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Trypanosoma cruzi receptors for human transferrin and their role

Maria F. Lima and Fernando Villalta

Division of Biomedical Sciences, School of Graduate Studies, Meharry Medical College, Nashville, TN, U.S.A.

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Trypanosoma cruzi amastigotes present receptors for human transferrin as indicated by the saturable binding of ^{125}I-transferrin to this form of the parasite. Computerized Scatchard analysis revealed one class of receptors present at 8.1×10^4 receptors per amastigote with a K_d of 2.82 μM. Immunofluorescence studies indicate that more than 90% of amastigotes bind human transferrin, whereas trypomastigotes do not. Iron is required for amastigote growth in cell-free medium since deferoxamine, an iron chelator, inhibits amastigote growth. Amastigote growth is restored when deferoxamine is removed from the medium. ^{59}Fe-transferrin, which bound to amastigotes at 4°C for 1 h, was readily dissociated from the parasite surface upon treatment with acid. However, this treatment did not disrupt binding that occurred at 37°C for 1 h. Amastigote growth in cell-free medium is inhibited in ferrotransferrin-depleted serum, and addition of ferrotransferrin but not apotransferrin restores parasite growth. Western blots of solubilized amastigote membranes probed with anti-human transferrin receptor antibody recognize a protein of 200 kDa. This protein is present on the amastigote cell surface; therefore, human transferrin seems to interact with a 200-kDa surface amastigote protein receptor. Iron, which is essential for amastigote growth, thus appears to be delivered to *T. cruzi* amastigotes by transferrin receptor-mediated endocytosis.

Key words: *Trypanosoma cruzi*; Amastigote receptors for human transferrin; Iron delivery from ferrotransferrin, Endocytosis, receptor-mediated; Ferrotransferrin-dependent amastigote growth.

Introduction

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 [1]. *T. cruzi* has a complex life cycle and is present in its mammalian hosts as both the intracellular amastigote and the extracellular host–cell invasive bloodstream trypomastigote form. The amastigote form is the multiplicative stage in the mammalian host and transforms into the trypomastigote form intracellularly.

The basic mechanisms whereby *T. cruzi* amastigotes take up micronutrients from the host to multiply in mammalian cells are largely unknown. An understanding of these basic mechanisms may facilitate the formulation of rational strategies for blocking the multiplication of the intracellular form of this human parasite in the mammalian phase of the cycle.

We have been investigating the involvement of host proteins in the transport of micronutrients to amastigotes. Transferrin is a major iron transport protein of mammalian cells [2] and transferrin receptors have been found in virtually all cells and in increased amounts during cell proliferation [3–5]. Since amastigotes are the multiplicative form of the parasite in the mammalian host we have investigated in this work the binding of human transferrin to *T. cruzi* amastigotes and the consequences of this interaction.

Correspondence address: F. Villalta, Division of Biomedical Sciences, Meharry Medical College, 1005 D.B. Todd, Jr. Boulevard, Nasnville, TN, U.S.A.

Abbreviations: A-AP, Avidin alkaline phosphatase; CHAPS, 3-[(3-cholamidopropyl) dimethyl-ammonio]-1-propane sulfonate); HBSS, Hanks' Balanced Salt solution; PBS, phosphate-buffered saline, pH 7.2; PMSF, phenylmethylsulfonyl fluoride; NHS-biotin, *N*-hydroxisuccinimide biotin; TLCK, *N*-*p*-tosyl-L-lysine-chloromethyl ketone

Materials and Methods

Parasites. The Tulahuen strain of *T. cruzi* was used in this work. Blood trypomastigotes isolated from mice by chromatography on a diethylaminoethyl cellulose column [6] were used to infect Vero cell cultures [7]. Amastigotes released from these infected cells were isolated on a metrizamide gradient [7] and grown in modified ML-15HA medium [7] without hemin and supplemented with 10% fetal bovine serum (Gibco Laboratories, Grand Island, NY). Parasites were washed by centrifugation with Hanks' balanced salt solution (Gibco) and resuspended at the appropriate concentrations in media as described below.

Radioiodination of transferrin. Chromatographically purified human ferrotransferrin was obtained from Sigma (St. Louis, MO). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [8] of this protein revealed a sharp band of 81 kDa. Transferrin (1 mg) was mixed with 1 mCi of ^{125}I (specific activity, 17 ml^{-1} I; ICN Biochemicals, Irvine, CA) in Iodogen (Pierce, Rockford, IL)-coated tubes [9] at room temperature for 15 min. Unbound radioactivity was removed by gel filtration through Sephadex G-25 (Pharmacia, Piscataway, NJ). Radiolabeled transferrin was concentrated by ultrafiltration in Centrisart I tubes (Vangard International, Neptune, NJ). The specific activity of the concentrated ^{125}I-labeled transferrin was 2.1. $\times 10^6$ – 5.5 $\times 10^6$ cpm (μg protein)$^{-1}$.

Labeling of apotransferrin with $^{59}FeCl$. Purified human apotransferrin (Sigma) was labeled with $^{59}FeCl$ by the nitriloacetic acid method as described [10]. Briefly, apotransferrin (8 mg) was dissolved in 0.25 M Tris-Cl buffer in the presence of 0.1 M $NaHCO_3$. This solution was mixed with 100 mM disodium nitriloacetate and 0.125 mCi $^{59}FeCl$ (specific activity 29.3 mCi ml^{-1} Fe at 0.341 mg Fe ml^{-1}; ICN Biochemicals) for 1 h at room temperature. Unbound radioactivity was removed by gel filtration through Sephadex G-25. ^{59}Fe-labeled transferrin was concentrated by ultrafiltration as described above. Transferrin preparations were routinely found to be fully saturated since the ratio A_{280}/A_{450} was 0.1 which is consistent with full saturation [11]. The labeling conditions did not form high molecular weight aggregates of transferrin, since SDS-PAGE of ^{59}Fe-labeled transferrin presented one sharp band of 81 kDa.

Binding assays. The binding of ^{125}I-transferrin to amastigotes was carried out in 1.5-ml Eppendorf tubes, precoated with 20% bovine serum albumin (BSA Sigma). 50-μl portions of the amastigote suspension (4×10^6 amastigotes ml^{-1} in Hanks' Balanced Salt solution, pH 7.2, supplemented with 1% BSA, HBSS-BSA) were added to the tubes, followed by 50 μl of HBSS-BSA containing increasing concentrations of ^{125}I-labeled transferrin (50 – 600 μg ml^{-1} in HBSS-BSA) and 50 μl of HBSS-BSA alone, or containing 100-fold excess unlabeled transferrin to determine non-specific binding. Each point was done in triplicate. The tubes were incubated at room temperature with constant shaking for 1 h. Unbound radiolabeled transferrin was removed by three washes with HBSS by centrifugation at 4°C and the radioactivity associated with the cellular pellet was determined. Specific binding was determined by subtracting non-specific binding (determined in the presence of excess unlabeled transferrin) from the total amount of counts. The binding data was analyzed by the ENZFITTER computer program as described [12].

In addition, binding of human transferrin to the surface of the parasite was evaluated by indirect immunofluorescence [13,14]. Amastigotes or culture trypomastigotes obtained from myoblasts [15,16] were fixed with 0.25% formaldehyde and then incubated (37°C, 30 min) with phosphate buffered saline (PBS) supplemented with 1% BSA alone (control) of containing 1000–2000 μg transferrin ml^{-1}. After several washes, these preparations were incubated (37°C, 30 min) with heat-inactivated normal rabbit serum, washed, and then incubated (37°C, 30 min) with a solution of goat anti-transferrin IgG. After several washes the slides were incubated (37°C, 30 min) with a 1:10 dilution of fluorescein-labeled rabbit anti-goat IgG (Cappel, West Chester, PA) in PBS. The slides were then washed, air-dried and examined with a fluorescence microscope.

Binding to and dissociation of ^{59}Fe-transferrin from amastigotes. Triplicate groups of Eppendorf tubes (1.5 ml) received 50-μl portions of the amastigote suspension (2×10^7 amastigotes ml^{-1} in HBSS) followed by 50 μl of HBSS containing 400 μg ml^{-1} of ^{59}Fe-labeled transferrin or HBSS alone for 1 h at 4°C or 37°C. Unbound [^{59}Fe]transferrin was removed by centrifugation at 4°C. To half of the amastigote samples, 50 μl of 0.25 M acetic acid in 0.5 M NaCl was added for 5 s at 4°C, conditions that were found to dissociate bound transferrin from its receptor on mammalian cells [11]. The pH of the cultures was immediately neutralized to 7.0 by the addition of 1 M sodium acetate. The other half of the amastigote samples received 50 μl of HBSS for 5 s. Cells were centrifuged in an Eppendorf centrifuge at 13000 × *g* for 5 min and radioactivity associated with the pellets was determined in a gamma counter. This short treatment of amastigotes with acetic acid did not affect the morphology of the parasite, the amastigote concentration in the pellet or their ability to grow in ML-15HA.

Biotinylation of amastigotes. Amastigote cell surface proteins were biotinylated by adding a 2.6 mM final concentration of *N*-hydroxisuccinimide biotin (NHS-Biotin) (Bio-Rad, Richmond, CA), dissolved in dimethyl formamide, to 5×10^8 parasites suspended in PBS for 15 min at room temperature [15,16]. Amastigotes retained similar morphology throughout the procedure as observed by optical microscopy. Parasites were then washed three times with cold PBS, and solubilized with 0.8% 3-[(3-cholamidopropyl)-dimethylamonio]-1-propane sulfonate (CHAPS) [15,16] in PBS in the presence of proteinase inhibitors (1 mM phenylmethylsulfonyl fluoride (PMSF) 1 mM *p*-tosyl-L-lysinechloromethylketone (TLCK) 2.8 μg ml^{-1} aprotinin). Solubilized amastigotes were centrifuged at 13000 × *g* at 4°C for 5 min to remove debris.

Western blots. 10 μg of biotinylated parasite samples obtained as described above were resolved by SDS-PAGE, using a 10% acrylamide gel, in the presence or absence of B-mercaptoethanol, according to Laemmli [8] in a mini PROTEAN II cell (Bio-Rad). Gels were then electroblotted onto 0.45-μm nitrocellulose membranes (Bio-Rad) for 1 h at 4°C in a mini-Trans-Blot cell (Bio-Rad). Biotinylated molecular weight markers (Bio-Rad) were included in the gels to determine the relative molecular weight of parasite samples. The nitrocellulose membranes were then stained with avidin-alkaline phosphatase (A-AP; Bio-Rad), and developed.

Immunoblots. The molecular weight of amastigote surface proteins recognized by anti-human transferrin receptor antibody was investigated with immunoblots. Membrane-enriched preparations were prepared as described in detail [17], subjected to SDS-PAGE, and blotted onto nitrocellulose membranes as described above. The nitrocellulose strips were then reacted with a 5% solution of non-fat dry milk in PBS for 1 h at room temperature, and incubated with a 1:50 dilution of the IgG monoclonal B$_3$/25 specific for the human transferrin receptor (Boehringer-Mannheim, CA) in a 5% solution of non-fat dry milk in PBS overnight at 4°C. Membranes were then washed with Tris-buffered saline solution, pH 7.5, containing 0.2% Tween (TTBS), and incubated with a 1:100 dilution of goat anti-mouse IgG labeled with alkaline phosphatase (Bio-Rad) for 1 h at room temperature. Nitrocellulose membranes were washed twice for 5 min with TTBS, twice for 5 min with Tris-buffered saline, pH 7.5 (TBS), and developed.

Amastigote growth in the presence and absence of deferoxamine. Triplicate aliquots of amastigote suspensions were incubated in flat-bottomed microculture wells (Limbro, New Haven, CT) containing 0.1-ml portion of amastigote suspension (2×10^7 organisms ml^{-1} in ML-15HA medium). 0.01 ml of deferoxamine solution (Desferal mesylate, Ciba-Geigy, Summit, NJ) at 2.5 mg ml^{-1} in ML-15HA and 0.79 ml of ML-15HA medium supplemented with 10% FBS. Control cultures received 0.01 ml of ML-15HA medium instead of deferoxamine. Amastigote cultures were incubated at 37°C in a 5% CO_2-in-air-incubator saturated with water vapor as described [7]. The concentration of amastigotes was determined microscopically with a Neubauer hemocytometer at 24 h intervals. At the end of the experiments,

deferoxamine was removed from amastigote cultures by washing the cells three times with HBSS and resuspending the parasites in fresh ML-15HA medium. These cultures were incubated in the same conditions as described above and amastigote growth was determined microscopically.

Amastigote growth in medium supplemented with human serum depleted of transferrin and supplemented with ferrotransferrin or apotransferrin. In these experiments, heat-inactivated serum from healthy volunteers was used to supplement ML-15HA medium [7]. Human serum was depleted of transferrin by passing through an affinity chromatography Affi-Gel Hz column (Bio-Rad) coupled to goat anti-human transferrin IgG. The coupling of this antibody (3 mg ml^{-1}) to Affi-Gel Hz was performed as described by the manufacturer. SDS-PAGE of the run-off material stained wit silver stain did not show the 81-kDa band corresponding to human transferrin. In addition, no band was observed by immunodiffusion when this material was incubated with goat anti-human transferrin IgG. Control human serum was treated similarly but eluted from a column containing an irrelevant goat anti-human IgG coupled to Affi-gel Hz. In contrast, human serum chromatographed under these conditions presented the typical 81-kDa band of transferrin. Ferrotransferrin (100% Fe-saturated human transferrin) and apotransferrin (unsaturated human transferrin) were purchased from Sigma. ML-15HA medium [7] does not contain ferrotransferrin, apotransferrin or any source of iron.

Amastigote cultures were washed three times with HBSS and triplicate aliquots of parasite suspensions were incubated in plastic microculture plates (Limbro) containing 0.02 ml of amastigote suspension (1.55×10^8 organisms ml^{-1} in ML-15HA medium), and either 0.9 ml of ML-15HA supplemented with 10% human transferrin-depleted human serum as described above, 0.9 ml of ML-15HA supplemented with 10% human serum, or 0.9 ml of ML-15HA supplemented with 10% transferrin-depleted human serum and 3 mg ml^{-1} of ferrotransferrin or apotransferrin. The cultures were incubated as described above and amastigote growth was evaluated in a hemocytometer [7].

Presentation of results and statistics. The results presented in the tables and figures of this paper are typically representative of three independent experiments with the same design. Differences were considered to be significant if $P \leq 0.05$ as determined by Student's *t*-test.

Results

Binding of labeled transferrin to amastigotes. The binding of ^{125}I-transferrin to *T. cruzi* amastigotes was specific and saturable, indicating the presence of transferrin receptors in this form of the parasite (Fig. 1). As indicated by the insert of Fig. 1, computerized Scatchard analysis [12] of the binding data indicates that there is one class of receptors for transferrin present at 8.4×10^4 per amastigote with a K_d of 2.82 μM. Preincubation of amastigotes with excess (100-fold) cold transferrin resulted in a dramatic inhibition of subsequent ^{125}I-transferrin binding. A 1000-fold excess of bovine serum albumin had no effect on ^{125}I-transferrin binding.

The results of immunofluorescence studies showed that at physiological concentrations of human transferrin almost all amastigotes bound this protein but trypomastigotes did not (Table I). Controls performed in the absence of transferrin did not show fluorescence associated with parasites. Similar experiments carried out with live preparations of these two mammalian stages of *T. cruzi* had similar results (data not shown).

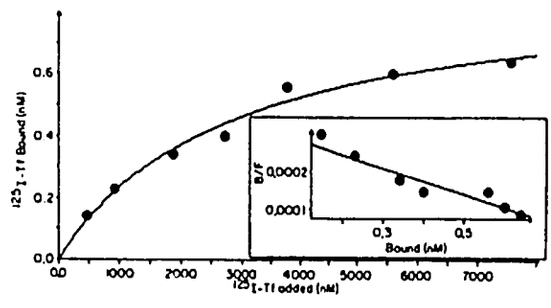

Fig. 1. Specific binding of ^{125}I-labeled human transferrin to *T. cruzi* amastigotes. Insert is a Scatchard analysis of the binding data. These results are typically representative of three independent experiments. Binding assays and computerized analysis of the data were performed as described in Materials and Methods.

TABLE I

Presence of receptors for human transferrin on the surface of mammalian stages of *T. cruzi*

Stage of *T. cruzi*	Transferrin concentration (μg ml^{-1})	Percentage of fluorescent cells
Amastigote	0	0.0 ± 0.0
Amastigote	1000	75.9 ± 0.4
Amastigote	2000	94.8 ± 2.5
Trypomastigote	0	0.0 ± 0.0
Trypomastigote	1000	0.0 ± 0.0
Trypomastigote	2000	0.0 ± 0.0

This set of results is representative of three independent experiments with the same protocol.

Binding of [^{59}Fe]transferrin to and dissociation from amastigotes. To distinguish cell surface-bound from internalized ligand, we examined the release of [^{59}Fe]transferrin from amastigotes by acid [11]. These conditions release [^{59}Fe] transferrin from the transferrin receptor complex in mammalian cells [11]. As shown in Table II, [^{59}Fe]transferrin, which bound to amastigotes under conditions in which ligand-receptor complex internalization does not occur (4°C for 1 h), was readily dissociated from the parasite surface. However, [^{59}Fe]transferrin bound to amastigotes at 37°C for 1 h was not dissociated by acid treatment, indicating that transferrin had attained an intracellular location where it was no longer susceptible to dissociation from its receptor.

TABLE II

Binding to and dissociation of [^{59}FE]transferrin from *T. cruzi* amastigotes

Binding temperature	Acid treatment	Radioactivity associated with amastigotes (cpm)
4°C	Yes	282 ± 27
4°C	No	3993 ± 131*
37°C	Yes	4281 ± 168
37°C	No	5480 ± 1571

[^{59}Fe]Transferrin was incubated with amastigotes, as described in Materials and Methods, at either 4°C or 37°C. After removing the unbound ligand, half the samples were treated with acid for 5 s at 4°C; the pH was immediately neutralized to 7.0 and the radioactivity associated with the amastigotes was determined. Differences between cultures with or without acid treatment were statistically significant. ($P>0.05$).

Identification of an amastigote surface protein that cross reacts with the human transferrin receptor. We found that a protein of 200 kDa is recognized by antibodies against human transferrin receptors when amastigote membrane preparations were resolved by SDS-PAGE under non-reducing conditions, blotted onto nitrocellulose membranes, probed with the B$_3$/25 monoclonal antibody specific for the human transferrin receptor and incubated with goat anti-mouse IgG labeled with alkaline phosphatase (Fig. 2, lane B). No band was present when the blots were incubated with mouse anti-human IgG (Fig. 2, lane C) or when blots of trypomastigote membranes obtained as described above were treated with this monoclonal antibody and with goat anti-mouse IgG (Fig. 2, lane D). A protein of same molecular weight is present on the cell surface of amastigotes, as indicated by either Western blots of biotinylated amastigote surface proteins (Fig. 2, lane A) or autoradiography of radioiodinated surface proteins of amastigotes (results not shown).

Effect of deferoxamine and ferrotransferrin on amastigote growth in cell-free medium. The medium used to evaluate amastigote growth was a modified ML-15HA [7] without hemin. ML-15HA medium does not contain ferrotransferrin or any source of iron [7]. In addition, amastigotes do not use hemin as a source of iron (Villalta, F. and Lima, F., unpublished observations). In these experiments, media were supplemented with heat-inactivated human serum as a source of ferrotransferrin to allow amastigote growth.

Our results indicate that deferoxamine, an iron chelator, dramatically inhibited amastigote growth with respect to control cultures when it was added to the medium (Fig. 3). Amastigote growth was restored when deferoxamine was removed from the medium and amastigotes were resuspended in ML-15HA, without hemin, supplemented with 10% human serum (results not shown), indicating that Fe-depleted parasites are viable but unable to grow until a source of iron is restored.

Our results also indicate that ML-15HA supplemented with human serum that had been depleted of ferrotransferrin was not able to support amastigote growth (Fig. 4). The addition of ferrotransferrin restored this growth, while the addition of apotransferrin did not (Fig. 4).

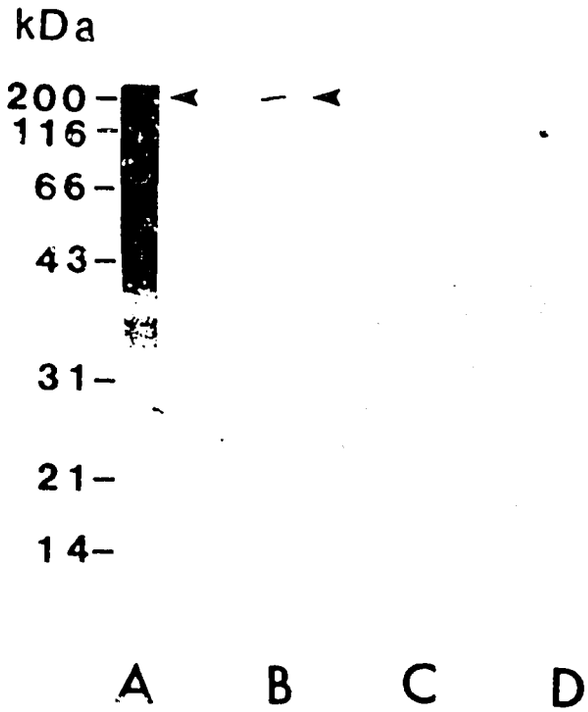

Fig. 2. Identification of an amastigote surface protein that cross reacts with the human transferrin receptor. (A) SDS-PAGE of biotinylated surface proteins of *T. cruzi* amastigotes, blotted onto nitrocellulose membranes, and reacted with A-AP. (B) SDS-PAGE of *T. cruzi* amastigote membranes, blotted onto nitrocellulose, and probed with monoclonal antibody B*y*25 specific for the human transferrin receptor and developed. (C) SDS-PAGE of *T. cruzi* amastigote membranes, blotted onto nitrocellulose, and probed with mouse anti-human IgG. (D) SDS-PAGE of *T. cruzi* trypomastigote membranes, blotted onto nitrocellulose, and probed with monoclonal antibody B*y*25. Arrows indicate the 200-kDa protein recognized by the monoclonal antibody B*y*25. Biotinylated molecular standards (Bio-Rad) are indicated in kilodaltons.

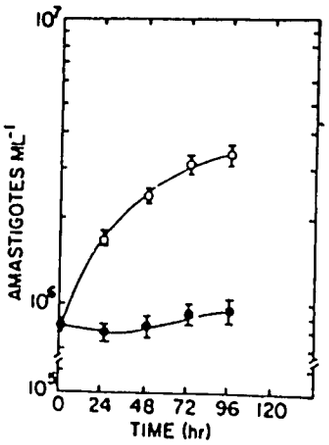

Fig. 3. Effect of deferoxamine on *T. cruzi* amastigote growth. Amastigotes were incubated in ML-15HA supplemented with 10% FBS (○) or in ML-15HA supplemented with 10% FBS and deferoxamine (●). Amastigote growth was evaluated microscopically as described in Material and Methods.

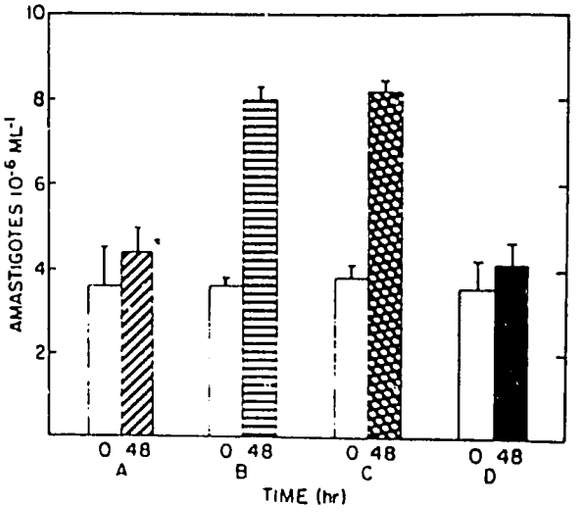

Fig. 4. *T. cruzi* amastigote growth in medium supplemented with transferrin-depleted human serum in the presence or absence of exogenous ferrotransferrin. (A) Amastigotes incubated for 48 h at 37°C in modified ML-15HA supplemented with transferrin-depleted human serum. (B) Amastigotes incubated in modified ML-15HA containing transferrin-depleted human serum supplemented with ferrotransferrin. (C) Amastigotes incubated in modified ML-15HA supplemented with normal human serum. (D) Amastigotes incubated in modified ML-15HA containing transferrin-depleted human serum supplemented with apotransferrin. The initial amastigote concentrations (open bars) and the parasite concentration after 48 h of growth were determined microscopically as described in Materials and Methods.

Discussion

This study shows that *T. cruzi* amastigotes present receptors for human transferrin. In the presence of physiological concentrations of ferrotransferrin, this ligand binds to its receptor, which appears to be a host cross-reacting protein of 200 kDa. Iron, which is essential for amastigote growth, would then be delivered from ferrotransferrin to amastigotes by receptor-mediated endocytosis.

The binding of ^{125}I-transferrin to amastigotes is concentration-dependent, saturable and specific,

thus meeting the criteria for a biological receptor. Unlike mammalian cells, which present only one type of receptor for transferrin with a K_d of 10^{-9}M and an apparent molecular weight of 180 kDa [18,19], amastigotes appear to present also one class of receptors but with a different K_d of 2.82×10^{-6}M and an apparent molecular weight of 200000. A protein of 93 kDa has been found to function as a transferrin receptor on *Plasmodium falciparum* [20], but there has been some controversy in these findings [21]. The fact that trypomastigotes, the invasive but non-dividing form of *T. cruzi*, do not present receptors for this protein, would suggest that receptors for transferrin could be involved in some aspect of parasite growth and differentiation, as has been shown for mammalian cells. Normal mammalian cells, for example, express fewer transferrin receptors than cancerous or stimulated (rapidly proliferating) cells [22]. This has been attributed to the different iron needs of these cells, since iron is needed for cell division [23], RNA polymerases [24], and initiation and maintenance of DNA synthesis [25]. Whether the absence and presence of transferrin receptors in trypomastigotes and amastigotes, respectively, is also due to the different iron requirements of these forms of the parasite, or the expression of transferrin receptors induces a signal for parasite differentiation, is not known.

Amastigotes require iron for their growth in cell-free medium, as shown in this work by the ability of deferoxamine, an iron chelator, to suppress parasite growth. Two of our findings indicate that transferrin could be supplying amastigotes with iron: (a) amastigotes are unable to grow in medium supplemented with transferrin-depleted human serum, but grow to control levels when this deficient medium is supplemented with physiological concentrations of ferrotransferrin; (b) amastigotes are able to internalize ferrotransferrin, as shown by the inability of a mild acid treatment to remove ferrotransferrin bound to amastigotes at 37°C as opposed to its dissociation when parallel experiments were performed at 4°C.

Host hypoferric responses occur during infection of mice with *T. cruzi [26], and it was speculated that this may be due to the transfer of iron to sites of intracellular parasite multiplication, since depletion of host intracellular iron stores reduces parasite pathogenicity [26]. These in vivo results would agree with our in vitro observations, reported in this paper, and explain the mechanisms by which the multiplicative forms of the parasite take iron from the host. T. cruzi* might compete for iron from ferrotransferrin with the host, since patients with Chagas' disease present severe anemia with low levels of iron in plasma [27]. It is possible that the intracellular forms of *T. cruzi* could take ferrotransferrin from the cytoplasm of the cell in the early steps of ferrotransferrin-host cell receptor internalization. Amastigotes could bind ferrotransferrin from vesicles containing the ferrotransferrin-receptor complex, since this form of the parasite, which is free in the cytoplasm, can secrete proteases [28] which might digest these vesicles and release the ferrotransferrin from the complex. Alternatively, amastigotes could bind ferrotransferrin when the parasites are released from bursting infected cells. Whether or not *T. cruzi* amastigotes can take iron from ferritin is unknown. These possibilities are currently under investigation in our laboratory.

Since *T. cruzi* requires an intracellular location to multiply and disseminate in the body, a detailed investigation of the peculiar mechanisms by which this intracellular human parasite takes iron from the host could offer a novel approach to blocking the infection.

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THE 38TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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THURSDAY MORNING, DECEMBER 14

SCIENTIFIC SESSION V: VIRUS VACCINES (Continued)

10:00 463 T-CELL DETERMINANTS ON LASSA VIRUS GLYCOPROTEIN (GP-C). V.J. La Posta* and G.A. Cole. University of Maryland at Baltimore, Baltimore, MD.

10:15 464 NEUTRALIZING ANTIBODY FOLLOWING FORMALIN INACTIVATED HEPATITIS A VACCINE: PERSISTENCE OF ANTIBODY AND NEUTRALIZATION OF ISOLATES FROM THREE CONTINENTS. L.N. Binn*, M. Sjogren, R.H. Marchwicki, C. Hoke, and W. Bancroft. Walter Reed Army Institute of Research, Washington, DC.

SCIENTIFIC SESSION W: KINETOPLASTIDIA - BIOCHEMISTRY, MOLECULAR BIOLOGY AND CHEMOTHERAPY

8:00 AM - 10:30 AM — Nautilus II - III

Chairpersons: K.P. Chang and A. Clarkson

Time	Abstract	
8:00	465	CHARACTERIZATION OF AN RNA VIRUS FROM THE PARASITE LEISHMANIA. B. Widmer, A.M. Comeau, D.B. Furlong, D.F. Wirth and J.L. Patterson. Harvard Medical School and Harvard School of Public Health, Boston, MA.
8:15	466	MONOCLONAL ANTIBODIES AGAINST T. CRUZI NEURAMINIDASE REVEAL ENZYME POLYMORPHISM AMONG DIFFERENT STRAINS, RECOGNIZE A SUBSET OF PARASITES AND ENHANCE INFECTION IN VITRO. R.P. Prioli* and M.E.A. Pereira. New England Medical Center Hospitals, Boston, MA.
8:30	467	INHIBITION OF ANTIMONY-RESISTANT LEISHMANIA WITH A BIS (BENZYL) POLYAMINE ANALOG. R.J. Baumann*, A.J. Bitonti, P.P. McCann, W.L. Hanson, and A. Sjoerdsma. Merrel Dow Research Institute, Cincinnati, OH and the University of Georgia, Athens, GA.
8:45	468	EFFECTS OF ETHER ANALOGUES OF LYSOPHOSPHOLIPIDS ON LEISHMANIA. B.Z. Ngwenya* and J. Wiltshire-Scott. Hahnemann University School of Medicine, Philadelphia, PA.
9:00	469	METABOLIC PREADAPTATION OF METACYCLIC AFRICAN TRYPANOSOMES FOR THE MAMMALIAN BLOODSTREAM. J. Kiaira, W.R. Fish, and E.J. Bienen. University of Nairobi and International Laboratory for Research on Animal Diseases, Nairobi, KENYA.
9:15	470	MITOCHONDRIAL PROTON MOTIVE FORCE IN BLOODSTREAM AFRICAN TRYPANOSOMES DEMONSTRATED BY RHODAMINE 123. M. Saric*, E.J. Bienen, G. Pollakis, R.W. Grady, and A.B. Clarkson, Jr. New York University Medical Center, New York, NY; ILRAD, Nairobi, KENYA; and Cornell University Medical Center, New York, NY.

SCIENTIFIC SESSION W: KINETOPLASTIDIA (Continued)

9:30 471 INVOLVEMENT OF HUMAN TRANSFERRIN IN THE TRANSFER OF IRON TO TRYPANOSOMA CRUZI. M.F. Lima* and F. Villalta. Meharry Medical College, Nashville, TN.

9:45 472 IDENTIFICATION OF A GENE WHICH IS DIFFERENTIALLY EXPRESSED DURING DEVELOPMENT OF TRYPANOSOMA RHODESIENSE FROM BLOODSTREAM TO PROCYCLIC TRYPOMASTIGOTES. L.E. Wirtz, D.A. Sylvester, and G.C. Hill. Meharry Medical College, Nashville, TN.

10:00 473 DNA METHYLATION PATTERNS OF TRYPANOSOMA BRUCEI BRUCEI DURING DFMO INDUCED TRANSFORMATION IN VIVO. B.F. Giffin* and S.J. Wunderle. University of Dayton, Dayton, OH.

10:15 474 DIFFERENTIAL SENSITIVITY OF TRYPANOSOMA B. RHODESIENSE CLINICAL ISOLATES TO DIFLUOROMETHYLORNITHINE AND ARSENICALS. C.J. Bacchi*, H. Nathan, N. Yarlett, P. Sayer, A. Njogu, P.P. McCann, A.J. Bitonti, and A.B. Clarkson, Jr. Pace University, New York, NY; Kenya Trypanosomiasis Research Institute (KETRI), Muguga, KENYA; Merrell Dow Research Institute, Cincinnati, OH; New York University Medical Center, New York, NY.

SCIENTIFIC SESSION X: SCHISTOSOMIASIS - ANTIGENS AND IMMUNOGENS

8:30 AM - 10:30 AM — Sea Pearl III - VI

Chairpersons: S.L. James and D.G. Colley

Time Abstract

8:30 475 IDENTIFICATION AND CHARACTERIZATION OF GLYCOSYL-PHOSPHATIDYLINOSITOL - LINKED SCHISTOSOMA MANSONI ADULT WORM IMMUNOGENS. S.Y. Sauma* and M. Strand. Johns Hopkins University School of Medicine, Baltimore, MD.

8:45 476 EVIDENCE OF CROSS-REACTIVE, SHARED IDIOTYPES ON ANTI-SEA ANTIBODIES FROM HUMANS AND MICE WITH SCHISTOSOMIASIS. M.A. Montesano*, G.L. Freeman, G. Gazzinelli and D.G. Colley. Universidad Federale Juiz de Fora, Juiz de Fora, MG, BRAZIL and VA Medical Center and Vanderbilt University School of Medicine, Nashville, TN.

9:00 477 CHARACTERIZATION OF TRIOSE PHOSPHATE ISOMERASE cDNA AND GENOMIC CLONES FROM SCHISTOSOMA MANSONI. M.G. Reis*, A. Gross, D. Harn and C. Shoemaker. Harvard School of Public Health, Boston, MA.

9:15 478 PEPTIDE FRAGMENTS OF RECOMBINANT SCHISTOSOME TRIOSE PHOSPHATE ISOMERASE RECOGNIZED BY ANTI-28 KD ANTIBODIES. D.A. Harn*, and W. Gu. Harvard School of Public Health, Boston, MA.

W: KINETOPLASTIDIA - BIOCHEMISTRY, MOLECULAR BIOLOGY AND CHEMOTHERAPY

471 INVOLVEMENT OF HUMAN TRANSFERRIN IN THE TRANSFER OF IRON TO TRYPANOSOMA CRUZI.

M.F. Lima* and F. Villalta, Division of Biomedical Sciences, Meharry Medical College, Nashville, TN.

The basic mechanisms by which the obligate intracellular human protozoan Trypanosoma cruzi takes up micronutrients from the host to multiply in mammalian cells are not known. An understanding of these processes may facilitate the formulation of strategies for blocking the mammalian phase of the parasite cycle. We have been investigating the consequences of the interaction of host iron-binding proteins with amastigote forms of the parasite. We have found that amastigotes present receptors for human transferrin and that these receptors are developmentally regulated. Iron is required for amastigote growth in cell-free medium. We have also found that amastigote growth in cell-free medium is inhibited when parasites are grown in media supplemented with ferro-transferrin depleted serum. Addition of ferro-transferrin but not apotransferrin restored parasite growth. ^{59}Fe-transferrin bound to amastigotes at 4°C for 1 hour was readily dissociated from the parasite surface upon mild acid treatment. However, this treatment did not disrupt binding that occurred at 37°C, indicating that transferrin had obtained an intracellular location. Western blots of solubilized amastigote membranes probed with anti-human transferrin receptor IgG indicate that a protein of molecular weight 200kDa, which is present on the amastigote cell surface, interacts with transferrin. These results indicate that iron appears to be delivered to T. cruzi amastigotes by transferrin receptor mediated endocytosis. (Supported by USAID grant DAN-5053-G-SS-8052-00)

472 IDENTIFICATION OF A GENE WHICH IS DIFFERENTIALLY EXPRESSED DURING DEVELOPMENT OF *TRYPANOSOMA RHODESIENSE* FROM BLOODSTREAM TO PROCYCLIC TRYPOMASTIGOTES

L.E. Wirtz, D.A. Sylvester, and *G.C. Hill. Meharry Medical College, Nashville, TN.

Differentiation of bloodstream trypomastigotes of *Trypanosoma rhodesiense* to procyclic forms is induced by introduction into culture at 27°C. We sought to identify genes whose expression is altered upon this environmental shift and which are therefore implicated in either the activation or execution of the new developmental program. We report here on the molecular cloning of one such developmentally regulated gene from *T.rhodesiense* and on features of its expression during differentiation. In Northern analyses, insert from this clone detects a 1.6 kb p[A+] transcript which is ten to thirty fold more abundant in established procyclics than in bloodstream forms. The kinetics with which this transcript accumulates in steady-state RNA during differentiation were examined by probing Northerns of total RNA isolated from transforming organisms at different timepoints after introduction into culture. An increase above blood stage levels is observed within 0.5 hours of the environmental shift. Steady-state levels peak at 50 fold bloodstream levels about 24 hours before 50% of the population have acquired a procyclic morphology. The transcript fails to accumulate in the absence of protein synthesis, pointing to a requirement for the *de novo* synthesis of regulatory factors following the shift. The transcript is also detected and shows similar regulation in *T.brucei* strains. In addition, we have identified a nontransforming *T.brucei* strain in which transcripts remain at the bloodstream level for greater than 24 hours after introduction into culture, until viability is lost, suggesting that this variant is blocked early in the developmental program. Preliminary DNA sequence data reveal no significant homology to known proteins or genes. A more complete dissection of the role of this gene in differentiation including characterization of the gene product and of its level of regulation is underway and should contribute to our understanding of what regulatory mechanisms come into play to orchestrate transitions between developmental stages as these parasites differentiate during their life cycle. Supported by NIH grant AI-21159.