INFECTION AND IMMUNITY, Nov. 1986, p. 456–464
0019-9567/86/110456-09$02.00/0

Vol. 54, No. 2

Antibody-Independent Interactions of Fibronectin, C1q, and Human Neutrophils with *Treponema pallidum*

ROBERT E. BAUGHN

*The Departments of Microbiology and Immunology and of Dermatology, Baylor College of Medicine, Houston, Texas 77030, and the Syphillis Research Laboratory, Veterans Administration Medical Center, Houston, Texas 77211**

Received 10 April 1986/Accepted 25 July 1986

Although recent evidence suggests that fibronectin may be involved in the attachment of treponemes to mammalian cells, its possible role in promoting phagocytosis of *Treponema pallidum* has not been investigated. In the present study, we examined the antibody-independent interactions of fibronectin, C1q, and human polymorphonuclear leukocytes with *T. pallidum*. Binding of [^{125}I]fibronectin was specific and saturable with an affinity constant of approximately 2×10^7 M^{-1}. The number of binding sites per treponeme at 37°C, irrespective of the mammalian source of fibronectin, was between 2,500 and 7,500, with a mean of approximately 4,700. Binding of [^{125}I]C1q to *T. pallidum*, in the absence of antibodies to the organism, also was saturable and specific. Pretreatment of treponemes with C1q enhanced binding of soluble [^{125}I]fibronectin two- to threefold and also increased attachment of ^{125}I-surface-labeled treponemes to fibronectin-coated surfaces. Treatment of ^{125}I-labeled *T. pallidum* with fibronectin alone, or together with C1q, however, did not enhance surface phagocytosis by neutrophils.

Fibronectin (Fn), a large glycoprotein with a molecular mass of approximately 440 kilodaltons (kDa), is known to interact with eucaryotic cells, several bacterial species, and a number of macromolecules including C1q (reviewed in reference 32). Following activation of C1 by the classical complement pathway, C1r and C1s are dissociated from C1q so that the collagenlike region of the latter is exposed and capable of interacting with C1q receptors on polymorphonuclear leukocytes (PMNs), monocytes, null cells, and B lymphocytes or with plasma Fn (8, 20, 27, 33, 44–46).

Recent studies (3, 15, 35, 49, 53–55) examining the interaction between Fn and *Treponema pallidum* have suggested that attachment may be an important determinant in the pathogenesis of early syphilis. Unfortunately, discrepancies exist concerning the binding affinity constant (K_a) and the number of receptors per organism; reported findings for both parameters differ by as much as 3 orders of magnitude (49, 53). Steiner and Sell (49) used rabbit Fn and Percoll-gradient-purified treponemes, whereas Thomas et al. (53) used human Fn and treponemes purified by a different technique, making it difficult to directly compare results. Although some of those studies assessed the role of Fn in the binding of *T. pallidum* to cultured cells, such as SF1Ep and HEp-2 cells, interactions with PMNs and mononuclear leukocytes have not been studied. Such studies would appear to be essential for a better understanding of the pathogenicity of *T. pallidum*, since neutrophils constitute the first line of defense of the body, and since an opsonic role for Fn in promoting phagocytosis has been described (16, 37, 38, 43, 57, 58).

The overall objective of the present study was to determine whether C1q, in an antibody-independent system, could augment Fn-treponemal interactions alone and in concert with PMNs. Such experiments were considered a prerequisite to studies designed to assess the role of Fn in promoting the phagocytosis of immunoglobulin G (IgG)-coated *T. pallidum*.

MATERIALS AND METHODS

Organisms. *T. pallidum* Nichols, originally obtained from the Centers for Disease Control, Atlanta, Ga., and free from contamination with the passenger pleural-effusion agent (14), was passaged by intratesticular injection in New Zealand white male rabbits. Organisms for challenge and for most immunologic studies were extracted from infected testes and separated from host tissue contaminants by gradient density centrifugation with 40% Percoll (Pharmacia Fine Chemicals, Piscataway, N.J.) containing 0.1 mM dithiothreitol (18) as previously described (4). For comparative purposes, after extraction and the two centrifugational washes at $754 \times g$ for 10 min necessary to remove host cell contaminants, treponemes were harvested on a 0.8% Methacel (Dow Chemical Co., Midland, Mich.)–50% Hypaque (Winthrop Laboratories, Div. Sterling Drug, Inc., New York, N.Y.) gradient at $650 \times g$ for 20 min (1, 53). The supernatant fluid containing the treponemes was removed, and the organisms were pelleted at $17,000 \times g$ for 15 min. For sodium dodecyl sulfate-polyacrylamide gel electrophoresis, treponemal pellets were suspended in 10 mM Tris-bartial buffer (pH 8.6; ionic strength, 0.2) containing 15 mM sodium azide, 200 kIU of aprotinin (Trasylol; FBA Pharmaceuticals, New York, N.Y.) per ml, and 1 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, Mo.) and stored at −70°C. These suspensions were subsequently adjusted to contain 4 mg of protein per ml (9), diluted 1:2 in Tris hydrochloride reducing buffer (0.5 M Tris [pH 6.8], 2% sodium dodecyl sulfate, and 2% 2-mercaptoethanol containing 0.1% tracking dye [bromphenol blue]), and heated at 63°C for 30 min. For Fn- and C1q-binding assays, pellets that had undergone the Percoll or Methacel-Hypaque purification steps were suspended in 0.02 M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 7.4), containing 0.15 M sodium chloride and 0.1% bovine serum albumin (BSA), counted by dark-field microscopy, and used immediately.

Suspensions of avirulent spirochetes, *Treponema refringens* and *Treponema phagedenis*, biotype Reiter, were prepared by cultivation in Spirolate brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10% normal rabbit serum or with 10% fetal calf serum, at 35°C for 5 to 7 days (6). Organisms were harvested by centrifugation at 10,000 × *g* for 25 min, washed four times with phosphate-buffered saline (PBS; pH 7.4), and finally resuspended in either Tris hydrochloride reducing buffer or 0.02 M HEPES buffer.

Staphylococcus aureus Cowan I was used for comparative purposes in several experiments. Cells for those studies were grown in tryptic soy broth (BBL) for 18 h, harvested by centrifugation, and resuspended in 0.02 M HEPES buffer.

Purification of C1q and Fn. C1q was isolated from human serum with ion exchange chromatography and gel filtration by the methods of Tenner et al. (51). The C1q was hemolytically active, contained no detectable immunoglobulins by radial immunodiffusion, and was homogeneous on unreduced sodium dodecyl sulfate-polyacrylamide gels. Affinity-purified human Fn and bovine Fn were obtained from Calbiochem-Behring, La Jolla, Calif. Fn also was purified from rabbit and human plasma by gelatin affinity chromatography with Affi-gel gelatin (Bio-Rad Laboratories, Richmond, Calif.). Fn concentrations were measured spectrophotometrically, and aggregates were removed by centrifugation at 10,000 × *g* for 15 min, snap frozen at concentrations of 10 to 15 mg/ml, and stored at −70°C. Materials were analyzed by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing and reducing conditions to verify purity.

Purification of Fn cell-binding fragments. Cell-binding fragments or domains of unlabeled Fn were isolated under conditions similar to those described by Thomas et al. (53), based on the original method of Hayashi and Yamada (19). After trypsinization (tolysulfonyl phenylalanyl chloromethyl ketone-treated trypsin; Sigma), Fn digests were subjected to preparative chromatography on DEAE cellulose and gelatin-agarose, and fractions were obtained by elution with 0.13 M NaCl from a final chromatographic step with heparin-agarose. The 66- to 75-kDa fragments were finally enriched by electroelution from 3-mm-thick preparative 10% acrylamide gels (6). Unstained bands in appropriate regions were sliced, removed, and subjected to electroelution at 10 mA per channel overnight in 10 mM ammonium bicarbonate buffer. Collection of materials was in membrane bags with 10,000-molecular-weight cutoffs. The electroeluted materials were then concentrated to approximately 2 mg/ml by using Centricon centrifugal microconcentrators (Amicon Corp., Lexington, Mass.). The purity was verified by reelectrophoresing the materials on 10% gels.

Immunologic reagents. Sheep antisera to human Fn and goat antiserum to human C1q were obtained from Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill. Goat and rabbit antiserum to Fn and goat antiserum to human C1q were obtained from Cooper Biomedical, Inc., Malvern, Pa. Affinity-purified IgG fractions of goat or sheep antibodies, or both, to a variety of rabbit proteins (IgA, IgG, IgM, C1q, and Fn) were obtained from Miles Scientific and Cooper. Goat antibodies to human Fn and mouse monoclonal antibodies (MAbs), types 1, 2, and 3, to human Fn components were obtained from Calbiochem-Behring. The type 1 MAb reacts with whole Fn and various heparin-binding fragments derived from the COOH-terminal heparin-binding site, the type 2 MAb binds to an antigenic determinant in the region of the Fn molecule that interacts with cells, and the type 3 MAb binds to a determinant near the gelatin-binding region of the molecule. Types 2 and 3 cross-react with bovine Fn.

Radiolabeling. For use in radioimmunoassay (RIA), 1 mg of affinity-purified goat or sheep IgG antibodies (or both) against individual rabbit or human proteins were labeled with 1 mCi of ^{125}I-labeled Na (Amersham Corp., Arlington Heights, Ill.) by using iodobeads (Pierce Chemical Co., Rockford, Ill.) (26), as described previously (5). Fn was iodinated either by the lactoperoxidase method (28) or with iodobeads (26). Nearly identical results were obtained with both procedures; approximately 93 to 96% of the radioactivity in both types of preparations could be precipitated with specific anti-Fn antibodies. The specific activity of labeled Fn preparations was between 5 × 10^6 and 10^8 cpm/μg. C1q also was iodinated by using iodobeads; specific activites typically ranged between 5 × 10^4 and 10^6 cpm/μg. Iodinated C1q and Fn, if not used immediately after labeling, were stored at −70°C for no more than 4 days. Purity of labeled Fn and C1q was verified on Western blots of sodium dodecyl sulfate gels. Radiolabeled C1q was hemolytically active and capable of binding to immune complexes.

Bacterial strains also were surface labeled with ^{125}I-labeled Na by using iodobeads. After removal of the beads, labeled organisms were separated from unbound label by centrifugation at 17,000 × *g* for 20 min. For comparison purposes, treponemes were intrinsically labeled with [^{35}S]methionine (Amersham) by the procedure of Moskophidis and Muller (30) without modification.

Estimation of endogenous host proteins bound to *T. pallidum*. The procedures for solid-phase RIA as performed in our laboratory, have been described in detail previously (6). In an attempt to estimate the extent of endogenous host proteins binding to *T. pallidum*, triplicate wells of polyvinyl microtiter plates were coated with 100 μl of 15 twofold dilutions of various rabbit protein standards (IgA, IgG, IgM, C1q, and Fn). Dilutions for the coating of these standard plates were made in carbonate buffer (pH 9.6) and concentrations ranged between 20 μg and 0.12 ng/ml; plates were incubated overnight at 4°C. Test materials in these assays consisted of treponemes harvested by both the Percoll and Methacel-Hypaque gradient techniques as well as organisms obtained at the end of the preparative low-speed centrifugation steps in the extraction scheme. After dark-field microscopic counts, suspensions were diluted in PBS to contain 10^8 and 5 × 10^7 treponemes per ml. Samples (50 μl) of each of the test suspensions were added to triplicate wells of test plates and air dried. The test plates were subsequently fixed with 70 μl of 10% ethanol and air dried again. All plates were washed twice with PBS-Tween, and wells were filled with 0.1% BSA in PBS and incubated for 30 min to block nonspecific sites. After three additional washes with PBS-Tween, the wells were filled with 100 μl of the appropriate ^{125}I-labeled second antibody in 0.1% BSA in PBS. After 2 h of incubation at 4°C, the plates were washed six times with PBS-Tween and dried, and the wells were cut apart and counted in a gamma counter. Appropriate controls for the standard and test plates and determination of nonspecific binding consisted of carbonate buffer without standard proteins and PBS without organisms, respectively. The nanogram concentration of each host protein bound endogenously was subsequently estimated from the appropriate standard curve.

Because we were interested in ultimately studying the role of C1q in an antibody-independent system, an alternative method was used to establish the fact that Percoll-purified

treponemes were relatively free from contamination with host immunoglobulins. Western blots of *T. pallidum* (described below) were incubated for 2 h with iodinated goat IgGs directed against the various heavy chains of rabbit immunoglobulins. After being washed, blots were counted in a gamma counter and subsequently sliced into 1-mm slices for profiles of counts per minute per slice (4–6). Controls in these assays consisted of Western blots of purified rabbit immunoglobulins developed with the same iodinated second antibodies and processed under identical conditions.

Fn- and C1q-binding assays. Binding of Fn to *T. pallidum* was performed in triplicate according to the methods described by Proctor et al. (37) with the following modifications. HEPES buffer (0.8 ml) containing BSA was added to conical, plastic Microfuge tubes (Beckman Instruments, Inc., Fullerton, Calif.) and allowed to stand at 37°C for 15 min. HEPES buffer (0.1 ml), containing 5×10^7 *T. pallidum* or 5×10^7 avirulent treponemes, was added to tubes and gently vortexed before addition of increasing concentrations of [^{125}I]Fn in 0.1 ml of HEPES buffer to make a final volume of 1 ml. Unless otherwise noted, all reactions were carried out at 37°C at a pH of 7.4. Separation of free and bound Fn was accomplished by pelleting treponemes, removing the supernatant fluids, slicing off the bottom of the tubes, and counting the pellets in a gamma counter. Tubes containing excess unlabeled Fn were used to determine nonspecific binding levels; these were then subtracted to estimate the actual amounts of Fn bound. In those experiments involving pretreatment of treponemes with C1q, 5×10^8 cells were incubated in buffer alone or HEPES buffer containing 20 or 100 nM unlabeled C1q for 30 min at 37°C, before performing binding assays with 30 μg of radiolabeled Fn per ml.

C1q-binding assays were carried out under similar conditions except that mixtures of treponemes and labeled C1q in HEPES buffer containing 0.15 mM Ca^{2+} and 0.5 mM Mg^{2+} were incubated for 30-min intervals at 37°C.

Identification of Fn fragments binding to treponemes. Radiolabeled Fn (1 ml containing 0.5 μg) in PBS was incubated with 0.1 μg of trypsin at 25°C for 1 h, at which time digestion was stopped by adding 10 μl of 0.1 M phenylmethylsulfonyl fluoride. Aliquots also were incubated with 0.02 μg of Cathepsin D (Sigma; EC 3.4.23.5) for 2 to 6 h at 30°C (41). Those digestions were stopped with 0.01 μg of pepstatin (Sigma). Samples (100 ml) of labeled degradation products were incubated with 10 μl containing 2×10^7 bacterial cells for 1 h at 37°C, followed by centrifugation at $10,000 \times g$ for 20 min. Pellets were washed twice in PBS and bound, and labeled fragments were released by boiling for 1 min in electrophoresis sample buffer (16). After centrifugation, supernatant fluids were subjected to electrophoresis, Western blotting, and direct autoradiography as described below. The concentrations of phenylmethylsulfonyl fluoride and pepstatin used in these studies were capable of inactivating the respective degradative enzymes without substantially altering the Coomassie blue-stained profiles of *T. pallidum*.

Electrophoretic transfer blotting (Western blot) technique. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described by Laemmli (22) was performed with 3% stacking gels and separating gels of 10% acrylamide or 8 to 20% gradient gels as previously described (4–6). Resolved proteins from materials run on gels were electrophoretically transferred to nitrocellulose papers by our modifications (4–6) of the method originally described by Towbin et al. (56).

Attachment of radiolabeled organisms to Fn-coated cover slips. Glass cover slips (22 by 10 mm) were overlaid with 100 μl of Fn (300 μg/ml), incubated for 1 h at 37°C, incubated in a solution of ovalbumin in PBS (1 mg/ml), rinsed with PBS, and used in attachment assays. Control cover slips were coated with BSA alone. The cover slips were not allowed to dry. Surface-labeled treponemes (100 μl containing 10^6 or 10^7 cells) were incubated on cover slips at 37°C. After incubation, cover slips were thoroughly rinsed with PBS and transferred to glass tubes for counting.

PMNs. Collection of PMNs from peripheral blood of healthy adults in our laboratory has been described in detail (31). Briefly, heparinized blood (1 U of preservative-free heparin per ml of blood) was allowed to sediment in 6% dextran (dextran T-70; molecular weight, 70,000; Pharmacia) at $160 \times g$ for 35 min. Erythrocytes were lysed by gently washing and shaking the pellet in a solution of 0.87 g of NH_4Cl per dl. All cells were then washed in Hanks balanced salt solution containing 0.15 mM Ca^{2+}, 0.5 mM Mg^{2+}, and 0.1% BSA, counted in a hemacytometer, and diluted to the desired concentration.

Phagocytic assays. In an initial series of experiments, phagocytosis of ^{35}S- and ^{125}I-radiolabeled *T. pallidum* by human PMNs was measured by using a suspension assay. RPMI 1640 (200 ml; GIBCO Laboratories, Grand Island, N.Y.) supplemented with 2% BSA and containing 2×10^6 radiolabeled treponemes was dispensed into polypropylene vials (Beckman). An equal volume of the same medium containing 2×10^5 PMNs (final treponeme/cell ratio, 10:1) was added to each vial, followed by incubation with vigorous agitation in a shaking water bath at 37°C. Reactions were stopped at 5, 15, and 30 min by the addition of 2.5 ml of cold PBS to each vial. Cell-associated treponemes were collected by a series of three washings in cold PBS at $160 \times g$ for 10 min, and the pellets were counted.

For most of the phagocytic assays, however, we used a modification of the surface phagocytosis method (59) originally described by Wood et al. (61). Briefly, surface-iodinated organisms were added to wells of 24-well tissue culture dishes and incubated at 37°C for 4 h. The addition of 1 ml containing 2.5×10^6 treponemes per ml (approximately 0.5 to 1 cpm per organism) usually resulted in approximately 10^6 adherent ^{125}I-surface-labeled bacteria. Approximately 10^5 human neutrophils in 100 μl of RPMI 1640 was added to triplicate wells for quantitation of phagocytosis. After appropriate time intervals (5, 15, and 30 min) at 37°C in a stationary position, extracellular bacteria were separated from phagocyte-associated organisms by differential centrifugation and washed twice, and pellets were counted on a gamma counter. Phagocytosis was expressed as the percent uptake of the total population of bacteria in each well. Determination of the percent leukocyte-associated radioactivity in the experiments with *S. aureus* was simplified by treatment with the muralytic antibiotic lysostaphin for 10 minutes at 4°C before differential centrifugation.

Statistical analysis. All data were stored in data bases, and statistical analyses performed with an EPISTAT statistical program (Tracy L. Gustafson, Round Rock, Tex.), with the following exceptions. Scatchard analyses (42) were performed with the EBDA/LIGAND program (Grant McPherson, Victorian College of Pharmacy Ltd., Victoria, Australia) and *t* tests were evaluated with the SAS PROC TTEST program (48).

RESULTS

Estimation of endogenous host proteins bound to *T. pallidum* after extraction by different techniques. Although the

earlier work by Hanff et al. (18) established that Percoll-purified treponemes were relatively free of contamination by host proteins, we felt compelled to document those findings before examining the roles of C1q and Fn in what we hoped would constitute an antibody-independent phagocytic system. Table 1 shows the results obtained with RIA in three experiments, each representing *T. pallidum* extracted from a different rabbit. Values shown represent estimated means ± standard error based on at least four replicate determinations. Treponemes extracted from infected testes and merely washed twice at 754 × *g*, to remove gross cellular debris, were so heavily contaminated with Fn, IgG, and albumin that it was necessary to centrifuge the organisms at 17,000 × *g* before using them in RIAs. The Methacel-Hypaque gradient technique appeared to remove 60 to 75% of the albumin, 35 to 65% of the IgG, and approximately 75% of the Fn associated with organisms subjected solely to centrifugation steps. In contrast, substantial quantities (>97%) of the endogenously bound host proteins were removed by the Percoll purification step. The level of IgA contamination was less than 0.01 ng per 10^8 organisms in all instances (data not shown). Qualitative Western blot analyses with ^{125}I-labeled antibodies against individual rabbit immunoglobulin heavy chains (data not shown) substantiated the RIA results.

Binding of [^{125}I]Fn to treponemes. In an initial series of experiments, 30-μg amounts of ^{125}I-labeled human Fn were incubated at 37°C with 5 × 10^7 *T. pallidum*, *T. refringens*, or *T. phagedenis* biotype Reiter in 1-ml volumes of HEPES-buffered saline. The results of four experiments with *T. pallidum* and a representative experiment with *T. phagedenis* biotype Reiter are shown in Fig. 1. Each point represents the mean specific binding value of triplicate determinations after subtraction of nonspecific binding. Nonspecific binding values in these single-point assays were determined in 10-fold excesses of unlabeled Fn (300 μg), roughly equal to the normal plasma level. In each instance, binding reached saturation within the first 20 min of incubation. Additional experiments with both avirulent organisms yielded results similar to the kinetics shown in the lower portion of Fig. 1 with *T. phagedenis* biotype Reiter. At this particular concentration, *T. pallidum* routinely bound substantially greater amounts of Fn than the avirulent treponemes. On the basis of these experiments, an incubation time of 20 min at 37°C was chosen for subsequent studies to ensure maximal binding at physiological temperatures.

TABLE 1. Association of endogenous host proteins with *T. pallidum* extracted by different procedures

Host protein	Mean amt (ng) of protein/10^8 organisms ± SE[a]		
	Wash[b]	M-H[c]	Percoll[d]
Expt 1			
Albumin	11.35 ± 1.34	2.98 ± 0.32	0.08 ± 0.01
IgG	3.24 ± 0.42	1.17 ± 0.22	0.08 ± 0.02
IgM	0.37 ± 0.05	0.11 ± 0.03	<0.01
C1q	<0.02	<0.01	<0.01
Fn	8.20 ± 0.94	2.15 ± 0.28	0.14 ± 0.03
Expt 2			
Albumin	9.43 ± 1.12	3.68 ± 0.44	0.05 ± 0.01
IgG	1.66 ± 0.23	0.93 ± 0.08	0.14 ± 0.02
IgM	<0.58 ± 0.08	<0.03	<0.01
Clq	<0.04 ± 0.02	<0.01	<0.01
Fn	6.85 ± 0.83	1.46 ± 0.21	0.22 ± 0.04
Expt 3			
Albumin	15.75 ± 1.39	6.09 ± 0.74	<0.03
IgG	2.79 ± 0.42	1.82 ± 0.22	0.06 ± 0.01
IgM	0.21 ± 0.04	0.09 ± 0.02	<0.01
C1q	<0.01	<0.01	<0.01
Fn	11.30 ± 1.53	2.93 ± 0.37	0.34 ± 0.06

[a] Means were based on at least four replicate determinations.
[b] Wash, Two washes at 754 × *g* and one wash at 17,000 × *g*.
[c] M-H, Methacel-Hypaque gradient purification.
[d] Percoll, Percoll density gradient purification.

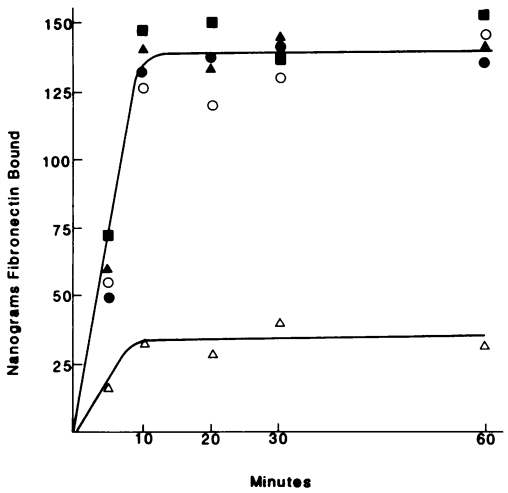

FIG. 1. Kinetics of [^{125}I]Fn binding to *T. pallidum* and to *T. phagedenis* biotype Reiter. Organisms (5 × 10^7) in 1-ml volumes were exposed to 30 μg of radiolabeled Fn at 37°C for indicated times. Each point represents the mean of triplicate determinations in four experiments with *T. pallidum* (○, ●, ▲, ■) or a representative experiment with *T. phagedenis* biotype Reiter (△). Line estimations for each organism were determined by the least squares method.

Binding of ^{125}I-labeled Fn from different mammalian species to Percoll-purified *T. pallidum* was subsequently quantitated as a function of increasing ligand concentration. Pooled results of several studies carried out under various conditions are shown in Table 2. The concentrations bound represent specific binding after subtraction of nonspecific binding values. Nonspecific binding was determined by dilution of [^{125}I]Fn in a 50-fold excess of unlabeled Fn. Linear plots obtained by Scatchard analyses of the binding data suggested the presence of only one type of binding site. Appreciable effects on either of these binding parameters were not noted with the use of other species of labeled Fn, with the addition of Ca^{2+} and Mg^{2+}, or with treponemes

TABLE 2. Binding of Fn to *T. pallidum*[a]

Medium and temp (°C)	Fn source	No. of expts	Apparent K_a ± SD × 10^7 M^{-1}	Receptor sites/ treponeme
HEPES buffer, 37	Human	3	1.75 ± 0.35	5,926 ± 2,121
	Rabbit	2	2.05 ± 0.51	4,775 ± 1,493
	Bovine	2	1.53 ± 0.42	3,528 ± 1,047
HEPES buffer, 25	Human	2	2.41 ± 0.54	6,843 ± 2,326
	Rabbit	2	1.67 ± 0.48	6,149 ± 1,844
HEPES buffer with Ca^{2+} and Mg^{2+}, 37	Human	3	2.17 ± 0.56	4,833 ± 1,842
	Rabbit	2	2.63 ± 0.64	5,108 ± 1,733

[a] Specific binding values corrected for nonspecific binding by dilution of [^{125}I]Fn in a 50-fold excess of unlabeled Fn as described in the text. Triplicate determinations were carried out for each concentration of [^{125}I]Fn in each experiment.

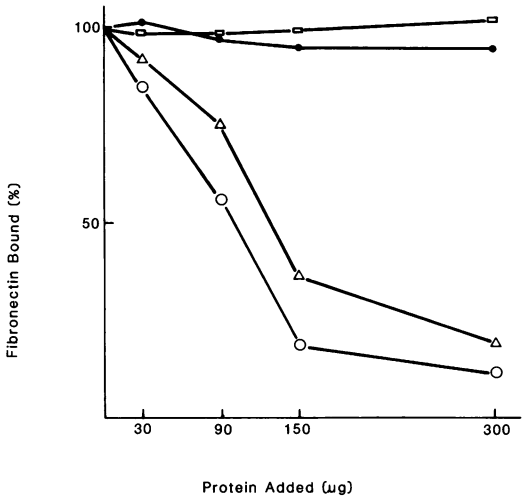

FIG. 2. Displacement of [^{125}I]Fn by addition of unlabeled proteins. *S. pallidum* (5×10^7) was incubated in the presence of [^{125}I]Fn at 37°C for 20 min, followed by washing and a 1-h incubation in the presence of an excess of unlabeled Fn (○), unlabeled cell-binding fragments (△), or unlabeled C1q (●). Radioactivity associated with treponemes was determined, and the data are expressed as percentages of controls (incubations in the absence of unlabeled proteins).

harvested by the alternative (Methacel-Hypaque) method (data not shown). A slight but insignificant increase in the number of receptor sites per treponeme was noted when experiments were carried out under aphysiologic conditions at 25°C (human Fn, $P = 0.53$; rabbit Fn, $P = 0.37$).

To assess reversibility of binding, a prerequisite for Scatchard analysis, increasing amounts of unlabeled human Fn were added to displace ^{125}I-human Fn bound by *T. pallidum*. The representative study (Fig. 2) suggested that roughly 82 and 88% of bound Fn could be displaced within 1 h by 5- and 10-fold excesses of unlabeled Fn, respectively. Purified 66-kDa cell-binding fragments were also capable of reversing binding. Purified C1q and normal human IgG (data not shown), however, could not displace bound [^{125}I]Fn.

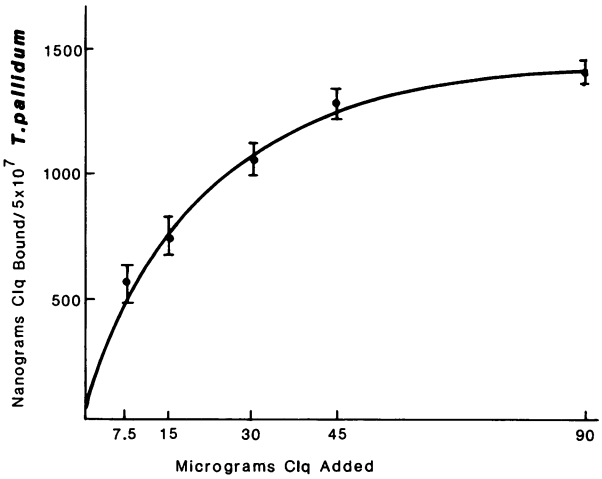

FIG. 3. Antibody-independent binding of C1q to *T. pallidum*. *T. pallidum* (5×10^7 cells per ml) was exposed to increasing concentrations of ^{125}I-labeled C1q for 30 min at 37°C. Shown are representative results from three experiments with Percoll-purified organisms.

Attempts also were made to quantitate binding of ^{125}I-labeled human and rabbit Fn to *T. phagedenis* biotype Reiter as a function of increasing ligand concentration. In contrast to the single-point assays (Fig. 1) with a 10-fold excess of unlabeled Fn, the results obtained in these studies in the presence of a 50-fold excess of unlabeled Fn were erroneous; specific binding values at several concentration points, regardless of the source or species of labeled Fn, were reduced to levels equal to or below nonspecific binding values.

Antibody-independent binding of C1q to *T. pallidum*. The ability of *T. pallidum* to bind [^{125}I]C1q in an immunoglobulin-free system also was examined. In a series of preliminary studies with 30 μg of [^{125}I]C1q, saturation was achieved within 15 min at 37°C, whereas less C1q bound to *T. pallidum* at 25°C (data not shown). The results of a representative study in which *T. pallidum* was exposed to increasing concentrations of [^{125}I]C1q at 37°C for 30-min intervals is shown in Fig. 3. As shown, binding of [^{125}I]C1q was saturable; concentrations bound represent specific binding following subtraction of nonspecific binding values determined in a 50-fold excess of unlabeled C1q. Binding was also specific in that addition of unlabeled C1q displaced 80 to 90% of [^{125}I]C1q already bound to treponemes (data not shown).

Effect of C1q pretreatment on Fn. *T. pallidum* was incubated with 20 and 100 nM of C1q and washed, and the ability to bind [^{125}I]Fn was examined in another series of single-point assays. The addition of Ca^{2+} and Mg^{2+} had no effect ($P > 0.2$) on binding of either species of Fn (Table 3). In these two representative experiments run on the same day with the same sources of radiolabeled Fn, Methacel-Hypaque-harvested organisms appeared to bind approximately twice as much Fn as did Percoll-purified treponemes. Pretreatment of *T. pallidum* with 20 nM C1q enhanced Fn binding approximately twofold, regardless of the gradient method used for harvesting organisms. Pretreatment at the higher concentration (100 nM) increased Fn binding significantly ($P < 0.0095$), suggesting that the degree of enhancement was dependent on the amount of C1q used for pretreatment.

Although earlier studies examined binding of *T. pallidum* to Fn-coated surfaces (15, 35, 49, 53–55), they did not address the question of whether C1q pretreatment could enhance Fn binding, as recently demonstrated with other microorganisms (47). In an attempt to resolve this issue and extend the experiments described above, we pretreated surface-labeled treponemes with C1q, washed them, and examined their ability to attach to Fn-coated cover slips.

TABLE 3. Effect of C1q pretreatment on binding of Fn by *T. pallidum*

Pretreatment	Amt (ng) of [^{125}I]Fn bound/5 × 10^7 organisms (*P*)			
	Percoll[a]		M-H[b]	
	Human	Rabbit	Human	Rabbit
HEPES buffer without Ca^{2+} and Mg^{2+}	128 ± 14.3	139 ± 11.8	214 ± 26.3	190 ± 17.4
HEPES buffer with Ca^{2+} and Mg^{2+}	115 ± 9.8 (0.28)	122 ± 15.3 (0.20)	234 ± 21.6 (0.37)	215 ± 22.3 (0.19)
20 nM C1q	247 ± 30.5 (0.0036)	256 ± 27.5 (0.0024)	422 ± 34.8 (0.0014)	347 ± 39.2 (0.0069)
100 nM C1q	296 ± 33.7 (0.0014)	381 ± 44.3 (<0.0008)	461 ± 38.1 (<0.0009)	378 ± 56.5 (<0.0095)

[a] Percoll, Percoll density gradient purification.
[b] M-H, Methacel-Hypaque gradient purification.

Results of a representative experiment are shown in Table 4. The disparity in counts per minute per organism between ^{125}I-surface-labeled treponemes and those intrinsically labeled with ^{35}S prevented direct comparisons with comparable numbers per cover slip; attempts to use 10^7 ^{125}I-labeled organisms resulted in control counts binding to BSA in excess of 12,000 cpm. Furthermore, Methacel-Hypaque-purified treponemes could not be satisfactorily surface labeled with ^{125}I-labeled Na; attempts resulted in preferential labeling of contaminating IgG and albumin. Pretreatment of *T. pallidum* with C1q significantly enhanced the ability of the organism to interact with Fn (rows 4 and 6). Other cover slips for control purposes were exposed to goat anti-human Fn or MAbs directed against different Fn fragments for 30 min at 37°C before washing and adding labeled treponemes. Whereas pretreatment with MAbs against the heparin-binding fragment (type 1) and the gelatin-binding fragment (type 3) exerted minor effects, the polyclonal goat antibodies and the type 2 MAb, directed against the cell-binding fragment, substantially inhibited attachment (>74% in all instances).

Inability of C1q and Fn pretreatments to enhance surface phagocytosis of *T. pallidum*. To determine whether C1q and Fn pretreatment can effect PMN ingestion of *T. pallidum*, surface phagocytosis was assessed by using a modification of the methods originally described by Wood et al. (61). The choice of this procedure over that of suspension assays was based on a series of preliminary studies with various multiplicities of infection in the absence of normal human serum as an opsonin. Results of those experiments (data not shown) indicated that the surface phagocytosis assays were much more reproducible on different days with PMNs from different donors. After attachment of radiolabeled *T. pallidum* to plastic surfaces, organisms were exposed to Fn alone, or after pretreatment with C1q, they were washed and then incubated with PMNs. Although this is not shown in Table 5, exposure to C1q or Fn or both did not significantly affect the number of adherent ^{125}I-surface-labeled organisms before the addition of PMNs; all wells contained approximately 10^6 treponemes at the end of the 4-h attachment period, regardless of additions during the last hour. Except for the positive controls with pooled normal human serum, none of the pretreatment conditions exerted an effect on phagocytosis at the end of 30 min. Effects also were not noted at the end of 5- or 15-min incubation periods in these or similar experiments with rabbit or bovine Fn (data not shown).

TABLE 4. Effects of antibody and C1q pretreatments on attachment of *T. pallidum* to Fn-coated cover slips[a]

Pretreatment	Protein on cover slip	10^6 ^{125}I-labeled Percoll-purified organisms bound	10^7 ^{35}S-labeled Methacel-Hypaque-purified organisms bound
Buffer alone	BSA	943 ± 120 (4.7)[b]	159 ± 20 (7.4)
	Fn	20,176 ± 1,981 (100)	2,150 ± 244 (100)
20 nM C1q	BSA	1,037 ± 182 (5.1)	131 ± 24 (6.1)
	Fn	27,043 ± 3,904 (134)	2,752 ± 307 (128)
100 nM C1q	BSA	1,211 ± 202 (6.0)	114 ± 19 (5.3)
	Fn	36,114 ± 4,790 (179)	3,375 ± 411 (157)
Anti-Fn	Fn	4,661 ± 681 (23.1)	417 ± 73 (19.4)
MAb type 1	Fn	9,362 ± 1,473 (46.4)	1,142 ± 219 (53.1)
MAb type 2	Fn	4,473 ± 653 (22.2)	568 ± 83 (26.4)
MAb type 3	Fn	14,633 ± 2,097 (81.5)	1,821 ± 243 (84.7)

[a] Suspensions (100 μl) containing 10^6 or 10^7 labeled organisms were added to each of five cover slips after pretreatment as described in the text and incubated at 37°C for 1 h. 10^6 ^{125}I-labeled, Percoll-purified treponemes represent 6.4×10^5 cpm whereas 10^7 ^{35}S-labeled, Methacel-Hypaque-purified organisms represent 9.4×10^4 cpm.

[b] Values in parentheses represent percentages as compared with BSA or Fn.

TABLE 5. Failure of Fn and C1q pretreatment to enhance phagocytosis by human PMNs

Pretreatment condition	Phagocytosis[a]	
	Expt 1	Expt 2
Control	170,635 ± 29,060 (18.2 ± 3.1)	140,760 ± 25,092 (23.0 ± 4.1)
10% Pooled, normal[b] human serum	422,840 ± 59,990 (45.1 ± 6.4)	242,965 ± 44,065 (39.7 ± 7.2)
HEPES buffer with Ca^{2+} and Mg^{2+} (1 h)[c]	200,638 ± 44,065 (21.3 ± 4.7)	123,624 ± 20,969 (20.2 ± 3.3)
HEPES buffer (30 min)[b] and 300 μg of Fn (30 min)	180,012 ± 20,626 (19.2 ± 2.2)	115,668 ± 17,748 (18.9 ± 2.9)
20 nM C1q (30 min)[c] and 300 μg of Fn (30 min)	204,388 ± 23,439 (21.7 ± 2.5)	136,476 ± 19,584 (22.3 ± 3.2)
100 nM C1q (30 min)[c] and 300 μg of Fn (30 min)	191,262 ± 31,877 (20.3 ± 3.4)	144,432 ± 16,524 (23.6 ± 2.7)

[a] Phagocytosis, mean ± standard error of triplicate determinations of leukocyte-associated radioactivity at the end of 30 min. Numbers in parentheses represent percentages phagocytosed.

[b] Not a pretreatment condition; PMNs were diluted in RPMI 1640 containing 10% pooled, heat-inactivated human serum.

[c] Pretreatment conditions before addition of PMN diluted in RPMI 1640 without an opsonin source.

Western blot analysis of *T. pallidum*-Fn interactions. In an attempt to identify domains of the Fn molecule binding to *T. pallidum*, peptides generated by enzymatic digestion of ^{125}I-labeled human Fn were incubated with organisms. Trypsin and cathepsin D digestion resulted in substantially different Western blot profiles of enzymatic fragments of [^{125}I]Fn (Fig. 4, lanes e and f, respectively). Of those labeled tryptic fragments, *T. pallidum* bound domains with molecular weights of 76, 66, 48, 43, 35, and 16 kDa (lane g). *T. phagedenis* biotype Reiter and *T. refringens* appeared to bind the same four lower-molecular-size fragments, but not the 76- and 66-kDa domains (data not shown). Comparative studies with *S. aureus* Cowan I revealed only one band, a 28-kDa fragment (lane h). In contrast to the studies with trypsin fragments, *T. pallidum* appeared to bind cathepsin D-generated domains with higher molecular weights (peptides of 140, 90, 76, 66, and 43-kDa [lane i]). With the exception of the 43-kDa material, those radiolabeled fragments were not bound by either of the avirulent organisms (data not shown). A Western blot of purified C1q, blocked with BSA and sequentially exposed to unlabeled Fn and ^{125}I-surface-labeled *T. pallidum*, is shown in lane j. Lane k shows a representative blot of electroeluted, enriched cell-binding domains exposed to ^{125}I-labeled *T. pallidum*; on blots of 7.5% gels, two distinct bands, 76 and 66 kDa, were evident (data not shown). A single band in the 87-kDa region was evident in Western blots of *T. pallidum* peptides exposed to ^{125}I-labeled cell-binding fragments (lane l). Western blots of *T. pallidum* exposed to unlabeled Fn or unlabeled, purified cell-binding fragments and subsequently developed

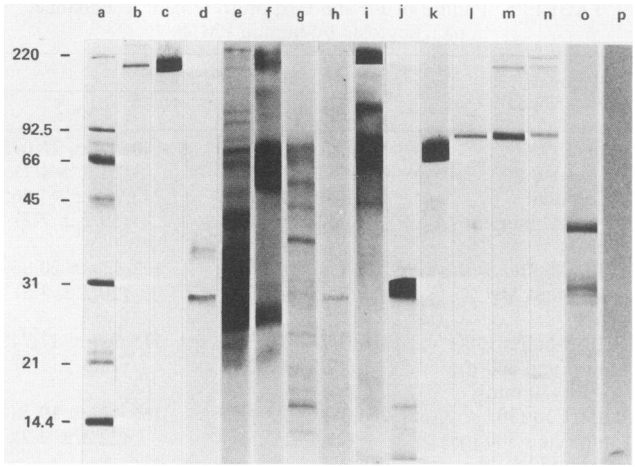

FIG. 4. Western blot analysis of *T. pallidum*-Fn interactions. Lanes: a, Coomassie blue-stained standards (numbers at left indicate kilodaltons); b, Coomassie blue-stained human Fn; c, [^{125}I]Fn blot; d, [^{125}I]C1q blot; e, trypsin digest of [^{125}I]Fn; f, cathepsin D digest of ^{125}I; g, tryptic fragments of [^{125}I]Fn bound to *T. pallidum*; h, tryptic fragments of [^{125}I]Fn bound to *S. aureus* Cowan I; i, cathepsin D digest of [^{125}I]Fn bound to *T. pallidum*; j, C1q exposed to unlabeled Fn and then developed with ^{125}I-labeled *T. pallidum*; k, purified cell-binding fragments exposed to ^{125}I-labeled *T. pallidum*; l, *T. pallidum* proteins exposed to ^{125}I-labeled cell-binding fragments; m, *T. pallidum* proteins exposed to unlabeled Fn and then developed with ^{125}I-labeled type 2 MAb against cell-binding domains; n, *T. pallidum* proteins exposed to unlabeled, purified cell-binding fragments and then developed with ^{125}I-labeled type 2 MAb; o, purified flagellar proteins of *T. pallidum*, stained with Coomassie blue; p, *T. pallidum* flagellar proteins exposed to ^{125}I-labeled Fn.

with ^{125}I-labeled MAb type 2 directed against cell-binding domain, lanes m and n, respectively, also revealed single bands in the 87-kDa region. Purified flagella of *T. pallidum*, which we and others (34) have shown to consist of predominantly 37-, 34-, and 33-kDa polypeptides (lane o) failed to bind ^{125}I-labeled Fn (lane p) or purified cell-binding fragments (data not shown).

DISCUSSION

Although attachment of organisms to host cells probably constitutes the first step in the pathogenesis of syphilis, with Fn playing one of the key roles, attachment is also regarded as the first of two steps in the phagocytic process. Unanswered questions concerning the possible opsonic role that Fn might play in the phagocytosis of treponemes by neutrophils and whether antibody-independent binding of C1q to *T. pallidum* could mediate Fn binding led us to undertake the present investigation.

Our results indicate that purified C1q, in the absence of a specific antibody, is capable of binding to *T. pallidum*. C1q binding by virulent treponemes was specific, saturable, and reversible. Overall, these results are in agreement with previous studies concerned with binding of C1q to both gram-positive and gram-negative bacteria (7, 12, 13, 24, 25, 47, 52).

Pretreatment of *T. pallidum* with C1q resulted in enhanced binding of Fn (Table 3) and also increased attachment of organisms to Fn-coated surfaces but not to surface-bound albumin (Table 4). Enhancement appeared to be dependent on the amount of C1q used for precoating, irrespective of the gradient method used for harvesting organisms. While these results are in general agreement with previous studies with *Escherichia coli* and *Staphylococcus* spp. (47), they also extend the recent study by Thomas et al. (55). In that study, Fn pretreatment of treponemes led to enhanced attachment to Fn-coated surfaces. Thus, it would appear that Fn binding to *T. pallidum* can be mediated via C1q-Fn as well as through Fn-Fn interactions.

Fn and C1q pretreatment of *T. pallidum*, however, did not appear to enhance or even promote phagocytosis by neutrophils in an in vitro assay system. Thus, although binding of Fn to treponemes may be related to the pathogenesis of *T. pallidum* infections, we did not obtain evidence which would suggest an opsonic role for this glycoprotein in an antibody-independent system. Except for two studies (23, 40) implicating Fn as a possible opsonin, considerable evidence suggests that Fn does not enhance phagocytosis, despite its importance in adherence (37, 38, 43, 57, 58). It is possible that optimal opsonizatin requires substrate-bound Fn and that satisfactory conditions are not met by the mere addition of soluble Fn. Alternatively, the phagocytic activity of PMNs may require synergistic action between other cofactors and Fn, similar to that required for stimulation of neutrophil migration (17).

Before we undertook the studies aimed at determining whether C1q could exert an effect on Fn binding, experiments were carried out to: (i) verify that treponemes were relatively free of contaminating immunoglobulins so that they could be used in antibody-independent assay systems; and (ii) attempt to resolve discrepancies in the existing literature concerning Fn-*T. pallidum* interactions. In agreement with the earlier work of Hanff et al. (18), Percoll-density-gradient-purified treponemes were relatively free of contaminating, endogenous host proteins (Table 1). Organisms which were not subjected to either gradient purification technique, however, were heavily contaminated with albumin, IgG, and Fn.

Binding of Fn to *T. pallidum* as characterized in our initial studies was specific, since excess unlabeled Fn inhibited binding of [^{125}I]Fn whereas other proteins tested, including C1q, had virtually no effect. Binding of Fn by *T. pallidum* appeared to involve a single class of receptors which bound Fn with a K_a of approximately 2×10^7 M^{-1}. When Fn was used as a ligand, the number of receptor molecules per cell was calculated to be approximately 5×10^3. Although these estimates are in good agreement with the recent results of Steiner and Sell (49), they differ radically from the parameters calculated by Thomas et al. (53). The number of receptors per treponeme in the latter study was estimated to be 4.3×10^6, nearly 3 orders of magnitude greater. Although Scatchard analysis in the present study was corrected for nonspecific binding (11), determined in 50-fold excesses of unlabeled Fn, it is not clear whether similar corrections were made in the study of Thomas et al. Except for the fact that Methacel-Hypaque-harvested organisms bound more Fn than did Percoll-purified treponemes in single-point assays (Table 3), discrepancies in binding could not be attributed solely to differences in purification steps, incubation, temperatures, or mammalian sources of Fn (Table 2).

Failure of Fn to act as an opsonin for *T. pallidum* may be related to the manner in which the organism binds to the macromolecule. Thomas et al. (53) have shown that, unlike *S. aureus*, *T. pallidum* appears to bind the mammalian cell-binding site of Fn. Evidence in the present study con-

firms that finding. Additionally, our 87-kDa protein, which appears to be the only treponemal antigen consistently present in purified immune complexes of animal or human origin (4, 5, 21), in all likelihood corresponds to their P_1. Their P_2 antigen, however, with a molecular mass of 37 kDa, would appear to be unrelated to our major flagellar protein with a similar molecular mass (5) in that the latter failed to bind Fn (Fig. 4). Blots of enzymatically digested fragments of radiolabeled fragments bound to *T. pallidum* suggested that several domains other than the C-terminal peptides of 66 and 75 kDa (41) were capable of binding nonspecifically (lanes g and i); binding of the 48-, 43-, 35-, and 16-kDa radiolabeled fragments was inhibited in the presence of excess, unlabeled, intact Fn (data not shown).

Thomas et al. (55) recently postulated that unique Fn-Fn interactions occur between Fn molecules on treponemes and those on coated surfaces and that the tropism of Fn-primed *T. pallidum* to the extracellular matrix may help explain why dissemination of the disease occurs. Our major concern with this hypothesis is that it does not take into consideration the fact that vigorous humoral responses precede dissemination of both the natural disease in humans and the experimental disease in intravenously infected rabbits. These humoral responses also appear to include autoimmune phenomena; anti-Fn antibodies in the sera of patients with secondary syphilis (5) and anti-Fn (15) and anti-creatine kinase (50) antibodies in the sera of rabbits with experimental syphilis. Conformational changes in Fn (2, 60), particularly near pH 7, are known to favor Fn-Fn interactions. Furthermore, conformational changes via binding to *T. pallidum* may favor the induction of autoimmune responses (5, 15). Aside from the role that these autoimmune antibodies appear to play in the formation of immune complexes (6), one must question whether they enhance clearance of treponemes coated with endogenous host antigens by phagocytic cells or conversely act more as blocking antibodies. The degree of saturation with Fn and the presence of antibodies directed against Fn, bound by the organism, might be expected to impact directly with clearance by PMNs and other phagocytic cells. With a high, irreversible K_a, one might expect complete saturation to the point where such interactions would result in decreased, rather than facilitated, phagocytosis, theoretically precluding formation of an opsonic bridge as recently postulated by Proctor et al. (39). Such a hypothesis, however, would also appear to be dependent on the treponemal receptor binding both of the cell-binding, COOH-terminal ends which are linked by a disulfide bond (29) or would result in conformational changes so that the other cell-binding terminal end is not recognized by the PMN. Arguments against such a hypothesis are the dimeric nature of the molecule, the fact that conformational changes appear to involve the free NH_2 terminal domains (60), and the lower, reversible K_as obtained in the present study.

Finally, as mentioned above, it is possible that Fn-C1q-*T. pallidum* represents an insufficient signal for PMNs. In this regard, Brown (10) in a recent review has asserted that enhanced PMN phagocytosis requires at least two signals in addition to recognition of an opsonized target. PMNs had to be exposed to a chemotaxin before interaction with Fn, and the two signals could not be reversed. Further studies with phorbol myristic acid or formylmethionyl-leucyl-phenylalanine, which appear to be required for enhanced phagocytosis of Fn-C3b/C3bi erythrocytes (36) and Fn-*S. aureus* (R. A. Proctor, personal communication) by PMN, might clarify the role, if any, that Fn plays in phagocytosis of *T. pallidum*.

ACKNOWLEDGMENTS

I thank I. M. Campbell for assistance in preparing this manuscript and C. B. Adams for diligent technical assistance. I also thank Bret M. Steiner, University of Wisconsin, and Thomas J. Fitzgerald, University of Minnesota, for their critical evaluation of the manuscript.

This work was supported by research funds from the Veterans Administration Medical Center, Houston, Tex.

LITERATURE CITED

1. **Alderete, J. F., and J. B. Baseman.** 1979. Surface-associated host proteins on virulent *Treponema pallidum*. Infect. Immun. **26**:1048–1056.
2. **Alexander, S. S., Jr., G. Colonna, and H. Edelhock.** 1979. The structure and stability of human plasma cold-insoluble globulin. J. Biol. Chem. **254**:1501–1505.
3. **Baseman, J. B., and E. C. Hayes.** 1980. Molecular characterization of receptor binding proteins and immunogens of virulent *Treponema pallidum*. J. Exp. Med. **151**:573–586.
4. **Baughn, R. E., C. B. Adams, and D. M. Musher.** 1983. Circulating immune complexes in experimental syphilis: identification of treponemal antigens and specific antibodies to treponemal antigens in isolated complexes. Infect. Immun. **42**:585–593.
5. **Baughn, R. E., M. C. McNeely, J. L. Jorizzo, and D. M. Musher.** 1986. Characterization of the antigenic determinants and host components in immune complexes from patients with secondary syphilis. J. Immunol. **136**:1406–1414.
6. **Baughn, R. E., and D. M. Musher.** 1985. Radioimmunoassays for the detection of antibodies to treponemal polypeptide antigens in serum. J. Clin. Microbiol. **21**:922–929.
7. **Betz, S. J., and H. Isliker.** 1981. Antibody-independent interactions between *Escherichia coli* J5 and human complement components. J. Immunol. **127**:1748–1753.
8. **Bing, D. H., S. Almeda, H. Isliker, J. Lahav, and R. O. Hynes.** 1982. Fibronectin binds to the C1q component of complement components. Proc. Natl. Acad. Sci. USA **79**:4198–4201.
9. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
10. **Brown, E. J.** 1986. The role of extracellular matrix proteins in the control of phagocytosis. J. Leukocyte Biol. **39**:579–591.
11. **Chamness, G. C., and W. L. McGuire.** 1975. Scatchard plots: common errors in correction and interpretation. Steroids **26**:538–542.
12. **Clas, F., J. R. Golecki, and M. Loos.** 1984. Electron microscopic study showing antibody-independent binding of C1q, a subcomponent of the first component of complement, to serum-sensitive salmonellae. Infect. Immun. **45**:795–797.
13. **Clas, F., and M. Loos.** 1981. Antibody-independent binding of the first component of complement (C1) and its subcomponent C1q to the S and R forms of *Salmonella minnesota*. Infect. Immun. **31**:1138–1144.
14. **Fennestad, K. L., L. Brunn, and E. Wedo.** 1980. Pleural effusion disease agent as passenger of *Treponema pallidum* suspensions from rabbits. Br. J. Vener. Dis. **56**:198–203.
15. **Fitzgerald, T. J., and L. A. Repesh.** 1985. Interactions of fibronectin with *Treponema pallidum*. Genitourin. Med. **61**:147–155.
16. **Froman, G., L. M. Switalski, A. Fairs, T. Wadstrom, and M. Hook.** 1984. Binding of *Escherichia coli* to fibronectin: a mechanism of tissue adherence. J. Biol. Chem. **259**:14899–14905.
17. **Hakansson, L., and P. Venge.** 1985. The combined action of hyaluronic acid and fibronectin stimulates neutrophil migration. J. Immunol. **135**:2735–2739.
18. **Hanff, P. A., S. J. Norris, M. A. Lovett, and J. N. Miller.** 1984. Purification of *Treponema pallidum*, Nichols strain, by percoll density gradient centrifugation. Sex. Transm. Dis. **11**:275–286.
19. **Hayashi, M., and K. M. Yamada.** 1983. Domain structure of the carboxyl-terminal half of human plasma fibronectin. J. Biol. Chem. **258**:3332–3340.
20. **Ingham, K. C., S. A. Brew, and S. Miekka.** 1983. Interaction of plasma fibronectin with gelatin and C1q. Mol. Immunol. **20**:287–295.

21. **Jorizzo, J. L., M. C. McNeely, R. E. Baughn, A. R. Solomon, T. Cavallo, and E. B. Smith.** 1986. Role of circulating immune complexes in human secondary syphilis. J. Infect. Dis. **153**:1014–1022.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **277**:680–685.
23. **Lanser, M. E., and T. M. Saba.** 1981. Fibronectin as a co-factor necessary for optimal granulocyte phagocytosis of *Staphylococcus aureus*. RES J. Reticuloendothel. Soc. **30**:415–424.
24. **Leist-Welsh, P., and A. B. Bjornson.** 1982. Immunoglobulin-independent utilization of the classical pathway in opsonophagocytosis of *Escherichia coli* by human peripheral leukocytes. J. Immunol. **128**:2643–2651.
25. **Loos, M., B. Wellek, R. Thesen, and W. Opferkuch.** 1978. Antibody-independent interaction of the first component of complement with gram-negative bacteria. Infect. Immun. **22**:5–9.
26. **Markwell, M. A.** 1982. A new solid-state reagent to iodinate proteins. I. Conditions for the efficient labeling of anti-serum. Anal. Biochem. **122**:427–432.
27. **Menzel, E. J., J. S. Smolen, L. Liotta, and K. B. M. Reid.** 1981. Interaction of fibronectin with C1q and its collagen-like reagent (CLF). FEBS Lett. **129**:188–192.
28. **Morrison, M.** 1980. Lactoperoxidase-catalyzed iodination as a tool for investigation of proteins. Methods Enzymol. **70**:214–220.
29. **Mosher, D. F.** 1980. Fibronectin. Prog. Hemostasis Thromb. **5**:111–151.
30. **Moskophidis, M., and F. Muller.** 1984. Molecular analysis of immunoglobulins M and G immune response to protein antigens of *Treponema pallidum* in human syphilis. Infect. Immun. **43**:127–132.
31. **Musher, D. M., M. Hague-Park, F. Gyorkey, D. C. Anderson, and R. E. Baughn.** 1983. The interaction between *T. pallidum* and human polymorphonuclear leukocytes. J. Infect. Dis. **147**:77–86.
32. **Pearlstein, E., L. Gold, and A. Garcia-Pardo.** 1980. Fibronectin: a review of its structure and biological activity. Mol. Cell. Biochem. **29**:103–128.
33. **Pearlstein, E., J. Sorvillo, and I. Gigli.** 1982. The interaction of human plasma fibronectin with a subunit of the first component of complement. J. Immunol. **128**:2036–2039.
34. **Penn, C. W., M. J. Bailey, A. Cockayne.** 1985. The axial filament antigen of *Treponema pallidum*. Immunology **54**:635–641.
35. **Peterson, K. M., J. B. Baseman, and J. F. Alderete.** 1983. *Treponema pallidum* receptor binding proteins interact with fibronectin. J. Exp. Med. **157**:1958–1970.
36. **Pommier, C. G., J. J. O'Shea, T. Chused, K. Yancey, M. Frank, P. Takahashi, and E. J. Brown.** 1984. Studies on the fibronectin receptors of human peripheral blood leukocytes. J. Exp. Med. **159**:137–151.
37. **Proctor, R. A., D. F. Mosher, and P. J. Olbrantz.** 1982. Fibronectin binding to *Staphylococcus aureus*. J. Biol. Chem. **257**:14788–14794.
38. **Proctor, R. A., E. Prendergast, and D. F. Mosher.** 1982. Fibronectin mediates attachment of *Staphylococcus aureus* to human neutrophils. Blood **59**:681–687.
39. **Proctor, R. A., J. A. Textor, J. M. Vann, and D. F. Mosher.** 1985. Role of fibronectin in human monocyte and macrophage bactericidal activity. Infect. Immun. **47**:629–637.
40. **Raynor, R. H., J. E. Doran, and A. C. Reese.** 1981. Polymorphonuclear leukocyte monolayer system for studies of phagocytosis. RES J. Reticuloendothel. Soc. **29**:441–449.
41. **Richter, H., and H. Hormann.** 1983. A large cathepsin D-derived fragment from the central part of the fibronectin subunit chains. FEBS Lett. **155**:317–320.
42. **Scatchard, G.** 1949. The attraction of proteins from small molecules and ions. Ann. N.Y. Acad. Sci. **51**:660–666.
43. **Simpson, W. A., D. L. Hasty, J. M. Mason, and E. H. Beachey.** 1982. Fibronectin-mediated binding of group A streptococci to human polymorphonuclear leukocytes. Infect. Immun. **37**:805–810.
44. **Sorvillo, J., I. Gigli, and E. Pearlstein.** 1983. Requirements for the binding of human plasma fibronectin in the C1q subunit of the first component of complement. J. Immunol. **131**:1400–1404.
45. **Sorvillo, J., I. Gigli, and E. Pearlstein.** 1985. Fibronectin-binding to complement component C1q: localization of their respective binding sites. Biochem. J. **226**:207–215.
46. **Sorvillo, J. M., I. Gigli, and E. Pearlstein.** 1986. The effect of fibronectin on the processing of C1q- and C3b/bi-coated immune complexes by peripheral blood monocytes. J. Immunol. **136**:1023–1026.
47. **Sorvillo, J. M., and E. Pearlstein.** 1985. C1q, a subunit of the first component of complement, enhances binding of plasma fibronectin to bacteria. Infect. Immun. **49**:664–669.
48. **Statistical Analysis System Institute.** 1982. SAS user's guide, 1982 ed., p. 217. Statistical Analysis System Institute, Cary, N.C.
49. **Steiner, B. M., and S. Sell.** 1985. Characterization of the interaction between fibronectin and *T. pallidum*. Curr. Microbiol. **12**:157–162.
50. **Strugnell, R. A., W. F. Williams, G. Raines, J. S. Pedersen, L. A. Drummond, B. H. Toh, and S. Faine.** 1986. Autoantibodies to creatine kinase in rabbits infected with *Treponema pallidum*. J. Immunol. **136**:667–671.
51. **Tenner, A. J., P. H. Lesavre, and N. R. Cooper.** 1981. Purification and radiolabeling of human C1q. J. Immunol. **127**:648–653.
52. **Tenner, A. J., R. J. Ziccardi, and N. R. Cooper.** 1984. Antibody-independent C1 activation by *E. coli*. J. Immunol. **133**:886–891.
53. **Thomas, D. D., J. B. Baseman, and J. F. Alderete.** 1985. Fibronectin mediates *Treponema pallidum* cytadherence through recognition of fibronectin cell-binding domain. J. Exp. Med. **161**:514–525.
54. **Thomas, D. D., J. B. Baseman, and J. F. Alderete.** 1985. Putative *Treponema pallidum* cytadhesins share a common functional domain. Infect. Immun. **49**:833–835.
55. **Thomas, D. D., J. B. Baseman, and J. F. Alderete.** 1986. Enhanced levels of attachment of fibronectin-primed *Treponema pallidum* to extracellular matrix. Infect. Immun. **52**:736–741.
56. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
57. **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.
58. **Verbrugh, H. A., P. K. Peterson, D. E. Smith, B.-Y. T. Nguyen, J. R. Hoidal, B. J. Wilkinson, J. Verhoef, and L. T. Furcht.** 1981. Human fibronectin binding to staphylocccal surface protein and its relative inefficiency in promoting phagocytosis by human polymorphonuclear leukocytes, monocytes, and alveolar macrophages. Infect. Immun. **33**:811–819.
59. **Verhoef, J., P. K. Peterson, and P. G. Quie.** 1977. Kinetics of staphylococcal opsonization, attachment, ingestion and killing by human polymorphonuclear leukocytes: a quantitative assay using ^{3}H-thymidine labeled bacteria. J. Immunol. Meth. **14**:303–309.
60. **Williams, E. C., P. A. Janmey, J. D. Ferry, and D. F. Mosher.** 1982. Conformational states of fibronectin. J. Biol. Chem. **257**:14973–14978.
61. **Wood, W. E., M. R. Smith, W. P. Perry, and J. W. Berry.** 1951. Studies on the cellular immunology of acute bacteremia. I. Intravascular leukocytic reaction and surface phagocytosis. J. Exp. Med. **94**:521–535.