-binding domain was estimated to be  $4.3 \times 10^6$  and  $1.1 \times 10^6$  per spirochete, respectively. These data indicate that both fibronectin and cell-binding domain adhere to *T. pallidum* with comparable avidity in a receptor-ligand type manner.

*Cell-binding Fraction and Fibronectin Promote Equivalent Levels of T. pallidum Adherence to Coated Surfaces.* Finally, we wanted to see whether treponemes attached to cell-binding domain, as was demonstrated for fibronectin. Fig. 8 demonstrates that the same level of attachment of radiolabeled treponemes was achieved on coverslips coated with cell-binding domain as was observed for treponemes incubated on fibronectin-coated coverslips. Only low levels of  $^{35}\text{S}$ -treponemes were associated with BSA-coated coverslips under identical experimental conditions. These data indicate that the cell-binding domain polypeptide is functionally equivalent to the whole fibronectin molecule in mediating host cytheadherence.

### Discussion

The preferential and avid association of plasma proteins with live *T. pallidum*, but not with the avirulent Reiter treponeme, suggests that acquisition of host macromolecules is a distinguishing property of virulent treponemes (6). Fibronectin selectively binds to *T. pallidum* and appears to serve as an affinity matrix and anchoring mechanism for tip-mediated treponemal attachment to host cell surfaces. Data presented in this report strongly support the view that fibronectin alone is involved in cytheadherence (Table I) and are in agreement with the concept that *T. pallidum*-cell interactions are mediated through receptor-ligand events (6).

Fibronectin is a multifunctional glycoprotein with well-defined structure-function domains (7, 10, 17, 18, 24). The dimer molecule is composed of polypeptide subunits with molecular weights of  $\sim 220$  K, linked by a disulfide bridge (7). Physicochemical studies reveal that the protein consists of relatively protease-resistant fragments which bind to macromolecules such as gelatin and heparin. In addition, a fibronectin fragment that promotes cellular attachment has been characterized, and this cell-binding domain appears to associate with eukaryotic cell surface receptors (17).

To better understand the recognition events between *T. pallidum* ligands and

fibronectin, we used immunological probes and biochemical techniques to dissect the molecular interaction of *T. pallidum* with fibronectin. Monoclonal antibody to cell-binding domain diminished treponemal attachment to fibronectin-coated coverslips (Table II) to the same extent as polyclonal antifibronectin sera, implicating the cell-binding domain as the relevant fibronectin protein region involved in cytoadsorption. In contrast, antibody to gelatin-binding domain had little effect on treponemal attachment. The inhibition of surface parasitism by monoclonal antibody to heparin-binding domain can be explained on the basis of antibody crossreactions with heparin-binding sites known to reside within or adjacent to the cell-binding region (17).

Purification of the cell-binding domain yielded a highly enriched preparation containing a 68 K mol wt fragment (Figs. 2 and 4) (17). Motile treponemes adhere to this peptide (Fig. 8) at levels equal to the entire fibronectin molecule. The observation that *T. pallidum* has high affinity, saturable binding sites (Fig. 5) for the cell-binding domain of fibronectin is indicative of specific receptor-ligand type reactions between host fibronectin and the putative treponemal adhesin molecules (15). Efficient competition of labeled cell-binding domain could be achieved only with homologous unlabeled protein (Fig. 6). Furthermore, the cell-binding domain proved to be the best competitor of iodinated fibronectin binding to *T. pallidum* organisms (Fig. 3). Collectively, the data strongly implicate a specific treponeme cell-binding domain (fibronectin) recognition event. Finally, Scatchard analysis provided  $K_d$  for both reactions on the order of  $10^{-7}$  M, consistent with the high affinity binding of these organisms to host cells (3, 5, 6).

This may be the first report of a pathogenic microorganism, such as *T. pallidum*, associating with the cell-binding domain region of host cellular fibronectin. While previous reports demonstrate that yeast, viruses, and other bacteria bind the fibronectin holoprotein (25–27), our data clearly show binding by the syphilis spirochete to a specialized cell-binding region of the fibronectin molecule. The dimeric nature of fibronectin would suggest that virulent treponemes may associate with an accessible cell-binding domain region not occupied by its cell surface receptor (17). Furthermore, it is possible that pathogens such as *Trypanosoma cruzi* (28), which also appears to exploit host cell fibronectin for cytoadherence, may do so in a similar manner. These data demonstrate the unique aspects of this host-parasite interaction and illustrate the strategy used by the syphilis spirochete in an initial event in the infectious process.

The finding that *T. pallidum* specifically recognizes the cell-binding domain polypeptide of fibronectin may now permit precise identification and purification of the implicated treponemal adhesion molecules. Importantly, future structure-function characterization of the treponemal ligands may provide valuable data for development of vaccines and diagnostic probes. For example, defined polypeptide antigens with biologic functions such as those involved in cellular parasitism might be used as vaccines to elicit antibodies that prevent the initial cytoadherence event. We are hopeful that our studies of the parasitism of host cells by *T. pallidum* at a molecular-biochemical level will lead to an increased understanding of localized and disseminated disease.

### Summary

The specificity of the interaction between *Treponema pallidum* and fibronectin was demonstrated. Treatment of host cells with only antifibronectin sera and not anticollagen or antilaminin sera, inhibited treponemal cytoadsorption. Incubation of fibronectin-coated coverslips with monoclonal antibody to the cell-binding domain of fibronectin reduced treponemal attachment to the same extent as antifibronectin serum. Both iodinated fibronectin and iodinated cell-binding domain bound to *T. pallidum* in a saturable manner. Specificity of the *T. pallidum* association with the cell-binding domain was demonstrated in competition experiments in which cell-binding domain was the most effective inhibitor of the binding of either radioiodinated cell-binding domain or fibronectin to *T. pallidum*. Scatchard analysis gave  $K_d$  on the order of  $10^{-7}$  M for both cell-binding domain and fibronectin binding to *T. pallidum*, consistent with the high affinity interaction of these organisms with host cell surfaces. Finally, the same level of attachment of treponemes was achieved on coverslips coated with cell-binding domain as that observed for organisms incubated with fibronectin, indicating that the cell-binding domain polypeptide is functionally identical to fibronectin in mediating *T. pallidum* adherence.

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