INFECTION AND IMMUNITY, Apr. 1989, p. 1030–1034
0019-9567/89/041030-05$02.00/0

Vol. 57, No. 4

Fibronectin Increases *Trypanosoma cruzi* Amastigote Binding to and Uptake by Murine Macrophages and Human Monocytes

EDOUARD L. NOISIN AND FERNANDO VILLALTA*

Division of Biomedical Sciences, School of Graduate Studies, Meharry Medical College, Nashville, Tennessee 37208

Received 28 October 1988/Accepted 3 January 1989

***Trypanosoma cruzi* amastigotes present receptors for human fibronectin as indicated by the saturable binding of [^{125}I]fibronectin to this form of the parasite. Scatchard analysis indicates that the number of fibronectin receptors per amastigote was 1.3×10^3 with a K_d of approximately 2.3 nM. Addition of physiological concentrations of fibronectin to amastigote-macrophage cocultures significantly increased the binding of amastigotes to murine macrophages. This increase was evidenced in both the number of amastigotes bound to macrophages and the percentage of macrophages containing bound amastigotes. The uptake of amastigotes by either murine macrophages or human blood monocytes was also increased in the presence of exogenous fibronectin. The increase induced by fibronectin was blocked when amastigotes were pretreated with the RGDS tetrapeptide of the fibronectin cell attachment site. Furthermore, the ability of fibronectin to enhance amastigote binding to and uptake by macrophages was inhibited by the F(ab')$_2$ fragment of anti-fibronectin immunoglobulin G (IgG) but not by an irrelevant anti-human IgG F(ab')$_2$ fragment. Pretreatment of either amastigotes or macrophages with fibronectin also resulted in a significant increase in amastigote binding to and uptake by macrophages. These results suggest that fibronectin may play a role in facilitating the attachment and ingestion of *T. cruzi* amastigotes by macrophages and monocytes in chagasic tissue lesions.**

Trypanosoma cruzi, a protozoan affecting millions of people in South and Central America, requires an intracellular location to multiply and amplify the disease in mammalian hosts (2). Macrophages and monocytes interact with and ingest amastigotes, the multiplicative form of the parasite, at inflammatory sites (7). The processes by which inflammatory cells kill amastigote forms of *T. cruzi* have been reported (20–23), but the mechanisms by which this form of the parasite binds to and is ingested by macrophages and monocytes are poorly understood. Macrophage-monocyte interactions with amastigotes are important because these phagocytic cells play a role in the immunomodulation of the host response to *T. cruzi* (S. A. Howard and F. Villalta, manuscript in preparation).

Fibronectin (Fn) is a glycoprotein produced by both macrophages and monocytes (1, 6) and is secreted in increased amounts during inflammatory processes (12, 14). These cells are found to be present in inflammatory sites containing *T. cruzi* amastigotes (7) and also to participate in the destruction of this form of the parasite (20–23). In this work, we investigate the effect of this glycoprotein on the binding and uptake of amastigotes by murine macrophages and human monocytes.

MATERIALS AND METHODS

Animals. Crl:CD-1(ICR)BR Swiss mice aged 7 weeks from Charles River Breeding Laboratories, Inc. (Raleigh, N.C.) were used as a source of mouse peritoneal macrophages.

Parasites. The Tulahuen strain of *T. cruzi* was used in this work. Blood trypomastigotes isolated from infected mice by chromatography on a diethylaminoethyl-cellulose column (9) were used to infect Vero cell cultures (19). Amastigotes released from these infected cells were isolated on a metrizamide gradient (19) and grown in ML-15HA medium as described previously (19). Amastigotes were also isolated from the spleens of infected mice (9). No enzymes were used in this procedure. The latter amastigotes were purified in a linear sucrose gradient (9) followed by a metrizamide gradient (19). Amastigotes grown in ML-15HA or isolated from the spleens of infected mice were washed by centrifugation with Dulbecco modified minimal essential medium supplemented with 100 U of penicillin and 100 μg of streptomycin per ml (DMEM; GIBCO Laboratories, Grand Island, N.Y.). To study the interaction between amastigotes and macrophages or monocytes, suspensions of parasites were adjusted to 1×10^7 organisms per ml in DMEM supplemented with 1% bovine serum albumin (DMEM-BSA; Sigma Chemical Co., St. Louis, Mo.).

Radioiodination of fibronectin. Purified human plasma Fn was obtained from Calbiochem-Behring, San Diego, Calif. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8) of this Fn revealed a sharp band of 220 kilodaltons. Fn (1 mg) was mixed with 2 mCi of [^{125}I]Na (specific activity, 17 Ci/mg of I; ICN Biochemicals, Irvine, Calif.) in Iodogen (Pierce Chemical Co., Rockford, Ill.)-coated tubes (4) at room temperature for 15 min. Unbound radioactivity was removed by gel filtration through Sephadex G-25 (Pharmacia, Inc., Piscataway, N.J.). Radiolabeled Fn was concentrated by ultrafiltration in Centrisart I tubes (Vangard International, Inc., Neptune, N.J.). The specific activity of the concentrated ^{125}I-labeled Fn was 1×10^6 to 2×10^6 cpm/μg.

Binding assays. Triplicate groups of Eppendorf tubes (1.5 ml) precoated with 20% BSA received 50-μl portions of the amastigote suspension (4×10^6 amastigotes/ml in phosphate-buffered saline [pH 7.2] plus 1% BSA) followed by 50 μl of phosphate-buffered saline–BSA containing increasing concentrations of ^{125}I-labeled Fn (0.9 to 30.0 μg/ml in phosphate-buffered saline–BSA) and 50 μl of phosphate-buffered saline–BSA alone or containing 100-fold-excess unlabeled Fn to determine nonspecific binding. The tubes were incubated at room temperature with constant shaking for 1 h. Unbound radiolabeled Fn was removed by centrifugation at 4°C. Specific binding was determined by subtracting nonspecific binding from the total amount of counts bound.

* Corresponding author.

Mouse peritoneal macrophage and human blood monocyte cultures. Mice were killed by excess ether anesthesia and then injected intraperitoneally with 5 ml of sterile DMEM supplemented with 10% heat-inactivated fetal bovine serum (DMEM + FBS; GIBCO Laboratories, Grand Island, N.Y.) containing 10 U of heparin per ml. The methods for collecting and processing the peritoneal cells and for preparing resident mouse peritoneal macrophage monolayers have been described (24). Human mononuclear cells were obtained from blood by density gradient centrifugation on Sepracell-MN (Sepratech Corp., Oklahoma City, Okla.) using the whole-blood separation procedure described by the manufacturer. The band containing mononuclear cells was washed in DMEM and suspended in DMEM-fetal bovine serum, and human blood monocytes were separated by adherence to the bottom of Lab-Tek tissue culture chambers (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) for 2 h. Macrophages and monocyte monolayers consisted of >98% nonspecific esterase-positive cells with typical macrophage morphology. Adherent mouse peritoneal macrophages were further incubated overnight in DMEM-fetal bovine serum at 37°C in an atmosphere of 5% CO_2, whereas human blood monocytes were incubated for 2 h under similar conditions before the parasites were added.

Measurement of amastigote binding to macrophages. Mouse peritoneal macrophage monolayers were washed with DMEM at 4°C immediately prior to adding Fn and untreated or Fn-treated parasites suspended in DMEM-BSA. The parasite:macrophage ratio used was 6:1. The cocultures were incubated at 4°C for 3 h, and the nonbound amastigotes were removed by washing with cold DMEM. After fixation with absolute methanol and staining with Giemsa, the percentage of cells containing bound *T. cruzi* amastigotes and the number of amastigotes bound per 100 cells were microscopically determined. Independent experiments were performed in triplicate.

Measurement of amastigote uptake by mouse peritoneal macrophages or human blood monocytes. The procedure to measure amastigote uptake by human blood monocytes and mouse peritoneal macrophages has been described (21, 24). Briefly, mouse peritoneal macrophages or human blood monocyte monolayers were washed with DMEM. These cultures then received 100 μl of amastigote suspension in DMEM-BSA and 100 μl of DMEM-BSA or DMEM-BSA supplemented with selected concentrations of Fn, goat $F(ab')_2$ fragment of anti-human Fn immunoglobulin G (IgG) (Cooper Biomedical, Inc., Malvern, Pa.), goat $F(ab')_2$ fragment of anti-human IgG (Sigma, St. Louis, Mo.), or a combination of these agents. The cocultures were incubated in 5% CO_2 at 37°C for 2 h. Under these conditions, the majority of cell-bound amastigotes were ingested by macrophages (21, 24, 25) or monocytes (21), as further confirmed by electron microscopy studies (results not shown). The unbound parasites were removed by washing each culture chamber with DMEM. The cocultures were fixed, stained, and counted as described above.

Pretreatment of amastigotes and macrophages with Fn or RGDS. Amastigotes or macrophages were washed with DMEM and preincubated at 4°C for 1 h with DMEM-BSA or DMEM-BSA containing Fn (200 μg/ml). In some experiments, amastigotes were also pretreated with the RGDS tetrapeptide (2 mg/ml in DMEM-BSA) of the Fn cell attachment site (CalBiochem). Amastigotes and macrophages were then washed with DMEM. The parasite suspension was adjusted to a concentration of 1×10^7 organisms per ml in DMEM-BSA and incubated with Fn-treated or untreated

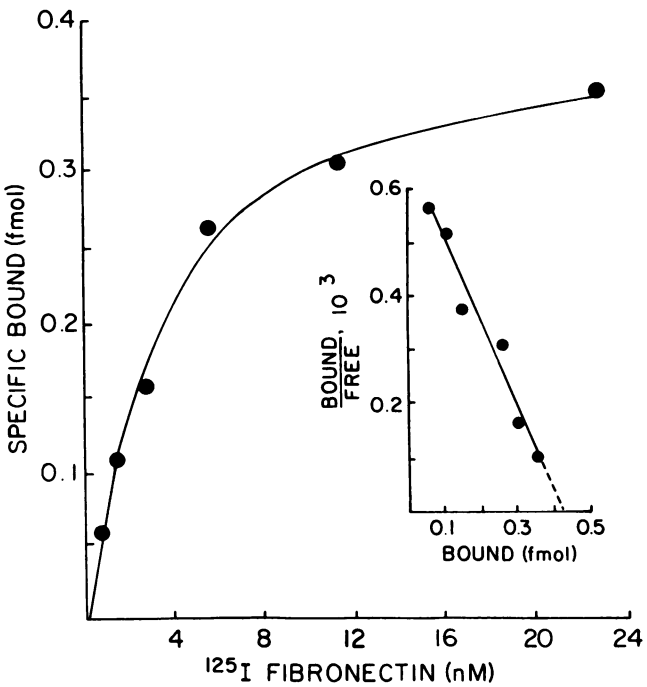

FIG. 1. Specific binding of ^{125}I-labeled human Fn to *T. cruzi* amastigotes. Insert is a Scatchard analysis of the binding data. This is a representative experiment from three independent experiments with similar results. Binding assays and analysis of the data were performed as described in Materials and Methods.

macrophage counterparts. Amastigotes that were pretreated with the RGDS tetrapeptide were added to macrophage or monocyte cultures containing DMEM-BSA or DMEM-BSA supplemented with Fn (200 μg/ml). Experiments were conducted as described above. The selected concentration of RGDS did not affect the ability of macrophages to exclude trypan blue and ingest latex beads. In addition, this concentration of RGDS did not affect the morphology of the parasite and its ability to grow in cell-free medium.

Presentation of results. All results in this work were obtained from triplicate values and represent two to five independent experiments with identical protocols. Results are expressed as the mean ± standard deviation. Differences were considered to be statistically significant if $P \leq 0.05$ by the Student *t* test.

RESULTS

Binding of radiolabeled Fn to *T. cruzi* amastigotes. The binding of radiolabeled human Fn to *T. cruzi* amastigotes was specific and saturable, indicating the presence of Fn receptors on this form of the parasite (Fig. 1). As indicated in the insert of Fig. 1, Scatchard analysis (17) of the binding data indicates that there is a single class of receptors for Fn present at 1.33×10^3 per amastigote with a K_d of 2.3 nM. The RGDS tetrapeptide at the concentration of 4.6 mM inhibited 50% of the binding of ^{125}I-labeled Fn to amastigotes.

Effect of Fn on the binding to and uptake of *T. cruzi* amastigotes by murine macrophages and human blood monocytes. In binding experiments performed at 4°C, pretreatment of either macrophages or amastigotes with Fn (Table 1) or addition of Fn to amastigote-macrophage cocultures

TABLE 1. Pretreatment of either *T. cruzi* amastigotes or macrophages with Fn increased binding of amastigotes to macrophages[a]

Cell and pretreatment	% Macrophages associated with amastigotes[b]	No. of amastigotes bound per 100 macrophages[b]
Amastigote		
DMEM-BSA	24.6 ± 2.9	25.8 ± 1.6
Fn	33.3 ± 1.8 (35.4)[c]	36.3 ± 6.0 (40.7)[c]
Macrophage		
DMEM-BSA	25.6 ± 1.6	27.2 ± 1.3
Fn	34.6 ± 1.9 (35.2)[c]	39.8 ± 2.6 (46.3)[c]

[a] Amastigotes and macrophages were each pretreated with either DMEM-BSA or Fn (200 μg/ml in DMEM-BSA), washed with DMEM, and incubated with their counterparts at 4°C for 3 h.

[b] Mean ± standard deviation of triplicate cocultures of a representative experiment.

[c] Differences between these values and the corresponding control values were statistically significant ($P \leq 0.05$). Percent change with respect to control values is given in parentheses.

(Table 2) resulted in increased binding of amastigotes to macrophages, as evidenced by a significant increase in both the percentage of macrophages containing bound amastigotes and the number of amastigotes bound to 100 macrophages. Furthermore, the increase in cell binding observed in the presence of Fn was specifically blocked by an anti-human Fn $F(ab')_2$ fragment but not by an anti-human $F(ab')_2$ IgG fragment (Table 2).

Experiments designed to evaluate the uptake of amastigotes by macrophages and monocytes were performed at 37°C. The addition of different concentrations of Fn to macrophage-amastigote cocultures resulted in increased uptake of parasites by macrophages, as evidenced by a significant increase in both the percentage of macrophages containing amastigotes and the number of parasites per 100 macrophages (Table 3). The effect was dose dependent, with 200 μg of Fn per ml giving the maximal effect on amastigote-macrophage interactions (Table 3). In addition, the enhancing effect of Fn on amastigote-macrophage interactions was also observed at concentrations up to 500 μg/ml. The ability of Fn to increase parasite uptake by macrophages varied occasionally between different experiments of similar protocols. However, the increase in amastigote uptake by macrophages was always observed in the presence of Fn.

TABLE 2. Anti-Fn $F(ab')_2$ specifically inhibited the enhancing effect of Fn on the binding of amastigotes to macrophages[a]

Coculture conditions	% Macrophages containing amastigotes[b]	No. of amastigotes bound per 100 macrophages[b]
DMEM-BSA	17.1 ± 0.1	19.0 ± 1.6
Fn	23.2 ± 0.1[c] (35.7)	24.3 ± 0.7[c] (27.9)
Anti-Fn $F(ab')_2$	17.1 ± 0.2 (0.0)	18.5 ± 0.4 (−2.6)
Fn + anti-Fn $F(ab')_2$	18.2 ± 1.3 (6.4)	19.7 ± 0.1 (3.7)
Anti-IgG $F(ab')_2$	17.4 ± 1.1 (1.8)	19.1 ± 2.0 (0.5)
Fn + anti-IgG $F(ab')_2$	21.8 ± 1.3[c] (27.5)	23.4 ± 0.6[c] (23.2)

[a] Fibronectin, antibodies, or both were added to amastigote-macrophage cocultures and incubated at 4°C for 3 h.

[b] Mean ± standard deviation of triplicate cocultures of a representative experiment. Percent change with respect to control values is given in parentheses.

[c] Differences between these values (Fn, antibodies, or both in DMEM-BSA) and the corresponding control values (DMEM-BSA) were statistically significant ($P \leq 0.05$).

TABLE 3. Addition of exogenous Fn to macrophage-*T. cruzi* amastigote cocultures increased parasite uptake by macrophages[a]

Fn (μg/ml)	% Macrophages containing amastigotes[b]	No. of amastigotes per 100 macrophages[b]
0.0	16.8 ± 3.1	28.3 ± 3.5
25.0	25.9 ± 2.3 (54.2)[c]	37.1 ± 3.9 (31.1)[c]
100.0	27.0 ± 2.3 (60.7)[c]	42.3 ± 2.9 (49.5)[c]
200.0	32.7 ± 3.2 (94.6)[c]	49.6 ± 1.1 (75.3)[c]

[a] Amastigotes were added to macrophage monolayers containing different concentrations of Fn and incubated at 37°C for 2 h.

[b] Mean ± standard deviation of triplicate cocultures of a representative experiment.

[c] Differences between these values and the corresponding control values obtained in the absence of Fn were statistically significant ($P \leq 0.05$). Percent change with respect to control values is given in parentheses.

Pretreatment of either macrophages or amastigotes with Fn also caused a significant increase in the uptake of parasites by macrophages (Table 4). Furthermore, the enhancing effect of Fn on amastigote uptake by macrophages could be inhibited by an antihuman Fn $F(ab')_2$ fragment but not by an anti-human IgG $F(ab')_2$ fragment (Table 5). The presence of either $F(ab')_2$ fragment in the absence of Fn did not significantly alter parasite-host cell association with respect to control values (Table 5). Pretreatment of amastigotes with the tetrapeptide RGDS (that contains the Fn cell attachment site) (15, 16, 27) inhibited the enhancing effect of Fn on parasite uptake by macrophages (Table 6).

In the presence of Fn, human blood monocytes showed a significant increase in their ability to take up amastigotes (Table 7). The ability of Fn to enhance amastigote uptake by monocytes was inhibited when amastigotes were pretreated with RGDS. In the absence of Fn, RGDS had no effect on the ability of monocytes to take up amastigotes with respect to control values (Table 7).

The ability of macrophages to take up amastigotes isolated from the spleens of infected mice was significantly enhanced by Fn. In a representative experiment, addition of Fn caused a statistically significant increase in both the percentage of macrophages containing amastigotes (24.8 ± 0.5) and the number of amastigotes per 100 macrophages (27.6 ± 0.5) when these values were compared with control values (16.8 ± 1.6 and 18.4 ± 1.1, respectively). The enhancing effect of Fn in these experiments was similar to that observed in

TABLE 4. Pretreatment of either *T. cruzi* amastigotes or macrophages with Fn increased the uptake of amastigotes by macrophages[a]

Cell and pretreatment	% Macrophages containing amastigotes[b]	No. of amastigotes per 100 macrophages[b]
Amastigote		
DMEM-BSA	20.1 ± 2.2	25.3 ± 2.2
Fn	26.9 ± 1.8 (33.8)[c]	33.0 ± 0.6 (30.4)[c]
Macrophage		
DMEM-BSA	20.2 ± 1.3	24.4 ± 2.4
Fn	25.2 ± 0.4 (24.8)[c]	32.8 ± 2.1 (34.4)[c]

[a] Amastigotes and macrophages were each pretreated with either DMEM-BSA or Fn (200 μg/ml in DMEM-BSA), washed with DMEM, and exposed to their counterparts at 37°C for 2 h.

[b] Mean ± standard deviation of triplicate cocultures of a representative experiment.

[c] Differences between these values and the corresponding control values were statistically significant ($P \leq 0.05$). Percent change with respect to control values is given in parentheses.

TABLE 5. Anti-human Fn $F(ab')_2$ fragment specifically inhibited the enhancing effect of Fn on the uptake of *T. cruzi* amastigotes by macrophages[a]

Conditions	% Macrophages containing amastigotes[b]	No. of amastigotes per 100 macrophages[b]
DMEM-BSA	19.7 ± 1.7	22.0 ± 3.4
Fn	30.6 ± 0.1 (55.3)[c]	40.0 ± 0.1 (81.8)[c]
Anti-Fn $F(ab')_2$	18.8 ± 2.8 (−4.6)	22.0 ± 2.5 (0.0)
Fn + anti-Fn $F(ab')_2$	18.3 ± 0.6 (−7.1)	22.1 ± 0.5 (0.5)
Anti-IgG $F(ab')_2$	20.4 ± 1.6 (3.6)	24.8 ± 5.5 (12.7)
Fn + anti-IgG $F(ab')_2$	27.2 ± 4.0 (38.1)[c]	33.4 ± 1.8 (51.8)[c]

[a] Fn, antibodies, or both were added to amastigote-macrophage cocultures, and the cocultures were incubated at 37°C for 2 h.
[b] Mean ± standard deviation of triplicate cocultures of a representative experiment. Percent change with respect to control values is given in parentheses.
[c] Differences between these values and the corresponding control values (DMEM-BSA) were statistically significant ($P \leq 0.05$).

cocultures containing amastigotes derived from infected mammalian cell monolayers.

DISCUSSION

This study shows that *T. cruzi* amastigotes present receptors for human fibronectin (Fig. 1). In the presence of physiological concentrations of fibronectin, these receptors appear to increase the binding to and uptake of amastigotes by both murine macrophages and human monocytes. The enhancing effect of Fn on amastigote binding to and uptake by these two types of phagocytic cells appears to be specific since anti-human Fn $F(ab')_2$ fragments can inhibit it (Tables 1 and 5), apparently by interacting with the Fn molecule.

The fact that pretreatment of either macrophage or *T. cruzi* amastigotes with Fn intensifies the degree of parasite binding to and uptake by macrophages indicates that Fn receptors on both amastigotes (as stated previously) and macrophages (27) are responsible for the effect of Fn on amastigote-phagocyte cell interactions. This finding suggests that *T. cruzi* amastigote binding to and uptake by mononuclear phagocytic cells is mediated in part by the interaction of amastigote-bound Fn with macrophage and monocyte Fn

TABLE 6. Pretreatment of *T. cruzi* amastigotes with RGDS inhibited the enhancing effect of Fn on amastigote uptake by macrophages[a]

Amastigote pretreatment (addition of Fn to cocultures[b])	% Macrophages containing amastigotes[c]	No. of amastigotes per 100 macrophages[c]
DMEM-BSA (−)	9.8 ± 0.1	10.0 ± 0.4
DMEM-BSA (+)	19.8 ± 2.9 (102.0)[d]	20.8 ± 3.5 (108.0)[d]
RGDS (−)	7.5 ± 1.1 (−23.5)[d]	7.3 ± 1.3 (−27.0)[d]
RGDS (+)	7.7 ± 1.1 (−21.4)[d]	7.9 ± 0.8 (−21.0)[d]

[a] Amastigotes were preincubated with either DMEM-BSA or RGDS (2 mg/ml in DMEM-BSA) for 1 h at 4°C and, after washing, were added to macrophage cultures containing DMEM-BSA or Fn (200 μg/ml in DMEM-BSA).
[b] Symbols indicate addition (+) or no addition (−) of Fn.
[c] Mean ± standard deviation of triplicate cocultures of a representative experiment.
[d] Differences between these values and the corresponding control values (cocultures in the absence of Fn-containing amastigotes pretreated with DMEM-BSA) were statistically significant ($P \leq 0.05$). Percent change with respect to control values is given in parentheses.

TABLE 7. Pretreatment of *T. cruzi* amastigotes with RGDS inhibited the enhancing effect of Fn on amastigote uptake by human blood monocytes[a]

Amastigote pretreatment (addition of Fn to cocultures[b])	% Monocytes containing amastigotes[c]	No. of amastigotes per 100 monocytes[c]
DMEM-BSA (−)	36.4 ± 6.4	45.2 ± 9.1
DMEM-BSA (+)	54.5 ± 6.1 (49.7)[d]	88.1 ± 7.6 (94.9)[d]
RGDS (−)	34.8 ± 5.7 (−4.4)[d]	45.2 ± 6.5 (0.0)[d]
RGDS (+)	33.2 ± 2.0 (−8.8)[d]	42.9 ± 4.7 (−5.1)[d]

[a] Amastigotes were preincubated either with DMEM-BSA or RGDS (2 mg/ml in DMEM-BSA) for 1 h at 4°C and, after washing, were added to human blood monocyte cultures that contained DMEM-BSA or Fn (200 μg/ml in DMEM-BSA).
[b] Symbols indicate addition (+) or no addition (−) of Fn.
[c] Mean ± standard deviation of triplicate cocultures of a representative experiment.
[d] Differences between these values and the corresponding control values (cocultures in the absence of Fn-containing amastigotes pretreated with DMEM-BSA) were statistically significant ($P \leq 0.05$). Percent change with respect to control values is given in parentheses.

receptors or vice versa by a bridging phenomenon. However, since anti-human Fn $F(ab')_2$ fragment did not significantly affect the binding and uptake of amastigotes in the absence of Fn, it is likely that Fn is not the only ligand involved in *T. cruzi* amastigote-macrophage or amastigote-monocyte interactions.

The fact that pretreatment of *T. cruzi* amastigotes with RGDS inhibited the effect of Fn on amastigote uptake by either murine macrophages or human monocytes (Tables 6 and 7) suggests that the enhancing effect of Fn is mediated by the Fn cell attachment site (RGDS). Macrophage uptake of amastigotes isolated from the spleens of infected mice was also enhanced by Fn, suggesting that this interaction may occur in vivo.

Macrophages and monocytes, cellular components of the inflammatory reaction that interact with amastigotes (20–23), present receptors for Fn (5, 27). This molecule is present in elevated amounts during the inflammatory process (12, 14). It is possible that *T. cruzi* amastigotes could acquire host Fn during this process. Our results indicate that physiological concentrations of Fn enhance the binding and uptake of amastigotes by either macrophages or monocytes and suggest that, under these conditions, these two types of phagocytic cells could interact with *T. cruzi* amastigotes.

Of interest is the fact that human lactoferrin, a glycoprotein also secreted in increased amounts during inflammatory processes, is able to enhance *T. cruzi* amastigote-macrophage or amastigote-monocyte interactions (10), suggesting that these interactions may depend upon complex processes.

The observation that inflammatory cells surrounding tissue lesions contain amastigotes (7) and the fact that macrophages, monocytes, eosinophils, and neutrophils take up and destroy these forms of the parasite released from mammalian cells (20–23) indicate that these cells may be involved in the reduction of tissue forms of the parasite observed in the chronic phase of the disease. Recent observations indicate that when pure populations of amastigotes growing in logarithmic phase are ingested by inflammatory cells, these forms of the parasite are destroyed and digested (20–23). When macrophages ingest amastigotes contaminated with small amounts of transitional forms in differentiation from amastigotes to trypomastigotes or trypomastigotes, macrophages destroy and digest amastigotes, but the few transi-

tional forms and trypomastigotes can establish a late weak infection (3).

Our findings that *T. cruzi* amastigotes present receptors for Fn, together with the observations of others that trypomastigote (11, 26) and epimastigote (13) forms of the same parasite present receptors for this molecule, indicate that Fn receptors are present on all stages of this parasite, regardless of their invasive capacities. This molecule may therefore play a significant role in increasing the attachment and incorporation of intracellular forms of *T. cruzi*, released from bursting infected cells, by macrophages and monocytes at the level of chagasic tissue lesions.

ACKNOWLEDGMENTS

This work was supported by Public Health Service Grant 1-R29-AI-25637-01A1 from the National Institutes of Health and by grant DAN-5053-G-SS-7076-00 from the United States Agency for International Development.

E.L.N. is supported by Minority Biomedical Research Support fellowship grant RR08037 from the National Institutes of Health.

LITERATURE CITED

1. **Alitalo, K., T. Hovi, and A. Vaheri.** 1980. Fibronectin is produced by human macrophages. J. Exp. Med. **151**:602–613.
2. **Brener, Z.** 1973. Biology of *Trypanosoma cruzi*. Annu. Rev. Microbiol. **27**:347–382.
3. **Carvalho, R. M., M. N. L. Mirelles, W. de Souza, and W. Leon.** 1981. Isolation of the intracellular stage of *Trypanosoma cruzi* and its interaction with mouse macrophages in vitro. Infect. Immun. **33**:546–554.
4. **Fraker, P. J., and J. C. Speck.** 1978. Protein and cell membrane iodination with a sparingly soluble chloroamide 1,3,4,6-trachloro-3-6 diphenylglycoluril. Biochem. Biophys. Res. Commun. **80**:849–857.
5. **Hosein, B., and C. Bianco.** 1985. Monocyte receptors for fibronectin characterized by a monoclonal antibody that interferes with receptor activity. J. Exp. Med. **162**:157–170.
6. **Johansson, S., K. Rubin, M. Hook, T. Ahlgren, and R. Seljelid.** 1979. *In vitro* biosynthesis of cold insoluble globulin (fibronectin) by mouse peritoneal macrophages. FEBS Lett. **105**:313–316.
7. **Koberle, F.** 1968. Chagas' disease and Chagas' syndromes: the pathology of American trypanosomiasis. Adv. Parasitol. **6**: 63–116.
8. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
9. **Leon, W., F. Villalta, T. Queiroz, and A. Szarfman.** 1979. Antibody-induced capping on the intracellular stage of *Trypanosoma cruzi*. Infect. Immun. **26**:1218–1220.
10. **Lima, M. F., and F. Kierszenbaum.** 1985. Lactoferrin effects on phagocytic cell function. I. Increased uptake and killing of an intracellular parasite by murine macrophages and human monocytes. J. Immunol. **134**:4176–4183.
11. **Ouaissi, M. A., D. Afchain, A. Capron, and J. A. Grimaud.** 1984. Fibronectin receptors on *Trypanosoma cruzi* trypomastigotes and their biological function. Nature (London) **308**:380–382.
12. **Owens, M. R., and C. D. Chimino.** 1982. Synthesis of fibronectin by the isolated perfused rat liver. Blood **59**:1305–1309.
13. **Peyrol, S., M. A. Ouaissi, A. Capron, and J. A. Grimaud.** 1987. *Trypanosoma cruzi*: ultrastructural visualization of fibronectin bound to culture cells. Exp. Parasitol. **63**:112–114.
14. **Pick-Kober, K. H., D. Munker, and A. M. Gressner.** 1986. Fibronectin is synthesized as an acute phase reactant in rat hepatocytes. J. Clin. Chem. Clin. Biochem. **24**:521–528.
15. **Pierschbacher, M. D., E. G. Hayman, and E. Ruoslahti.** 1983. Synthetic peptide with cell attachment activity of fibronectin. Proc. Natl. Acad. Sci. USA **80**:1224–1227.
16. **Pierschbacher, M. D., and E. Ruoslahti.** 1984. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature (London) **309**:30–33.
17. **Scatchard, G.** 1949. The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. **51**:660–672.
18. **Villalta, F., and W. Leon.** 1979. Effect of purification by DEAE-cellulose column on infectivity of *Trypanosoma cruzi* blood forms. J. Parasitol. **65**:188–189.
19. **Villalta, F., and F. Kierszenbaum.** 1982. Growth of isolated amastigotes of *Trypanosoma cruzi* in cell-free medium. J. Protozool. **29**:570–576.
20. **Villalta, F., and F. Kierszenbaum.** 1983. Role of polymorphonuclear cells in Chagas' disease. I. Uptake and mechanisms of destruction of intracellular forms of *Trypanosoma cruzi* by human neutrophils. J. Immunol. **131**:1504–1510.
21. **Villalta, F., and F. Kierszenbaum.** 1984. Role of inflammatory cells in Chagas' disease. II. Interactions of mouse macrophages and human monocytes with intracellular forms of *Trypanosoma cruzi*: uptake and mechanisms of destruction. J. Immunol. **133**:3338–3343.
22. **Villalta, F., and F. Kierszenbaum.** 1984. Role of inflammatory cells in Chagas' diesease. I. Uptake and mechanisms of destruction of intracellular forms of *Trypanosoma cruzi* by human eosinophils. J. Immunol. **132**:2053–2058.
23. **Villalta, F., and F. Kierszenbaum.** 1986. Effects of human colony-stimulating factor on the uptake and destruction of a pathogenic parasite (*Trypanosoma cruzi*) by human neutrophils. J. Immunol. **137**:1703–1707.
24. **Villalta, F., and F. Kierszenbaum.** 1983. Role of cell surface mannose residues in host cell invasion by *Trypanosoma cruzi*. Biochim. Biophys. Acta **736**:39–44.
25. **Villalta, F., and F. Kierszenbaum.** 1984. Host cell invasion by *Trypanosoma cruzi*: role of cell surface galactose residues. Biochem. Biophys. Res. Commun. **119**:228–235.
26. **Wirth, J. J., and F. Kierszenbaum.** 1984. Fibronectin enhances macrophage association with invasive forms of *Trypanosoma cruzi*. J. Immunol. **133**:460–464.
27. **Wright, S. D., and B. Meyer.** 1985. Fibronectin receptor of macrophages recognizes the sequence Arg-Gly-Asp-Ser. J. Exp. Med. **162**:762–767.