The infective trypomastigote stage of Trypanosoma cruzi expresses a set of surface glycoproteins that are known collectively as Tc85 and belong to the gp85/trans-sialidase supergene family. A member of this family, Tc85–11, with adhesive properties to laminin and cell surfaces was recently cloned. In this report, the Tc85–11 domain for cell binding and its corresponding receptor on epithelial cell LLC-MK2 are described. Using synthetic peptides corresponding to the Tc85–11 carboxy-terminal segment, we show that the mammalian cell-binding domain colocalizes to the most conserved motif of the trypanosome gp85/trans-sialidase supergene family (VTXNVFLYNR). Even though Tc85–11 binds to laminin, the 19-residue cell-binding peptide (peptide J) does not contain the laminin-binding site, because it does not bind to laminin or inhibit cell binding to this glycoprotein. The host cell receptor for the peptide was characterized as cytokeratin 18. Addition of anti-cytokeratin antibodies to the culture medium significantly inhibited the infection of epithelial cells by T. cruzi. Tc85–11 is a multiadhesive glycoprotein, encoding at least two different binding sites, one for laminin and one for cytokeratin 18, that allow the parasite to overcome the barriers imposed by cell membranes, extracellular matrices, and basal laminae to reach the definitive host cell. This is the first description of a direct interaction between cytokeratin and a protozoan parasite.

Chagas' disease is a chronic and incapacitating illness, caused by the protozoan parasite Trypanosoma cruzi when infective trypomastigotes invade host cells (1). The protozoan is transmitted to humans by wound contamination with insect feces during blood sucking. A particularly important portal of entry is the ocular conjunctiva that is put in contact with contaminated insect feces by involuntary scratching from nearby bites on a sleeping person's face, leading to a periorbital swelling known as Romão's sign. Other forms of transmission such as blood transfusion, congenital transmission, and breast feeding are also important, particularly in northern hemisphere regions that received intense migratory currents from Ibero-American countries. In recent years, 85–90-kDa parasite surface proteins have been implicated in host cell invasion by different investigators (2–7). Our laboratory was the first to describe trypomastigote-specific 85-kDa surface glycoproteins, suggesting their role in host cell invasion by the parasite (6–10). These proteins, collectively denominated Tc85, form a population of heterogeneous glycosylphosphatidylinositol-anchored surface glycoproteins with similar molecular masses but different electric charges (7–8, 11). Tc85 proteins belong to the gp85/trans-sialidase gene superfam (12) and share common motifs with bacterial neuraminidases (1, 12–13). Interestingly, all members of the superfam contain a conserved sequence (VTXNVFLYNR) (12) upstream from the carboxyl terminus and absent in bacterial neuraminidases. The infection of at least one member of the Tc85 family in parasite-host cell interactions is indicated by the observation that the monoclonal antibody H1A10, which specifically recognizes Tc85 glycoproteins, inhibits host cell invasion by the parasite in vitro by 50–90% (6, 7). An acidic 786-amino acid member of the Tc85 family (Tc85–11) and a recombinant fusion protein of the monoclonal antibody H1A10 epitope-containing carboxyl-terminal segment of Tc85–11 (Tc85–1) both showed adhesive properties to isolated laminin and to entire cells (10).

The high plasticity of the cytoskeleton is often exploited by pathogens to enter non-phagocytic cells. Increasing evidence has been provided for the expression of cytoskeletal proteins on cell surfaces that serve as receptors for different ligands. For example, intermediate filament proteins belonging to the cytokeratin family are expressed on the cell surface and act as receptors for bacteria as well as for plasminogen and tissue plasminogen activator, high molecular weight kininogen, and thrombin-antithrombin complexes (14–20).

The present work demonstrates that the conserved common sequence VTXNVFLYNR of the gp85 glycoprotein/trans-sialidase supergene family is a mammalian cell-binding domain. Its host cell receptor for this motif was purified and characterized as cytokeratin 18 (CK18) present on the surface of LLC-MK2
cells (monkey kidney epithelial cells). Because Tc85 also binds to laminin (10), the results presented herein suggest that the Tc85 family is composed of multiahesive glycoproteins that bind to different receptor molecules either located on the cell surface or belonging to components of the extracellular matrix.

**EXPERIMENTAL PROCEDURES**

**Parasite Strain and Culture—** T. cruzi strain Y was used throughout. Culture conditions for parasites and mammalian cells are described elsewhere (21).

**Peptide Synthesis—** Peptides were synthesized in an automated bench top simultaneous multiple solid phase synthesizer (PSSM 8 system from Shimadzu) using the Fmoc (N-(9-fluorenyl)methoxycarbonyl) procedure. The synthesized peptides were deprotected and purified by semipreparative HPLC using an Econosil C-18 column (10 μm, 22.5 x 150 mm) and a two-solvent system: (A) triﬂuoroacetic acid/H2O (1:1000) and (B) triﬂuoroacetic acid/MeCN/H2O (1:900:100). The peptides were separated at a flow rate of 7 ml/min and a gradient from 0 (or 30) to 50 (or 60%) of solvent B. Analytical HPLC was performed using a binary HPLC system (Shimadzu) with an SPD-10AV detector, coupled to an Ultrasphere C-18 column (5 μm, 4.6 x 150 mm). The peptides were separated at a flow rate of 5 ml/min and a gradient from 0 to 100% of solvent B. The purity of obtained peptides was checked by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) spectroscopy in the reflectron mode (ToF-Spec-E from Micromass, Manchester, UK) and by amino acid sequencing, performed with a Shimadzu sequencer, model PPSQ-20 (22).

**Binding of Cells to Synthetic Peptides—** In a 24-well plate, 40 μg of each peptide in 200 μl of 10% MeSO were dripped overnight at 37 °C with agitation, washed with PBS (140 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.3), and incubated for 2 h with 1% BSA/PBS. LLC-MK2 cells were cultured as described (6) in a 75-cm² bottle, removed by trypsin, and resuspended in 5 ml of DME medium supplemented with 10% FCS. The cells were incubated for 1 h at 37 °C in 50-ml polyethylene tubes (Corning) and washed twice with DME medium to remove FCS. Then, 1 x 10⁵ cells in 0.5 ml of DME medium were added to the peptide-coated wells and incubated for 1 h at 37 °C. The cells were washed three times with DME medium and analyzed using an inverted microscope. In binding competition assays, LLC-MK2 cells were preincubated for 15 min with the peptide of interest and added to peptide-coated wells. After incubation and washing as described, the number of bound cells was quantified following staining with crystal violet (23).

**Binding of 125I- Peptide J to Mammalian Cells—** Peptide J was radiolabeled with 125I (Amersham Pharmacia Biotech) using the chloramine-T method (24) and purified by reverse-phase HPLC, resulting in a specific activity of 1 x 10⁶ cpm μg⁻¹. LLC-MK2 cells were cultured as described above, and 6 x 10⁵ cells were incubated for 2 h on ice in the presence of increasing concentrations of the radiolabeled peptide. Non-specific binding was determined in the presence of a 100-fold excess of unlabeled peptide J. The reaction mixtures were washed three times to remove unbound radiolabeled ligand, and cells were lysed in 1% SDS for 10 min and directly assayed for radioactivity by scintillation counting. Each experiment was performed in triplicate.

**Isolation and Biotinylation of Cell Surface Proteins—** LLC-MK2 and K562 cells were collected as described, washed three times with PBS, and biotinylated with the EZ-Link-Sulfo-NHS-biotinylation kit (Pierce) as recommended by the manufacturer. Plasma membranes were prepared (25) and solubilized in 100 mM β-octyl glucoside for 2 h at 4 °C.

**Affinity Chromatography—** Peptide J was synthesized with an additional cysteine at the amino terminus. One mg of peptide J was coupled to a solid matrix (UltraLink™ iodoacetyl, Pierce) and used for affinity chromatography experiments. The supernatants from solubilizations in β-octyl glucoside were incubated overnight with the peptide J affinity gel at 4 °C with agitation. The gel was loaded into a column, and the column was washed with 40 volumes of 25 mM β-octyl glucoside in incubation buffer. The gel was then washed with 1 x 10⁶ μg/ml of peptide J in incubation buffer, followed by agitation for 1 h at room temperature. The column was washed again with 40 column volumes of PBS and then incubated with 8 μl urea as above. The collected fractions were dialyzed, concentrated, and analyzed by SDS-PAGE (26) in 9% gels.

**Western Blot—** Following analysis by SDS-PAGE, proteins were transferred to a supported nitrocellulose membrane using 25 mM Tris, 150 mM glycine, and 20% methanol (pH 8.3) as transfer buffer. The blots were blocked with 3% BSA in TBST (Tris-buffered saline, 10 mM Tris, pH 7.5, 150 mM NaCl) containing 0.05% Tween 20 and 0.03% Triton X-100 and incubated for 2 h at room temperature with Extravidin-peroxidase (Sigma) or anti-PAN-cytokeratin antibody (Sigma), as recommended by the manufacturer. The bound complexes were then visualized with 3, 4, 5, 6, 8, 10, 13, and 18. The membrane was washed with TBST and, when necessary, incubated with the secondary antibody conjugated to peroxidase. The reaction was developed with the ECL kit (Amersham Pharmacia Biotech).

**RESULTS**

**Definition of the Tc85-1 Cell-binding Site—** To define the Tc85-1 cell-binding site, we synthesized 11 peptides with five amino acid overlaps that spanned the carboxyl-terminal segment of the recombinant Tc85-11 protein. These were used to coat the surface of 24-spot plates and mediate LLC-
MK₂ cell adhesion. As shown in Fig. 1A, the cells only adhered significantly to the well coated with peptide J (19 amino acids) that contained the VTVTNVFLYNR motif. This motif is highly conserved and present in all members of the gp85/trans-sialidase superfamily. This observation implicates the importance of that common sequence in the binding of members of the gp85/trans-sialidase supergene family to their host cell receptors (Fig. 1B).

Other cell lines that are invaded by *T. cruzi* were tested for their affinity for peptide J. In addition to LLC-MK₂ cells, tumor cells (B16F10), human umbilical cord endothelial cells (ECV), macrophage-like cells (J774), mouse fibroblast cells (3T3), and mouse pheochromocytoma cells (PC-12) bound to peptide J (data not shown). Consistent with a crucial function of peptide J in cell invasion, mouse erythrocytes and K562 erythroleukemia cells (27) that are not invaded by *T. cruzi* did not bind to peptide J.

Additional evidence for the physiological relevance of peptide J binding to mammalian cells is that a 10⁻⁶ M concentration of this peptide inhibited the binding of the recombinant Tc85–11 protein to the host cell. As opposed to Tc85–11, peptide J does not bind to laminin; nor does it inhibit cell binding to laminin. Furthermore, it was established that peptide J does not bind to cells at the same receptor used by laminin or to laminin on its cell-binding domain, because different concentrations of this glycoprotein did not affect LLC-MK₂ adhesion to peptide J. The combined data strongly suggest that the Tc85–11 recombinant protein is a molecule with multiple adhesion sites, specific for different ligands of the vertebrate host cell.

Radioiodinated peptide J binds to LLC-MK₂ cells in a specific, saturable manner, as shown by nonlinear saturation analysis (Fig. 2). The data suggest 1.66 ± 0.16 × 10⁶ binding sites with a *K₅* of 175 ± 56 nM. The number of binding sites is comparable with that determined for plasminogen binding to CK8 (15). Interestingly, cytokeratin 8 associates with CK18 to form an intermediate filament heteropolymer in several cell types.

**Mapping of the Amino Acids Required for the Binding of**

**Fig. 1.** The most conserved sequence of the gp85/trans-sialidase superfamily binds to LLC-MK₂ cells. A, each well of a 24-well plate was coated with 40 µg of peptides A–K corresponding to the 131 amino acids of the carboxyl terminus of Tc85–11 in the presence of BSA to diminish unspecific binding and then incubated with 1 × 10⁵ LLC-MK₂ cells in 0.5 ml of DME medium for 1 h at 37 °C. The wells were washed three times with DME medium, and analysis by inverted microscopy revealed that cells adhered significantly only to the well coated with peptide J but not to wells coated with BSA or other peptides. The
Peptide J to the Host Cell by Alanine Scanning—To identify the minimal sequence that is relevant for the binding of peptide J to the host cell, truncated peptides were constructed spanning the whole sequence of peptide J. Cells were layered on peptide J-coated plates, and truncated peptides were checked for their competing ability for cell adhesion. The minimal inhibitory sequence was VTNVFLYNRPL (data not shown). To identify the residues responsible for this binding, each amino acid of the minimal inhibitory sequence was consecutively substituted by alanine, and the modified sequences were tested for their inhibitory effects in adhesion assays of peptide J to cells. It was observed that substitution in some positions resulted in the loss of the inhibitory effect on the binding of cells to peptide J (Fig. 3). These experiments strongly indicate that the amino acid sequence VTXVFLYXR, conserved in most members of the 85-kDa trypomastigote surface glycoprotein family, is essential for parasite-cell interaction. In 40 analyzed sequences (Fig. 1B), LYXR was present in all members of the family, whereas the first Val residue that is found in 80% of the sequences was substituted in the remaining sequences by Leu or Ala, which are also apolar amino acids. Threonine, at position 2, showed a smaller degree of conservation (38%), most often being replaced by other polar residues: Ser (15%), Asn (20%), and Lys (15%). The valine at position 4 is again highly conserved (95%), and Leu substitutes Phe at position 5 in 38% of the molecules.

Identification of the Host Cell Receptor for the Truncated Common Sequence of Tc85—To characterize the host cell receptor for peptide J, the peptide was coupled to an affinity matrix and used for purification of the receptor, employing chromatographic methods. The affinity matrix was incubated with solubilized membranes from biotinylated LLC-MK2 cells. The 8 M urea eluates of the LLC-MK2 cell extracts revealed a biotinylated 45-kDa molecule that was detected by Western blot (Fig. 4).

To obtain further evidence that the 45-kDa molecule is the host cell receptor, radiiodinated peptide J was chemically cross-linked with LLC-MK2 cells. For a negative control, we also performed the experiment using K562 cells, which were neither infected by T. cruzi nor adhered to surfaces coated with peptide J. As an additional control, an alanine-substituted peptide, peptide J-Ala (VTNVFYNRPL), that does not inhibit cell binding to peptide J was radiolabeled and cross-linked to LLC-MK2 cells. Solubilization and separation of plasma membrane proteins by SDS-PAGE, a protein migrating with a molecular mass of 45 KDa was detected only in the LLC-MK2 cell extract (Fig. 5). The labeling of the 45-kDa protein was specific, because it could be inhibited by a 100-fold molar excess of unlabeled peptide. As expected, no specific labeling of K562 cells by 125I-peptide J was observed, and the peptide J-Ala did not bind to LLC-MK2 cells. These results strongly suggest that a 45-kDa molecule present on LLC-MK2 cells is involved in adhesion of the parasite to these cells.

Cytokeratin 18 Is a Host Cell-binding Site for the Most Conserved Domain of the Tc85 Family—Purified 45-kDa biotinylated protein fractions, as described in Fig. 4, were digested with trypsin, and the peptides were analyzed by mass spectrometry. The identified peptides from three independent experiments indicated that the 45-kDa molecule was biotinylated CK18. In agreement with the mass spectrometry analysis, the isolated protein comigrated with authentic cytokeratin 18 with an apparent molecular mass of 45 kDa and a pI of 5.4 in a two-dimensional SDS-PAGE (data not shown). To further confirm these results, LLC-MK2 and K562 plasma membrane extracts were incubated with the peptide J affinity column, and after elution with 1 M NaCl and 8 M urea, the eluates were...
fractions of LLC-MK2 and K562 cells were prepared and incubated for 2 h on ice with \( ^{125}\text{I}-\text{peptide J} \) or \( ^{125}\text{I}-\text{peptide J-Ala} \) in the presence (+) and absence (−) of a 100-fold excess of unlabeled peptide. The receptor-ligand complexes were separated by centrifugation, and chemical cross-linking was performed with ethyl-3-(3-dimethylaminopropyl)carbodiimide as described under “Experimental Procedures.” After washing and solubilization, proteins were separated by SDS-PAGE, and \( ^{125}\text{I}-\text{peptide J-protein complexes} \) were detected by autoradiography. The arrow marks the 45-kDa region. Identical results were obtained in six independent experiments.

CK18 Is Present on the Surface of Intact LLC-MK2 Cells and Binds to Trypomastigotes—Intact LLC-MK2 and K562 cells were tested for the presence of cytokeratin by immunofluorescence microscopy with fluorescent anti-CK18-specific antibody (Fig. 7). Whereas CK18 is present in the cytoplasm of both cell lines, only LLC-MK2 cells express cytokeratin on the surface. Moreover, \( ^{125}\text{I}-\text{peptide J} \) binds in a specific manner to trypomastigotes (Fig. 8) but not to epimastigotes, the noninvasive developmental form of \( T. cruzi \) (data not shown).

\( ^{125}\text{I}-\text{peptide J} \) and \( ^{125}\text{I}-\text{peptide J-Ala} \) were incubated with LLC-MK2 cell membrane fractions from K562 and LLC-MK2 cells were incubated with the peptide J affinity gel. The columns were washed with PBS and eluted with 1 M NaCl and 8 M urea. The collected fractions were analyzed by SDS-PAGE, and \( ^{125}\text{I}-\text{peptide J-protein complexes} \) were detected by autoradiography. The arrow marks the 45-kDa region. No 45-kDa molecule was eluted from columns that were saturated with BSA instead of peptide J.

analyzed by SDS-PAGE and tested by Western blot with anti-PAN-cytokeratin antibody. As shown in Fig. 6A, a cytokeratin molecule of 45 kDa is present only in 8 M eluates of LLC-MK2 cells. As expected, no K562 cytokeratin could be eluted from the peptide J column. Furthermore, \( ^{125}\text{I}-\text{CK18} \) bound to the peptide J affinity column and showed the same elution pattern as CK18 from LLC-MK2 cells (Fig. 6B). Control BSA columns did not bind CK18. The fact that the anti-PAN-cytokeratin antibody, which recognized many proteins in the cell extract, was able to recognize only CK18 in the column eluate suggests a highly specific binding.

Fig. 5. A 45-kDa host cell surface molecule is specifically labeled by \( ^{125}\text{I}-\text{peptide J} \) but not by \( ^{125}\text{I}-\text{peptide J-Ala} \). Membrane fractions of LLC-MK2 and K562 cells were prepared and incubated for 2 h on ice with \( ^{125}\text{I}-\text{peptide J} \) (Pep J) or \( ^{125}\text{I}-\text{peptide J-Ala} \) (Pep J-Ala) in the presence (+) and absence (−) of a 100-fold excess of unlabeled peptide. The receptor-ligand complexes were separated from free ligands by centrifugation, and chemical cross-linking was performed with ethyl-3-(3-dimethylaminopropyl)carbodiimide as described under “Experimental Procedures.” After washing and solubilization, proteins were separated by SDS-PAGE, and \( ^{125}\text{I}-\text{peptide J-protein complexes} \) were detected by autoradiography. The arrow marks the 45-kDa region. Identical results were obtained in six independent experiments.

Fig. 7. CK18 is expressed on the surface of LLC-MK2 but not on K562 cells. Viable, impermeabilized LLC-MK2 cells adhered to peptide J- or FCS-coated wells, and K562 cells were fixed with 4% paraformaldehyde and incubated with a specific anti-CK18 antibody followed by fluorescein isothiocyanate anti-mouse antibody IgG. LLC-MK2 cells showed a patchy fluorescent pattern of cytokeratin, whereas no fluorescence was observed in viable, impermeabilized K562 cells. When the cells were permeabilized with 0.05% saponin, a diffuse cytoplasmic fluorescence pattern of cytokeratin was observed in LLC-MK2 and K562 cells. The light colored panels show phase contrast, and the darker colored panels show immunofluorescence images. Similar results were observed in three independent experiments.
S.D. of two experiments.

The data show the average of triplicate determinations over nitrocellulose filters that were previously saturated with 0.1% purified trypomastigotes were incubated with 1 × 10^6 cpm of ^125I-CK18 in the presence (black bar) and absence (white bar) of a 20-fold excess of unlabeled CK18. The incubation mixtures were separated by filtration over nitrocellulose filters that were previously saturated with 0.1% BSA, and the filter-bound radioactivity was quantified using scintillation counting. The data show the average of triplicate determinations ± S.D. of two experiments.

**DISCUSSION**

*T. cruzi* invades non-phagocytic cells in an energy-dependent manner (28) by a mechanism different from phagocytosis. Invasion is preceded by an adhesion step involving surface molecules from both the parasite and the host cell. Members of the Tc85 glycoprotein family, expressed on the surface of the infective form of *T. cruzi*, were first suggested to be involved in the invasion process (29). Members of the Tc85 family represent 1–2% of the *T. cruzi* genome, with highly redundant and simultaneously expressed members, a stumbling block that eliminates the possibility of employing genetic approaches to their functional analysis. It is our working hypothesis that the gp85/trans-sialidase gene family, in addition to members coding for trans-sialidase activity, comprises a family coding for adhesion proteins, with several of its members interacting with specific ligands. It is worth noting that the 3.5-h half-life of the Tc85 family is considerably short (35). This fast turnover could facilitate the progression of the parasite from blood vessels to the cells if different continuously expressed subsets of the family bound to different ligands on cell surfaces, extracellular matrices, and basal laminae. It would seem likely that cruzipain (36, 37) and other proteases (38) could be operative in breaking the successive protein-protein interactions, thus facilitating parasite progression. An additional, but not exclusive, possibility is that individual members of the Tc85 family may interact with two or more ligands on the cell surface.

The latter hypothesis is favored by our data, because Tc85–11 has two different binding sites. One of these sites for cruzipain binding block that eliminates the possibility of employing genetic approaches to their functional analysis. It is our working hypothesis that the gp85/trans-sialidase gene family, in addition to members coding for trans-sialidase activity, comprises a family coding for adhesion proteins, with several of its members interacting with specific ligands. It is worth noting that the 3.5-h half-life of the Tc85 family is considerably short (35). This fast turnover could facilitate the progression of the parasite from blood vessels to the cells if different continuously expressed subsets of the family bound to different ligands on cell surfaces, extracellular matrices, and basal laminae. It would seem likely that cruzipain (36, 37) and other proteases (38) could be operative in breaking the successive protein-protein interactions, thus facilitating parasite progression. An additional, but not exclusive, possibility is that individual members of the Tc85 family may interact with two or more ligands on the cell surface.

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for similar motifs in other pathogen-host cell interactions un-

The view that cytokeratins and other intracellular proteins are confined solely to the cytosol has recently undergone revi-

In summary, the results herein presented favor the hypothesis that the conserved common sequence of the gp85/trans-sialidase family is an important docking domain to the host cell surface, although other sites should not be ruled out (10, 27). Along with growing evidence for surface expression of intracellular proteins and the expression of cytokeratins also in epithelial and other tissues, our data indicate CK18 as a putative mammalian cell receptor for T. cruzi and/or a binding protein that is necessary for further receptor activation. Other studies have shown that transforming growth factor β receptors are required for the infection of mammalian cells by T. cruzi (45), but the parasite ligand is unknown. Interestingly, cells treated with epidermal growth factor plus transforming growth factor β express higher levels of CK18 (46). The fact that CK18 can be phosphorylated (47) suggests an involvement of cytokeratin in the intracellular signaling induced by T. cruzi (48).

Previous incubation of LLC-MK2 cells with peptide J or Tc85–11 increases cell invasion by T. cruzi, suggesting a role for the gp85/trans-sialidase family as signaling molecules that enhance receptiveness of the host cell for the parasite by a yet unknown mechanism. Members of this family possessing a relatively short half-life (35) are constitutively shed into culture medium (49), suggesting that contact between the surface of the parasite or shed Tc85 proteins and CK18 in the mammalian cell may promote signaling events in the host cell, thus facilitating T. cruzi infection.

T. cruzi internalization requires host cell lysosome recruitment, inducing localized clustering and fusion of host cell lysosomes with the plasma membrane at the site of trypomastigote attachment. This process requires host cell [Ca2+]i transients and transient rearrangement of actin microfilaments, which might facilitate lysosome access to the plasma membrane during parasite invasion (48). Because other filaments are connected to actin microfilaments in the cytosol, it is possible that parasite binding to CK18 may also influence the lysosome migration process. It is worth noting that thrombin-anti-thrombin complexes, which are internalized via the CK18 receptor on the surface of hepatocytes, are degraded by lysosomes (17).

T. cruzi infection is a complex process involving several host and parasite molecules in the recognition process as well as the involvement of enzymatic reactions and bivalent ions. The present study indicates a new and physiologically relevant role for the most conserved sequence of the gp85/trans-sialidase super gene family. We have shown that this sequence is involved in host cell binding during the infection process and that CK18 is a putative trypomastigote receptor on epithelial cells.

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REFERENCES

1. Colli, W. (1993) PASEB J. 7, 1257–1264
2. Burleigh, B. A., and Andrews, N. W. (1993) Annu. Rev. Microbiol. 47, 175–200
3. Ortega-Barria, E., and Pereira, M. E. A. (1992) Infect. Agents Dis. 1, 136–145
4. Ouaissi, M. A., Cornette, J., Afnahn, D., Capron, A., Gras-Masse, H., and Tartar, A. (1986) Science 234, 653–657
5. Ramirez, M. I., Ruiz, B., Areh, E., Silveira, J. F., and Yoshida, N. (1993) Infect. Immun. 61, 3636–3641
6. Alves, M. J. M., Abuin, G., Kuwajima, V. J., and Colli, W. (1986) Mol. Biochem. Parasitol. 21, 75–82
7. Abuin, G., Colli, W., Souza, W., and Alves, M. J. M. (1989) Mol. Biochem. Parasitol. 35, 229–238
8. Katzin, A. M., and Colli, W. (1983) Biochim. Biophys. Acta 727, 403–411
9. Giardino, R., Chamas, R., Veiga, S. S., Colli, W., and Alves, M. J. M. (1994) Mol. Biochem. Parasitol. 65, 85–94
10. Giardino, R., Fouts, D. L., Tevari, D., Colli, W., Manning, J. E., and Alves, M. J. M. (1999) J. Biol. Chem. 274, 3461–3468
11. Andrews, N. W., Katzin, A. M., and Colli, W. (1984) Eur. J. Biochem. 140, 599–604
12. Cruz, G. A. M., and Takeda, G. B. (1993) Annu. Rev. Microbiol. 47, 385–411
13. Schenkmann, S., and Eichinger, D. (1993) Parasitol. Today 9, 218–222
14. Hembrough, T. A., Vasudevan, J., Allietta, M. M., Glass II, W. F., and Gonias, S. L. (1995) J. Cell Sci. 108, 1071–1082
15. Hembrough, T. A., Li, L., and Gonias, S. L. (1996) J. Biol. Chem. 271, 25684–25691
16. Hembrough, T. A., Kralovich, K. R., Li, L., and Gonias, S. L. (1996) Biochem. J. 317, 763–769
17. Wells, M. J., Hatton, M. W., Hewlett, B., Podor, T. J., Sheffield, W. P., and Blajchman, M. A. (1997) J. Biol. Chem. 272, 28574–28581
18. Hasman, A. A. K., Zisman, T., and Schmaier, A. H. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 6801–6805
19. Sajian, U. S., Sylvester, F. A., and Forstner, J. F. (2000) Infect. Immun. 68, 1787–1792
20. Tamura, G. S., and Nittayajarn, A. (2000) Infect. Immun. 68, 2129–2134
21. Andrews, N. W., and Colli, W. (1982) J. Protoc. 14, 447–451
22. Bates, S. A., and Alberico, F. (2000) Solidphase Synthesis: A Practical Guide, Marcel Dekker, Inc., New York
23. Morla, A., Zhang, Z., and Roosdela, E. (1994) Nature 367, 193–196
24. Roth, J. (1975) Methods Enzymol. 27, 223–233
25. Borrow, P., and Oldstone, M. B. A. (1992) J. Virol. 66, 7270–7281
26. Lednicky, U. K. (1978) Nature 277, 680–685
27. Ruiz, R. C., Favoreto, S. Jr., Dorta, M. L., Oshiro, M. E., Ferreira, A. T., Manque, P. M., and Yoshida, N. (1996) Biochem. J. 329, 505–511
28. Schenkmann, S., Robbins, E. S., and Nussenzeew, V. (1991) Infect. Immun. 59, 645–654
29. Zingales, B., Andrews, N. W., Kuwajima, V. J., and Colli, W. (1982) Mol. Biochem. Parasitol. 6, 111–124
30. Boschetti, M. A., Piras, M. M., Henriquez, D., and Piras, R. (1997) Mol. Biochem. Parasitol. 24, 175–184
31. Lima, M. F., and Villafita, F. (1989) Mol. Biochem. Parasitol. 33, 159–170
32. Araguth, M. F., Rodrigues, M. M., and Yoshida, N. (1988) Parasite Immunol. (Off.) 10, 707–712
33. Ouaissi, M. A., Cornette, J., and Capron, A. (1986) Mol. Biochem. Parasitol. 19, 201–211
34. Frasch, A. C. (2000) Parasitol. Today 16, 282–286
35. Abuin, G., Colli, W., and Alves, M. J. M. (1996) Braz. J. Med. Biol. Res. 29, 335–341
36. Cazzulo, J. J., Stoka, V., and Turk, V. (1997) Biol. Chem. 378, 1–10
37. Schafstein, J., Schmitz, V., Morandi, V., Capella, M. M., Lima, A. P., Morrot, A., Juliano, L., and Muller-Esterl, W. (2000) J. Exp. Med. 192, 1289–1300
38. Santana, J. M., Grellet, P., Schrivel, J., and Teixeira, A. R. (1997) Biochem. J.
39. Hynes, R. O. (1992) Cell 69, 11–25
40. Haas, T. A., and Plow, E. F. (1994) Curr. Opin. Cell Biol. 6, 656–662
41. Cavallesco, R., and Pereira, M. E. A. (1988) J. Immunol. 140, 617–625
42. Miettinen, M., and Fetsch, J. F. (2000) Hum. Pathol. 31, 1062–1067
43. Mattey, D. L., Nixon, N., Wynn-Jones, C., and Dawes, P. T. (1993) Br. J. Rheumatol. 32, 676–682
44. Morianau, J., Pett, J. W., Riordan, J. F., and Vallee, B. L. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3815–3819
45. Ming, M., Ewen, M. E., and Pereira, M. E. A. (1995) Cell 82, 287–296
46. Sánchez, A., Pagan, R., Alvarez, A. M., Roncero, C., Vilario, S., Benito, M., and Fabregat, I. (1998) Exp. Cell Res. 242, 27–37
47. Ku, N. O., Liao, J., and Omary, M. B. (1998) EMBO J. 17, 1892–1906
48. Burleigh, B. A., and Andrews, N. W. (1998) Curr. Opin. Microbiol. 1, 461–465
49. Gonçalves, M. F., Umezawa, E. S., Katzin, A. M., de Souza, W., Alves, M. J. M., Zingales, B., and Colli, W. (1991) Exp. Parasitol. 72, 43–53.
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