Trypanosoma cruzi Amastigote Adhesion to Macrophages Is Facilitated by the Mannose Receptor

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Summary

Trypanosoma cruzi is an obligate intracellular protozoan parasite. The mammalian stage of the parasite life cycle describes amastigotes as an intracellular form that replicates, and trypomastigotes as an extracellular form that disseminates and invades cells. Recent studies, however, have demonstrated that amastigotes circulate in the blood of infected mammals and can invade mammalian cells. In this report, a *T. cruzi* surface glycoprotein gene, SA85-1.1, was expressed as an immunoglobulin chimera, and this recombinant globulin was used to screen normal mouse tissues for adhesive interactions. This approach identified a subset of macrophages in the skin and peripheral lymph node that bind the *T. cruzi* surface glycoproteins through the mannose receptor. To further examine the *T. cruzi* mannose receptor carbohydrate ligands, the interaction between *T. cruzi* and the mannose-binding protein, a mammalian lectin with similar carbohydrate binding specificities as the mannose receptor, was examined. These studies demonstrated that the mannose-binding protein recognized amastigotes, but not trypomastigotes or epimastigotes, and suggested that amastigotes would also be recognized by the mannose receptor. Therefore, amastigote adhesion to macrophages was investigated, and these experiments demonstrated that the mannose receptor contributes to amastigote adhesion. The data identify the first mammalian lectins that bind to *T. cruzi*, and are involved in *T. cruzi* invasion of mammalian cells. The data suggest that amastigotes and trypomastigotes may have developed different mechanisms to adhere to and invade host cells. In addition, it has been established that IFN-γ–activated macrophages express low levels of the mannose receptor and are trypanocidal; this suggests that the interaction between amastigotes and the mannose receptor enables amastigotes to increase their adherence with a population of macrophages that are nontrypanocidal and permissive for their intracellular replication.

Trypanosoma cruzi, which causes Chagas Disease or American Trypanosomiasis, is an obligate intracellular protozoan parasite of mammals. Infection frequently begins when reduviid bugs deposit metacyclic trypomastigotes on the skin. The parasites penetrate the skin through abrasions, and disseminate in the lymphatics and circulation (1). Trypomastigotes may transform into amastigotes in the extracellular milieu (2), and recent experiments indicate that both amastigotes and trypomastigotes invade mammalian cells (3). After the initial invasion of the cells, parasites are found within lysosomes, from which they escape to replicate in the cytoplasm (4). Following replication, some amastigotes transform into trypomastigotes. Both amastigotes and trypomastigotes are released from dying cells to potentially invade other cells. During the infection, parasites are found proliferating within macrophages; IFN-γ–activated macrophages, however, are trypanocidal (5).

The macrophage mannose receptor (MR)[1], a C-type lectin, is a membrane glycoprotein that is constitutively recycled between the plasma membrane and the endosomal compartment (6). The binding of ligands to the MR is calcium dependent, and sensitive to pH; this pH sensitivity facilitates the release of ligands in the endosomal compartment (6). The MR preferentially binds to glycoconjugates terminating in mannose, fucose, or *N*-acetylglucosamine (6). Few host glycoconjugates have these terminal carbohydrates, whereas many microorganism glycoconjugates do, which enables the MR to bind microorganisms and inter-

[1]*Abbreviations used in this paper:* LIT, liver infusion/tryptone media; MBP, mannose-binding proteins; m.o.i., multiplicity of infection; MR, mannose receptor; Rg, recombinant globulin; RPMI-BH, RPMI with 0.2% BSA and 10 mM Hepes; TBS, tris-buffered saline with 2 mM $CaCl_2$ and 2 mM $MgCl_2$.

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nalize them into the endosomal compartment of macrophages (6).

Mannose-binding proteins (MBP) are C-type lectins that circulate in the blood of mammals. MBPs have a similar carbohydrate binding specificity as the MR, as MBPs also bind to glycoconjugates terminating in mannose, fucose, or *N*-acetylglucosamine (7). The MBPs may be considered as "ante" antibodies; they distinguish between the oligosaccharides that decorate a variety of pathogenic microorganisms (including yeasts, bacteria, and viruses) from self glycoproteins (8). The analogy of MBP to antibodies extends to the ability of MBP to directly opsonize microorganisms or to initiate activation of the complement cascade (9).

Studies have indicated that the *T. cruzi* surface glycoproteins facilitate adhesion and invasion of mammalian cells (10–18). Many of the parasite surface glycoproteins are members of a superfamily defined by homology to sialidase (19–27). A minority of these glycoproteins function as the parasite sialidase/*trans*-sialidase (19, 24, 26, 27). We have previously defined the SA85-1 (surface antigen, 85 kD) glycoprotein family that is both a subset of the sialidase superfamily, and a subset of the major amastigote and trypomastigote surface glycoproteins of ~85 kD (23, 28). Biochemical analysis has indicated that the SA85-1 glycoproteins lack sialidase/*trans*-sialidase activity, raising the possibility that they function as the previously described adhesion molecules, capable of binding to the mammalian cell surface (29). To investigate this possibility, we have expressed two SA85-1 genes, SA85-1.1 and SA85-1.2, as immunoglobulin chimeras to identify tissues and cells of tissues that may bind these *T. cruzi* surface glycoproteins. We report here that this approach has defined an interaction between amastigotes and both the macrophage MR and the MBP. The interaction with the MR facilitates the adhesion of amastigotes to macrophages. IFN-γ down-regulates MR expression; this suggests that the interaction between amastigotes and the MR will favor parasite invasion and replication in macrophages that have not been activated by IFN-γ.

Materials and Methods

T. cruzi. *T. cruzi* CL strain subclone three was used (30). Amastigotes and trypomastigotes were obtained from culture supernatants of infected rat 3T3 cells grown in DME (Bio Whittaker Inc., Walkersville, MD) with 10% calf serum (Hyclone Laboratories Inc., Logan, UT). In these supernatants, the amastigote/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 liver infusion/tryptone medium (LIT) at 37°C for 24 h (3). These axenically derived amastigotes were pelleted by centrifugation at 1,000 *g* for 5 min (3). Before use in subsequent experiments, all parasites were washed three times in media without supplemental serum. LIT media was prepared by mixing 0.025 g/l hemin (bovine, type I; Sigma Chemical Co., St. Louis, MO) dissolved in 5 ml of 0.01 M NaOH with a 900 ml solution containing 4.0 g NaCl, 0.4 g KCl, 8.0 g Na_2HPO_4, 2.0 g glucose, 5.0 g Liver Infusion (Difco Laboratories Inc., Detroit, MI), and 5.0 g tryptose (Difco Laboratories Inc.). These amastigotes were contaminated with <1% trypomastigotes.

Mice and Rabbits. Female BALB/c mice were purchased from Bantin and Kingman, Inc. (Fremont, CA). New Zealand White rabbits were purchased from R and R Rabbitry (Stanwood, WA).

Reagents. mAbs F4/80 (the gift of C. B. Wilson, University of Washington, Seattle, WA) and N418 were used in the form of culture medium from exhaustively grown hybridomas (31, 32); whereas, mAbs M1/70 and M3/84 were purified from culture supernatants (33, 34). mAb MOMA-2 supernatant was purchased from Harlan Bioproducts for Science, Inc. (Indianapolis, IN) (35). SER-4 mAb was the gift of Dr. P. Crocker (John Radcliffe Hospital, Oxford, UK) (36). The anti-mouse MR antibody was the gift of Dr. P. Stahl (Washington University, St. Louis). The anti-human MBP mAb was the gift of Dr. R. A. B. Ezekowitz (Harvard University, Boston) (37). Human IgG mAb (Sigma Chemical Co.) and rat IgG mAb (Zymed Laboratories, Inc., South San Francisco, CA) secreted by myeloma cell lines were used as immunohistochemical controls. MOPC-21 mAb, secreted by a murine myeloma cell line was a gift from D. Lewis (University of Washington, Seattle, WA).

Anti–SA85-1.1 antibodies were produced by immunization with SA85-1.1 protein expressed in *E. coli* and isolated from inclusion bodies as described (23). A rabbit was repeatedly immunized with 100 μg of this SA85-1.1 protein, and anti–SA85-1.1 antibodies were affinity purified from rabbit serum with a SA85-1.1 affinity column as described (23). These anti–SA85-1 antibodies specifically recognize SA85-1 recombinant proteins in ELISA and Western blots (not shown). To generate the anti–SA85-1 mAb, a mouse was immunized with the same SA85-1.1 protein. Standard procedures were followed to produce hybridomas and to isolate the resulting mAb (38). The anti–SA85-1 mAb recognized both trypomastigotes and amastigotes in flow cytometry, and recombinant SA85-1 proteins in ELISA (not shown). The anti–SA85-1 mAb and the polyclonal anti–SA85-1 antibodies both appear to detect a similar broad band in Western analysis of *T. cruzi* mammalian stage lysates following one-dimensional SDS-PAGE (not shown).

FITC-conjugated goat anti–human antibodies were purchased from Tago Immunologicals (Burlingame, CA) and Jackson Immunologics (West Grove, PA). FITC-conjugated goat anti–rabbit and anti–rat antibodies were purchased from Tago. Biotin-conjugated goat anti–rat and anti–hamster were purchased from Caltag Labs (South San Francisco, CA). Texas Red-conjugated strepavidin was purchased from Molecular Probes (Eugene, OR).

To create SA85-1–immunoglobulin fusion proteins, PCR was used to amplify the SA85-1.1 and SA85-1.2 DNA from plasmids as previously described (23); synthetic oligonucleotides designed to allow fusion to the human IgG1 artificial spliced donor sequences were used (39). To amplify the DNA, 30 cycles were conducted, consisting of 30 s at 94°C, 2 min at 45°C, and 3 min at 72°C using the buffer recommended by the enzyme vendors (Cetus, Emeryville, CA). To construct SA85-1.1 recombinant globulin (Rg), the forward primer was GCGAAGCTTCCGCCGAGGCCCAACATG, and the reverse primer was GCGCAGATCTCTCCCGTACGCGGAAAC; the sequence allowed the SA85-1.1 fragment to be inserted as a HindIII to BglII fragment into HindIII- and BamHI-digested IgG expression vector. To facilitate the secretion of the SA85-1.2 immunoglobulin fusion protein, the SA85-1.2 DNA fragment was fused to the CD5 secretory signal sequence in the immunoglobulin expression vector. To construct SA85-1.2 Rg, the forward primer was GCGC-

CGGTACCGTCGACATTGACGGGTGTC, and the reverse primer was CGCGAGATCTCTCCCGTACGCGGAACCAGC; the sequence allowed the SA85-1.2 fragment to be inserted as a KpnI to BglII fragment into KpnI- and BamHI-digested vector.

The SA85-1 Rg expression plasmids were transfected into COS cells using DEAE dextran as previously described (40). 24 h after transfection, cells were trypsinized, seeded onto fresh 100-mm dishes, and allowed to grow for 7 d in 10 ml of DME. On the fourth day 10 additional ml of DME was added. Supernatants were harvested, centrifuged to remove cells and debris, and passed over a protein A column (Repligen, Cambridge, MA). The SA85-1 Rgs were eluted from the column with citrate buffer, pH 3.0, dialyzed into PBS, and stored at 4°C.

Radiolabeled SA85-1 Rgs were produced by the addition of 0.25 mCi of [^{35}S]methionine and [^{35}S]cysteine (NEN, Boston, MA) in DME lacking methionine, to COS cell cultures 24 h after transfection with expression plasmids. After overnight incubation the supernatants were harvested, cells and debris were removed by centrifugation, incubated with protein A–Sepharose (Pharmacia LKB Biotechnology Inc., Piscataway, NJ), washed, and eluted with SDS-PAGE sample load buffer with and without DTT.

The SA85-1.1 Rg with a mutated Fc domain to prevent binding to Fc receptors was constructed by digesting SA85-1.1 Rg with MluI and BglII, and inserting the SA85-1.1 DNA fragment into the expression vector with the mutated Fc domain (41, 42).

Recombinant human MBP (the gift of R. A. B. Ezekowitz, Harvard University, Boston) was purified by mannan chromatography from tissue culture supernatants of mouse myeloma cells that express a transfected human MBP gene (43).

Carbohydrates were purchased from Sigma Chemical Co.

12-mm round coverslips (Fisher Scientific, Pittsburgh, PA) were washed by incubation in Chromerge (Manostat Corp., New York, NY), followed by rinsing with water, and storage in 95% ethanol (44).

Acid-washed coverslips, to be derivatized with mannan or BSA, were placed in 24-well plastic tissue culture plate (Corning Glass Works, Corning, NY) and 0.3 ml of 0.1 mg/ml poly-L-lysine was added. After 30-min incubation at room temperature, the coverslips were washed and incubated with 2.5% glutaraldehyde for 15 min at room temperature. The coverslips were then washed, aspirated dry, and 0.3 ml of PBS containing either 50 mg/ml BSA or mannan with 10 mg/ml 1-ethyl-3-(di-methyl-amino-propyl) carbodiimide was added to the coverslips. After 60 min at room temperature, the coverslips were washed and incubated in PBS with 1.5 g/100 ml glycine and 1 mg/ml BSA at 4°C overnight. Before use the coverslips were washed with PBS (44).

Immunohistochemical Procedures. The techniques used for light immunohistochemistry have been described in detail (45). For light microscopic immunohistochemical analyses, frozen sections of tissue were mounted on amino alkylsilane-treated slides and allowed to air dry for at least 30 min before fixation (20 min in acetone at −20°C) (46). After rinsing in Tris-buffered saline with 2 mM $CaCl_2$ and 2 mM $MgCl_2$ (TBS), the sections were incubated for 1 h at room temperature with antibodies or recombinant proteins (20 μg/ml or 2 μg/slide). Antibodies and SA85-1 recombinant proteins were diluted into TBS with 10% normal mouse serum and 10% normal goat serum. After several washes in TBS, slides were incubated with fluorochrome-conjugated antibodies, or rabbit anti–SA85-1.1 antibodies for 1 h at room temperature. If necessary slides were washed again with TBS, and incubated with Texas red-conjugated strepavidin or FITC-conjugated anti-rabbit antibodies. Slides were washed a final time with TBS, mounted with coverslips, and photographed using a Microphot FXA (Nikon, Tokyo, Japan).

In experiments investigating the effect of cation concentrations, slides with LNs were fixed with acetone, washed with TBS, and incubated at room temperature for 1 h with TBS containing 10% mouse serum, 10% goat serum, and 10% BSA. Slides were then washed with TBS containing the appropriate concentration of cation, and then incubated with SA85-1.1 Rg diluted in TBS containing the appropriate concentration of cation. The remainder of the staining was performed as described above. Similarly, to determine the effects of pH on the binding of SA85-1.1 Rg, slides were initially incubated with TBS containing 10% mouse serum, 10% goat serum, and 10% BSA. Slides were then washed in glycine-buffered saline of the appropriate pH, and SA85-1.1 Rg was diluted in the glycine-buffered saline of the appropriate pH .

Biochemical Procedures. Lysates of mammalian stage parasites were prepared by removing culture media from infected rat 3T3 cells; debris was removed with a centrifugation at 50 *g* for 5 min. Parasites were then pelleted by centrifugation at 2,000 *g* for 15 min, and washed three times in serum-free media. The final pellet was then resuspended at 2×10^8 amastigotes and 2×10^8 trypomastigotes/ml in 10 mM tris, pH 8.0, 1% NP-40, PMSF 100 μg/ml, pepstatin 1 μg/ml, and leupeptin 1 μg/ml. The suspension was incubated on ice for 10 min, and then centrifuged at 16,000 *g* for 10 min at 4°C.

PNGase F treatment was performed by mixing 10 μl of lysate with 2 μl 10× denaturation buffer (New England Biolabs, Beverly, MA), and 8 μl of water, and heated at 100°C for 10 min. The reaction was cooled on ice, and then 3 μl of 10× reaction buffer (New England Biolabs), 3 μl of 10% NP-40, and 4 μl of PNGase F (4000 U; New England Biolabs) was added. The reaction was then incubated at 37°C for 16 h. Half of the reaction was subjected to SDS-PAGE and Western analysis. The mock sample was prepared in the same manner in parallel, except that PBS was substituted for the 4 μl of PNGase F.

SDS-PAGE was performed following standard procedures (47). Pore exclusion limit electrophoresis gels were 4–15% acrylamide (3–12% glycerol) gradient in 0.5× tris-buffered EDTA (48). Electrophoresis was continued for 18 h at 4°C (20 V/cm) (48). Thyroglobulin (669 kD), ferritin (440 kD), catalase (232 kD), aldolase (158 kD), and bovine serum albumin (67 kD) were used as molecular size standards (Pharmacia).

Western analysis followed protocols for semidry transfer (49). Bound antibodies were detected using a rabbit anti–mouse IgG antibody conjugated to horseradish peroxidase (Zymed Laboratories, Inc.), followed by enhanced chemiluminescence (Amersham Corp., Arlington Heights, IL) and exposure to x-ray film, or with an anti-mouse IgG antibody conjugated to alkaline phosphatase (Promega Corp., Madison, WI), and reacted with bromochloroindolyl phosphate/nitro blue tetrazolium (Bio-Rad Labs., Richmond, CA) (49). The anti–SA85-1 mAb was used at 10 μg/ml. The anti–SA85-1 antibodies were used at 10 μg/ml.

Gels for autoradiograms were incubated with Enhance (NEN) according to the manufacturers instructions.

Flow Cytometry. Parasites were incubated with human recombinant MBP or control proteins in 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, NJ) in PBS, washed three more times in PBS, and then incubated with an anti-human MBP mAb for 30 min at 0°C (37). The bound mAb

was detected with a goat anti-mouse Ig antibody conjugated to FITC (Tago Inc., Burlingame, CA). The fixed parasites were then subjected to flow cytometry on a FACScan (Becton Dickinson, San Jose, CA). A minimum of 10,000 events were collected and the data was analyzed with ReproMan (TrueFacts Inc., Seattle, WA).

Macrophages. J774E macrophages were the gift of Dr. P. Stahl (50). J774A.1 macrophage were the gift of Dr. W. Van Voorhis (University of Washington, Seattle). Both J774 cell lines were grown in DMEM α-media (GIBCO BRL, Gaithersburg, MD) supplemented with 10% FCS (Hyclone). The macrophages were grown on 12-mm coverslips for adhesion assays.

Human macrophages were derived from peripheral blood monocytes. 15 ml of blood from human volunteers, mixed with 0.3 ml of 10% K_2EDTA, was combined with an equal volume of normal saline, and was then underlaid with 10 ml Ficoll-Paque (Pharmacia) and centrifuged at 500 *g* at 18°C for 20 min. The PBMC at the interface were removed, mixed with HBSS, and centrifuged at 1200 rpm at 18°C for 10 min. The PBMC were washed with HBSS and centrifuged as above three more times. The PBMC were resuspended in serum-free media (GIBCO BRL) at 2.5 × 10^6 cells/ml, and 10 ml of this cell suspension was placed in Teflon beakers (Savillex Corp., Minnetonka, MN), and cultured at 37°C in a 5% CO_2 incubator for 3–7 d. 5 ml of media was replaced on alternate days. On the day of each experiment, loosely adherent PBMC were discarded by swirling the beakers and pouring off the media. Then 5 ml of ice-cold RPMI containing 0.2% BSA and 10 mM Hepes (RPMI-BH) was added to the beakers, incubated on ice for 30 min, and the adherent PBMC were loosened by repeated pipetting with a control drop pasteur pipette. The cells were collected and washed twice with RPMI-BH. The PBMC were resuspended at 1 × 10^5/ml and 100 μl were layered onto the surface of a 12-mm acid-washed coverslip. After 1 h the coverslips were washed with RPMI-BH six times to remove loosely adherent PBMC (44).

Thioglycollate-elicited mouse peritoneal macrophages were isolated by injecting Balb/c mice with 1 ml of Brewer's Thioglycollate (Difco Laboratories, Inc.) in the peritoneum. 3 d later the peritoneum was lavaged with 5 ml PBS to obtain the macrophages. The macrophages were then washed three times, resuspended in RPMI containing 10% FCS at 1 × 10^5 macrophages/ml, and 100 μl of cell suspension was plated onto 12-mm acid-washed coverslips and cultured at 37°C for 1 h. The coverslips were then washed three times to remove loosely adherent cells.

Adhesion Assays. Macrophages adherent on 12-mm glass coverslips were placed in individual wells containing RPMI only. Amastigotes were added in RPMI in the absence or presence of inhibitors of the MR (mannan or mannose-BSA; Sigma Chemical Co.) or controls (glucan or galactose-BSA; Sigma Chemical Co.). To permit the addition of pure populations of amastigotes, axenically derived amastigotes were used, which were isolated as described above (3). After 1–2 h at 37°C, the free amastigotes were removed by washing and the cells were fixed, made permeable, and stained with 4′,6-diamidino-2-phenylindole (Sigma Chemical Co.). In the fluorescent microscope (Microphot Fx, Nikon) 200 macrophages were scored for association with amastigotes. Amastigotes were detected by the characteristic fluorescent staining pattern of the kinetoplast. Phase microscopy was used to confirm that the amastigotes were in contact with a macrophage. This assay did not attempt to differentiate between amastigotes that have adhered to the macrophage surface or invaded the macrophage. An investigator not informed of sample treatment counted each sample in triplicate. Macrophages were scored for being associated with amastigotes, and for being associated with greater than one amastigote.

Statistical Methods. Data are expressed as mean ± standard error of the mean. Differences between experimental groups were assessed by a paired two-sample Student's *t* test. Statistical testing was accomplished with the Microsoft Excel 5.0 statistical package (Microsoft Corporation, Redmond, WA).

Results

SA85-1 Surface Proteins are Glycosylated and Expressed as Dimers. To begin to characterize the glycosylation of the SA85-1 glycoproteins, mammalian-stage parasite lysates were subjected to PNGase F (peptide: *N*-glycosidase F) treatment or mock treatment, followed by SDS-PAGE and Western analysis with an anti–SA85-1 mAb (Fig. 1 *a*). The data indicated that the glycosidase removed 20 kD of N-linked oligosaccharides from the SA85-1 glycoproteins.

To further examine the structure of the SA85-1 glycoproteins, mammalian stage lysates and culture supernatants containing spontaneously shed mammalian stage surface glycoproteins were subjected to native PAGE (pore exclusion limit analysis) and SDS-PAGE followed by Western analysis. Native PAGE demonstrated that the SA85-1 glycoproteins were expressed and shed as 200-kD dimers (Fig. 1 *b*); SDS-PAGE revealed that the SA85-1 glycoproteins migrated at 100 kD using nonreducing and reducing conditions (Fig. 1 *c*). Together the data suggested that the SA85-1 glycoproteins are synthesized and secreted as noncovalent dimers.

Preparation of Soluble SA85-1 Immunoglobulin Fusion Proteins. To investigate the interactions of the SA85-1 glycoproteins with mammalian tissue, two SA85-1 genes

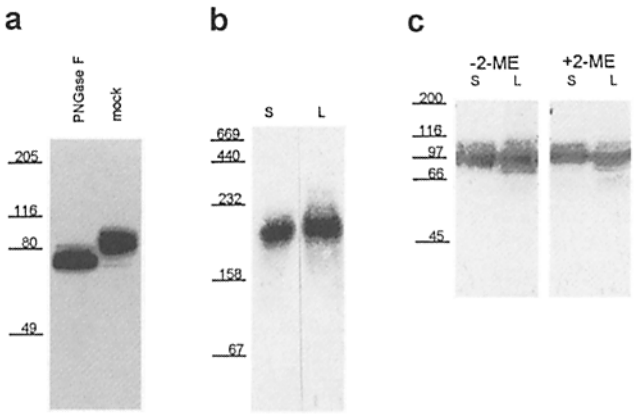

Figure 1. Structural analysis of SA85-1 proteins. (*a*) The SA85-1 proteins are glycosylated. Mammalian-stage lysates were subjected to PNGase F treatment or mock treatment, followed by SDS-PAGE and Western analysis using an anti–SA85-1 mAb. (*b*) The SA85-1 glycoproteins are expressed and secreted as dimers. 3 μg of spontaneously shed (*S*) or mammalian-stage lysate (*L*) were electrophoresed on a 4–15% nondenaturing polyacrylamide gel until the limit of migration was reached, followed by Western analysis using an anti–SA85-1 mAb. (*c*) The SA85-1 glycoprotein dimers are not dependent on sulfhydryl-bonds. 3 μg of spontaneously shed (*S*) or mammalian-stage lysate (*L*) were subjected to SDS-PAGE (7.5% gel) using nonreducing (−2-ME) or reducing (+2-ME) conditions, followed by Western analysis using an anti–SA85-1 antibody.

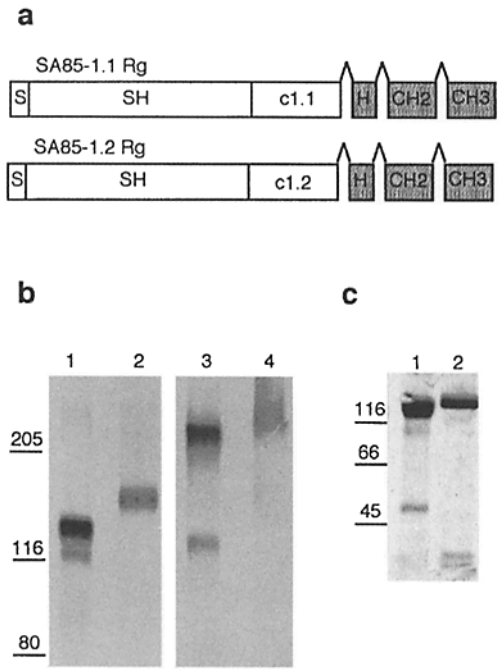

Figure 2. Schematic diagram, structure, and purification of SA85-1 Rgs. (*a*) Structure of SA85-1.1 Rg and SA85-1.2 Rg genes. The SA85-1 domains are represented by boxes. *S*, secretory signal sequence; *SH*, sialidase homology domain; and *C*, carboxyl-terminal domain. Antibody exons (human IgG1) are stippled, and introns are connected by solid lines. H, CH2, and CH3 denote the IgG hinge, CH2, and CH3 constant region exons, respectively. (*b*) SA85-1.1 Rg and SA85-1.2 Rg form dimers. SA85-1.1 Rg and SA85-1.2 Rg radiolabeled with [^{35}S]methionine were subjected to SDS-PAGE (6% gel); lane *1* (SA85-1.1 Rg) and lane *2* (SA85-1.2 Rg) reducing conditions; and lane *3* (SA85-1.1 Rg) and lane *4* (SA85-1.2 Rg) nonreducing conditions. (*c*) Commassie blue stained protein A column purified SA85-1.1 Rg (lane *1*) and SA85-1.2 Rg (lane *2*) following SDS-PAGE (7.5% gel); samples were exposed to reducing conditions.

were expressed as immunoglobulin fusion glycoproteins using COS cells. These immunoglobulin chimeras were produced by genetic fusion of the SA85-1.1 (genomic DNA and cDNA hybrid segment) and SA85-1.2 (genomic DNA segment) to the genomic DNA segment encoding human IgG1 (Fig. 2 *a*) (23). Expression plasmids encoding these constructs directed the production of soluble SA85-1 Rg dimers into the supernatant of transfected COS cells (Fig. 2 *b*). The SA85-1 Rgs were purified from the COS cell supernatants with protein A (Fig. 2 *c*).

Soluble SA85-1.1 Recombinant Proteins React with Skin and LN Macrophages. Immunohistochemistry was used to identify mammalian tissues and cells which react with SA85-1 Rgs. SA85-1.1 Rg binding was detected to scattered cells in the skin dermis (Fig 3 *a*). No staining of SA85-1.2 Rg to the skin thin section was observed (Fig. 3 *b*); similarly an irrelevant human IgG1 antibody did not stain the skin (Fig. 3 *c*). In the peripheral LN, SA85-1.1 Rg was observed to stain subcapsular sinuses and T cell-dependent areas, and not to stain B cell follicles (Fig. 4, *a–d*). Often, the SA85-1.1 Rg$^+$ cells appeared as groups of cells that involved and surrounded the peripheral LN lymphatics that link the capsule to the medulla (interfollicular sinuses) (Fig. 4, *a–d*). Again, no staining by SA85-1.2 Rg (Fig. 4 *e*), or an irrelevant human IgG1 antibody (Fig. 4 *f*) was observed. The staining of SA85-1.1 Rg to subcapsular and interfollicular sinuses suggested an interaction with macrophages, a prominent cell type of these locations.

To define the cell lineage of the binding cells (SA85-1.1 Rg$^+$ cells), two color immunofluorescence with cell lineage-specific mAbs and SA85-1.1 Rg was performed (not shown). In skin, SA85-1.1 Rg staining colocalized with some dermal macrophages identified by mAb F4/80 and M1/70 (not shown) (31, 51, 52). There was no colocalization with epidermal Langerhans cells detected by mAb F4/80 (not shown) (51).

In peripheral LN all SA85-1.1 Rg$^+$ cells were also stained by the macrophage specific mAbs M1/70, M3/84, and MOMA-2 (not shown) (35, 52, 53). Some SA85-1.1 Rg$^+$ cells colocalized with F4/80$^+$ macrophages, and some did not (not shown) (31, 51, 52). There was also colocalization of SA85-1.1 Rg$^+$ cells and the SER-4 mAb which detects a subset of macrophage in the LN (not shown) (36, 54). There was no detectable colocalization with the dendritic cell specific mAb N418 (not shown) (32).

Labeling macrophages with SA85-1.1 Rg was not the result of Fc interactions. These immunohistochemical stud-

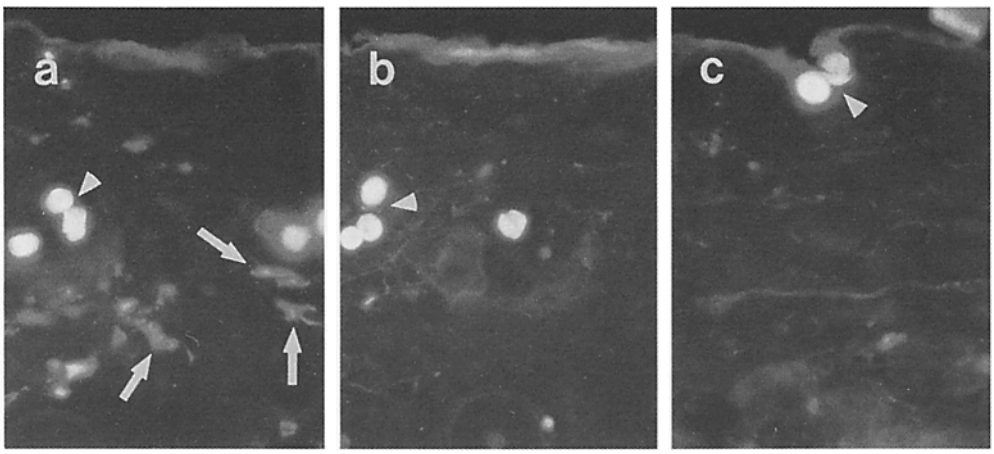

Figure 3. Reactivity of SA85-1.1 Rg with cells of the skin. (*a*) SA85-1.1 Rg staining of dermal cells. Arrows indicate scattered labeled cells, and arrowheads indicate autofluorescence of hair shafts. (*b*) Staining with SA85-1.2 Rg, and (*c*) with an irrelevant human IgG1 antibody. Only autofluorescence of hair shafts and connective tissue is visible. ×1160.

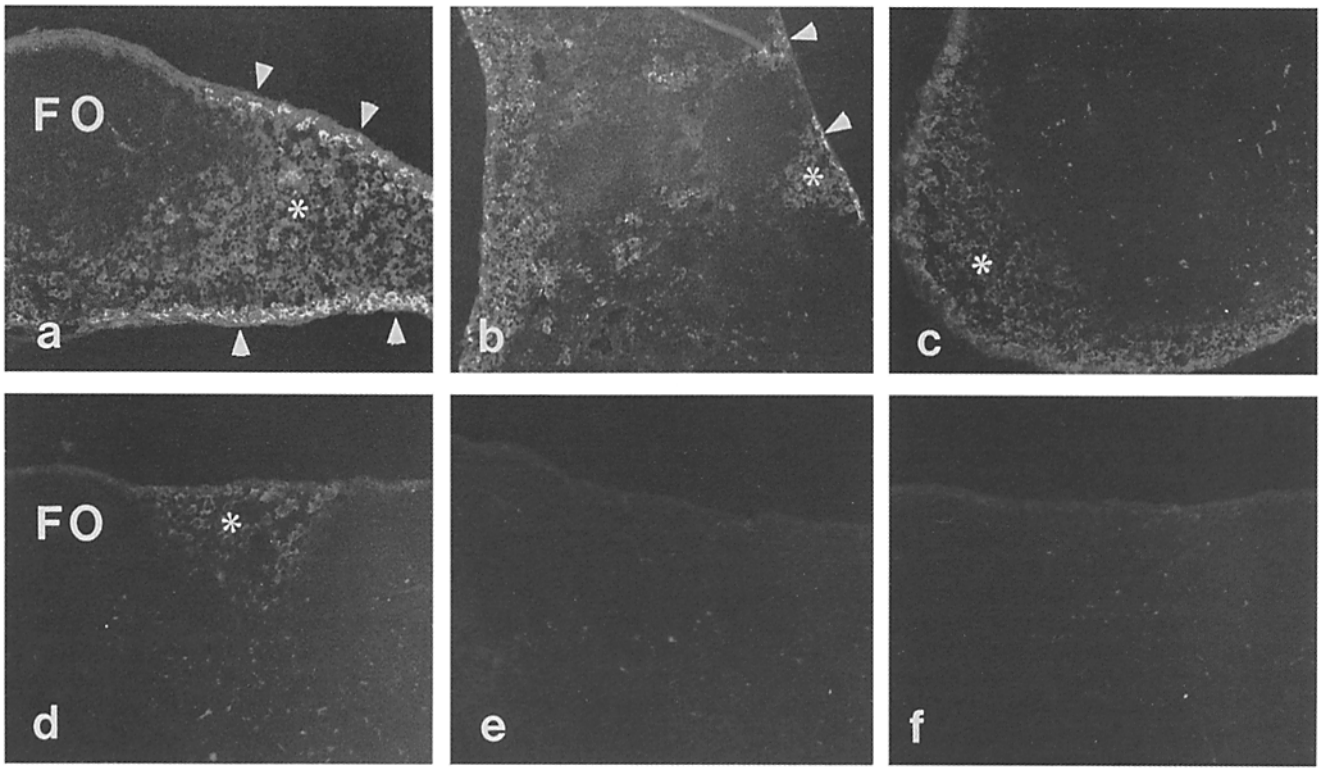

Figure 4. Reactivity of SA85-1.1 Rg with peripheral LN. (*a–d*) SA85-1.1 Rg localizes to subcapsular and interfollicular regions, sparing follicles. (*FO*) Follicles. (*Arrowheads*) Subcapsular sinuses. (*) Interfollicular sinuses. Staining with SA85-1.2 Rg (*e*) and an irrelevant human IgG1 antibody (*f*). ×330, except *b* ×280.

ies were performed in the presence of 10% normal mouse serum to prevent detectable FcR interactions. Using these conditions, neither SA85-1.2 Rg nor an irrelevant human IgG1 bound to the tissues indicating that nonspecific human Fc interactions were not occurring (Figs. 3 and 4). In addition, the SA85-1.1 gene was expressed fused to a human IgG1 Fc that has been genetically modified to prevent the binding to Fc receptors (called SA85-1.1 Rg-m) (41, 42). The binding of SA85-1.1 Rg-m and SA85-1.1 Rg to peripheral LN serial thin sections was indistinguishable (not shown).

Carbohydrates Block the Binding of Soluble SA85-1 Glycoproteins. Previous studies indicated that mannose, galactose, *N*-acetyl-D-glucosamine and mannan inhibit the adhesion and invasion of *T. cruzi* into mammalian cells, suggesting that carbohydrates were critical to infection (55–58). Therefore, the binding of SA85-1.1 Rg to LN macrophages was examined in the presence of these sugars and others. Specific carbohydrates (mannose, fucose, *N*-acetyl-D-glucosamine, and mannan) were shown to inhibit the binding, suggesting that carbohydrates effected the interaction (not shown). Pretreatment of the lymph node with mannan, a polymer of mannose, followed by washing to remove the free mannan, and then incubation of the lymph node with SA85-1.1 Rg, blocked the binding of SA85-1.1 Rg (not shown). This result indicates that mannan and other carbohydrates which block ligand binding, recognize a lectin expressed by the LN macrophages. The adhesion studies suggest that the carbohydrates of SA85-1.1 Rg also bind to this LN lectin.

The Binding Properties of Soluble SA85-1.1 to LN Macrophages Suggest an Interaction with the Mannose Receptor. The pattern of carbohydrate inhibition of SA85-1.1 Rg lectin binding was consistent with the lectin's being the MR (6). If SA85-1.1 Rg carbohydrates were binding to the MR, then the binding would be calcium dependent and sensitive to acidic pH (6). The binding of SA85-1.1 Rg to peripheral LN was examined in the presence of varying concentrations of $CaCl_2$, and at different H^+ concentrations. SA85-1.1 Rg binding was detected with $CaCl_2$ concentrations between 2 mM and 100 μM (not shown). Weak binding was observed with 50 μM $CaCl_2$, and no binding with 10 μM $CaCl_2$ (not shown) or 0 μM $CaCl_2$. Other divalent cations (magnesium and manganese) could not substitute for the calcium requirement (not shown). At pH 5.5 the SA85-1.1 Rg binding was similar to that observed at neutral pH (not shown). No binding was observed at pH 5.0 and lower, or above pH 9.0 (not shown). These data are consistent with SA85-1.1 Rg binding to the MR.

To further examine the possibility that SA85-1.1 Rg was binding to the MR, immunohistochemical analysis was performed with SA85-1.1 Rg and an anti-MR antibody. Colocalization was noted between SA85-1.1 Rg and the anti-MR antibody in peripheral LN (not shown). This result supported the previous data that indicated that SA85-1.1 Rg was binding to the MR.

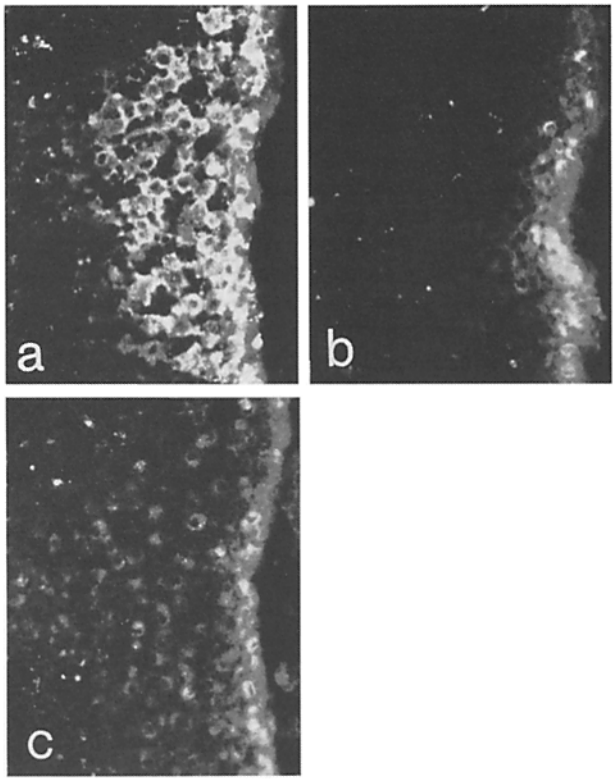

Figure 5. The reactivity of SA85-1.1 Rg with LN is inhibited by mammalian-stage surface glycoproteins. SA85-1.1 Rg binding to LN thin sections is shown following pretreatment with 5 μg/ml spontaneously shed surface glycoproteins in the presence of EDTA (*a*) or in the absence of 10 mM EDTA (*b*). Staining with an irrelevant human IgG1 antibody (*c*). ×660.

The Binding of Parasite-synthesized Surface Glycoproteins to LN Macrophages Blocks the Binding of SA85-1.1 Rg and Is Calcium-Dependent. To examine if parasite-synthesized glycoproteins bound to LN macrophages in the same manner as SA85-1.1 Rg, initial efforts were made to detect binding of spontaneously shed mammalian stage surface glycoproteins, which include the SA85-1 glycoproteins (23), with various anti–SA85-1 antibodies and mAbs. These studies were difficult to interpret because of high background binding of antibodies to the LN thin sections. As an alternate approach, spontaneously shed mammalian stage surface glycoproteins were used to pretreat LN thin sections, which were subsequently stained with SA85-1.1 Rg. The pretreatment at a concentration of 5 μg/ml of protein was found to completely block the binding of SA85-1.1 Rg (Fig. 5 *b*). If the pretreatment with spontaneously shed glycoproteins was done in the presence of EDTA, then the binding of SA85-1.1 Rg was not inhibited (Fig. 5 *a*). Although the glycosylation of SA85-1.1 Rg by COS cells is not likely to be identical to the glycosylation of SA85-1 glycoproteins by *T. cruzi*, these results suggested that in the presence of calcium, the parasite-synthesized glycoproteins bound the MR, and inhibited the binding of SA85-1.1 Rg.

Amastigotes, but Not Trypomastigotes, Are Recognized by the MBP. Because both the MR and the MBPs bind glyco-

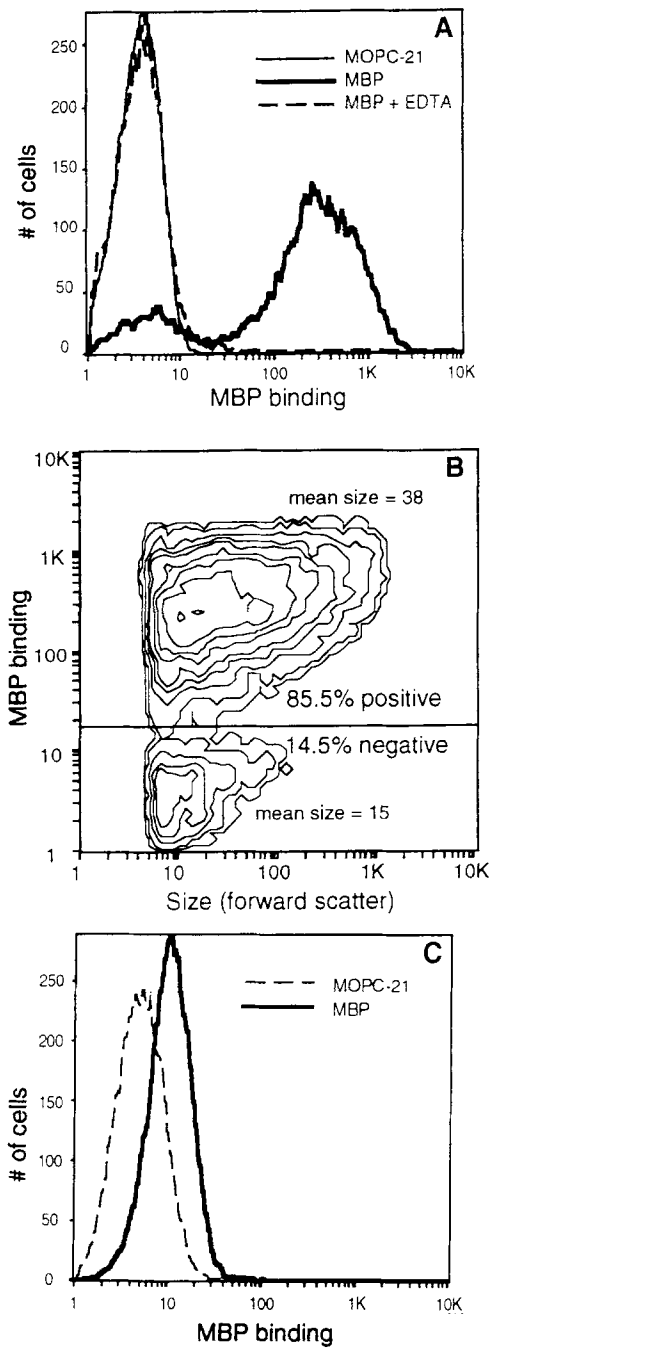

Figure 6. Flow cytometry of live amastigotes indicating MBP binding. (*a*) MBP binds to a subset of mammalian-stage parasites. Live tissue culture-derived parasites were incubated with 10 μg/ml of MBP or MOPC-21, washed and fixed, and the bound MBP was detected with a murine anti-MBP mAb, and an anti-murine Ig antibody conjugated with FITC. Inhibition of MBP binding was demonstrated by the addition of 10 mM EDTA. MOPC-21 mAb was used as an irrelevant protein control. (*b*) The mammalian-stage parasites that bind MBP are twice the relative size as the nonbinding parasites. The data in *A* are displayed with relative size (inferred from forward scatter) on the X-axis, and MBP binding on the Y-axis. Contour intervals represent the relative number of parasites. (*c*) MBP binds epimastigotes weakly compared to mammalian stage parasites. Live axenic derived epimastigotes were incubated with MBP and antibodies as described in *A*.

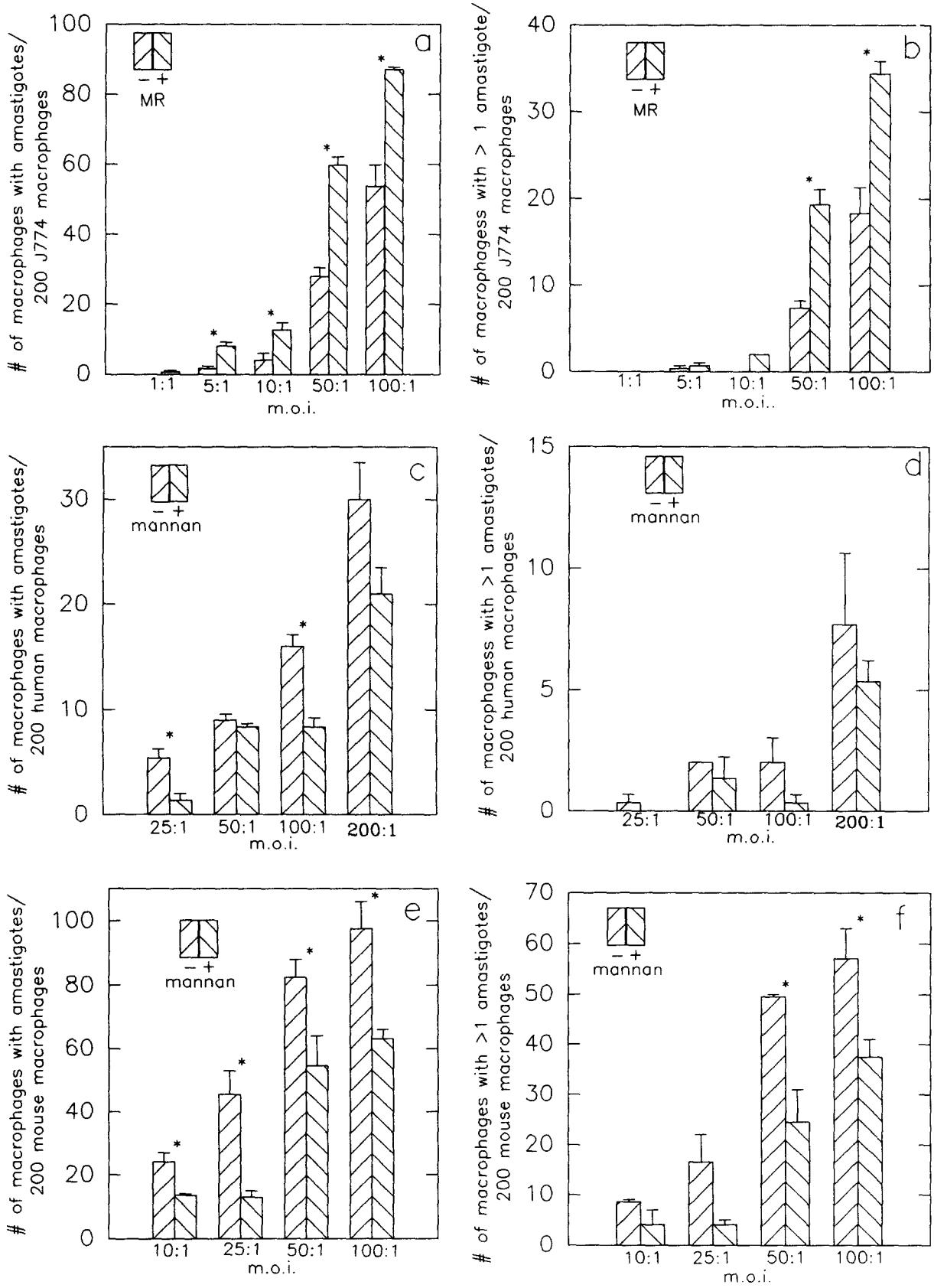
a
MR
# of macrophages with amastigotes/ 200 J774 macrophages
m.o.i.
1:1
5:1
10:1
50:1
100:1
b
MR
# of macrophagess with > 1 amastigote/ 200 J774 macrophages
m.o.i..
c
mannan
# of macrophages with amastigotes/ 200 human macrophages
m.o.i.
25:1
50:1
100:1
200:1
d
mannan
# of macrophagess with >1 amastigotes/ 200 human macrophages
m.o.i.
e
mannan
# of macrophages with amastigotes/ 200 mouse macrophages
m.o.i.
10:1
25:1
50:1
100:1
f
mannan
# of macrophages with >1 amastigotes/ 200 mouse macrophages
m.o.i.

Table 1. *Amastigote Association with J774 Macrophages**

Condition	% Association‡ of macrophages with ⩾ 1 amastigote (mean ± SE)	% Association‡ of macrophages with >1 amastigote (mean ± SE)	*n*
MR^+, Mannan (1 mg/ml)	41 ± 5§	45 ± 8§	6
MR^+, Glucan (1 mg/ml)	237 ± 6‖	352 ± 38‖	1
MR^-, No inhibitors	42 ± 9§	60 ± 9§	9
MR^-, mannan (1 mg/ml)	136 ± 32‖	149 ± 43‖	3

*J774 macrophages were incubated under serum-free conditions with amastigotes in the presence of mannan, glucan or media alone. Data are the mean ± SE from the indicated number of independent experiments.

‡% association = (amastigotes associated with J774 macrophages using different conditions/amastigotes associated with MR^+ J774E macrophages using media only) × 100.

§ $P < 0.05$ comparing amastigote association with the indicated macrophages and conditions to MR^+ macrophages in the absence of inhibitors.

‖Association was increased rather than decreased.

conjugates with terminal mannose, fucose, and *N*-acetyl-D-glucosamine, soluble MBP was used to examine *T. cruzi* for the expression of these terminal carbohydrates (Fig. 6). In Fig. 6 *a* MBP binds to a subset of mammalian stage parasites as demonstrated by a shift in the fluorescent intensity of greater than 50-fold (Fig. 6 *a*). This binding was inhibited by the omission of calcium (not shown), or the addition of EDTA (Fig. 6 *a*) indicating that the interaction was calcium dependent. The mammalian-stage parasites that bound MBP (85.5%) had a relative size (inferred from forward light scatter) of 38, as compared to a relative size of 15 for the nonbinding parasites (14.5%) (Fig. 6 *b*). These data suggest that the spherical amastigotes with greater forward scatter properties bound MBP, and that the elongated trypomastigotes did not. Fluorescent microscopy confirmed that only amastigotes bound MBP, whereas trypomastigotes did not (not shown). In addition, MBP bound epimastigotes weakly (a twofold shift in fluorescent intensity [Fig. 6 *c*]) compared to the MBP binding to mammalian stage parasites (Fig. 6 *a*). The data demonstrated that live amastigotes bind MBP, and that expression of the MBP ligand is developmentally regulated.

The incubation of *T. cruzi* trypomastigotes in LIT media for 24 h results in their conversion to amastigotes; these amastigotes are morphologically and antigenically identical to amastigotes that develop intracellularly (3). To further examine the developmental regulation of the MBP ligand, a mixed population of amastigotes and trypomastigotes was converted to amastigotes in LIT media, and the expression of the MBP ligand was monitored with flow cytometry. At 0 h, both amastigotes and trypomastigotes were detected (not shown), and only the amastigotes bound MBP (not shown). After the 24-h incubation in LIT, the cells were >99% amastigotes by phase-contrast microscopy, and 95% amastigotes by flow cytometry forward scatter analysis (not shown). The flow cytometry revealed that the amastigote population uniformly bound MBP (not shown), and that the mean fluorescent intensity of MBP binding increased from 10.7 in the 0-h amastigotes, to 19.8 in the 24-h amastigotes (not shown). The data demonstrated that axenically derived amastigotes express the MBP ligand, and further demonstrated that the MBP ligand is developmentally regulated.

The MR Facilitates the Adhesion of Amastigotes to Macrophages. The flow cytometry studies demonstrated an interaction between amastigotes and MBP, and suggested that amastigotes would also bind to the MR. An interaction between amastigotes and the MR may facilitate amastigote entry into macrophages; therefore, the adhesion of amastigotes to a variety of macrophages was investigated (Fig. 7 and Tables 1–4).

Amastigote adhesion to the J774E macrophage cell line (MR^+) and the J774A macrophage cell line (MR^-) were compared at different multiplicities of infection (m.o.i.); this experiment revealed that more amastigotes associated with the MR^+ macrophages (Fig. 7, *a* and *b*). Because the amount of amastigote association with macrophages varied in independent experiments, the cumulative data from multiple experiments is presented as percent association (Tables 1–4). The cumulative data using the J774 cell lines revealed that the MR^- macrophages were associated with 42% less amastigotes than the MR^+ macrophages (Table 1). Mannan, a ligand of the MR, inhibited the association of amastigotes with the MR^+ macrophages, whereas glucan

Figure 7. The MR contributes to the association of amastigotes with macrophages. Macrophages were incubated with amastigotes at different m.o.i. (amastigotes:macrophage) for 2 h, washed, fixed, and then scored for association with ⩾1 amastigote (*left*) or >1 amastigote (*right*): (*a*) and (*b*) J774 MR^+ and MR^- macrophages; (*c*) and (*d*) human monocyte-derived macrophages with mannan (1 mg/ml) and without mannan; (*e* and *f*) mouse thioglycollate elicited peritoneal macrophages with mannan (1 mg/ml) and without mannan. The data represent the mean ± the standard error of the mean from three determinations. *, $P < 0.05$ comparing (*a* and *b*) amastigote association with MR^+ macrophages to amastigote association with MR^- macrophages; and (*c–f*) amastigote association with macrophages in the presence of added carbohydrates to amastigote association with macrophages in the absence of added carbohydrates.

Table 2. *Amastigote Association with Human Macrophages**

Condition	% Association‡ of macrophages with ≥ 1 amastigote (mean ± SE)	% Association‡ of macrophages > 1 amastigote (mean ± SE)	*n*
Mannan (1 mg/ml)	46 ± 8§	26 ± 12§	15
Mannan (0.5 mg/ml)	14 ± 8	29 ± 14	1
Mannan (0.2 mg/ml)	13 ± 7§	21 ± 21	2
Glucan (1 mg/ml)	237 ± 63‖	188 ± 43‖	3
Glucan (0.5 mg/ml)	290 ± 138‖	455 ± 184‖	2
Glucan (0.2 mg/ml)	200 ± 1.2‖	265 ± 65‖	2
Mannose-BSA (0.2 mg/ml)	29 ± 12§	10 ± 4§	3
Mannose-BSA (0.02 mg/ml)	46 ± 19	29 ± 10	1
Mannose-BSA (0.002 mg/ml)	63 ± 10	86 ± 0	1
Galactose-BSA (0.2 mg/ml)	97 ± 19	171 ± 42‖	1
Galactose-BSA (0.02 mg/ml)	94 ± 0	86 ± 25	1

*Human monocyte-derived macrophages were incubated under serum-free conditions with amastigotes in the presence of mannan, glucan, mannose-BSA, galactose-BSA, or media alone. Data are the mean ± SE from the indicated number of independent experiements.
‡% association = (amastigotes associated with human macrophages using different conditions/amastigotes associated with human macrophages using media only) ×100.
§ $P < 0.05$ comparing amastigote association with macrophages in the presence of various inhibitors to amastigote association with macrophages in the absence of inhibitors.
‖ Association was increased rather than decreased.

did not (Table 1). In addition, mannan did not inhibit the MR^- cell line (Table 1). These initial studies suggested that the MR facilitates amastigote adhesion to macrophages. It is unclear why glucan augmented the interaction of amastigotes with the macrophages. Because amastigotes did adhere to MR^- macrophages, and mannan did not completely block the association of amastigotes with MR^+ macrophages, other receptors are likely to be involved in adhesion (Table 1).

Both human macrophages derived from peripheral blood monocytes, and thioglycollate-elicited mouse peritoneal macrophages, express high levels of MR; these cells were also used to investigate amastigote invasion (6). First, mannan was shown to inhibit the association of amastigotes

Table 3. *Amastigote Association with Mouse Macrophages**

Condition	% Association‡ of macrophages with ≥ 1 amastigote (mean ± SE)	% Association‡ of macrophages > 1 amastigote (mean ± SE)	*n*
Mannan (1 mg/ml)	49 ± 9§	47 ± 7§	5
Mannan (0.5 mg/ml)	67 ± 2	100 ± 0	1
Mannan (0.2 mg/ml)	52 ± 6	47 ± 17	1
Glucan (1 mg/ml)	171 ± 21‖	192 ± 25‖	1
Glucan (0.5 mg/ml)	98 ± 17	125 ± 25‖	1
Glucan (0.2 mg/ml)	108 ± 4‖	117 ± 33‖	1

*Thioglycollate elicited mouse peritoneal macrophages were incubated under serum-free conditions with amastigotes in the presence of mannan, glucan, or media alone. Data are the mean ± SE from the indicated number of independent experiments.
‡% association = (amastigotes associated with murine macrophages using different conditions/amastigotes associated with murine macrophages using media only) ×100.
§ $P < 0.05$ comparing amastigote association with macrophages in the presence of various inhibitors to amastigote association with macrophages in the absence of inhibitors.
‖ Association was increased rather than decreased.

Table 4. *Amastigote Association with Mouse Macrophages Is Inhibited by Modulation of the MR on a Mannan Substrate**

Condition	% Association‡ of macrophages with ≥1 amastigote (mean ± SE)	% Association‡ of macrophages with >1 amastigote (mean ± SE)	*n*
Mannan coated coverslip	63 ± 6	60 ± 11	2

*Thioglycollate elicited mouse peritoneal macrophages were plated on glass coverslips coated with mannan (50 mg/ml) or BSA (30 mg/ml) for 2 h. The slides were then washed and incubated under serum-free conditions with amastigotes in media alone. Data are the mean ± SE from the indicated number of independent experiments.

‡% association = (amastigotes associated with murine macrophages grown on mannan-coated coverslips/amastigotes associated with murine macrophages grown on BSA-coated coverslips) ×100.

with these macrophages at different m.o.i. (Fig. 7, *c–f*). Cumulative data with the human macrophages demonstrated inhibition of amastigote association in the presence of mannan or mannose-BSA (Table 2). With murine macrophages, the data demonstrated inhibition of amastigote association in the presence of mannan (Table 3). In addition, the plating of murine macrophages on mannan-coated coverslips, which results in the modulation of the MR to the basal surface of the macrophages where it is not accessible to the amastigotes for adhesion, reduced amastigote association (Table 4). Again, it is unclear why glucan and galactose-BSA augmented the associations of amastigotes with human and mouse macrophages (Tables 2 and 3). These studies demonstrated that the MR contributes to amastigote association with macrophages, and suggested that the MR may facilitate amastigote invasion of a subset of macrophages.

Discussion

Descriptions of the *T. cruzi* life cycle have described amastigotes as an intracellular replicative form (1). Recent studies, however, have demonstrated that amastigotes circulate in the blood stream of infected mammals during the acute phase (2), and that culture-derived amastigotes are resistant to complement-mediated lysis (59), are capable of invading macrophages in vitro, and are infective to mice in vivo (3). Although the role of extracellular amastigotes during the life cycle of the parasite remains unclear, these reports suggest that amastigotes are not limited to intracellular replication; rather, amastigotes may contribute to the dissemination of the parasite in the mammalian host and to the invasion of mammalian cells. The molecular interactions involved in amastigote invasion of mammalian cells are poorly defined. In this report we have demonstrated that amastigotes interact with two mammalian lectins with similar carbohydrate recognition specificity, the MBP and the macrophage MR. We have also demonstrated that the MR facilitates amastigote uptake into macrophages. This report identifies the first mammalian lectins (the MBP and the MR) to bind to *T. cruzi*, and the first mammalian lectin (the MR) to facilitate *T. cruzi* invasion of mammalian cells.

A definitive function for many of the *T. cruzi* surface glycoproteins, including the SA85-1 glycoproteins, has been difficult to demonstrate. The data in Fig. 1 demonstrated that the SA85-1 glycoproteins are glycosylated and expressed as dimers. Previous studies demonstrated that the SA85-1 glycoproteins displayed extensive diversity at the amino acid level, and homology to sialidases (23, 28). Together, these data suggested that the SA85-1 glycoproteins may function as a family of lectins binding to a variety of sialic acid containing oligosaccharides on the surface of host cells. To explore this possibility, SA85-1 Rg genes were assembled, expressed in COS cells as glycosylated dimers (Fig. 2), and the glycoproteins used to screen normal mouse tissues for adhesive interactions (Figs. 3 and 4). These experiments demonstrated an interaction between the carbohydrates of SA85-1.1 Rg and the MR; no adhesive interaction was observed with SA85-1.2 Rg. It is unclear why an interaction with SA85-1.2 Rg was not observed. Both SA85-1.1 and SA85-1.2 encode potential *N*-linked glycosylation sites (10 and 11, respectively). It is possible that SA85-1.2 Rg is glycosylated differently than SA85-1.1 Rg. Alternatively, SA85-1.2 Rg may fold in such a way that prevents its carbohydrate moieties from binding to the MR.

Previous studies have demonstrated that a known ligand of the MR, high-mannose oligosaccharides, are a major component of the *T. cruzi* surface glycoproteins, supporting the possibility that these surface glycoproteins, including the SA85-1 glycoproteins, would be a ligand of the MR (7, 60). Further evidence that the SA85-1 and related glycoproteins bind the MR was provided by the experiments that demonstrated that the MBP, a calcium-dependent lectin with similar carbohydrate binding specificities as the MR, bound amastigotes (Fig. 6). In addition, experiments used MBP-affinity chromatography to demonstrate that the SA85-1 glycoproteins and related major surface glycoproteins, expressed by amastigotes bind to MBP (not shown). An interaction between the parasite-synthesized surface glycoproteins and the MR was further supported by the immunohistochemical data that indicate that parasite-synthesized surface glycoproteins, which include the SA85-1 glycoproteins, bound the MR, and prevented the binding of SA85-1.1 Rg (Fig. 7). Finally, the flow cytometry data indicate that the MBP and MR carbohydrate ligands are expressed in a parasite stage-specific manner (Fig. 6). Thus it appears that both amastigotes and COS cells glycosylate some SA85-1 glycoproteins such that they bind to the

MBP and the MR. The glycosylation mechanisms enabling these different cell types to achieve a related glycosylation pattern remain unknown. Taken together, these data indicate that amastigote surface glycoproteins, including the SA85-1 glycoproteins, are ligands of the MBP and the MR, and suggest that the MR may facilitate adhesion of amastigotes to macrophages.

The adhesion assays demonstrated that amastigote association with macrophages was increased by the MR. The data also indicate that other receptors contribute to the amastigote adhesion to macrophages, as amastigotes adhered to the MR^- J774A macrophages, and amastigotes adhered to macrophages in the presence of high concentrations of MR inhibitors. A previous study has demonstrated that fibronectin increased amastigote association to resident murine peritoneal macrophages and human monocytes, suggesting that fibronectin may have contributed to the binding of amastigotes to macrophages in our assays (61). In addition, these experiments were performed in the absence of complement; because amastigotes are resistant to the lytic effects of complement (59), it is possible that complement binding to amastigotes would contribute to their opsonization by host cells.

In the adhesion assays, the augmented association of amastigotes with macrophages in the presence of glucan or galactose-BSA suggested that these compounds may simultaneously bind to receptors on both the macrophages and the amastigotes, which would form a bridge between them, and increase their association. The β-glucan receptor, and the Mac-2 protein (a galactose-specific lectin) are macrophage receptors which may participate in this proposed interaction (62).

The macrophage MR recycles between the plasma membrane and the lysosomal compartment. Two physiologic functions for this molecule have been proposed: molecular scavenging and host defense (6). An example of molecular scavenging is the binding of lysosomal hydrolases in the extracellular space at sites of inflammation, and the return of these enzymes to the macrophage lysosomal compartment (6). In host defense, the MR appears to function by binding to terminal mannose residues on the surface of microorganisms that results in their phagocytosis into the lysosomal compartment. Many microorganisms express terminal mannose residues, which would enable the MR to bind and facilitate their uptake as part of an innate immune response (6). Previous studies have indicated that the MR facilitates the phagocytosis of *Candida* species (63), *Saccharomyces cerevisiae* (64), *Aspergillus fumigatus* (65), *Pseudomonas aeruginosa* (44), and *Pneumocystis carinii* (66). In addition, the MR facilitates the uptake of *Leishmania donovani* and pathogenic strains of *Mycobacterium tuberculosis*, two organisms that proliferate within macrophages (67, 68). Other studies have suggested that HIV may gain entry into macrophages by binding to the MR through carbohydrate residues of gp120 (69). Our data indicate that *T. cruzi* amastigotes bind the MR, and that the surface glycoproteins, including the SA85-1 glycoproteins, are ligands of this interaction.

In this report, SA85-1.1 Rg was detected binding to the MR expressed by a subset of macrophages in the skin and peripheral LN (Figs. 3 and 4), while no binding was detected in cells in the brain, thymus, heart, spleen, liver, small intestine, and kidney (not shown). An explanation for this observed binding pattern is that the MR is expressed at higher levels by individual macrophages in skin and LN, which permitted the immunohistochemical detection of SA85-1.1 Rg binding. Previous studies investigating the expression of the MR by individual macrophages have not examined the skin or LNs (6). The brightest staining SA85-1.1 Rg^+ macrophages were detected in the subcapsular and interfollicular sinuses of the peripheral LN, where afferent lymph enters the LN (Fig. 4). Increased expression of the MR in macrophages of the skin and peripheral LN subcapsular and interfollicular sinuses would facilitate the proposed MR function in host defense, as microorganisms, including *T. cruzi*, commonly infect mammals through the skin, and disseminate to peripheral LNs via the lymphatics.

T. cruzi evasion of the host immune response is facilitated by its ability to replicate in macrophages. Past studies, however, have demonstrated that macrophages activated with IFN-γ are trypanocidal (5). In addition, IFN-γ downregulates the expression of the MR (6). Therefore, our data suggest that adherence of *T. cruzi* amastigotes mediated by the MR may select macrophages that have not been activated with IFN-γ, and that are permissive for parasite replication. The data also indicate that following infection of the skin, a common route of *T. cruzi* infection, and dissemination to regional LNs, amastigotes will encounter a subset of macrophages that express high levels of the MR and are permissive for their replication.

The experiments demonstrating that amastigotes are resistant to complement (59), and that amastigotes circulate in the blood of infected mammals (2), suggest the possibility that MBP, a circulating serum protein, may act as an opsonin and enhance clearance of the parasite. MBP may be considered a member of the collectin family; collectins are a family of molecules that have a collagen tail, and a carboxyl-terminal lectin domain (70). The collagen tail binds to the collectin receptor found on a variety of mammalian cells including leukocytes, endothelial cells, fibroblasts, and some epithelial cells; the lectin domain recognizes carbohydrates on a wide variety of microorganisms including *Leishmania* (71), yeasts, certain gram-negative and gram-positive bacteria, the hemagglutinin of certain strains of influenza virus, and the HIV gp120 (9). MBP interactions with microorganisms may result in opsonization and clearance by a variety of cells that express collectin receptors (9). Alternatively, after binding microorganisms, MBP may initiate complement activation via the classic or alternative pathways (72, 73). In addition, MBP may initiate complement activation through a novel serine protease, the MBP-associated protease (74).

Previous studies have demonstrated different patterns of plant lectin binding to amastigotes and trypomastigotes indicating that differential glycosylation occurs during the parasite life cycle (75). Consistent with these studies, recent reports have indicated that the *trans*-sialidase expressed by

trypomastigotes creates a sialic acid containing ligand that participates in trypomastigote invasion of mammalian cells; amastigotes do not express the *trans*-sialidase or the sialic acid ligand (76). In addition, ultrastructural studies have indicated that amastigotes bind to different regions of mammalian cells than trypomastigotes, suggesting that amastigote and trypomastigote interactions with host cells are different during cellular invasion (77). Our results, using the human MBP as a probe, further demonstrate that amastigote glycosidases generate oligosaccharide ligands that are distinct from those found on the surface of trypomastigotes (Fig. 6).

The surface glycoproteins and the SA85-1 glycoproteins display extensive diversity at the amino acid level, and it has been suggested that this diversity contributes to the parasite's broad host range by enabling a wide variety of interactions with distinct cell types. Previous studies have indicated that some 85-kD surface glycoproteins participate in adhesion interactions with carbohydrates, fibronectin, and laminin, and that these interactions contribute to host cell invasion (15, 16, 18). Other studies have indicated that some of these surface glycoproteins function by disrupting the complement system (78, 79). Although amino acid diversity may play a role in adhesion and entry of amastigotes into cells, our work suggests that the patterns of oligosaccharides expressed on these surface glycoproteins may provide a generic ligand for collectins like the MBP. One could envisage that MBPs, which are found in all mammalian sera, may function to recognize the stage-specific glycosylation of the major surface glycoproteins of amastigotes, and thereby enhance clearance into a wide variety of cells that express collectin receptors. This clearance mechanism, together with the ability of amastigotes to resist complement-mediated lysis (59), may enable the passively motile amastigotes to achieve a different cellular tropism than the actively motile trypomastigotes. Similarly, interactions with the MR would enable amastigotes to enter non-trypanocidal macrophages. Although circulating MBP may bind amastigotes and block some interactions with the MR, it is possible that early in the infection, amastigotes proliferating in the skin and peripheral LNs, where they have not encountered MBP, are able to bind the MR of macrophages, whereas later in the infection, amastigotes circulating in the blood, are recognized by MBP and are cleared by a variety of cells expressing the collectin receptor. These lectin interactions would provide another example of how parasites have exploited host defense mechanisms to their advantage.

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References

1. WHO Technical Report Series. 1991. Control of Chagas Disease. World Health Organization, Geneva. 95 pp.
2. Andrews, N.W., K.-S. Hong, E.S. Robbins, and V. Nussenzweig. 1987. Stage-specific surface antigens expressed during the morphogenesis of vertebrate forms of *Trypanosoma cruzi*. *Exp. Parasitol.* 64:474–484.
3. Ley, V., N.W. Andrews, E.S. Robbins, and V. Nussenzweig. 1988. Amastigotes of *Trypanosoma cruzi* sustain an infective cycle in mammalian cells. *J. Exp. Med.* 168:649–659.
4. Hall, B.F. 1993. *Trypanosoma cruzi*: mechanisms for entry into host cells. *Semin. Cell Biol.* 4:323–333.
5. Reed, S.G. 1988. In vivo administration of recombinant IFN-γ induces macrophage activation, and prevents acute disease, immune suppression, and death in experimental *Trypanosoma cruzi* infections. *J. Immunol.* 140:4342–4347.
6. Pontow, S.E., V. Kery, and P.D. Stahl. Mannose receptor. 1992. *Int. Rev. Cytology.* 137B:221–243.
7. Taylor, M.E. 1993. Recognition of complex carbohydrates by the macrophage mannose receptor. *Biochem. Soc. Trans.* 21:468–473.
8. Ezekowitz, R.A.B. 1991. Ante-antibody immunity. *In* Current Biology. P. Newmark, editor. Current Biology Ltd., London. 60–62.
9. Sastry, K., and R.A. Ezekowitz. 1993. Collectins: pattern recognition molecules involved in first line host defense. *Current Opin. Immunol.* 4:59–66.
10. Zingales, B., N.W. Andrews, V.Y. Kuwajima, and W. Colli. 1982. Cell surface antigens of *Trypanosoma cruzi*: possible correlation with the interiorization process in mammalian cells. *Mol. Biochem. Parasitol.* 6:111–124.

11. Katzin, A.M., and W. Colli. 1983. Lectin receptors in *Trypanosoma cruzi*: a *N*-acetyl-D-glucosamine-containing surface glycoprotein specific for the trypomastigote stage. *Biochim. Biophys. Acta.* 772:403–411.
12. Andrews, N.W., A.M. Katzin, and W. Colli. 1984. Mapping of surface glycoproteins of *Trypanosoma cruzi* by two-dimensional electrophoresis: A correlation with the cell invasion capacity. *Eur. J. Biochem.* 140:599–604.
13. Zingales, B., A.M. Katzin, M.V. Arruda, and W. Colli. 1985. Correlation of tunicamycin-sensitive surface glycoproteins from *Trypanosoma cruzi* with parasite interiorization into mammalian cells. *Mol. Biochem. Parasitol.* 16:21–34.
14. Alves, M.J.M., G. Abuin, V.Y. Kuwajima, and W. Colli. 1986. Partial inhibition of trypomastigote entry into cultured mammalian cells by monoclonal antibodies against a surface glycoprotein of *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.* 21: 75–82.
15. Ouaissi, M.A., J. Cornette, and A. Capron. 1986. Identification and isolation of *Trypanosoma cruzi* trypomastigote cell surface protein with properties expected of a fibronectin receptor. *Mol. Biochem. Parasitol.* 19:201–211.
16. Boschetti, M.A., M.W. Piras, D. Henriquez, and R. Piras. 1987. The interaction of a *Trypanosoma cruzi* surface protein with Vero cells and its relationship with parasite adhesion. *Mol. Biochem. Parasitol.* 24:175–184.
17. Abuin, G., W. Colli, W. de Souza, and M.J.M Alves. 1989. A surface antigen of *Trypanosoma cruzi* involved in cell invasion (Tc-85) is heterogeneous in expression and molecular constitution. *Mol. Biochem. Parasitol.* 35:229–237.
18. Giordano, R., R. Chammas, S.S. Veiga, W.C. Colli, and M.J.M. Alves. 1994. An acidic component of the heterogenous Tc-85 protein family from the surface of *Trypanosoma cruzi* is a laminin binding glycoprotein. *Mol. Biochem. Parasitol.* 65:85–94.
19. Campetella, O., D. Sanchez, J.J. Cazzulo, and A.C.C. Frasch. 1992. A superfamily of *Trypanosoma cruzi* surface antigens. *Parasitol. Today.* 8:378–381.
20. Fouts, D.L., B.J. Ruef, P.T. Ridley, R.A. Wrightsman, D.S. Peterson, and J.E. Manning. 1991. Nucleotide sequence and transcription of a trypomastigote surface antigen gene of *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.* 46:189–200.
21. Takle, G.B., and G.A.M. Cross. 1991. An 85-kilodalton surface antigen gene family of *Trypanosoma cruzi* encodes polypeptides homologous to bacterial neuraminidases. *Mol. Biochem. Parasitol.* 48:185–198.
22. Pollevick, G.D., J.L. Affranchino, A.C.C. Frasch, and D.O. Sanchez. 1991. The complete sequence of a shed acute-phase antigen of *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.* 47:247–250.
23. Kahn, S., T.G. Colbert, J.C. Wallace, N.A. Hoagland, and H. Eisen. 1991. The major 85-kDa surface antigen of the mammalian-stage forms of *Trypanosoma cruzi* is a family of sialidases. *Proc. Natl. Acad. Sci. USA.* 88:4481–4485.
24. Pereira, M.E.A., J.S. Mejia, E. Ortega-Barria, D. Matzilevich, and R.P. Prioli. 1991. The *Trypanosoma cruzi* neuraminidase contains sequences similar to bacterial neuraminidases, YWTD repeats of the low density lipoprotein receptor, and type III modules of fibronectin. *J. Exp. Med.* 174:179–191.
25. Van Voorhis, W.C., L. Barrett, R. Koelling, and A.G. Farr. 1993. FL-160 proteins of *Trypanosoma cruzi* are expressed from a multigene family and contain two distinct epitopes that mimic nervous tissues. *J. Exp. Med.* 178:681–694.
26. Uemura, H., S. Schenkman, V. Nussenzweig, and D. Eichinger. 1992. Only some members of a gene family in *Trypanosoma cruzi* encode proteins that express both trans-sialidase and neuraminidase activities. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:3837–3844.
27. Parodi, A.J., G.D. Pollevick, M. Mautner, A. Buschiazzo, D.O. Sanchez, and A.C.C. Frasch. 1992. Identification of the gene(s) coding for the *trans*-sialidase of *Trypanosoma cruzi*. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:1705–1710.
28. Kahn, S., W.C. Van Voorhis, and H. Eisen. 1990. The major 85-kD surface antigen of the mammalian form of *Trypanosoma cruzi* is encoded by a large heterogeneous family of simultaneously expressed genes. *J. Exp. Med.* 172:589–597.
29. Kahn, S., M. Kahn, W.C. Van Voorhis, A. Goshorn, A. Strand, N. Hoagland, H. Eisen, and S. Pennathur. 1993. SA85-1 proteins of *Trypanosoma cruzi* lack sialidase activity. *Mol. Biochem. Parasitol.* 60:149–152.
30. Plata, F., F.G. Pons, and H. Eisen. 1984. Antigenic polymorphism of *Trypanosoma cruzi*: clonal analysis of trypomastigote surface antigens. *Eur. J. Immunol.* 14:392–399.
31. Austyn, J.M., and S. Gordon. 1981. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. *Eur. J. Immunol.* 11:805–815.
32. Metlay, J.P., M.D. Witmar-Pack, R. Agger, M.T. Crowley, D.S Lawless, and R.M. Steinman. 1990. The distinct leukocyte integrins of mouse spleen dendritic cells as identified with new hamster monoclonal antibodies. *J. Exp. Med.* 171: 1753–1771.
33. Springer, T., G. Galfre, D.S. Secher, and C. Milstein. 1978. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. *Eur. J. Immunol.* 8:539–551.
34. Springer, T.A. 1981. Monoclonal antibody analysis of complex biological systems: combination of cell hybridization and immunoadsorbents in a novel cascade procedure and its application to the macrophage cell surface. *J. Biol. Chem.* 256: 3833–3839.
35. Kraal, G., M. Rep, and M. Janse. 1987. Macrophages in T and B cell compartments and other tissue macrophages recognized by monoclonal antibody MOMA-2. An immunohistochemical study. *Scand. J. Immunol.* 26:653–661.
36. Crocker, P.R., and S. Gordon. 1989. Mouse macrophage hemagglutinin (sheep erythrocyte receptor) with specificity for sialylated glycoconjugates characterized by a monoclonal antibody. *J. Exp. Med.* 169:1333–1346.
37. Schweinle, J.-E., M. Nishiyasu, T.Q. Ding, K. Sastry, S.D. Gillies, and R.A.B. Ezekowitz. 1993. Truncated forms of mannose-binding protein multimerize and bind to mannose-rich *Salmonella montevideo* but fail to activate complement *in vitro*. *J. Biol. Chem.* 268:364–370.
38. Harlow, E., and D. Lane. 1988. Antibodies, A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 139–239.
39. Aruffo, A., I. Stemenkovic, M. Melnick, C.B. Underhill, and B. Seed. 1990. CD44 is the principal cell surface receptor for hyaluronate. *Cell.* 61:1303–1313.
40. Aruffo, A. 1990. *In* Current Protocols in Molecular Biology. F.M. Ausabel, R. Brent, R.E. Kingstone, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl, editors. John Wiley and Sons, NY. 16.13.1–16.13.7.
41. Lund, J., G. Winter, P.T. Jones, J.D. Pound, T. Tanaka, M.R. Walker, P.J. Artymiuk, Y. Arata, D.R. Burton, R. Jefferis, and J.M. Woof. 1991. Human FcγRII interact with distinct but overlapping sites on human IgG. *J. Immunol.* 147: 2657–2662.

42. Hollenbaugh, D., L.S. Grosmaire, C.D. Kullas, N.J. Chalupny, S. Braesch-Andersen, R.J. Noellle, I. Stamenkovic, J.A. Ledbetter, and A. Aruffo. 1992. The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity. *EMBO J.* 11:4313–4321.
43. Super, M., S.D. Gillies, S. Foley, K. Sastry, J.-E. Schweinle, V.J. Silverman, and R.A.B. Ezekowitz. 1992. Distinct and overlapping functions of allelic forms of human mannose binding protein. *Nature Genet.* 2:50–55.
44. Speert, D.P., S.D. Wright, S.C. Silerstein, and B. Mah. 1988. Functional characterization of macrophage receptors for in vitro phagocytosis of unopsonized *Pseudomonas aeruginosa*. *J. Clin. Invest.* 82:872–879.
45. Farr, A.G., and P.K. Nakane. 1981. Immunohistochemistry with enzyme labeled antibodies: a brief review. *J. Immunol. Methods.* 47:129–144.
46. Rentrop, M., B. Knapp, H. Winter, and J. Schweizer. 1986. Aminoalkylsilane-treated glass slides as support for *in situ* hybridization of keratin cDNAs to frozen tissue sections under varying fixation and pretreatment conditions. *Histochem. J.* 18:271–276.
47. Gallagher, S.R., and J.A. Smith. 1990. Electrophoretic separation of proteins. *In* Current Protocols in Molecular Biology. F.M. Ausabel, R. Brent, R.E. Kingstone, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl, editors. John Wiley and Sons, NY. 10.2.1–10.2.21.
48. Clos, J., J.T. Westwood, P.B. Becker, S. Wilson, K. Lambert, and C. Wu. 1990. Molecular cloning and expression of a hexameric drosophila heat shock factor subject to negative regulation. *Cell.* 63:1085–1097.
49. Harlow, E., and D. Lane. 1988. Antibodies, A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 488–489.
50. Diment, S., M.S. Leech, and P.D. Stahl. 1987. Generation of macrophage variants with 5-azacytidine: selection for mannose receptor expression. *J. Leuk. Biol.* 42:485–490.
51. Hume, D.A., A.P. Robinson, G.G. Macpherson, and S. Gordon. 1983. The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80. *J. Exp. Med.* 158:1522–1536.
52. Witmar, M.D., and R.M. Steinman. 1984. The anatomy of peripheral lymphoid organs with emphasis on accessory cells: light-microscopic immunocytochemical studies of mouse spleen, lymph node, and Peyer's patch. *Amer. J. Anat.* 170: 465–481.
53. Flotte, T.J., T.A. Springer, and G.J. Thorbecke. 1983. Dendritic cell and macrophage staining by monoclonal antibodies in tissue sections and epidermal sheets. *Am. J. Pathol.* 111: 112–124.
54. van den Berg, T.K., J.J.P. Breve, J.G.M.C. Damoiseaux, E.A. Dopp, S. Kelm, P.R. Crocker, C.D. Dijkstra, and G. Kraal. 1992. Sialoadhesin on macrophages: its identification as a lymphocyte adhesion molecule. *J. Exp. Med.* 176:647–655.
55. Crane, M.S., and J.A. Dvorak. 1982. Influence of monosaccharides on the infection of vertebrate cells by *Trypanosoma cruzi* and *Toxoplasma gondii*. *Mol. Biochem. Parasitol.* 5:333–341.
56. Villalta, F., and F. Kierszenbaum. 1985. Role of surface *N*-acetyl glucosamine residues on host cell infection by *Trypanosoma cruzi*. *Biochim. Biophys. Acta.* 845:216–222.
57. 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.
58. 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.
59. Iida, K., M.B. Whitlow, and V. Nussenzweig. 1989. Amastigotes of *Trypanosoma cruzi* escape destruction by the terminal complement components. *J. Exp. Med.* 169:881–891.
60. Doyle, P., L. de la Canal, J.C. Engel, and A.J. Parodi. 1986. Characterization of the mechanism of protein glycosylation and the structure of glycoconjugates in tissue culture trypomastigotes and intracellular amastigotes of *Trypanosoma cruzi*. *Molec. Biochem. Parasitol.* 21:93–101.
61. Noisin, E.L., and F. Villalta. 1989. Fibronectin increases *Trypanosoma cruzi* amastigote binding to and uptake by murine macrophages and human monocytes. *Infect. Immunol.* 57: 1030–1034.
62. Stahl, P. 1992. The mannose receptor and other macrophage lectins. *Curr. Opin. Immunol.* 4:49–52.
63. Marodi, L., H.M. Korchak, and R.B. Johnston, Jr. 1991. Mechanisms of host defense against *Candida* species. I. Phagocytosis by monocytes and monocyte-derived macrophages. *J. Immunol.* 146:2783–2789.
64. Giaimis, J., Y. Lombard, P. Fonteneau, C.D. Muller, R. Levy, M. Makaya-kumba, J. Lazdins, and P. Poindron. 1993. Both mannose and β-glucan receptors are involved in phagocytosis of unopsonized, heat-killed *Saccharomyces cerevisiae* by murine macrophages. *J. Leuk. Biol.* 54:564–571.
65. Kan, V.L., and J.E. Bennett. 1988. Lectin-like attachment sites on murine pulmonary alveolar macrophages bind *Aspergillus fumigatus* conidia. *J. Infect. Dis.* 158:407–414.
66. Ezekowitz, R.A.B., D.J. Williams, H. Koziel, M.Y.K. Armstrong, A. Warner, F.F. Richards, and R.M. Rose. 1991. Uptake of *Pneumocystis carinii* mediated by the macrophage mannose receptor. *Nature (Lond.).* 351:155–158.
67. Blackwell, J.M., R.A.B. Ezekowitz, M.B. Roberts, J.Y. Channon, R.B. Sim, and S. Gordon. 1985. Macrophage complement and lectin-like receptors bind *Leishmania* in the absence of serum. *J. Exp. Med.* 162:324–331.
68. Schlesinger, L.S. 1993. Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by the mannose receptors in addition to complement receptors. *J. Immunol.* 150:2920–2930.
69. Larkin, M., R.A. Childs, T.J. Matthews, S. Thiel, T. Mizuochi, A.M. Lawson, J.S. Savill, C. Haslett, R. Diaz, and T. Feizi. 1989. Oligosaccharide-mediated interactions of the envelope glycoprotein gp120 of HIV-1 that are independent of CD4 recognition. *AIDS (Phila.).* 3:793–798.
70. Malhotra, R., J. Haurum, S. Thiel, and R.B. Sim. 1992. Interaction of C1q receptor with lung surfactant protein A. *Eur. J. Immunol.* 22:1437–1445.
71. Green, P.J., T. Feizi, M.S. Stoll, S. Thiel, A. Prescott, and M.J. McConville. 1994. Recognition of the major cell surface glycoconjugates of *Leishmania* parasites by the human serum mannan-binding protein. *Mol. Biochem. Parasitol.* 66: 319–328.
72. Lu, J., S. Thiel, H. Wiedemann, R. Timpl, and K.B.M. Reid. 1990. Binding of the pentamer/hexamer forms of mannan-binding protein to zymosan activates the proenzyme $C1r_2C1s_2$ complex, of the classical pathway of complement, without involvement of C1q. *J. Immunol.* 144:2287–2294.
73. Schweinle, J.E., R.A.B. Ezekowitz, A.J. Tenner, M. Kuhlman, and K.A. Joiner. 1989. Human mannose-binding protein activates the alternative complement pathway and en-

hances serum bactericidal activity on a mannose-rich isolate of *Salmonella*. *J. Clin. Invest.* 84:1821–1829.
74. Matsushita, M., and T. Fujita. 1993. Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J. Exp. Med.* 176:1497–1502.
75. Pereira, M.E.A., M.A. Loures, F. Villalta, and A.F.B. Andrade. 1980. Lectin receptors as markers for *Trypanosoma cruzi*. *J. Exp. Med.* 152:1375–1392.
76. Schenkman, S., and D. Eichinger. 1993. *Trypanosoma cruzi* trans-sialidase and cell invasion. *Parasitol. Today.* 9:218–222.
77. Mortara, R.A. 1991. *Trypanosoma cruzi*: amastigotes and trypomastigotes interact with different structures on the surface of HeLa cells. *Exp. Parasitol.* 73:1–14.
78. Joiner, K.A., W.D. da Silva, M.T. Rimoldi, C.H. Hammer, A. Sher, and T.L. Kipnis. 1988. Biochemical characterization of a factor produced by trypomastigotes of *Trypanosoma cruzi* that accelerates the decay of complement C3 convertases. *J. Biol. Chem.* 263:11327–11335.
79. Tambourgi, D.V., T.L. Kipnis, W.D. da Silva, K.A. Joiner, A. Sher, S. Haealth, B.F. Hall, and G.B. Ogden. 1993. A partial cDNA clone of trypomastigote decay-accelerating factor (T-DAF), a developmentally regulated complement inhibitor of *Trypanosoma cruzi*, has genetic and functional similarities to the human complement inhibitor DAF. *Infect. Immunol.* 61: 3656–3663.