

TREPONEMA PALLIDUM RECEPTOR BINDING PROTEINS INTERACT WITH FIBRONECTIN*

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An extensive literature exists that describes the complexity of the host-parasite relationship during syphilis. For example, a pronounced humoral response is elicited against biologically important treponemal antigens (1-7), but the role of antibody in protection remains speculative, especially since infected individuals can progress through the various stages of syphilis (6, 8) in the presence of high-titered antibody (1-5, 8-11). Inconsistencies in the activation of cellular mechanisms of immunity during early syphilis have also been reported (12-17).

Properties that apparently confer virulence potential to *T. pallidum* are being identified. For example, the ability of *T. pallidum* to selectively coat itself with host macromolecules (18, 19) may provide mechanisms by which this spirochete circumvents immune surveillance. In addition, acquisition of host macromolecules by *T. pallidum* may neutralize nonspecific defense mechanisms such as host proteases and at the same time contribute to the biosynthetic capabilities of the parasite (20). Another relevant observation concerns the ability of *T. pallidum* to attach to eucaryotic cells via a specialized tip structure (1, 3, 4, 21, 22) mediated by specific treponemal proteins (1, 3, 4) and host cell receptors (1, 4). This adherence event may permit colonization by *T. pallidum* of selective and privileged sites on targeted tissues.

Our recent emphasis has focused on the chemical and molecular interaction between *T. pallidum* and avidly bound host macromolecules. In preliminary studies, a plasma protein identified as fibronectin was found to readily associate with the treponemal outer membrane. Because of the importance of fibronectin in substratum adherence of host tissues (23-28), the possible role of this protein in treponemal surface parasitism was investigated.

Materials and Methods

Bacteria. The virulent Nichols strain of *T. pallidum*, cryopreserved in 10% dimethylsulfoxide in liquid nitrogen (29), was inoculated into rabbit testes as previously described (18, 30, 31). Treponemes were removed from minced testicular tissue excised at peak orchitis (11-14 d, postinoculation) by shaking in 15 ml of a salts-glucose treponemal medium under reducing conditions (18) for 15 min at room temperature. A treponemal suspension of $1.5-4.0 \times 10^8$

* Supported by Public Health service grant A1-15036, research career development award 1-K04-A1-00178 from the National Institute of Allergy and Infectious Diseases to JBB and by an institutional grant from The University of Texas Health Science Center at San Antonio to JFA.

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¹ Abbreviations used in this paper: BSA, bovine serum albumin; DME, Dulbecco's minimum essential medium; HEP-2, human epithelial cells; NP-40, Nonidet P40; PBS, phosphate-buffered saline; TCA, trichloroacetic acid; TN buffer, 50 mM Tris and 15 mM NaCl.

organisms per ml was clarified twice at 500 *g* for 15 min followed by removal of cellular debris by centrifugation on a cushion of 0.8% Methacel (Dow Corning Corp., Midland, MI)-50% Hypaque (Winthrop Laboratories, New York, NY) at 650 *g* for 20 min at room temperature. The supernatant containing highly motile treponemes was used for attachment assays (1, 22), or organisms were radiolabeled with [³⁵S]methionine as described below. For all other experiments, *T. pallidum* was pelleted at 17,000 *g* and washed twice in PBS (137 mM NaCl, 2.7 mM KCl, 4.6 mM Na₂PO₄, and 1.5 mM KH₂PO₄) to remove loosely bound host plasma proteins associated with the treponemal outer envelope (18). Organisms were then resuspended in PBS to the desired concentration. The avirulent spirochete, *Treponema phagedenis* biotype Reiter, was maintained by in vitro cultivation using Spirolate broth (BBL Microbiology Systems, Becton, Dickinson & Co., Cockeysville, MD) supplemented with 10% heat-inactivated rabbit serum (Kansas City Biologicals, Lanexa, KS) and kept at 34°C (22).

Radiolabeling of *T. pallidum* and Plasma Proteins. Approximately 1×10^9 motile treponemes in 10 ml of medium were radiolabeled with 1.5 mCi of [³⁵S]methionine (sp act 1,000 Ci/mmol; Amersham Corp., Arlington Heights, IL) for 3 h at 34°C under aerobic conditions (1, 2, 4). Then, labeled organisms were pelleted at 17,000 *g* and resuspended in PBS for use in attachment assays or were solubilized for affinity chromatography experiments.

Lactoperoxidase-catalyzed radioiodination of plasma proteins was a modification of the procedure of Garvey et al. (32). Briefly, 1 mCi of [¹²⁵I]NaI (Amersham Corp.) was mixed with a 1-ml protein preparation followed by addition of 100 µl of 1 mg/ml lactoperoxidase (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA) with gentle shaking at room temperature. To this was added 100 µl of freshly prepared H₂O₂ (Sigma Chemical Co., St. Louis, MO; 10 µl of stock 30% H₂O₂ in 50 ml PBS) followed by another 100 µl of H₂O₂ 15 min later. The iodination was stopped with cysteine after an additional 15-min incubation. Radioiodinated proteins were dialyzed using Spectrapor 10,000 mol wt cutoff dialysis tubing (Fisher Scientific, Houston, TX) for 3 d against several changes of PBS at 4°C. Efficiency of [¹²⁵I]-labeling was examined by trichloroacetic acid (TCA) precipitation (32). All protein determinations were performed using the Bradford reagents (Bio-Rad Laboratories, Richmond, CA) (33).

Fractionation of Normal Human Plasma. 25 ml of citrated human plasma containing 2,200 mg of protein were fractionated through cold ethanol precipitation procedures into eight defined fractions (Table I, sample no. 2-9) as described by Cohn et al. (34). Separation of each ethanol-precipitate was achieved by centrifugation at 17,000 *g*. All steps were carried out at recommended temperatures except that separation of precipitate I and III into fraction III-0 was performed at 0°C. All fractions were dialyzed against PBS and used immediately.

Fibronectin-depleted plasma and purified fibronectin were obtained by gelatin-agarose (Sigma Chemical Co.) chromatography on a 2- × 10-cm column. After equilibration of the column with PBS, pH 7.2, 10 ml of normal human plasma or fraction I + III-3 diluted with 10 ml PBS were added, and chromatography was performed at a rate of 10 ml/cm²/h at room temperature. The column was then extensively washed with PBS, and fibronectin eluted with a solution of 1.0 M sodium bromide in 0.05 M sodium acetate, pH 5.0 (28). Adherent fractions were analyzed by SDS-gel electrophoresis, and only fractions containing purified fibronectin were used.

Avid Binding of Radioiodinated Plasma Proteins to Intact Treponemes. 100 µl of PBS containing 5×10^9 parasites were added to siliconized (Sigmacote, Sigma Chemical Co.) microfuge tubes pretreated with 1% horse serum in order to reduce nonspecific binding. Then, 50 µl of radioiodinated plasma or plasma fractions were introduced and the final volume adjusted to 300 µl with PBS. After incubation for 30 min at 37°C with occasional gentle shaking, treponemes were pelleted at 17,000 *g* and washed twice in PBS. Organisms were finally resuspended in 900 µl cold PBS, transferred to another microfuge tube, and 100 µl of cold 100% TCA was added to generate a total acid-precipitated protein preparation (1, 18). After 12 h at 4°C the precipitate was washed with cold PBS and dissolved in 200 µl of electrophoresis buffer (60.5 mM Tris-hydrochloride [pH 6.8], 2% β-mercaptoethanol, 10% glycerol, 2% SDS, and 0.1% bromophenol blue). The sample was then boiled for 3 min and insoluble material removed by centrifugation. The resultant supernatant was analyzed in a Beckman gamma counter or used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

TABLE I
Comparative Acquisition of ^{125}I -Radiolabeled Plasma Protein Preparations by *T. pallidum* and
T. phagedenis Biotype Reiter

Number of sample	Protein preparation added to treponemes*	Composition ^{†‡}	cpm avidly bound (% of total cpm added)	
			<i>T. pallidum</i>	<i>T. phagedenis</i>
1.	Normal human plasma	—	1,900 (0.7)	Same as control [§]
2.	I + III-3	Plasminogen, fibronectin, fibrinogen	3,190,000 (7.6)	Same as control [§]
3.	II	γ -Globulins	20,800 (0.4)	Same as control [§]
4.	III-0	β -Lipoproteins, euglobulins, ceruloplasmin	34,600 (1.4)	Same as control [§]
5.	III-1,2	Prothrombin, isoagglutinins	18,000 (0.6)	Same as control [§]
6.	IV-1	α -Lipoproteins	19,500 (0.5)	Same as control [§]
7.	IV-6,7	β -Metal binding protein, α -2 mucoprotein, choline esterase, α -2 glycoprotein	13,700 (0.3)	Same as control [§]
8.	V	Albumin	6,700 (0.5)	Same as control [§]
9.	VI	α -1 Glycoprotein, small proteins and peptides	1,411,000 (0.4)	Same as control [§]
10.	Fibronectin	—	5,430,000 (1.3)	Same as control [§]

* 50 μl of radioiodinated plasma, specific Cohn fractions, or purified fibronectin were added to 100 μl containing 5×10^8 organisms and incubated at 37°C for 30 min as described in Materials and Methods.

[†] Specific activities for individual protein preparations (cpm per ng protein): 1. 2,700; 2. 2,800; 3. 900; 4. 800; 5. 520; 6. 700; 7. 750; 8. 500; 9. 5,600; 10. 4,500. Purified fibronectin was prepared from fraction I + III-3; Cohn fractions obtained as described (34) from normal human plasma.

[‡] Representative proteins in fraction as described by Cohn et al. (34).

[§] Tubes without organisms but handled identically as those with organisms were used to determine the level of nonspecific binding of radioactivity. Values never exceeded 1% the level detected for *T. pallidum* acquisition. *T. phagedenis* yielded cpm values equivalent to control tubes.

SDS-PAGE and Immunodetection of Fibronectin Avidly Bound to *T. pallidum*. Fibronectin was detected by electrophoretic blotting onto nitrocellulose of electrophoresed total TCA-precipitated proteins. Test samples containing both treponemal proteins and avidly associated host proteins were loaded onto individual wells of 3% and 7.5% acrylamide stacking and separating gels, respectively (1, 2, 4). Electrophoresis of proteins was performed on a 16-cm slab gel apparatus (Bio-Rad Laboratories, Richmond, CA) using an initial constant current of 15 mA per gel that was increased to 30 mA per gel upon penetration of the tracking dye into the separating gel. Electrophoretic transfer was performed by a modification of the method of Towbin et al. (35) using a Trans Blot cell (Bio-Rad Laboratories). Blotting was carried out at 300 mA for 18 h in a solution consisting of 25 mM Tris-base, 192 mM glycine, and 20% methanol, pH 8.3. The nitrocellulose blots were removed and incubated with 50 ml of 3% bovine serum albumin (BSA; Sigma Chemical Co.) prepared in Tris-NaCl (TN: 50 mM Tris and 154 mM NaCl) buffer, pH 7.5, for 1 h at 37°C with gentle shaking to block nonspecific binding sites. To this was added 0.5 ml of normal goat serum or monospecific hyperimmune goat antiserum to human fibronectin (N. L. Cappel Laboratories, West Chester, PA). After a 2-h incubation at room temperature, the blots were washed well with TN-0.05% Nonidet P40 (NP-40; Particle Data, Inc., Elmhurst, IL) followed by extensive washing with TN buffer alone. The blots were then incubated with 25 ml of TN-5% BSA containing 2.5×10^5 cpm per ml of [^{125}I] protein A (sp act 1.2×10^7 cpm/ μg) radiolabeled using a modified chloramine T-mediated iodination procedure (36). The nitrocellulose blots were again washed well in TN-0.05% NP-40 before air drying and exposure to x-ray film for autoradiography. Duplicate lanes from acrylamide gels were always stained after blotting to insure that efficient electrophoretic transfer of proteins had occurred.

Attachment of *T. pallidum* to Protein-coated Coverslips and HEp-2 Cells. Glass coverslips were incubated with 50 μ l of 1 mg/ml fibronectin or BSA in PBS. The protein solution was uniformly spread on the glass surfaces, allowed to dry for 1 h at room temperature, and the glass coverslips were rinsed three times by immersion in 100 ml of PBS. The glass surfaces retained a uniform layer of protein throughout the assay conditions as determined by indirect immunofluorescence microscopy using specific fluorescein-isothiocyanate-labeled rabbit anti-goat immunoglobulin against either goat anti-human fibronectin or goat anti-bovine serum albumin (N. T. Cappel Laboratories). Human epithelial cells (HEp-2) (ATCC, Rockville, MD) (1, 22) were maintained in Dulbecco's minimum essential medium (DME) supplemented with 10% fetal calf serum. Cells were grown at 37°C in an atmosphere of 5% CO₂. These cells stained for fibronectin using fluorescence microscopy. For treponemal attachment assays, 5×10^4 HEp-2 cells were seeded in Leighton tubes containing a single 9- $\times$ 35-mm coverslip, incubated for 24 h at 37°C and then at 34°C for no less than 6 h before the experiment. In certain cases fibronectin-coated coverslips and HEp-2 cells were treated at room temperature for 30 min with control serum or monospecific antifibronectin serum or monospecific serum raised against albumin or transferrin (N. L. Cappel Laboratories) each diluted 1:5 in DME, followed by washing with PBS.

1-ml suspensions of freshly extracted unlabeled *T. pallidum* organisms or treponemes radio-labeled with [³⁵S]methionine were added to coverslips or cell culture monolayers and incubated at 34°C for 2 h. The coverslips were then removed and rinsed three times in PBS (1, 22). The degree of HEp-2 cell surface parasitism was assessed by counting the number of treponemes attached to 20 individual HEp-2 cells that exhibited typical morphology (1, 22). Experiments were performed at least twice using triplicate test samples. Microscopy was accomplished with darkfield optics on a Leitz Ortholux II. Experiments with [³⁵S]methionine-labeled treponemes were processed similarly, except that the rinsed coverslip was placed in a vial with scintillation cocktail for measuring radioactivity.

Affinity Purification of Treponemal Proteins. A total of 2×10^{10} ³⁵S-labeled spirochetes washed at least three times in PBS were resuspended in 1 ml PBS containing 1.0% Zwittergent 3-12 (Calbiochem-Behring Corp.) detergent (37). Treponemes were solubilized by gentle homogenization and insoluble material clarified by centrifugation at 100,000 *g* for 30 min. The soluble treponemal extract was then diluted further with PBS to give a final Zwittergent concentration of 0.05%. Chromatography was performed on a 2- $\times$ 10-cm fibronectin-Sepharose (38) affinity column at 1 ml/cm²/h, followed by extensive sequential washing of the column with PBS, 2 M potassium bromide, and 10% SDS. Finally, the tightly bound, fibronectin-adherent material was displaced from the fibronectin-Sepharose matrix by boiling the beads for 3 min in 10 ml of electrophoresis solubilizing buffer. The beads were removed by centrifugation, and a 100- μ l aliquot containing released radioactivity evaluated by SDS-PAGE-fluorography (1, 3, 4).

Results

Acquisition of Plasma Proteins by Virulent Treponemes. The avid binding of radioiodinated plasma proteins by motile, washed *T. pallidum* organisms is illustrated in Fig. 1 (lane A). These ¹²⁵I-labeled proteins could not be removed from the parasite surface by repeated washing in PBS. Importantly, the avirulent spirochete, *T. phagedenis* biotype Reiter, did not bind any of the radioiodinated plasma proteins under identical experimental conditions (lane B) demonstrating the specific nature of *T. pallidum* acquisition of host macromolecules.

Since the identity of the acquired radioiodinated proteins could not be readily established through competition experiments using unlabeled preparations of co-migrating proteins such as immunoglobulin G or albumin, we performed Cohn fractionation of human plasma (34) to enrich for specific groups of proteins (Table I). We reasoned that these fractions might be useful in identification of proteins bound to *T. pallidum* outer envelopes. As seen in Table I, the ¹²⁵I-labeled fraction (sample no. 2) containing predominantly plasminogen, fibronectin, and fibrinogen resulted in levels of binding to *T. pallidum* greater than that detected for other Cohn fractions.

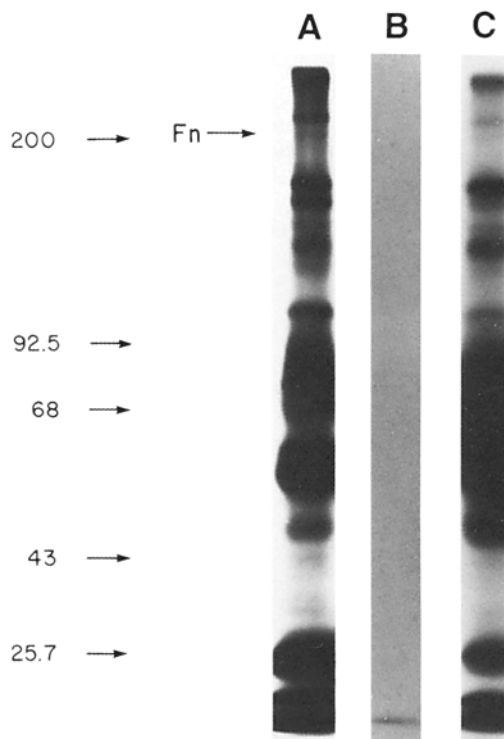


FIG. 1. Representative SDS-PAGE-autoradiography of total proteins after incubation of *T. pallidum* with radioiodinated normal human plasma. Lane A represents ^{125}I -labeled plasma proteins avidly bound to *T. pallidum*. Lane B shows the lack of plasma protein acquisition by the avirulent spirochete, *T. phagedenis* biotype Reiter, handled similarly. Lane C is a profile of ^{125}I -normal human plasma used in these acquisition assays. The location of fibronectin (Fn) was established by electrophoresis of ^{125}I -labeled purified human Fn under similar conditions. Mol wt markers ($\times 10^3$): myoglobin, 200; phosphorylase b, 92.5; bovine serum albumin, 68; ovalbumin, 43; and α -chymotrypsinogen, 25.7.

Consistent with the results presented in Fig. 1, the avirulent Reiter spirochete bound levels equal to control tubes without *T. pallidum*; these values usually represented $<1\%$ of the level bound by *T. pallidum* under similar conditions. Furthermore, ^{125}I -labeled fibronectin purified from fraction I + III-3 (see sample no. 10) was readily and avidly bound by *T. pallidum* but not by its avirulent counterpart.

Immunodetection of *T. pallidum*-acquired Fibronectin. The immunodetection of fibronectin on nitrocellulose blots of total TCA-precipitated treponemal proteins is shown in Fig. 2. Lane A reveals the immunodetection of purified plasma fibronectin with monospecific antifibronectin antibody and ^{125}I -protein A. Lane B shows the lack of reactivity of normal goat serum or ^{125}I -labeled protein A to an identical fibronectin blot. Lanes C and E demonstrate the binding of antifibronectin antibody to blots of total protein profiles from treponemes incubated with whole plasma and Cohn fraction I + III-3, respectively. Lane D shows the lack of binding of either normal goat serum or radioiodinated protein A to blotted *T. pallidum* proteins. As expected, fibronectin-depleted plasma incubated with motile parasites did not result in any detectable protein band (lane F). Finally, incubation of treponemes with purified fibronectin gave the profile shown in lane G.

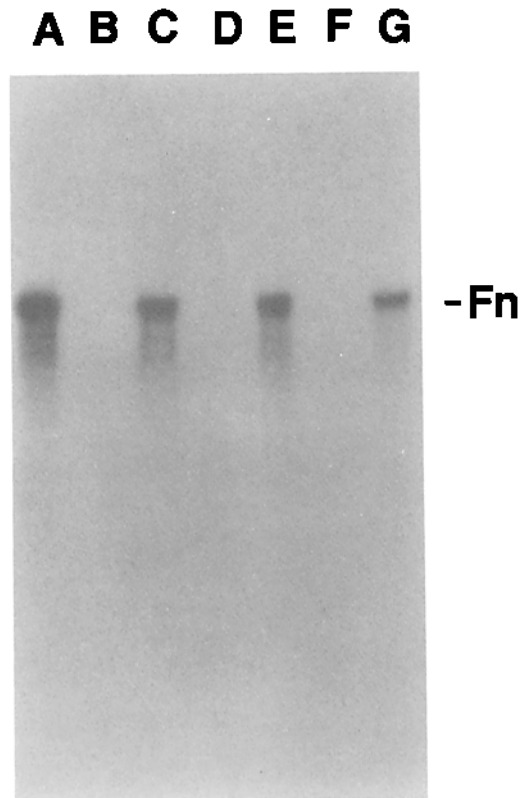


FIG. 2. Electrophoretic transfer and immunodetection of unlabeled fibronectin acquired by motile *T. pallidum* incubated with normal human plasma (lane C), Cohn fraction I + III-3 (lane E) and purified fibronectin (lane G). Lane A shows the immunodetection of purified fibronectin alone. Lane B demonstrates the lack of reactivity of normal goat serum and ^{125}I -protein A with fibronectin alone or with blotted *T. pallidum* proteins (lane D). Lane F shows the lack of detection using antifibronectin antibody and ^{125}I -protein A of *T. pallidum* incubated with plasma depleted of fibronectin. All procedures are as described in Materials and Methods.

Interaction of *T. pallidum* with Fibronectin-coated Coverslips. The binding of fibronectin to the outer envelope of *T. pallidum* prompted us to further investigate this association. Fig. 3 B demonstrates the tip-oriented adherence of motile treponemes to fibronectin-treated glass surfaces as visualized by darkfield microscopy. Incubation of motile treponemes with untreated or albumin-coated coverslips under similar conditions resulted in no detectable treponemal attachment (Fig. 3 A).

In addition, incubation of increasing numbers of ^{35}S -labeled parasites with fibronectin-treated glass surfaces yielded concomitantly increased levels of bound cpm when compared with untreated or bovine serum albumin-coated coverslips (Table II). Furthermore, incubation of the fibronectin-coated glass surface with antifibronectin serum before addition of radiolabeled *T. pallidum* significantly decreased treponemal binding to these coverslips (Table III). Treatment of the fibronectin coverslips with control serum or antiserum against albumin or transferrin did not diminish the extent of parasite binding. These data further suggested that the tip-oriented treponemal parasitism of host cells (1, 3, 4, 22) might be mediated by a fibronectinlike macromolecule.

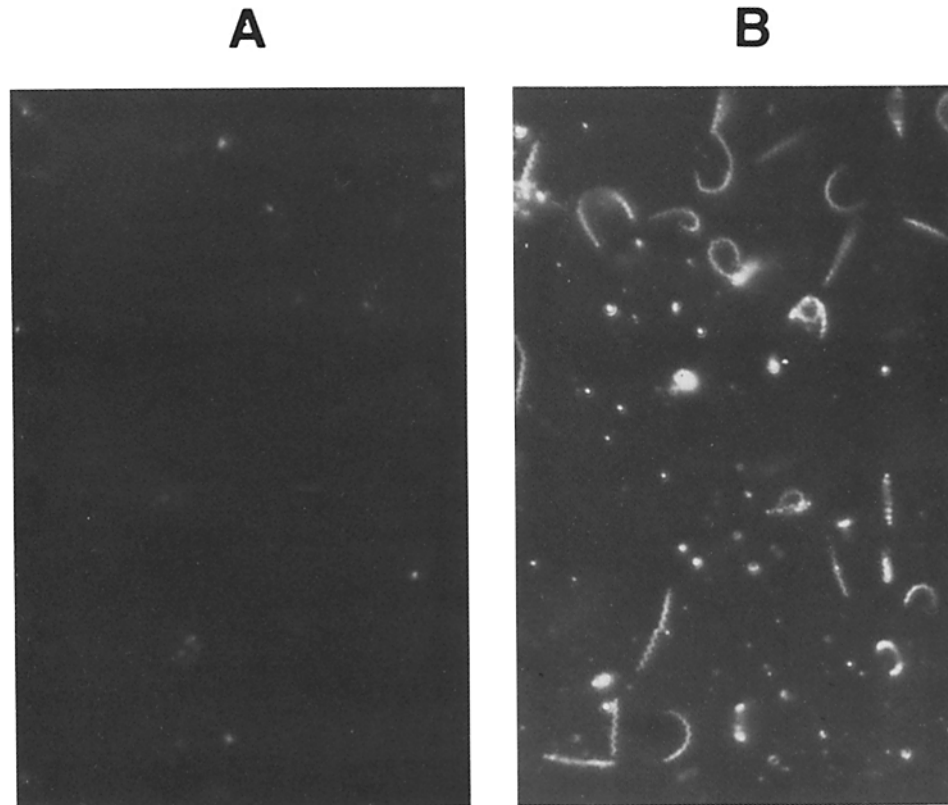


FIG. 3. A representative darkfield view of tip-mediated adherence of freshly harvested *T. pallidum* incubated with fibronectin-coated cover slips (B) and compared to the lack of attachment of treponemes from the same extraction to untreated or albumin-coated coverslips (A).

TABLE II
Adherence of [35 S]Methionine-labeled *T. pallidum** to Fibronectin-coated Coverslips

Treatment of coverslips [‡]	Recovered cpm [§]		
	Number of treponemes per ml added to coverslips		
	1.4×10^7	2.8×10^7	5.6×10^7
Control untreated	800 ± 63 (1.0)	825 ± 42 (1.0)	860 ± 150 (1.1)
Albumin	910 ± 80 (1.0)	930 ± 110 (1.0)	990 ± 170 (1.1)
Fibronectin	$1,800 \pm 430$ (1.0)	$3,800 \pm 760$ (2.1)	$8,200 \pm 840$ (4.5)

* Purification and radiolabeling of treponemes as described in Materials and Methods. 1 ml of motile organisms was added to Leighton tubes containing coverslips and incubation continued at 34°C for 2 h before extensive washing of coverslips in PBS for scintillation counting. 1.4×10^7 treponemes represent 70,000 cpm.

[‡] Coverslips were treated with respective plasma proteins for binding assays as described in Materials and Methods. A uniform layer of albumin or fibronectin was monitored by indirect immunofluorescence.

[§] Washed coverslips were placed in aqueous scintillation fluid for determination of cpm using a Beckman LS 7800 scintillation counting system.

^{||} Fold increase in cpm.

TABLE III
Specificity of Fibronectin Films in Attachment of ³⁵S-Labeled *T. pallidum**

Treatment [†]	Recovered cpm*	% of Control [‡]
Fetal calf serum	39,848 ± 276	100.0
Normal goat serum	41,338 ± 765	103.7
Antitransferrin	40,563 ± 827	101.7
Anti-BSA	40,929 ± 987	102.7
Antifibronectin	11,702 ± 359	29.4

* As described in Table II. A 1-ml suspension of 8×10^7 treponemes representing 850,000 cpm was added to coverslips treated with fibronectin and incubated at 34°C for 1 h.

[†] Fibronectin-coated coverslips were treated with 1.0 ml antiserum or control serum diluted 1:10 in PBS for 30 min at 37°C and washed three times with PBS before addition of treponemes.

[‡] Numbers calculated are based on fetal calf serum control value.

TABLE IV
Involvement of Fibronectin on In Vitro Adherence of *T. pallidum** to HEp-2 Cells

Experiment	Treatment [†]	Number of treponemes per cell (% of control) [‡]	
		A	B
1	Fetal calf serum	23 ± 4 (100.0)	19 ± 5 (100.0)
	Normal goat serum	22 ± 5 (95.6)	19 ± 4 (100.0)
	Anti-BSA	20 ± 5 (87.0)	17 ± 5 (89.4)
	Antifibronectin	11 ± 4 (47.8)	3 ± 2 (15.7)
Recovered cpm (% of control) [‡]			
2	Fetal calf serum	17,000 ± 960 (100.0)	ND
	Normal goat serum	16,661 ± 1351 (98.0)	ND
	Anti-BSA	16,969 ± 773 (99.8)	ND
	Antifibronectin	6,064 ± 2100 (35.6)	ND

* As described in Table II.

[†] A, HEp-2 cells were treated with 1.0 ml of antiserum or control serum reagents diluted 1:5 in DME for 30 min followed by three washes in DME; B, cells were sequentially treated twice with serum reagents and then washed with PBS before incubation with treponemes.

[‡] Leighton tube coverslips were scanned by darkfield microscopy and treponemal attachment quantitated as described in Materials and Methods. Approximately 100% of cells had adherent parasites. Percentages in parenthesis are relative to fetal calf serum control values.

[§] 5.6×10^7 radiolabeled treponemes representing 400,000 cpm were incubated with HEp-2 cells pretreated once with antiserum reagents. Coverslips were washed three times in DME and placed in scintillation fluid as described in Table I.

Role of Fibronectin in Treponemal Adherence to HEp-2 Cells. The possibility that cellular fibronectin synthesized by HEp-2 cells (24, 28) might serve as the receptor for tip-mediated attachment of intact, motile *T. pallidum* was then examined. The presence of fibronectin on HEp-2 cells was monitored by indirect immunofluorescence before the adherence assays. As seen in Table IV (Exp. 1 A), treatment of cell monolayers once with antifibronectin antibody before addition of freshly extracted treponemes resulted in a >50% reduction in attachment of organisms to host cell surfaces. Furthermore, treatment of HEp-2 cells with antifibronectin antiserum at least twice before addition of parasites almost totally abrogated treponemal attachment to HEp-2 cell monolayers (Table IV, Exp. 1 B). Results consistent with these observations were obtained when ³⁵S-radiolabeled treponemes were incubated with antifibronectin-

treated HEP-2 cell monolayers (Table IV, Exp. 2). A 65% reduction in recovered cpm was achieved with treatment of cells with antifibronectin serum reagent. These data reinforce the idea that cellular fibronectin may mediate treponemal attachment to host cells.

Affinity-purified Treponemal Surface Proteins. To examine the possible interaction of putative *T. pallidum* receptor-binding proteins (1, 3, 4, 22) with fibronectin, a Zwittergent-solubilized ^{35}S -labeled preparation of *T. pallidum* was chromatographed on fibronectin-Sepharose affinity column. Electrophoretic analysis of ^{35}S -labeled nonadherent and adherent column fractions is presented in Fig. 4. The pattern of [^{35}S] methionine-labeled proteins observed in the Zwittergent extract is shown in lane A and compared to a typical Coomassie Brilliant Blue stained profile of total TCA-precipitated *T. pallidum* proteins (lane D). Examination of nonadherent material revealed the selective removal by the fibronectin column of several radiolabeled proteins with molecular weights of 89,000, 29,000, and 25,000 (lane B). Most of the adherent ^{35}S -labeled material was only recovered from the fibronectin-Sepharose matrix by boiling in an SDS- β ME solubilizing buffer (lane C). This adsorbed material from intrinsically radiolabeled *T. pallidum* represented three prominent treponemal proteins with electrophoretic mobilities equal to those previously identified as proteins

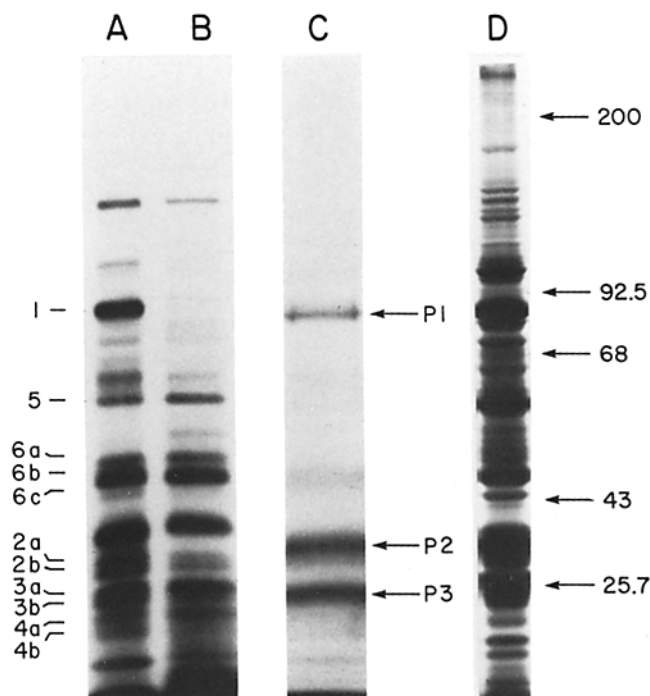


FIG. 4. SDS-PAGE-fluorography of intrinsically radiolabeled *T. pallidum* proteins after fibronectin-Sepharose affinity chromatography. Lane A shows the gel pattern of a Zwittergent extract of ^{35}S -labeled *T. pallidum* proteins. Lane B represents the eluted nonadherent fraction of radiolabeled treponemal proteins, whereas lane C is the protein profile of *T. pallidum* proteins bound selectively to the column. Lane D is a typical Coomassie Brilliant Blue stained profile of total TCA-precipitated *T. pallidum* proteins. Mol wt markers are as indicated in the legend of Fig. 1. Numbers indicate previously characterized *T. pallidum* proteins (1) and proteins 1, 2, and 3 are proteins implicated as host receptor-binding proteins (1, 4).

1, 2, and 3 (1, 4) and implicated as treponemal ligands binding to receptors on host cell surfaces (1, 3, 4, 22). The same results were obtained in experiments using ^{125}I -labeled organisms (1) (data not shown) confirming the surface origin of these treponemal, fibronectin-binding proteins.

Discussion

Evidence found by us and others suggest an important role in pathogenesis of *T. pallidum* adherence to host cells (1, 3, 4, 21, 22). The specialized tip structure on *T. pallidum* appears to serve as a functional organelle mediating this surface parasitism (1, 3, 4, 22), and structure-function studies of outer envelope *T. pallidum* proteins (1) have implicated three prominent, highly immunogenic treponemal proteins as potential ligands (1, 4). Because of the specific and complex nature of the attachment mechanism (1, 4, 22), it seemed that an understanding of the nature of host cell components that mediate treponemal adherence would further enhance and support the concept of membrane-membrane, ligand-receptor interactions between host and parasite.

We have been interested in examining the binding of host macromolecules to treponemal surfaces in order to clarify beneficial and/or detrimental biological properties conferred upon *T. pallidum* through the acquisition of host macromolecules. In this study, we present a strategy for the identification and characterization of human plasma proteins preferentially bound to *T. pallidum* (Fig. 1 and Table I). The observation that acquisition of specific plasma molecules was a property only of virulent treponemes (Fig. 1) led us to further study a potential function of these specifically acquired proteins. Because of the importance of fibronectin in cellular and tissue substratum adherence, the role of fibronectin on previously characterized biological properties of *T. pallidum* was examined.

Using a combination of microscopic, immunochemical, and cell culture techniques, we monitored the interaction of motile treponemes with fibronectin-coated glass coverslips and cell culture monolayers (Fig. 3 and Tables II-IV). In all cases, our observations were consistent with published reports (1, 3, 4, 22) on the tip-oriented attachment of *T. pallidum* to host cells. The specific nature of the *T. pallidum*-fibronectin interaction was established by (a) the selective binding of treponemes to coverslips coated with fibronectin (Table II); (b) the effective inhibition of *T. pallidum* binding to fibronectin-coated coverslips and cell monolayers previously treated with monospecific antifibronectin serum (Table III); and (c) the stoichiometric increased binding of ^{35}S -labeled organisms to fibronectin surfaces (Tables III and IV). These data establish a role for cellular fibronectin on *T. pallidum* adherence to host cells (Table IV). The inability of the avirulent Reiter spirochete either to acquire plasma proteins (Fig. 1 and Table I) or to parasitize eucaryotic cells (18, 21, 22) is consistent with the interpretations of these data.

Finally, chromatography of Zwittergent extract of intrinsically-labeled *T. pallidum* on a fibronectin-Sepharose column resulted in the selective purification of treponemal proteins 1, 2, and 3 (Fig. 4). These proteins have been previously found to reside on the parasite outer envelope and have been implicated, using a combination of molecular-biochemical approaches, as the ligands responsible for treponemal attachment to HEp-2 cells (1, 3, 22). Similar results were obtained using detergent preparations of radioiodinated *T. pallidum* reinforcing the surface origin of the fibronectin-

adherent treponemal proteins.

These data support and extend the concept of a ligand-receptor adherence mechanism in host cell surface parasitism by *T. pallidum* (1, 3, 4, 22). The role of fibronectin as a receptor on eucaryotic cells for *T. pallidum* adherence is consistent with published reports on the carbohydrate-proteinaceous nature of the cell components involved in this attachment mechanism (1, 4, 22). Furthermore, the possibility of continued host priming with these treponemal outer envelope proteins (4) is supported by the avid binding of proteins 1, 2, and 3 to fibronectin (Fig. 4) and the prolonged and highly selective humoral immune response to these proteins (1, 2).

The demonstration that *T. pallidum* ligands bind to fibronectin or fibronectinlike molecules on eucaryotic cell surfaces may permit a clearer understanding of the infectious process and how *T. pallidum* invades and colonizes specific tissue sites. The availability of purified and highly immunogenic treponemal ligands could lead to improved serodiagnostic probes and to studies on the mechanisms by which attachment proteins interact with host membranes at the molecular level. Also, this work along with observations by us (18) that other host macromolecules are avidly sequestered by the *T. pallidum* outer envelope may explain the frequent occurrence of autoimmune mechanisms and host cytopathology that are characteristic of syphilis (39). Ultimately, these studies may clarify the virulence of *T. pallidum* and host susceptibility and resistance to infection.

Summary

Analysis of plasma proteins avidly bound to *T. pallidum* surfaces revealed the ability of *T. pallidum* to acquire numerous host macromolecules. No acquisition was evident by the avirulent spirochete, *T. phagedenis* biotype Reiter. Western blotting technology using hyperimmune antifibronectin serum as a probe revealed the ability of virulent treponemes to avidly bind fibronectin from a complex medium such as plasma.

The specificity of the tiplike adherence of motile *T. pallidum* to fibronectin-coated glass surfaces and to fibronectin on HEp-2 cells was reinforced by the observation that pretreatment of coverslips or cell monolayers with monospecific antiserum against fibronectin substantially reduced *T. pallidum* attachment. The stoichiometric binding of *T. pallidum* to fibronectin-coated coverslips and the inability of unlabeled or ³⁵S-radiolabeled treponemes to interact with glass surfaces treated with other plasma proteins further established the specific nature of the interaction between virulent *T. pallidum* and fibronectin.

The avid association between three outer envelope proteins of *T. pallidum* and fibronectin was also demonstrated. These treponemal surface proteins have been previously identified as putative receptor-binding proteins responsible for *T. pallidum* parasitism of host cells. The data suggest that surface fibronectin mediates tip-oriented attachment of *T. pallidum* to host cells via a receptor-ligand mechanism of recognition.

Received for publication 17 February 1983.

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