Cloning of a Surface Membrane Glycoprotein Specific for the Infective Form of Trypanosoma cruzi Having Adhesive Properties to Laminin*

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Trypomastigotes of Trypanosoma cruzi express a set of surface glycoproteins known, collectively, as Tc-85. A monoclonal antibody to these proteins, named H1A10, inhibits (50–90%) in vitro parasite interiorization into host cells, thus implicating these glycoproteins in the infection process. Two DNA inserts, a genomic DNA fragment and a full-length cDNA encoding the H1A10 epitope, have now been cloned and characterized. Results show that both have high sequence identity with all reported members of the gp85/trans-sialidase gene family, although the H1A10 epitope exists only in the Tc-85 subset of the family. The epitope has been mapped by competition of antibody binding to a Tc-85 recombinant protein with peptides having sequences predicted by the Tc-85 DNA sequence, which contains also putative N-glycosylation sites and COOH-terminal glycosylphosphatidylinositol anchor insertion sites, as expected, since an N-glycan chain and a glycosylphosphatidylinositol anchor have been characterized previously in the Tc-85 subset. The protein encoded by the full-length cDNA insert binds to cells and in vitro to laminin, but not to gelatin or fibronectin, in a saturable manner.

For the first time it was possible to assign a defined ligand to a sequenced glycoprotein belonging to the gp85 family. This fact, together with the reported binding of family members to cell surfaces, reinforces the hypothesis that this family encodes glycoproteins with similar sequences but differing enough as to bind to different ligands and thus forming a family of adhesion glycoproteins enabling the parasite to overcome the barriers interposed by cell membranes, extracellular matrices, and basal laminae.

The trypomastigote form of Trypanosoma cruzi, which is the invasive form of the parasite for vertebrates and elicits in humans an ailment known as Chagas’ disease, has to overcome a formidable barrier in order to migrate from blood vessels, where they circulate, to the interior of the cells cytoplasm where they differentiate to the replicative amastigote forms (1–4). In addition to cell membranes and the extracellular matrix the parasite has to surpass the basal laminae, barriers found throughout the body surrounding organs and blood vessels (5–7). The most important macromolecules of the basal laminae are laminin, entactin, collagen-IV, and heparan sulfate. Laminins belong to a family of glycoproteins, heterotrimers of α, β, and γ chains, involved in the assembly and shape of basal laminae (8, 9). Their involvement with adhesion, spread, differentiation, and several other cell functions is well known and discussed elsewhere (10–12).

Our laboratory has implicated glycoproteins of 85,000 Da molecular mass as involved in host cell invasion by the parasite. They exist only in the invasive trypomastigote form and are absent from epimastigotes, the insect form of the parasite that is unable to invade vertebrate cells. These glycoproteins, collectively called Tc-85, form a population of heterogeneous GPI-anchored surface glycoproteins with similar molecular masses but different electric charges (13–15). Involvement of one or more of these family members with adhesion and/or internalization of the parasite to host cells is indicated by the observation that monoclonal antibody H1A10 (mAbH1A10), which recognizes and defines the Tc-85 glycoproteins, partially inhibits invasion of the host cell by the parasite in vitro (15, 16).

A trypomastigote specific surface 85 kDa glycoprotein, which binds to laminin-Sepharose (LBG, laminin-binding glycoprotein) and belongs to the most acidic components of the Tc-85 glycoprotein family, was reported as the putative laminin receptor (17). Anti-laminin antibodies also partially inhibit host cell invasion by trypomastigotes and laminin binds to the surface of the parasite in a specific and saturable manner, suggesting that it may be involved in the infection process (17). Interaction of pathogens with laminin, fibronectin, and extracellular matrix proteins have been described by others (18–22).

The present work describes the cloning of a full-length cDNA encoding a polypeptide recognized by mAbH1A10 with the expected properties of the LBG, thus showing that at least one of the glycoproteins

1 The abbreviations used are: GPI, glycosylphosphatidylinositol; ABTS, 2,2′-azino-di-[3-ethylbenzthiazoline sulfonate(6)] diammonium salt crystals; PBS, phosphate-buffered saline; BSA, bovine serum albumin; kb, kilobase pair(s); LBG, laminin-binding glycoprotein; mAb, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.
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the Tc-85 family members is an adhesion protein to a defined ligand. The corresponding cDNA insert has significant sequence identity with members of the gp85/trans-sialidase gene family of T. cruzi (23–25), raising the interesting hypothesis that several members of that superfamily may possess adhesive capability to different receptor molecules either located on the cell surface or belonging to components of the extracellular matrix.

EXPERIMENTAL PROCEDURES

Parasite Strain and Culture—Clone 3, isolated from the T. cruzi Peru strain by micromanipulation, was utilized for these studies. Growth and maintenance are described elsewhere (26). Properties and cloning conditions for the maBH1A10 are as reported (15, 16).

Nucleic Acid Isolation, Radiolabeling, Southern and Northern Transfers, and Restriction Enzymes—Parasite nuclear DNA, bacterial plasmid DNA, and phage DNA were isolated as described previously (27). Agarose gel electrophoresis of DNA, Southern and Northern transfers, hybridization, and filter washing were performed as described previously (28). DNA restriction fragments were radiolabeled with [α-32P]dATP by nick translation. All restriction enzymes were purchased from Boehringer Mannheim and used as recommended.

Screening of Libraries—A genomic DNA expression library constructed in phage λgt11 using DNA isolated from trypomastigotes was screened with the maBH1A10 as described below for Western blot analysis. A cDNA library constructed in λ ZAP II using trypomastigote poly(A) RNA was screened with Tc85-1 following PCR radiolabeling of the insert in Bluescript (Stratagene, Inc.) using the KS and SK primers as described below. DNA Subcloning and Fusion Protein Isolation—The insert from the λgt11 clone identified by reaction with maBH1A10 (Tc85-1) was isolated by digestion with EcoRI and gel electrophoresis. The insert was excised from the gel, purified, and subcloned into the plasmid Bluescript for sequencing and pGEX-1 for expression in E. coli cells (29). The recombinant Tc85-1 protein described in the text was obtained by PCR with a construct of the Tc85-1 insert subcloned as AvaI/EcoRI fragments into the polyhistidine fusion expression vector pTrHis-B (Invitrogen, Inc.) and expressed and purified as described by the manufacturer. The fragment was generated by PCR with the original subclone in Bluescript using the KS primer of the vector as the 3′ primer and a clone-specific primer, which introduced and AvaI site at the 5′ end of the insert (5′-GAATTCCCTAGGCGGAGAAAAC). The Tc85-1 insert was then subcloned into the plasmids Bluescript and pUC19 for sequencing and as a BamHI insert fragment into the polyhistidine fusion vector pET21 (Novagen, Inc.) for expression and purification of the protein. The BamHI XhoI insert was obtained by PCR with two synthetic oligonucleotides to introduce the restriction enzyme sites at the 5′ and 3′ ends, respectively (5′-CTTGGATCCCTAGGCGGAGAAAAC and 5′-GAGGATCCTAGGCGGAGAAAAC). The Tc85-1 insert was then subcloned into the expression vector pGEX-1 using a primer synthesized from sequences upstream of the glutathione S-transferase cloning site and on both 5′ and 3′ ends of subclones in the pTrHis expression vector with primers based upon sequences upstream and downstream of the multiple cloning site of the vector. Nucleotide sequence data were compiled and analyzed using the IBM Pestell Sequence Analysis programs for the IBM computer.

Peptide Synthesis—Peptides were synthesized with an Applied Biosystems 430A Synthesizer using ABI-fastmoc chemistry cycles. Peptides were cleaved from the resin using 90% trifluoroacetic acid and a scavenger. Cleaved peptides were filtered through a 0.45-μm filter, precipitated in cold ether, and the precipitate collected by centrifugation. Peptides were dried under nitrogen and purified by reverse phase high performance liquid chromatography over a Vydac-C18 column (1.0 × 55 cm) in 0.1% trifluoroacetic acid/H2O and 0.1% trifluoroacetic acid/acetonitrile. Peptide purity was determined from the column with a gradient of acetonitrile (0–100%) over 100 min at a flow rate of 4 ml/min. Absorption was monitored at 210 nm.

Inhibition of Binding of maBH1A10 with Synthetic Peptides—Microtiter wells were coated with Tc85-1 fusion protein (50 μl of a 10 μg/ml solution in PBS (PBS = 140 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.3)) for 1 h at room temperature, washed three times with PBS, and quenched with blocking buffer (0.2% BSA, 0.05% Tween 20 in PBS) for 2 h at room temperature. All peptides were prepared as 20 mM stock solutions in Me2SO. Coated microtiter wells were incubated overnight at 4 °C with 50 μl of a 2 μM solution of individual peptides in PBS + 10% Me2SO containing maBH1A10 (10 ng/ml), washed with PBS, incubated with peroxidase-conjugated anti-mouse sera for 1 h at room temperature, again PBS-washed, and developed with ABTS (Boehringer Mannheim). For direct binding of maBH1A10, microtiter wells were coated overnight at room temperature with 50 μl of a 100 μg/ml solution of each of the individual peptides in either 50 mM carbonate buffer, pH 9.6, or 50 mM acetate buffer, pH 4.0. The wells were washed, quenched with blocking buffer for 2 h at room temperature, and incubated with maBH1A10 in blocking buffer (1 μg/ml) overnight. Peptides were synthesized with Fastmoc chemistry and incubated with peroxidase-conjugated anti-mouse sera for 1 h at room temperature, washed with PBS, and developed with ABTS.

Cell Binding Assay—E. coli BL21 DE3 pLysS cells were transformed with the pET21-Tc85-11 construct and the cells grown on LBCC (Luria broth medium supplemented with 200 μg/ml carbenicillin and 35 μg/ml chloramphenicol) agar overnight at 37 °C/200 rpm. Several colonies were grown on 40 ml of LB medium and diluted with 30 ml of LB medium containing 0.5% glucose and 0.5 M NaCl, and further cultured for 30 min. The cells were harvested by centrifugation (5,000 × g/10 min at 4 °C), washed once with minimal M9 medium, resuspended in 40 ml of the same medium supplemented with a mixture of 18 amino acids, except methionine and cysteine (25–50 μg/ml each), and further cultured for 1 h at 37 °C/200 rpm. Induction was made with 1 mM isopropyl-1-thio-β-D-galactopyranoside for 10 min, followed by the addition of rifampicin (200 μg/ml) and induction at 42 °C in a water bath for 20 min. At this point 500 μCi of [35S]methionine were added, and the cells were grown for another 10 min at 37 °C/200 rpm, harvested by centrifugation (5,000 × g/10 min at 4 °C), lysed by the addition of 1 ml of lysis buffer (PBS containing 0.2% Nonidet P-40, 1 mM 1-lysozyme, and 1 mM each phenylmethylsulfonlfluoride and N-ε-tosyl-L-lysine chloromethyl ketone), and further incubated for 30 min at room temperature. The bacterial extract was clarified by centrifugation, (16,000 × g for 15 min), diluted with PBS-1.5% BSA (final concentration), and incubated with cells or extracellular matrix proteins. Proteins were incubated on 24-well plates (100 μl of 50 μg/ml in PBS) for 2 h at room temperature, washed with PBS, and incubated with PBS-3% BSA for 2 h, followed by the addition of the bacterial extract as described above. LC-MK cells were cultured overnight in 24-well plate (105 cells/well), washed with PBS, fixed with 2% glutaraldehyde in PBS for 1 h at room temperature, washed with PBS, and incubated with PBS-3% BSA for 2 h at room temperature, incubated overnight at 4 °C with the clarified cell extract containing 1.5% BSA in PBS, washed with PBS, and the radioactivity bound to the cell or to extracellular matrix pro-
teins was eluted with 100 μl of 1% SDS. The radioactivity was determined in a 10% trichloroacetic acid precipitate of a 20-μl aliquot, and the remaining eluate (80 μl) was concentrated on Speed-Vac and analyzed by SDS-PAGE, followed by exposure to x-ray film.

Laminin Binding Assay to the Tc85-1 Fusion Protein—Wells of microtiter plates were coated with 50 μl of purified Tc85-11 protein (10 μg/ml in PBS supplemented with 8% urea) for 2 h at room temperature, washed with PBS, blocked with PBS-1% BSA (2 h at room temperature), and incubated with increasing amounts of laminin in TCMM (TCMM = 25 mM Tris, pH 7.5, 50 mM NaCl, 5 mM CaCl₂, 5 mM MgCl₂, 1 mM MnCl₂, 1% BSA) overnight (at 4 °C). The wells were washed, incubated for 2 h at room temperature with anti-laminin rabbit serum, washed again, incubated with peroxidase-conjugated anti-rabbit sera for 1 h at room temperature, and developed with ortho-phenylenediamine (Sigma). Laminin was purified from Engelbreth-Holm-Swarm tumor, as described (17).

RESULTS

Isolation of a Genomic DNA (Tc85-1) Insert That Encodes the mAbH1A10 Epitope and Shares Identity with Members of the gp85/Trans-sialidase Gene Family—A genomic DNA library constructed in λgt11 was screened with the mAbH1A10 as described under “Experimental Procedures” and one positive clone, named H3.3, was selected. Restriction enzyme mapping analysis of the genomic DNA insert, from now on designated as Tc85-1, revealed a 0.4-kb insert whose complementary strands have been sequenced. Translation of either complementary strand of Tc85-1 resulted in a complete open reading frame. To identify which strand encoded the epitope recognized by mAbH1A10, the Tc85-1 was subcloned into pGEX-1 plasmid, and two bacterial cell lines containing the insert in opposite orientations were selected, grown, and induced. Western blot analysis showed that only one of the two orientations produced a 40-kDa protein recognized by mAbH1A10 (Fig. 1B). The nucleotide sequence of Tc85-1, with the conceptual amino acid translation, is shown in Fig. 1A.

To determine whether Tc85-1 encodes a polypeptide that could represent a portion of an 85-kDa trypomastigote-specific protein, the insert was subcloned into the pTrcHis vector, expressed in E. coli, and the fusion protein was enriched by affinity chromatography. Sera from immunized mice with the fusion protein were employed to develop Western blots containing epimastigote and trypomastigote lysates, as well as the fusion protein. As shown in Fig. 2, only the fusion protein and an 85-kDa trypomastigote-specific protein were recognized. This result is identical to that observed with mAbH1A10 and suggests that the Tc85-1 insert encodes a portion of an 85-kDa protein recognized by mAbH1A10.

A sequence identity search of the GenBank™ data base with the Tc85-1 sequence revealed a high percent identity with all members of the trans-sialidase supergene family (23, 24). Those with the highest percent identity were genes encoding trypomastigote-specific surface glycoproteins of M. tuberculosis. With 115,000–110,000 (Fig. 3). Consistent with the GenBank™ search and the Western blot results, the Tc85-1 insert hybridized with trypomastigote, but not epimastigote, poly(A)⁺ RNA of length 3.7 kb as well as with a large number of different size restriction fragments of a genomic Southern blot containing EcoRI- and BamHI-digested genomic DNA (data not shown).

Cloning and Sequencing the cDNA Insert Tc85-11—A trypomastigote cDNA library constructed in λ Zap II was screened with the Tc85-1 insert, and the clone that contained a full-length cDNA of size >2 kb encoding the mAbH1A10 epitope were selected, restricted, subcloned into the pTrcHis vector, and expressed in E. coli. One clone, named Tc85-11, encoded an expression product that reacted positive with mAbH1A10 by Western blot and was selected for this work. The whole coding region of the Tc85-11 gene was sequenced in both of the complementary strands by ExoIII/S1 nuclease partial digestion

(32). As expected, the DNA sequence has significant identity with Tc85-1 (89%; 118 identical amino acids out of 132) and with several previously described members of the gp85/trans-sialidase supergene family. Northern blot analysis shows that the Tc85-11 insert hybridizes to a 3.7-kb trypomastigote stage mRNA but not to epimastigote or LLC-MK₂ mRNA, as expected (Fig. 5A). Chromosome mapping resulted in hybridization of the Tc85-11 insert with 15 out of 20 chromosome bands of the CL Brener clone genome (Fig. 5B).

The open reading frame of 2,361 base pairs codes for a peptide of 786 amino acids with a molecular mass of 84,496 daltons, a theoretical pI of 5.00, and contains two ASP boxes (S-X-D-X-G-T-W) present in bacterial neuraminidases, as well as the V-T-V-X-N-V-F-L-Y-D-R sequence, both common to all members of the trans-sialidase supergene family of T. cruzi.
The presence of N-glycosylation and GPI anchor insertion sites is in accordance with previous observations showing that Tc-85 is a GPI-anchored membrane glycoprotein (33, 34) with N-linked carbohydrates (35, 36). The Tc85-11 polypeptide has 12 putative N-glycosylation sites as well as two signal peptides, one at the amino-terminal portion of the molecule, which directs the protein to the endoplasmic reticulum, and the other at the carboxyl-terminal portion for anchor insertion (shown in Fig. 4).

**Mapping the H1A10 Epitope in Tc85-1**—Peptides of length 15 amino acids, which overlap in sequence by 5 amino acids and span the Tc85-11 polypeptide, were synthesized and used to map the mAbH1A10 binding site by a competitive inhibition assay (Fig. 6A). Microtiter wells coated with the Tc85-11 fusion protein were incubated with mAbH1A10 in the presence of individual peptides, and inhibition of binding of mAbH1A10 to Tc85-1 was measured by ELISA. Only peptide G significantly inhibited the ability of the monoclonal antibody to react with Tc85-1, indicating that the epitope is contained within this 15-amino acid sequence (Fig. 6B). The same result was achieved when the peptides were immobilized and the direct binding of mAbH1A10 determined by ELISA. Only peptide G promoted the binding of mAbH1A10 in a reproducible and specific manner (data not shown). As shown in Fig. 6C, inhibition of the binding of mAbH1A10 to Tc85-1 by peptide G is clearly concentration-dependent, while peptide F shows no inhibition of binding of mAbH1A10 in the concentration range of 0.1 to 1.5 mM. Comparison of both Tc85-1 and Tc85-11 encoded peptides show that only peptides A, F, and G are 100% identical in both sequences. Peptides E and H have only one amino acid change at their ends when both sequences are compared (Fig. 6D). The peptide G sequence is contained in the most conserved region of both proteins, and as shown in Fig. 6C, only peptide G, but not peptide F, inhibits mAbH1A10 binding. Our result suggests that the peptide G-containing region is involved with mAbH1A10 binding to Tc85-1, and its position in the whole protein is indicated in Fig. 4.

**The Tc85-11 Protein Binds to the Host Cell**—Tc-85 is possibly involved in the process of invasion of host cells by the parasite. To verify whether the protein encoded by the Tc85-11 gene binds to the host cell, the coding sequence of the gene was subcloned (minus the first 26 amino acids comprising the amino-terminal signal peptide) into the pET21 vector, and the recombinant protein was expressed in *E. coli* as described under “Experimental Procedures.” Using this protocol, labeled Tc85-11 recombinant protein was produced by bacterial cells under the control of the rifampicin-insensitive T7 RNA polymerase, while expression of the other *E. coli* genes was inhibited by rifampicin, since they remained under the control of the bacterial polymerase. As judged by the total extract autoradiography (Fig. 7B, total extract), the labeling was restricted to the Tc85-11 protein, since the major product is clearly the 85-kDa polypeptide. Some bands, which appear below this band, are probably degradation products of the main protein. The 85-kDa polypeptide is also specifically immunoprecipitated by the mAbH1A10 (data not shown). Upon incubation of this extract with glutaraldehyde-fixed LLC-MK2, the bound radioactivity was eluted with SDS, and an aliquot was precipitated with trichloroacetic acid. Fig. 7A shows that binding to the cells was concentration dependent. The remainder of the eluted radioactivity was analyzed by SDS-PAGE and fluorography revealing an 85-kDa, as well as a 37-kDa, polypeptide (Fig. 7B). The 37-kDa polypeptide might be a proteolytic fragment of the 85-kDa polypeptide, which maintained a cell binding domain.

**The Tc85-11 Gene Codes for the LBG**—It has been shown previously that acidic components of Tc-85 family (pI values of 5.5–6.5) bind to laminin (17). Considering that the Tc85-11 protein binds to the host cell, and the predicted pI for the polypeptide is 5.00, an investigation was undertaken in order to show that laminin may mediate Tc85-11 attachment to the host cell. [35S]Methionine metabolically labeled Tc85-11 protein was incubated with laminin and other extracellular proteins. As shown in Fig. 8A, Tc85-11 binds specifically to laminin.

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**Fig. 2.** Western blots of epimastigote lysate (lanes 1, 4, and 7), trypomastigote lysate (lanes 2, 5, and 8), and pTrcHis/Tc85-1 fusion protein (lanes 3, 6, and 9) were reacted with: normal mouse serum (A), mouse anti-pTrcHis/Tc85-1 fusion protein (B), and mAbH1A10 (C). Anti-pTrcHis/Tc85-1 fusion protein reacts with a trypomastigote-specific 85-kDa protein (lane 5) as well as with the pTrcHis/Tc85-1 fusion protein (lane 6) but did not react with epimastigote protein (lane 4).

**Fig. 3.** Comparison of the predicted amino acid sequence of Tc85-1 with members of the gp85/trans-sialidase gene family. Amino acid differences are shown. Dashed lines represent deletions in the individual sequences.
nin, but not to gelatin or fibronectin previously immobilized on plastic wells. This result suggests that the Tc85-11 cDNA insert encodes the LBG gene. Interestingly, although the 37-kDa polypeptide bound to cells, it did not bind to laminin in vitro. This result suggests the possible existence of two different domains on Tc85-11, one for laminin and the other for another

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**Fig. 4.** DNA sequence and amino acid sequence of the open reading frame of the Tc85-11 cDNA insert (GenBank™ accession number AF085860). The *asterisk* indicates putative N-glycosylation sites. The two ASP boxes, the common sequence to all members of the gp85/trans-sialidase family, and the peptide G region are indicated by boxes. At the amino- and carboxyl-terminal two putative signal peptides are underlined with the arrows indicating the possible sites of cleavage. Sites for GPI anchor insertion can be seen at the carboxyl-terminal upstream of the hydrophobic sequence.

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FIG. 5. Northern blot analysis and chromosome location of the Tc85-11 insert. A, Northern blot analysis shows that the Tc85-11 cDNA insert hybridizes to trypomastigote mRNA (lane 3) but not to epimastigote (lane 2) or LLC-MK2 mRNA (lane 1). B, chromosome mapping of the Tc85-11 gene. A pulse-field gel of epimastigote of the clone CL Brener was stained with ethidium bromide (lane 1) and blotted to the nylon membrane. The blot was hybridized with a telomeric probe (lane 2), with the Tc88-11 cDNA insert (lane 3) and with a β-tubulin probe (lane 4). The numbers at the left side indicate the defined chromosome bands, and the arrows point to bands that hybridized with the Tc85-11 probe.

FIG. 6. Epitope mapping. A, peptides used in the competition experiment for epitope mapping. The figure shows their sequences and how they overlap. B, results of the competition experiment with the individual peptides and mAbH1A10. The amount of mAbH1A10 bound to the wells in the presence of each peptide was determined by ELISA. The values represent the intensity of reaction in relation to the control (mAbH1A10 in PBS 1% Me2SO equals 100% with a S.D. for this control always below 5%). The bars show the average of three distinct experiments. C, results showing the effect of concentration of peptide on the binding of mAbH1A10 to Tc85-1. mAbH1A10 was incubated with increasing concentrations of either peptide F or G in PBS 1% Me2SO and binding was measured by ELISA. D, comparison between Tc85-1- and Tc85-11-encoded peptides. Amino acid differences are shown. Dashed lines represent deletions in the individual sequences. The peptide G region is indicated.

receptor located on the host cell membrane. To further confirm that the Tc85-11 gene actually codes for a polypeptide with adhesive properties to laminin, the recombinant protein was purified from *E. coli* inclusion bodies and used to coat microtiter wells, which were then incubated with increasing concentrations of laminin. Laminin bound to Tc85-11 protein in a saturable manner (Fig. 8B).

**DISCUSSION**

After the pioneer reports on the existence of a specific glycoprotein(s) of 85 kDa, called Tc-85, on the surface of the infectious form of *T. cruzi* (13, 14, 37), and the finding that monoclonal antibodies against Tc-85 were able to inhibit partially the entrance of the parasite into host cells (16), several proteins of equivalent molecular weight were implicated in parasite-host cell interaction (38–40). Antibodies developed against proteins of similar size have been found to be inhibitors of parasite penetration (41), and an 85-kDa protein was described as a fibronectin receptor (19). No sequence data are available for most of the glycoproteins of that size (19, 38, 39), but it is likely that all may belong to the gp85/trans-sialidase family (cf. Refs. 23 and 24), since all gp85 genes cloned to date are members of this gene family (4).

This work shows that the Tc-85 glycoproteins, which bind to whey germ agglutinin (13) and are recognized by mAbH1A10
MK2 cells. Corresponding bound fractions eluted from the lysate, and a shows cell bound radioactivity with increasing concentrations of the bacterial material was eluted with 1% SDS. 

FIG. 7. Binding of Tc85-11 to LLC-MK2 cells. Metabolically labeled Tc85-11 protein was incubated with glutaraldehyde-fixed LLC-MK2 cells. An aliquot of the lysate was electrophoresed in SDS-PAGE (lane labeled “Total Extract”). The cells were thoroughly washed by flushing buffer on the wells, after which the bound material was eluted with 1% SDS. A shows an autoradiography of SDS-PAGE performed with the corresponding bound fractions eluted from the cells.

As genetic analysis of members of this family has previously shown putative sites for N-glycosylation as well as for GPI anchor insertion (cf. Refs. 23 and 24), it is safe to generalize that all members of the family are indeed membrane GPI-anchored glycoproteins, since an N-linked oligosaccharide anthera (35, 36) and a lysously phosphorylatedinositol anchor (33, 34) have been chemically demonstrated as part of the Tc-85 glycoprotein molecules. Another GPI-anchored glycoprotein from metacyclic trypomastigotes with adhesive properties to cells also belongs to the gp85/trans-sialidase family (40). The present work shows that at least one of the members of the Tc-85 subfamily binds to the host cell through laminin. This is the first time a chemically defined ligand is demonstrated for a member of the 85-kDa glycoprotein superfamily with adhesive properties.

Laminins are found in basal laminae, which are present as a continuous sheet surrounding each muscle fiber and pass through the synaptic cleft at the neuromuscular junction. Basal laminae are also found surrounding vessels and organs, beneath the epithelium and endothelium (5). Interestingly, muscle and the neuromuscular junction are the preferred sites of T. cruzi-induced lesions. During the beginning of infection, the parasite has to leave the site of infection (usually the insect bite wound) and reach the bloodstream. The interaction of T. cruzi with the extracellular matrix is thus a crucial step for the success of infection, as the parasite must leave the blood probably by adhering to endothelial cells and crossing the basal laminae as well as interacting with the extracellular connective milieu. Thus, the presence of a laminin receptor on the surface of the infective parasite can be very important during this stage.

Antibodies directed against integrins partially block parasite cell invasion, and the authors suggested that VLA-4 and VLA-5, both fibronectin receptors are involved, although other β1, but not β2, integrins also participate in the invasion process (42). It has been proposed that fibronectin could work as a bridge between the parasite and the cell (19), and it is likely that the same mechanism would be operative in the case of laminin reported herein.

It is our working hypothesis that the gp85/trans-sialidase gene family comprises a family coding for adhesion proteins, with several of its members interacting with specific ligands. Glycoproteins of about the same molecular mass have been described as a fibronectin ligand (19) and a cell adhesion molecule (39, 40), although a 60-kDa glycoprotein (penetrin), which promotes adhesion of trypomastigotes to heparin, heparan sulfate, and collagen, has also been described (2). The Tc85/LBG binds to laminin but not to fibronectin, showing the specificity of the interaction. Kahn et al. (43) have reported for the whole family the existence of at least 100 genes of about 2–2.5 kb representing at least 1% of the T. cruzi genome. It is worth noting that the 3.5 h half-life of the Tc85 family is considerably short (44). This fast turnover could facilitate the progression of the parasite from blood vessels to the cells if different continuously expressed subsets of the family would bind to different ligands on the cell surfaces, extracellular matrices, and basal laminae. Another possibility is that one single member may interact with two or more ligands on the surface of the cell. In multicellular organisms, it is common to find situations in which the same adhesion molecules interact with several proteins or cell surface receptors (45, 46). For instance, laminin binds to heparin, laminin itself, collagen-IV, entactin, integrins, and other putative non-integrin receptors (10, 11). Thus, during the migration through the basal lamina, the parasite can be covered with laminin, fibronectin, collagen, and/or hep-
arin, allowing contact between the nearest cell and the invasive trypomastigote through integrin receptors. Detachment from binding could be provided by shedding of the 85,000-Da glycoprotein adhesion molecule, a phenomenon that is constantly occurring (34, 47) and/or by proteases, which could cleave the matrix elements. At least two proteases that might play a role in \textit{T. cruzi} infection have been described, cruzipain (48, 49) and an 80-kDa collagenase (50). Shedding and protease digestion could help the parasite to be freed of the successive haptotactic interactions, allowing its migration through the basal lamina and the extracellular milieu.

Pathogen surface proteins belonging to multigenic families are frequently related with protection and escape from the immune system (51). For \textit{T. cruzi}, the gp85/ trans-sialidase family members have also been proposed to play a role in evasion of the immune system by the parasite (52, 53), which obviously does not exclude their proposed function as adhesion molecules.

The high amount of nuclear information that the parasite has kept through evolution in order to maintain highly similar genomic sequences is suggestive of the importance of that family for species survival. Thus, considering that \textit{T. cruzi}, as opposed to African trypanosomes, needs to enter cells in order to survive, it is conceivable that this parasite may have found a mechanism by which, making subtle sequence modifications in otherwise very similar genes coding for different gp85 proteins, it could adhere to different molecules of the cell membrane, the basal lamina, and the cell matrix. The molecular evolution of this family may have been driven on one side by the pressure of the host immune system and on the other by the ability of the parasite to enter host cells. Thus, parasites were selected not only by their capacity to survive in the bloodstream but also to invade cells more efficiently.

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Cloning of a Surface Membrane Glycoprotein Specific for the Infective Form of
*Trypanosoma cruzi* Having Adhesive Properties to Laminin
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