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The Major Surface Glycoproteins of *Trypanosoma cruzi* Amastigotes Are Ligands of the Human Serum Mannose-Binding Protein

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***Trypanosoma cruzi*, an obligate intracellular protozoan parasite, chronically infects mammals and causes Chagas' disease in humans. *T. cruzi* evasion of the mammalian immune response and establishment of chronic infection are poorly understood. During *T. cruzi* infection, amastigotes and trypomastigotes disseminate in the mammalian host and invade multiple cell types. Parasite surface carbohydrates and mammalian lectins have been implicated in the invasion of mammalian cells. A recent study has demonstrated that the human mannose-binding protein and the macrophage mannose receptor, two mammalian C-type lectins, bind to *T. cruzi* (S. J. Kahn, M. Wleklinski, A. Aruffo, A. Farr, D. Coder, and M. Kahn, J. Exp. Med. 182:1243–1258, 1995). In this report we identify the major surface glycoproteins, including the SA85-1 glycoproteins, as *T. cruzi* ligands of the mannose-binding protein. Further characterization of the interaction between the mannose-binding protein and *T. cruzi* demonstrates that (i) the SA85-1 glycoproteins are expressed by amastigotes and trypomastigotes but only amastigotes express the mannose-binding protein ligand, (ii) treatment of amastigotes with α-mannosidase inhibits the binding of mannose-binding protein, and (iii) amastigote binding of mannose-binding protein is stable despite the spontaneous shedding of some glycoproteins from its surface. Together, the data indicate that developmentally regulated glycosylation of surface glycoproteins controls the expression of ligands that affect the interactions between *T. cruzi* and mannose-binding protein. It has been established that the binding of mannose-binding protein to microorganisms facilitates their uptake into phagocytic cells. Preferential opsonization of amastigotes with mannose-binding proteins may account for their clearance from the circulation and may contribute to the parasite's ability to invade different cell types.**

Trypanosoma cruzi, an obligate intracellular parasite capable of invading a wide variety of mammalian cells, is the causative agent of Chagas' disease. During the acute phase of infection, the parasite enters the circulation and disseminates throughout the host. A chronic phase ensues in which the parasite is difficult to detect, yet it persists in the tissues and individuals remain infected for their lifetime. How the parasite evades the mammalian immune response is poorly understood.

Epimastigotes develop and replicate in the gut of blood-sucking insects and are spread to mammals as metacyclic trypomastigotes in the insect feces. In mammals, amastigotes replicate intracellularly, and some transform into trypomastigotes. Amastigotes and trypomastigotes are released from infected cells, and both forms circulate in the bloodstream and invade other cells (3, 22).

The surface glycoproteins of the mammalian-stage parasites are encoded by a superfamily of genes with sequence homology to sialidases (6, 11, 17, 28, 30, 32, 38–40). The major surface glycoproteins of approximately 85 kDa constitute a large subset of this superfamily. The SA85-1 glycoprotein family, encoded by more than 100 genes, is a subset of the major surface glycoproteins (17, 19). A definitive function for the SA85-1 glycoproteins and other surface proteins has been difficult to ascertain (18). Several studies have indicated that the surface glycoproteins contribute to adhesion and invasion of host cells (1, 2, 4, 5, 12, 21, 27, 42, 43). Some of the reports have indicated that the 85-kDa glycoproteins function as adhesion molecules, binding to extracellular proteins and carbohydrates (5, 12, 27). Other reports suggest that the carbohydrate portion of the 85-kDa proteins may act as a ligand and bind to host cell surface lectins to facilitate invasion (21, 43). Binding studies with plant lectins have demonstrated that the surface glycosylation of *T. cruzi* is stage specific (29). These findings suggest that the different parasite stages have developed unique lectin interactions with host cell surfaces.

Mannose-binding proteins (MBPs) are members of the ever-growing family of C-type lectins (8). The MBPs may be considered as "ante" antibodies as they distinguish between the carbohydrates of a variety of pathogenic microorganisms (including yeast, bacteria, and viruses) from self glycoproteins (10); in addition, MBP may directly opsonize microorganisms or initiate activation of the complement cascade (33). To date, a role for MBP in the pathogenesis of protozoan parasitic infections has not been explored. A recent report demonstrates that MBP binds strongly to amastigotes, does not bind to trypomastigotes, and binds weakly to epimastigotes (20). In this report, we have investigated the interaction of human MBP with *T. cruzi*. We found that (i) amastigote-expressed surface glycoproteins, including the SA85-1 glycoproteins, are ligands of MBP, (ii) mannose is a critical component of the MBP ligand, and (iii) MBP remains bound to the surface of

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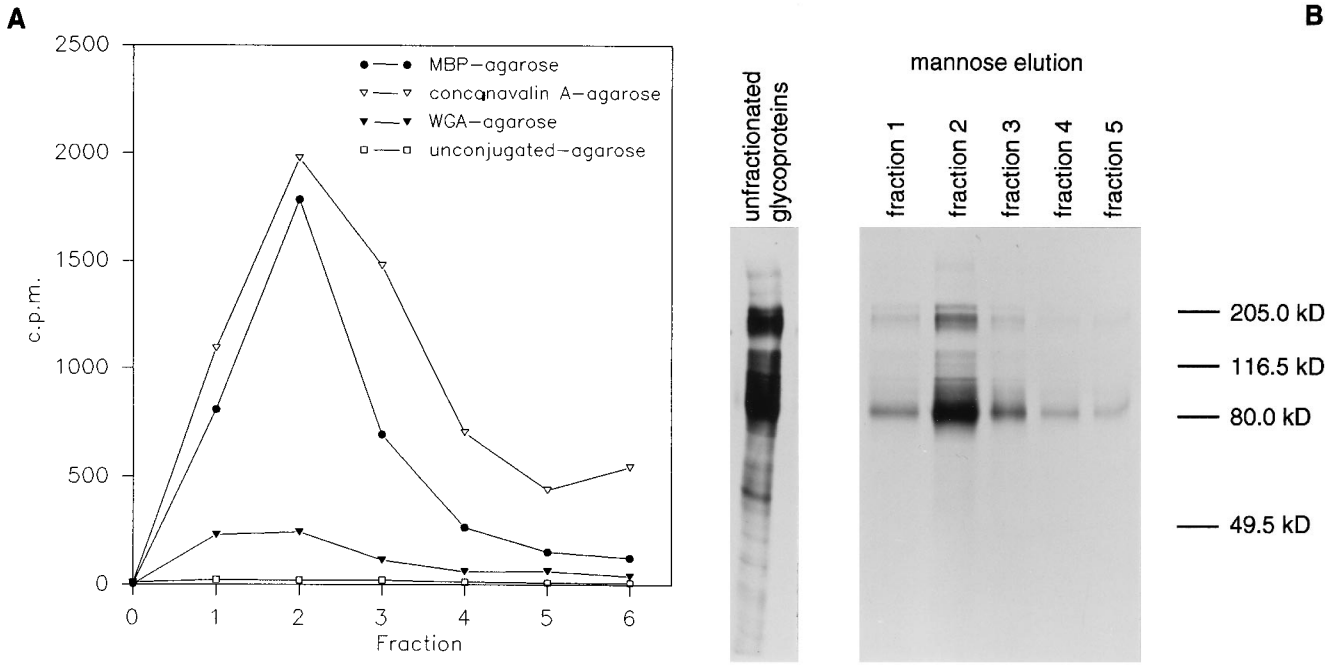

FIG. 1. MBP affinity chromatography of *T. cruzi* mammalian-stage glycoproteins. (A) Mammalian-stage glycoproteins bind to lectins. Equal amounts of unfractionated ^{35}S-labeled glycoproteins obtained from tissue culture conditioned medium (shed glycoproteins) were loaded onto an unconjugated-agarose column or lectin-agarose columns. The columns were washed with TBS, and then 200-μl fractions were collected in specific elution buffers described in Materials and Methods. Twenty microliters of each fraction was analyzed for the presence of ^{35}S-labeled glycoproteins. cpm, counts per minute. (B) Mammalian-stage glycoproteins with molecular masses of more than 80 kDa bind MBP. An equal portion of each fraction collected from the MBP column (panel 1A) was subjected to SDS-PAGE, fluorography, and autoradiography. Unfractionated ^{35}S-labeled glycoproteins (shed glycoproteins) were subjected to the same analysis.

amastigotes despite the spontaneous shedding of surface glycoproteins. This report identifies the first *T. cruzi* ligand of a mammalian lectin and suggests that MBP opsonization may facilitate amastigote entry into mammalian cells.

MATERIALS AND METHODS

T. cruzi. *T. cruzi* CL strain subclone three was used (31). Epimastigotes were grown in liver infusion-tryptone (LIT) medium with 10% fetal bovine serum (HyClone Laboratories, Inc., Logan, Utah). LIT medium is prepared by mixing 0.025 g of hemin (bovine, type I) (Sigma Chemical Co., St. Louis, Mo.) per ml dissolved in 5 ml of 0.01 M NaOH with a 900-ml solution containing 4.0 g of NaCl, 0.4 g of KCl, 8.0 g of Na_2HPO_4, 2.0 g of glucose, 5.0 g of liver infusion (Difco Laboratories Inc., Detroit, Mich.), and 5.0 g of tryptose (Difco Laboratories Inc.). Amastigotes (intracellular derived) and trypomastigotes were obtained from culture supernatants of infected rat 3T3 cells grown in Dulbecco's modified Eagle's medium (DME) (Bio Whittaker Inc., Walkersville, Md.) with 10% fetal bovine serum (HyClone Laboratories, Inc.). In these supernatants, the amastigote-to-trypomastigote ratio varied from 5:1 to 1:1. Axenically derived amastigotes were obtained by incubating amastigotes and trypomastigotes isolated from 3T3 culture supernatants in LIT medium at 37°C for 24 h (22). These axenic-derived amastigotes were pelleted by centrifugation at 1,000 × *g* for 5 min (22). Before use in subsequent experiments, all parasites were washed three times in medium without supplemental serum.

Anti-SA85-1 antibodies. Anti-SA85-1 antibodies were derived by repeated immunization of a rabbit with 100 μg of SA85-1.1 protein expressed in *Escherichia coli* and isolated from inclusion bodies (20). Anti-SA85-1.1 antibodies were affinity purified from rabbit serum with an SA85-1.1 affinity column (20).

Lectin chromatography. Supernatant containing surface glycoproteins was prepared by harvesting amastigotes and trypomastigotes in tissue culture supernatants from infected 3T3 cultures. 3T3 cells and cellular debris were removed by centrifugation at 200 × *g* for 5 min. Then the parasites in the supernatant were washed three times with DME, and 2×10^8 parasites per ml were incubated in DME without methionine (Gibco Laboratories, Grand Island, N.Y.) but with 10% dialyzed calf serum and a 200-μCi/ml final concentration of [^{35}S]methionine and [^{35}S]cysteine (1,200 Ci/mmol; Du Pont-New England Nuclear, Boston, Mass.) for 2 h at 37°C. Parasites were then washed three times in DME and incubated in DME (1.5×10^8 parasites per ml) for 4 h at 37°C; parasites were then removed by centrifugation, and supernatants were stored frozen at −80°C.

MBP-agarose was the gift of Pierce Chemical Co., Rockford, Ill. ^{35}S-labeled supernatants were subjected to MBP affinity chromatography. The MBP column was equilibrated with Tris-buffered saline (TBS) (50 mM Tris [pH 8.0], 150 mM NaCl, 2 mM $CaCl_2$). After application of the ^{35}S-labeled supernatants, the column was washed with TBS; then 50-μl fractions of TBS without $CaCl_2$ and with 10 mM EDTA, or fractions collected with TBS containing 100 mM mannose, were collected. Equal aliquots of each fraction were analyzed for radioactivity and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 7.5% gel. Fluorography of the gels was performed with Enhance according to the manufacturer's instructions (Du Pont-New England Nuclear).

Concanavalin A (ConA)-agarose, wheat germ agglutinin (WGA)-agarose, and unconjugated agarose were obtained from Sigma Chemical Co. The ConA-, WGA-, and unconjugated-agarose columns were equilibrated and washed with TBS. From the ConA-agarose column, 50-μl fractions of TBS with 100 mM methyl α-D-mannopyranoside were collected. From the WGA-agarose column, 50-μl fractions of TBS with 100 mM *N*-acetylglucosamine were collected. From the unconjugated-agarose column, 50-μl fractions of TBS without $CaCl_2$ and with 10 mM EDTA, TBS containing 100 mM mannose, or TBS containing 100 mM methyl α-D-mannopyranoside were collected. Carbohydrates were obtained from Sigma Chemical Co.

Supernatant containing nonradioactive shed surface glycoproteins was prepared by harvesting amastigotes and trypomastigotes from 3T3 cultures, removing 3T3 cells by centrifugation at 200 × *g* for 5 min, washing the parasites three times in DME without serum, and incubating 1.5×10^8 parasites per ml for 4 to 16 h in DME. Parasites were then removed by centrifugation, and supernatants were stored frozen at −80°C. These supernatants were subjected to chromatography on an MBP-agarose column; the bound glycoproteins were collected in TBS without $CaCl_2$ containing 10 mM EDTA. The concentrations of affinity-isolated glycoproteins were determined by the procedure of Bradford (Bio-Rad, Richmond, Calif.) by using bovine gamma globulin as a standard. The glycoproteins were subjected to SDS-PAGE and analyzed by Coomassie staining. The profile of these eluted nonradioactive glycoproteins appeared to be the same (not shown) as that of the [^{35}S]methionine-labeled glycoproteins which were eluted from the MBP-agarose column (see Fig. 1B).

Shed glycoproteins from rat 3T3 cells were derived by washing a 75-cm^2 monolayer of rat 3T3 cells with DME and then incubating the monolayer in DME for 16 h at 37°C.

Western blot (immunoblot) analysis. The proteins from a 7.5% polyacrylamide gel were transferred to a nylon membrane (Micron Separations Inc., Westboro, Mass.). The membrane was incubated with BLOTTO (phosphate-buffered saline [PBS] solution containing 5% nonfat milk and 0.02% azide)

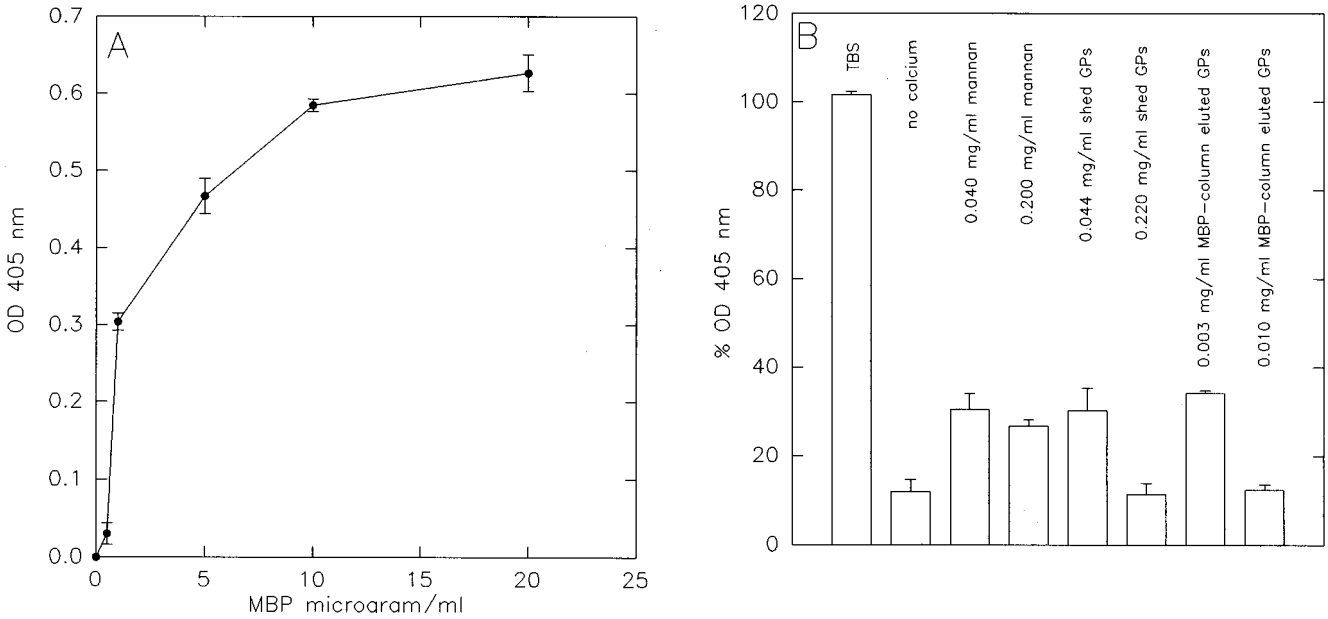

FIG. 2. ELISA demonstrating MBP binding to mammalian-stage parasites. (A) MBP binding to mammalian-stage parasites is saturable. Tissue culture-derived amastigotes and trypomastigotes were heat inactivated and allowed to adhere to ELISA wells; MBP was added at the indicated concentrations in the presence of TBS. Bound MBP was detected with a mouse MAb to MBP and a rabbit anti-murine antibody conjugated to horseradish peroxidase. OD, optical density. (B) MBP binding is inhibited by mammalian-stage glycoproteins. The ELISA was used as described above. MBP was present in all wells at 10 μg/ml in TBS. Mannan or *T. cruzi* glycoproteins were added as indicated. The binding was plotted as percent of OD_{405}. GP, glycoprotein.

overnight at 4°C and then incubated with anti-SA85-1 antibody. The membrane was then incubated with a goat anti-rabbit immunoglobulin (Ig) antibody conjugated to horseradish peroxidase, and the bound antibodies were detected by enhanced chemiluminescence according to manufacturer's instructions (Amersham, Arlington Heights, Ill.).

MBP. Recombinant human MBP was purified by mannan chromatography from tissue culture supernatants of mouse myeloma cells which express a transfected human MBP gene (37).

Enzyme-linked immunosorbent assay (ELISA). Mammalian-stage parasites from 3T3 cultures were heat killed at 55°C for 5 min (14). Parasites (10^6) were added to each well in PBS and incubated at 4°C overnight. The plates were then incubated with BLOTTO for 2 h at room temperature or longer at 4°C. Throughout the procedure, unless noted, plates were washed and reagents were incubated in TBS. The plates were then washed and incubated with MBP or control proteins for 1 h, washed and incubated with a mouse monoclonal antibody (MAb) to MBP (35), and washed and incubated with a rabbit anti-mouse Ig antibody conjugated to horseradish peroxidase (Zymed Laboratories Inc., South San Francisco, Calif.). The bound horseradish peroxidase-conjugated antibodies were detected with an ABTS Microwell Substrate System (Kirkegaard and Perry Laboratories Inc., Gaithersburg, Md.).

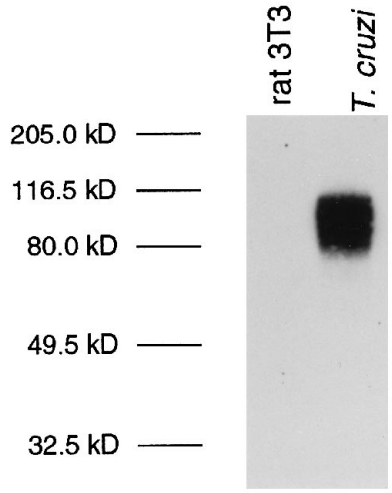

FIG. 3. SA85-1 glycoproteins are ligands of MBP. A total of 1.3 μg of *T. cruzi* glycoproteins was eluted from a MBP column with EDTA, and an equal amount of proteins derived from tissue culture supernatants of 3T3 cells was subjected to SDS-PAGE and transferred to a nylon membrane. The membrane was incubated with 10 μg of anti-SA85-1 antibody per ml, and the antibody reactivity was detected by enhanced chemiluminescence.

Flow cytometry. Parasites were incubated with human recombinant MBP or control proteins in Hanks balanced salt solution (HBSS). Unless indicated, HBSS was made with 2 mM $CaCl_2$ and without glucose and magnesium salts. Following incubation for 30 min at 0°C, the parasites were washed three times in HBSS, fixed with 4% paraformaldehyde (J. T. Baker Inc., Phillipsburg, N.J.) in PBS, washed three more times in PBS, and then incubated with an anti-human MBP MAb for 30 min at 0°C (35). The bound MAb was detected with a goat anti-mouse Ig antibody conjugated to fluorescein isothiocyanate (Tago Inc., Burlingame, Calif.). Bound SA85-1 antibodies were detected with goat anti-rabbit Ig antibody conjugated to R-phycoerythrin (Caltag Laboratories, South San Francisco, Calif.). The fixed parasites were then analyzed by flow cytometry on a FACScan (Becton Dickinson, San Jose, Calif.). A minimum of 10,000 events were collected, and the data were analyzed with ReproMan (TrueFacts Inc., Seattle, Wash.). MOPC-21, an IgG1 MAb secreted by a murine myeloma cell line, was a gift from David Lewis (University of Washington, Seattle).

α-Mannosidase from jack beans was obtained from Sigma Chemical Co. Live axenic-derived amastigotes were incubated at 37°C for 80 min in 200 μl of 150 mM NaCl, 50 mM sodium acetate, and 5 mM $CaCl_2$, with or without 0.7 U of α-mannosidase.

RESULTS

Previous studies have demonstrated that *T. cruzi* amastigotes bind MBP, and that some *T. cruzi* surface glycoproteins contain high-mannose oligosaccharides (7, 20). This suggested that *T. cruzi* surface glycoproteins may bind MBP. To investigate this possibility, lectin chromatography was performed with ^{35}S-labeled *T. cruzi* glycoproteins (16). Because the parasite spontaneously sheds its surface glycoproteins (16, 17), culture supernatants from ^{35}S-labeled amastigotes and trypomastigotes were passed over the lectin-agarose columns in the presence of calcium. Fractions were collected from the MBP column with buffers containing either 100 mM mannose (Fig. 1A) or 10 mM

EDTA (not shown); in both sets of fractions a sharp peak of radiolabeled material was observed. Previous studies using lectin chromatography have shown that tissue culture supernatant containing mammalian-stage glycoproteins bind ConA and WGA (16). To compare the binding of MBP with ConA and WGA, equal amounts of glycoproteins, derived from tissue culture supernatant, were subjected to ConA-agarose, WGA-agarose, and unconjugated-agarose chromatography, and again the collected fractions were examined for the presence of ^{35}S-labeled glycoproteins. Compared with the MBP chromatography, more ^{35}S-labeled glycoproteins bound the ConA-agarose column, while much less bound the WGA-agarose column (Fig. 1A). No radioactive material was collected from the unconjugated-agarose column (Fig. 1A).

An equal portion of each fraction collected from the MBP column with mannose was subjected to SDS-PAGE and fluorography, which revealed a major band that extended between 80 kDa and 100 kDa; a second strong band was detected at approximately 160 kDa (Fig. 1B). The SDS-PAGE results were essentially identical following collection of fractions in the presence of 10 mM EDTA (not shown). The presence of the band between 80 kDa and 100 kDa suggested that the major surface glycoproteins, with molecular masses of between 80 kDa and 100 kDa, were ligands of MBP.

An ELISA was developed to further examine the binding of MBP to *T. cruzi* and its surface glycoproteins. Heat-killed mammalian-stage parasites were used to coat the ELISA plate (14), and purified human recombinant MBP was shown to bind in a dose-dependent manner (Fig. 2A). This binding was dependent on calcium and was inhibited with mannan, confirming the specificity of the MBP interaction (Fig. 2B). In addition, 44 and 220 μg of *T. cruzi* glycoproteins per ml, derived from tissue culture supernatant, inhibited the binding by 70 and 90%, respectively (Fig. 2B). In comparison, 3 and 10 μg of glycoproteins per ml, eluted from the MBP column, inhibited the binding by 70 and 90%, respectively, indicating that the MBP chromatography enriched the inhibitory activity by more than 10-fold (Fig. 2B). The data were consistent with the possibility that the parasite MBP ligands are the surface glycoproteins and suggested that MBP may bind circulating *T. cruzi* during an infection.

The SA85-1 glycoproteins are encoded by a large gene family, and are a subset of the major mammalian-stage surface glycoproteins of 85 kDa (17, 19). To examine if SA85-1 glycoproteins were a ligand of MBP, glycoproteins derived from tissue culture supernatant were eluted from an MBP column with EDTA and subjected to Western analysis with an anti-SA85-1 glycoprotein antibody. This analysis revealed that SA85-1 glycoproteins were present in the MBP bound fraction (Fig. 3). The specificity of the antibodies was demonstrated by their lack of reactivity to proteins derived from rat 3T3 cells (Fig. 3). The data demonstrate that SA85-1 glycoproteins are one of the MBP ligands.

Previous studies have demonstrated that the SA85-1 glycoproteins were expressed by amastigotes and trypomastigotes but not epimastigotes (19). To confirm that SA85-1 glycoproteins are expressed by amastigotes and trypomastigotes but that MBP binds to amastigotes only, a mixed population of amastigotes and trypomastigotes was analyzed for MBP and anti-SA85-1 antibody binding. A subpopulation of parasites which bound both MBP and anti-SA85-1 antibody was identified (Fig. 4A, region 1). Analyzing these data by cell size revealed that these double positive parasites were amastigotes (the population with larger forward scatter), whereas the SA85-1 glycoprotein-expressing parasites were both amastigotes and trypomastigotes (Fig. 4B). Fluorescent microscopy

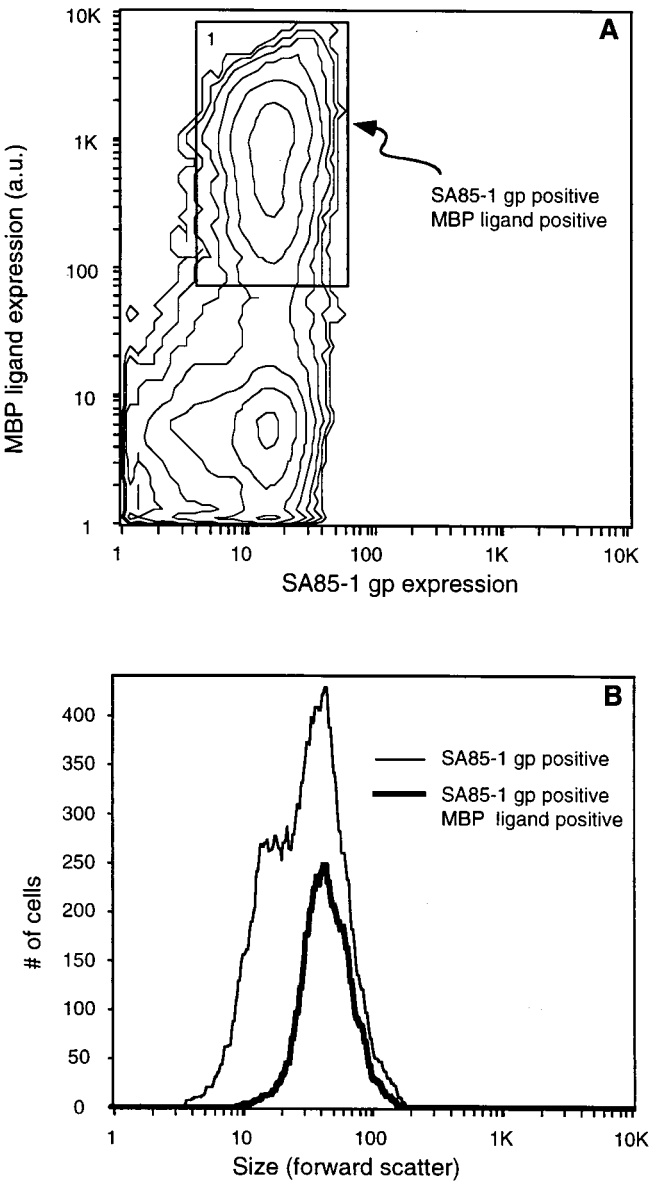

FIG. 4. Both amastigotes and trypomastigotes express SA85-1 glycoproteins, but only amastigotes bind MBP. (A) A subset of mammalian-stage parasites expresses SA85-1 glycoproteins and MBP ligands. Live parasites were washed and then incubated with 10 μg of MBP per ml at 0°C for 30 min. The parasites were then washed three times, fixed with 4% paraformaldehyde, washed, and then incubated with a murine MAb to MBP and a rabbit antibody to SA85-1 surface glycoproteins. The parasites were then washed and incubated with both anti-rabbit Ig antibody conjugated to fluorescein isothiocyanate and anti-murine antibody conjugated to R-phycoerythrin. SA85-1 glycoprotein expression is represented on the *x* axis, and MBP binding is represented on the *y* axis in arbitrary units (a.u.). The cells in region 1 express both the SA85-1 glycoproteins and the MBP ligands. (B) The parasites which express both SA85-1 glycoproteins and MBP ligands are amastigotes. Cell size is represented on the *x* axis, and number of cells is represented on the *y* axis. The cells in region 1 of panel A are represented by the heavy line, and the cells in panel A which express SA85-1 glycoproteins are represented by the light line. gp, glycoprotein.

confirmed that amastigotes bind MBP and that trypomastigotes do not (Fig. 5). The data suggest that amastigotes modify the SA85-1 surface glycoproteins differently than trypomastigotes and that these differences lead to developmental stage-specific interactions with MBP and possibly other host lectins.

To further analyze the interaction of MBP with amastigotes,

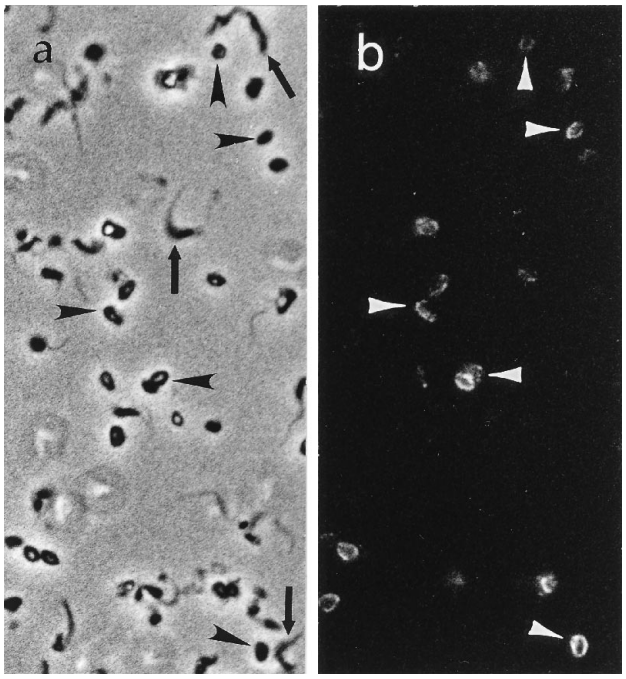

FIG. 5. Phase-contrast microscopy and fluorescent microscopy demonstrate that MBP binds amastigotes. Mammalian-stage parasites were washed and incubated with MBP (10 μg/ml); this was followed by incubation with a mouse anti-MBP MAb and incubation with an anti-mouse immunoglobulin antibody conjugated to fluorescein isothiocyanate. After staining, the parasites were allowed to adhere to a glass slide and were viewed by phase-contrast (a) or fluorescent (b) microscopy. Arrowheads indicate amastigotes in phase-contrast and fluorescent images; arrows indicate trypomastigotes in phase-contrast image. Magnification, ×2,590.

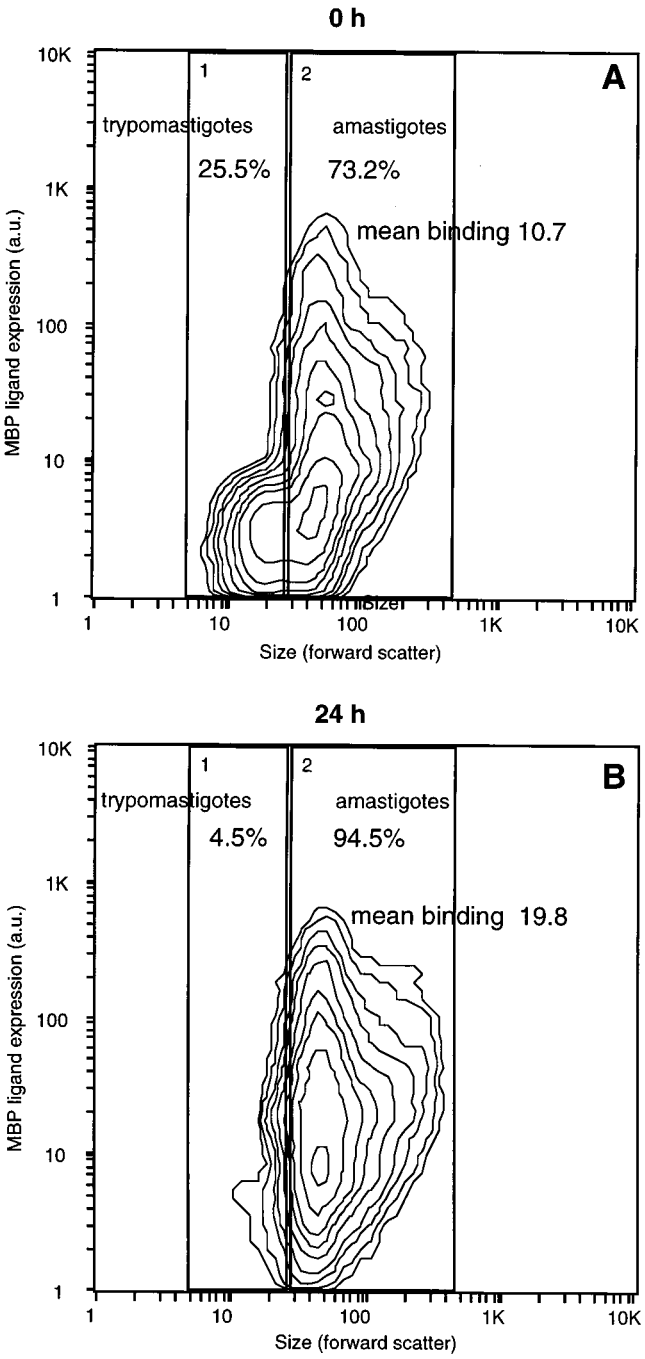

FIG. 6. Amastigotes generated in axenic culture bind MBP. (A) At 0 h in axenic conditions, amastigotes bind MBP, whereas trypomastigotes do not. MBP binding was performed as described in the legend for Fig. 4. The *x* axis represents relative size (inferred from forward scatter), and the *y* axis represents MBP binding in arbitrary units (a.u.). Gates 1 and 2 were drawn to demonstrate the two populations defined by relative size. (B) Conversion of trypomastigotes into amastigotes. After 24 h in axenic conditions, the trypomastigotes have converted into amastigotes, and the amastigotes demonstrated increased binding of MBP. Flow cytometry analysis is as described above.

homogeneous populations of amastigotes were generated by incubation of trypomastigotes in LIT medium for 24 h. Amastigotes generated by this procedure are morphologically and antigenically identical to amastigotes that develop intracellularly (22). To demonstrate that these amastigotes bind MBP, a mixed population of amastigotes and trypomastigotes from 3T3 tissue culture supernatants was converted to amastigotes in LIT medium, and the expression of the MBP ligand was monitored by flow cytometry. At 0 h, both amastigotes and trypomastigotes were present (Fig. 6A), and only the amastigotes bound MBP (Fig. 6A). After a 24-h incubation in LIT medium, the cells were >99% amastigotes as shown by phase-contrast microscopy (data not shown), and 95% amastigotes as shown by flow cytometry forward scatter analysis (Fig. 6B). Flow cytometry revealed that the amastigote population uniformly bound MBP (Fig. 6B) and that the mean fluorescent intensity of MBP binding increased from 10.7 in the amastigotes at 0 h (Fig. 6A, region 2) to 19.8 in the amastigotes at 24 h (Fig. 6B, region 2). The data demonstrated that axenically derived amastigotes express the MBP ligand and further demonstrated that the MBP ligand is developmentally regulated.

A common component of MBP ligands and the *T. cruzi* high-mannose-type oligosaccharides is α-linked mannose (7, 33). In an attempt to further characterize the *T. cruzi* ligand of MBP, axenically derived amastigotes were treated with α-mannosidase and examined for their ability to bind MBP. α-Mannosidase treatment diminished MBP binding (Fig. 7) compared with mock treatment (Fig. 7) or no treatment (data not shown). These data indicated that α-linked mannose was an essential component of the *T. cruzi* MBP ligand, and that expression of specific α-linked mannose oligosaccharides was restricted to amastigotes.

T. cruzi spontaneously sheds its surface glycoproteins (16, 17) and, therefore, may rapidly release the MBP ligand and bound MBP. To investigate the stability of bound MBP on the

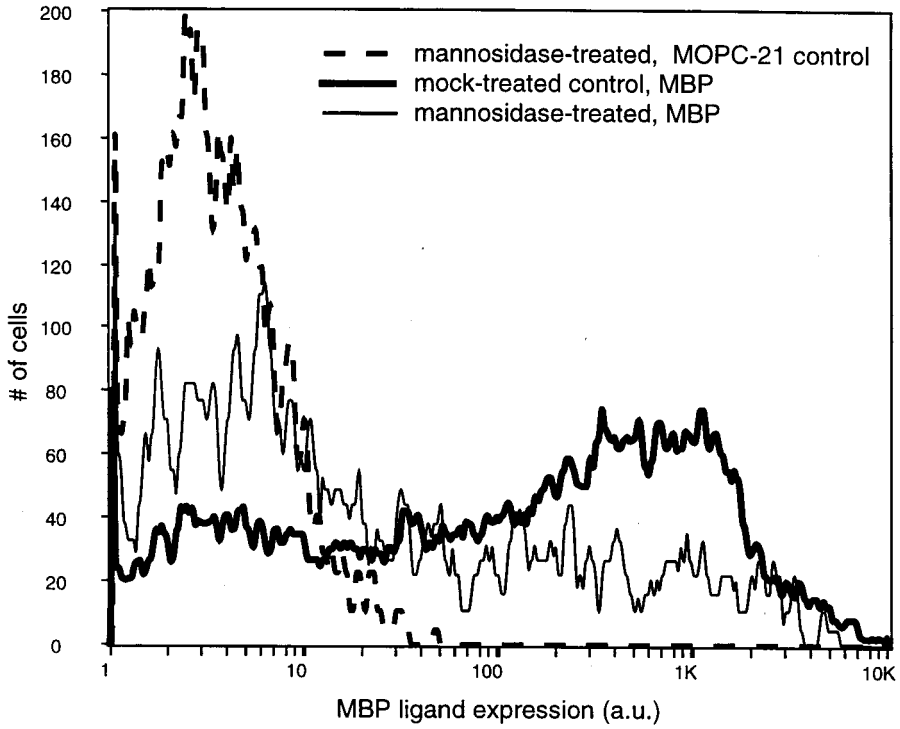

FIG. 7. Mannosidase treatment of amastigotes inhibits MBP binding. Axenically derived amastigotes were incubated at 37°C for 80 min in buffer only (mock-treated control) or in buffer with 0.7 U of α-mannosidase. These parasites were then stained with MBP and analyzed by flow cytometry analysis as described in the legend for Fig. 4. As an irrelevant protein control in one staining reaction, MOPC-21 (10 μg/ml) replaced MBP (10 μg/ml) during the primary incubation.

amastigote surface, axenically derived amastigotes were incubated with MBP at 0°C, washed to remove unbound MBP, shifted to 37°C, fixed with paraformaldehyde at different times, and examined for the presence of bound MBP. Over 1 h, although some loss of binding was observed, significant amounts of bound MBP were still detected (Fig. 8). These data suggest that the association of MBP with amastigotes is stable and that this interaction may contribute to their opsonization and cellular invasion during *T. cruzi* infection.

DISCUSSION

T. cruzi is an obligate intracellular parasite that is capable of infecting many mammalian species and of proliferating in a wide variety of host cells. Amastigotes are the intracellular replicative form of the parasite. Recent reports have demonstrated, however, that amastigotes circulate in the blood of infected mammals, are resistant to complement, and are capable of invading mammalian cells both in vitro and in vivo (3, 15, 22). These experimental findings indicate that amastigotes, in addition to trypomastigotes, have a blood-borne phase in the life cycle of the parasite. Exposure of amastigotes to serum proteins raises the possibility that serum factors may act as opsonins and enhance clearance of the parasite.

One molecule in the serum which may act as an opsonin is MBP, which is a carbohydrate recognition molecule involved in first-line host defense (33). We report here that MBP is able to bind to amastigote ligands that include the SA85-1 glycoproteins and other related surface glycoproteins (Fig. 1, 3, and 4). The MBP-amastigote interaction is stage specific (Fig. 4 to 6) and mannan inhibitable (Fig. 2B) and requires calcium (Fig. 2B). Mannosidase treatments significantly reduce this interaction (Fig. 7). Trypomastigotes also express SA85-1 glycoproteins but do not bind MBP (Fig. 4). These findings suggest an important role for stage-specific glycosylation in *T. cruzi*-host interactions.

MBP is a collectin; collectins are molecules that have a collagen tail and a carboxy-terminal lectin domain (24). The collagen tail is a ligand for the collectin receptor which is found on mammalian cells, including monocytes, endothelial cells, fibroblasts, and some epithelial cells (24); the lectin domain recognizes carbohydrates on *Leishmania* spp., yeast, bacteria, influenza virus, and human immunodeficiency virus (13, 33). The hepatic synthesis of MBP is increased as part of the acute-phase response to infections, which permits MBP to participate in immune surveillance within minutes of an infectious challenge (33). The interaction of MBP with microorganisms may result in either the clearance of the microorganisms by cells that express collectin receptors (33) or the initiation of complement activation via the classical pathway, the alternative pathway, or the novel MBP-associated protease pathway (23, 25, 36). MBP may provide immediate protection against microbial pathogens during the 1- to 3-day lag period required for induction of specific clonal immunity. To date no studies have defined a role for MBP during parasitic infections.

Ultrastructural studies have indicated that *T. cruzi* amastigotes and trypomastigotes bind to different regions of mammalian cells, which suggests that the two forms of the parasite express different surface components that may lead to different mechanisms of adhesion and invasion of cells (26). Evidence that amastigotes and trypomastigotes express different surface components comes from studies that demonstrated differential binding of plant lectins (29). In addition, the *T. cruzi trans*-sialidase, which is expressed by trypomastigotes and not amastigotes, decorates trypomastigotes with sialic acid ligands; some of these sialic acid ligands have been shown to participate

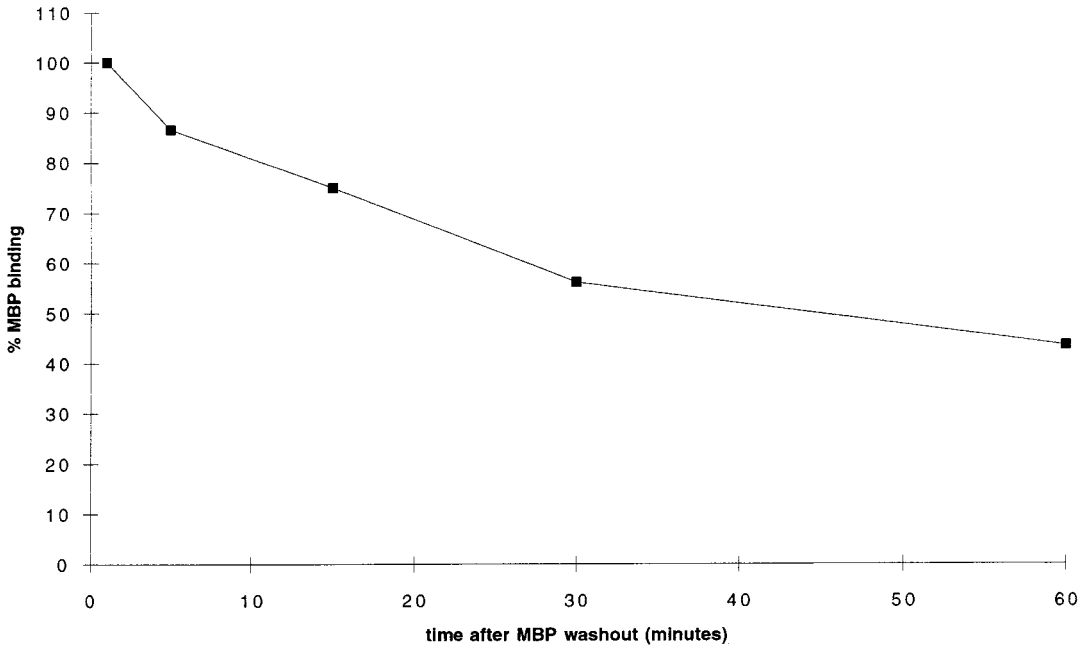

FIG. 8. MBP stably binds to the surface of live amastigotes. Live amastigotes were incubated with MBP (10 μg/ml) for 30 min at 0°C, washed, and placed at 37°C for different periods of time before fixation, staining, and processing for flow cytometry analysis as described in the legend for Fig. 4. The *x* axis represents time, and the *y* axis represents percent MBP binding.

in trypomastigote invasion of mammalian cells (34). Also, the mannose receptor, which has binding specificities similar to those of the MBP, has been shown to facilitate the uptake of amastigotes into macrophages (20). The results presented here suggest that differential glycosylation of amastigote and trypomastigote surface glycoproteins directs the binding of human serum MBP to amastigotes (Fig. 3 to 6) and that this interaction may contribute to amastigote-specific adhesion interactions with mammalian cells.

The binding of MBP to amastigote surface glycoproteins indicates that these glycoproteins are modified with sugars that have hexose rings in which the hydroxyl groups of the third and fourth carbons are in the equatorial position; these sugars may include mannose, *N*-acetylglucosamine, glucose, and fucose but exclude galactose and sialic acid (41). The decrease in binding of MBP after treatment with α-mannosidase indicates that mannose is a critical component of the ligand and suggests that the ligand is a high-mannose-type oligosaccharide linked to surface glycoproteins (Fig. 7).

Previous studies have indicated that all the developmental stages of *T. cruzi* express surface glycoproteins conjugated to high-mannose-type oligosaccharides, and that amastigotes express high-mannose-type oligosaccharides distinct from those expressed by trypomastigotes and epimastigotes (7). In addition, axenically derived amastigotes and intracellularly derived amastigotes express distinct high-mannose-type oligosaccharides (9). Our data demonstrate strong binding of MBP only to amastigotes and demonstrate differences in the degree of binding of MBP to axenically derived amastigotes (mean fluorescent intensity of 19.8) and intracellularly derived amastigotes (mean fluorescent intensity of 10.7) (Fig. 6). The data suggest that the differences in MBP binding to the parasite forms may be explained by variations in high-mannose-type oligosaccharide structures.

Surface glycoproteins isolated by MBP chromatography included one of the major surface glycoprotein families, the SA85-1 glycoproteins (Fig. 1B and 3). The surface glycoproteins and the SA85-1 glycoproteins are members of a superfamily of glycoproteins which display extensive diversity at the amino acid level. This diversity may contribute to the parasite's ability to adhere to and invade a variety of cell types. Previous studies have indicated that 85-kDa surface glycoproteins participate in adhesion interactions with carbohydrates, fibronectin, and laminin (5, 12, 27). Although amino acid diversity may play a role in adhesion of amastigotes to cells, this report suggests that the stage-specific glycosylation of surface proteins may provide an amastigote-specific ligand for collectins like the MBP. MBPs, which are found in all mammalian sera, may function to recognize the major surface glycoproteins of amastigotes and thereby enhance clearance into a variety of cells that express collectin receptors. This clearance mechanism may enable the amastigotes to achieve a different cellular tropism than trypomastigotes. In addition, extracellular amastigotes that bind collectins may initiate complement deposition and complement-mediated inflammation in the tissues. This complement-mediated inflammation may contribute to the chronic inflammation affecting a variety of tissues in Chagas' disease.

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