Infectivity of *Trypanosoma cruzi* strains is associated with differential expression of surface glycoproteins with differential Ca^{2+} signalling activity

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Mammalian cell invasion assays, using metacyclic trypomastigotes of *Trypanosoma cruzi* G and CL strains, showed that the CL strain enters target cells in several-fold higher numbers as compared with the G strain. Analysis of expression of surface glycoproteins in metacyclic forms of the two strains by iodination, immunoprecipitation and FACS, revealed that gp90, undetectable in the CL strain, is one of the major surface molecules in the G strain, that expression of gp82 is comparable in both strains and that gp35/50 is expressed at lower levels in the CL strain. Purified gp90 and gp35/50 bound more efficiently than gp82 to cultured HeLa cells. However, the intensity of the Ca^{2+} response triggered in HeLa cells by gp82 was significantly higher than that induced by gp35/50 or gp90. Most of the Ca^{2+} signalling activity of the metacyclic extract towards HeLa cells was due to gp82 and was inhibitable by gp82-specific monoclonal antibody 3F6. Ca^{2+} mobilization was also triggered in metacyclic trypomastigotes by host-cell components; it was mainly gp82-mediated and more intense in the CL than in the G strain. We propose that expression of gp90 and gp35/50 at high levels impairs binding of metacyclic forms to host cells through productive gp82-mediated interaction, which leads to the target-cell and parasite Ca^{2+} mobilization required for invasion. Analysis of metacyclic forms of eight additional *T. cruzi* strains corroborated the inverse correlation between infectivity and expression of gp90 and gp35/50.

INTRODUCTION

A number of studies have revealed that invasion of mammalian cells by *Trypanosoma cruzi*, the protozoan parasite that causes Chagas' disease, requires host-cell Ca^{2+} mobilization [1–5], an event triggered by specific parasite factors [3,6]. In metacyclic trypomastigotes, the *T. cruzi* developmental forms that initiate infection in mammalian hosts, we have identified a glycoprotein of 82 kDa (gp82) and a 35/50 kDa mucin-like glycoprotein (gp35/50), both of which have been implicated in the process of cell invasion [7,8], as molecules capable of inducing a Ca^{2+} signal in target cells [3].

The ability to enter host cells may differ significantly in metacyclic forms of different *T. cruzi* strains [8]. What determines such variability is not clear. Circumstantial evidence has suggested that it may result from differential expression of surface molecules in distinct strains [8]. Variation in expression of metacyclic trypomastigote surface glycoproteins that interact with mammalian cells, such as gp35/50 and gp90 [7,9], has been reported [10,11], but correlation with infectivity has not been established. One interesting possibility is that the differential Ca^{2+} signalling activity of the various parasite surface molecules, differentially expressed in *T. cruzi* strains, determines infectivity. To test that possibility, we examined in metacyclic trypomastigotes of different strains whether infectivity correlated with expression of surface molecules, in particular of gp35/50, gp82 and gp90, and whether these glycoproteins differed in their ability to induce a Ca^{2+} response in host cells.

In addition to Ca^{2+} mobilization in target cells, elevation in parasite Ca^{2+} concentration has been claimed to play a part in *T. cruzi* internalization [2,12]. We therefore compared the Ca^{2+} response triggered by host-cell components in metacyclic forms of *T. cruzi* strains displaying distinct cell-invasion capacity. Furthermore, we investigated whether gp90, gp35/50 or gp82 play a role in that response.

MATERIALS AND METHODS

Parasites, mammalian cells and cell-invasion assay

T. cruzi strains G [13] and CL [14] were used throughout this study. In one set of experiments we also used eight additional strains, namely: Y [15], F [16], Tulahuen [17], M226 [18], Costalimai [19] and the Venezuelan strains Dm28, Dm30 and Guafitas [10]. Parasites were maintained alternately in mice and in axenic cultures. Liver infusion tryptose medium [20] and Grace's medium (Gibco) were used respectively to grow parasites and to obtain cultures enriched in metacyclic forms, which were purified by passage through a DEAE-cellulose column, as described [21]. HeLa cells, the human carcinoma-derived epithelial cells, Vero cells, which are fibroblasts from kidney of African green monkey, and human leukaemic K562 cells [22] were grown at 37 °C in RPMI 1640 supplemented with 10 % (v/v) fetal calf serum, streptomycin (100 μg/ml) and penicillin (100 units/ml) in a humidified 5 % CO_2 atmosphere. Experiments of mammalian cell invasion by *T. cruzi* were performed essentially as previously described [23].

Monoclonal antibodies (mAbs)

mAbs 1G7, 3F6 and 2B10, directed respectively to *T. cruzi* metacyclic trypomastigote surface glycoproteins gp90, gp82 and gp35/50, and generated as described [11,21], were purified on a Protein A-Sepharose column (Pharmacia Biotech). Unrelated mAb 1C3, directed to *Leishmania amazonensis* gp63 [24], was a gift from Clara Lucia Barbieri (Universidade Federal de São Paulo).

Abbreviations used: $[Ca^{2+}]_i$, cytosolic free Ca^{2+}; GPI, glycosylphosphatidylinositol; mAb, monoclonal antibody.

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Purification of native and recombinant *T. cruzi* proteins and cell-binding assay

To isolate gp35/50 from metacyclic trypomastigotes, we followed the procedure described by Ruiz et al. [7]. Gp90 and gp82 were purified by affinity chromatography on immobilized mAbs 1G7 and 3F6 respectively, as described [25,26]. Recombinant proteins containing sequences of gp90 [27] or gp82 [28] were purified as detailed in [28], followed by electroelution [29]. Binding of purified molecules to HeLa cells was assayed by using para-formaldehyed-fixed cells [8]. *L. amazonensis* gp63 was provided by Clara Lucia Barbieri.

Flow cytometry

Live metacyclic trypomastigotes (5×10^6) were incubated for 1 h on ice with mAbs directed to metacyclic trypomastigote surface molecules, or with unrelated mAb 1C3. After three washings with PBS, the parasites were fixed with 2% paraformaldehyde in PBS for 30 min. The fixative was washed out and the parasites were incubated with fluorescein-labelled goat anti-immunoglobulin G for 1 h at room temperature. After two more washes, the number of fluorescent parasites was estimated with a Becton–Dickinson FACscan cytometer.

Determination of intracellular Ca^{2+} concentration

To measure mammalian cell cytosolic free Ca^{2+} ($[Ca^{2+}]_i$), we proceeded as follows. Cells were washed in Tyrode solution (137 mM NaCl/2.7 mM KCl/12 mM $NaHCO_3$/0.36 mM NaH_2PO_4/0.53 mM $MgCl_2$/1.36 mM $CaCl_2$/5.5 mM glucose), pH 7.6. After adjusting the concentration of cells to 2×10^6/ml, they were incubated with 5 M fura2 acetoxymethyl ester (Sigma) for 2 h at room temperature and non-incorporated fura 2 was washed out. Fluorescence was read in a fluorophotometer SPEX AR-CM system (Spex Industries, NJ, U.S.A.) with dual-wavelength excitation (340 and 380 nm) and emission at 510 nm. The increase in mammalian cell $[Ca^{2+}]_i$ resulting from the addition of *T. cruzi* extract or purified glycoprotein was calculated as described by Grynkiewicz et al. [30]. For each preparation we determined R_{max} and R_{min}, which correspond to the fluorescence ratio at 340 and 380 nm in the presence of saturating Ca^{2+} after treatment with 50 M digitonin, and in the absence of Ca^{2+} upon addition of 10 mM EGTA respectively. For determination of parasite $[Ca^{2+}]_i$, we followed the same procedure. Parasites, at 7×10^7 cells/ml, were washed in Tyrode solution before incorporation of fura 2 for 3 h at room temperature. Elevation in parasite $[Ca^{2+}]_i$ upon exposure to mammalian cell extract or mAbs directed to various *T. cruzi* surface molecules, was calculated as above. *T. cruzi* and mammalian cell extracts used in Ca^{2+} signalling experiments were prepared as follows: cells were sonicated on ice in an ultrasonic processor XL (2 pulses of 30 s each) in the presence of protease inhibitors (1 mM PMSF/1 mM iodoacetamide/25 μg/ml leupeptin). After ascertaining the disruption of cells under a phase-contrast microscope, the sonicated preparation was centrifuged at 12000 ***g*** for 5 min and the supernatant was collected.

Other methods

Surface iodination of parasites, immunoprecipitation and immunoblotting were carried out essentially as described previously [21,23].

RESULTS

Differential infectivity of metacyclic trypomastigotes of *T. cruzi* G and CL strains towards mammalian cells

Cell-invasion assays were performed by incubating HeLa or Vero cells with metacyclic trypomastigotes of G or CL strain for 3 h at 37 °C at a parasite:cell ratio of 10:1. After washing the cells in PBS and staining with Giemsa, the number of intracellular parasites was counted in a total of 500 cells. These experiments consistently showed that G and CL strains differ widely in their ability to invade mammalian cells, the CL strain always entering target cells in several-fold higher numbers as compared with the G strain. In ten independent assays, the means $\pm$ S.D. of intracellular parasites per 100 HeLa cells was 116.4 ± 17.9 and 14.4 ± 3.1 respectively for CL and G strain, and 92.8 ± 17.0 versus 14.8 ± 6.0 respectively in the case of Vero cells.

Surface molecules with distinct Ca^{2+} signalling activity are differentially expressed in G and CL strain metacyclic trypomastigotes

Firstly, we examined whether the metacyclic trypomastigote surface glycoproteins gp35/50, gp82 and gp90, known to interact with mammalian cells [7–9], were differently expressed in G and CL strains. The parasites were analysed by surface iodination, immunoprecipitation, immunoblotting and FACS. Labelling of metacyclic forms with ^{125}I, followed by SDS/PAGE analysis, revealed gp90 as one of the major surface components in the G strain but failed to detect gp90 in the CL strain (Figure 1A), a

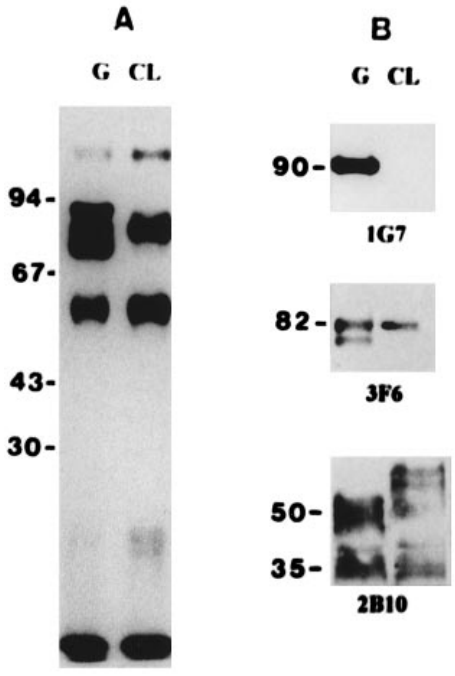

Figure 1 Surface molecules expressed in metacyclic forms of *T. cruzi* G and CL strains

Metacyclic forms were surface-iodinated and their detergent extracts were analysed by SDS/PAGE (**A**). Parasite extracts immunoprecipitated by mAbs 1G7 and 3F6 are shown in (**B**). Note the lack of a 90 kDa molecule in the CL strain. The 35/50 kDa molecules, resistant to iodination, were revealed in immunoblots using mAb 2B10

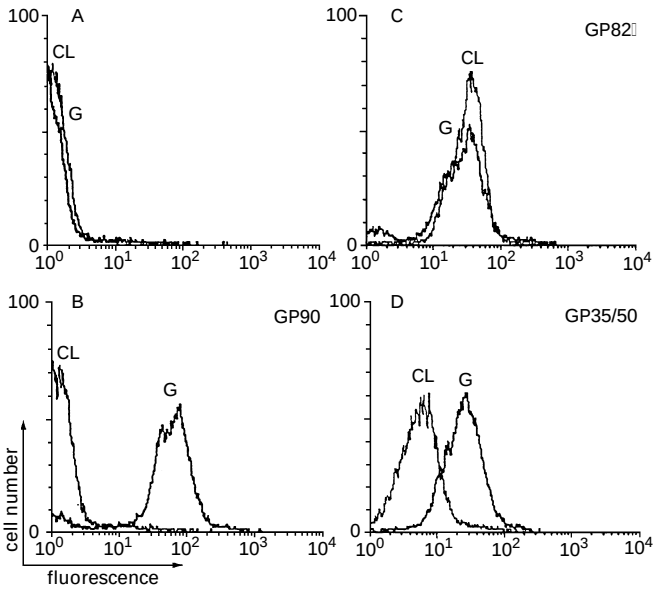

Figure 2 Flow cytometric analysis of expression of gp90, gp82 and gp35/50 in G and CL strain metacyclic trypomastigotes

Live parasites were incubated with unrelated mAb 1C3 (**A**), mAb 1G7 (**B**), mAb 3F6 (**C**) or mAb 2B10 (**D**), followed by reaction with fluorescent-labelled goat anti-mouse Ig, and were then analysed by FACS.

result compatible with that of FACS analysis (Figure 2B). As regards gp82, which in the G strain appears as a doublet band (Figure 1B), it is apparently expressed in comparable amounts in both strains, the difference being that a small proportion of G strain parasites do not express gp82 (Figure 2C). gp35/50, a highly glycosylated molecule not susceptible to iodination, was found to be expressed at lower levels in the CL strain, either in immunoblots or by flow cytometry (Figures 1B and 2D).

Next we compared the Ca^{2+} signalling effect of gp90, gp82 and gp35/50 on target cells. The glycoproteins were purified from G strain metacyclic trypomastigotes and their purity was ascertained by silver staining (Figure 3). Assays were then performed by adding 2.5 μg of purified molecules to HeLa cells preloaded with fura 2 and by measuring the elevation of intracellular Ca^{2+} concentration. As shown in Table 1, the intensity of the Ca^{2+} response triggered in HeLa cells by gp82 was significantly higher than that induced by gp35/50 or gp90.

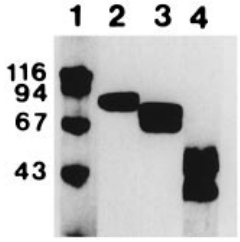

Figure 3 Analysis of purified metacyclic trypomastigote surface glycoproteins

gp90 (lane 2), gp82 (lane 3), gp35/50 (lane 4), purified from G strain metacyclic forms, were subjected to SDS/PAGE and the gel was silver stained. Molecular size markers (kDa) are shown in lane 1.

Table 1 Ca^{2+} mobilization induced in mammalian cells by *T. cruzi* molecules

Values correspond to means ± S.D. of four experiments. The difference between the gp82-induced signal and that triggered by other proteins was significant ($P < 0.05$) by Student's *t* test.

Addition to HeLa cells	Increase in $[Ca^{2+}]_i$ (nM)
Glycoprotein purified from *T. cruzi* metacyclic forms*	
gp90	42.2 ± 4.9
gp82	128.6 ± 18.0
gp35/50	52.0 ± 12.5
L. amazonensis gp63	36.5 ± 4.1

* Purified molecules (2.5 μg) were added to HeLa cells preloaded with fura 2.

The increase in $[Ca^{2+}]_i$ promoted by gp90 was very low, a little higher than that resulting from exposure of HeLa cells to unrelated *Leishmania* gp63 (Table 1). To examine the association between the Ca^{2+} signalling activity of gp90, gp82 and gp35/50 and the ability of these molecules to bind to target cells, binding assays were performed by adding the purified glycoprotein, at various concentrations, to paraformaldehyde-fixed HeLa cells. As shown in Figure 4(A), they all bound to HeLa cells in a dose-dependent and saturable manner, i.e. in a receptor-dependent manner, gp82 less efficiently than gp90 or gp35/50, indicating that efficiency of binding is dissociated from Ca^{2+} signal-inducing activity. Recombinant proteins, generated in bacteria, containing sequences of gp90 or gp82, bound to HeLa cells as effectively as the native glycoproteins (Figure 4B). We also tested the Ca^{2+} signalling activity, as well as the ability to bind to HeLa cells, of gp82 and gp35/50 purified from the CL strain. The results were similar to those of the G strain (results not shown).

In additional experiments, we determined the Ca^{2+} signalling activity of metacyclic trypomastigote extract towards mammalian cells and the relative contribution of gp90, gp82 and gp35/50 contained in the extract. The profile of gp90, gp82 and gp35/50 in the extract (Figure 5), which consisted of the supernatant of sonicated parasites after centrifugation at 12000 ***g*** for 5 min, was essentially the same as that of detergent-lysed parasites. Soluble components extracted from metacyclic forms, but not from non-infective epimastigotes, induced Ca^{2+} mobilization in HeLa and Vero cells, but not in K562 cells, which are resistant to *T. cruzi* infection (results not shown). When metacyclic trypomastigote extract was preincubated for 30 min at room temperature with mAb 3F6, and then added to HeLa cells, its Ca^{2+} signalling activity was considerably reduced (Figure 5). In three independent experiments, the average percentage inhibition by mAb 3F6 of HeLa cell $[Ca^{2+}]_i$ elevation, triggered by G and CL strain extract, was respectively of the order of 55 %–70 %. Inhibition by mAb 2B10 was in the range 16–25 %, whereas mAb 1G7 had no effect on CL strain activity and only a minor inhibitory effect on G strain activity (results not shown). All these results indicate that most of the Ca^{2+} signalling activity contained in the metacyclic trypomastigote extract comes from gp82, a molecule not expressed in epimastigotes [21] and postulated to be the main metacyclic surface molecule involved in target-cell invasion [8].

Differential Ca^{2+} response is induced in G and CL strain metacyclic trypomastigotes by host-cell components

To examine the possible difference in Ca^{2+} response triggered in G and CL strain metacyclic forms upon interaction with host cells, fura 2-loaded parasites were exposed to HeLa cell extract

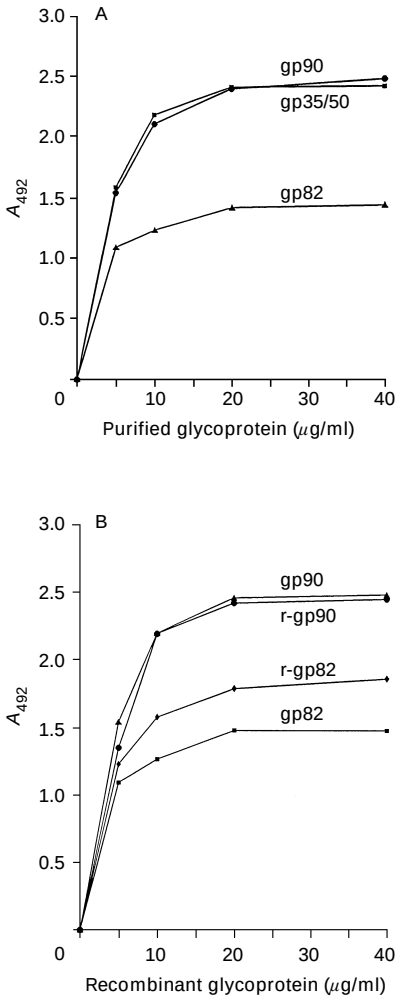

Figure 4 Binding of metacyclic trypomastigote surface glycoproteins and corresponding recombinant proteins to HeLa cells

Increasing concentrations of (**A**) purified glycoprotein or (**B**) recombinant proteins, containing the carboxy-proximal domain of gp90 (r-gp90) or the entire peptide sequence of gp82 (r-gp82), were added to wells in ELISA plates containing paraformaldehyde-fixed HeLa cells. After washes, the cells were sequentially incubated with mAbs to gp90, gp82 or gp35/50 and anti-mouse Ig conjugated to peroxidase. *o*-Phenylenediamine was used to reveal the bound enzyme. Values are means of the absorbance at 492 nm of triplicate samples.

and their $[Ca^{2+}]_i$ levels were measured. We invariably detected a Ca^{2+} response of higher intensity in CL strain than in G strain metacyclic forms (Figure 6). Significantly, epimastigotes failed to respond (Figure 6). Extracts of Vero cells, but not of K562 cells resistant to *T. cruzi* infection, triggered Ca^{2+} signalling in metacyclic trypomastigotes in a manner similar to HeLa cell extract (results not shown).

In an attempt to identify the parasite surface molecules that mediate Ca^{2+} response in metacyclic trypomastigotes, we tested whether mAbs 2B10, 3F6 and 1G7, by mimicking host-cell receptors for gp35/50, gp82 and gp90 respectively, could induce parasite Ca^{2+} mobilization. mAb, at a final concentration of 10 μg/ml, was added to fura 2-loaded parasites and their $[Ca^{2+}]_i$ levels were measured. As shown in Figure 7, mAb 3F6, and to a lesser extent mAb 2B10, but not mAb 1G7, triggered a Ca^{2+} response in both G and CL strain metacyclic trypomastigotes. The involvement of gp82 in parasite Ca^{2+} mobilization was confirmed in experiments in which addition of recombinant gp82 to HeLa cell extract drastically reduced its Ca^{2+} signalling effect towards CL strain metacyclic forms (results not shown). We were unable to detect any inhibitory effect of purified gp90 or gp35/50 on HeLa cell Ca^{2+} signalling activity towards G strain parasites (results not shown).

Expression of gp90 and gp35/50 inversely correlates with infectivity of metacyclic forms of different *T. cruzi* strains

Experiments performed with G and CL strain metacyclic trypomastigotes showed an inverse correlation between infectivity and expression of gp90 and gp35/50, suggesting that lack of gp90 and low-level expression of gp35/50 might be contributing to the high invasiveness of strains such as CL, possibly by facilitating interaction of gp82 with host cells. To test the validity of such an assumption, we extended our analysis of infectivity and expression of gp90, gp82 and gp35/50 to metacyclic forms of eight additional *T. cruzi* strains by using the various procedures described in previous sections. We found no quantitative differences in expression of gp82 between strains. In one highly invasive strain, Y, gp90 was undetectable and gp35/50 was expressed at low levels, whereas in all poorly invasive strains expression of gp90 and gp35/50 was similar to that of the G strain (Table 2). Of note is that one parasite strain, Dm28, with intermediate infectivity, differed from G strain primarily by expressing lower levels of gp90 on the surface (Table 2).

DISCUSSION

Our results suggest that the ability of metacyclic trypomastigotes of different *T. cruzi* strains to enter mammalian cells is determined by differential expression of a set of surface glycoproteins with differential Ca^{2+} signalling activity. We have found that metacyclic forms of poorly infective *T. cruzi* strains ubiquitously express gp90, a glycoprotein undetectable in highly invasive strains, and display higher levels of gp35/50 on the surface, whereas quantitative differences in gp82 expression between high and low invaders were not in evidence (Figures 1 and 2 and Table 2). The glycoproteins gp90, gp82 and gp35/50 were all capable of binding to target cells in a receptor-mediated fashion (Figure 4A), and induced in these cells an increase in $[Ca^{2+}]_i$ (Table 1), an event that is required for parasite internalization [1–5]. However, there was a difference in the intensity of the Ca^{2+} response, that triggered by gp82 being significantly higher than that induced by gp90 or gp35/50 (Table 1). These findings, added to the fact that most metacyclic trypomastigote Ca^{2+} signalling activity towards HeLa cells is inhibited by gp82-specific mAb 3F6 (Figure 5), suggest a central role for gp82 in host-cell Ca^{2+} signalling, which is consistent with the view that gp82 is the main surface molecule involved in metacyclic trypomastigote entry into mammalian cells [8]. We envisage from these observations that, in highly invasive CL-like strains, the predominant parasite–target cell interaction is gp82-mediated. In contrast, G-like strains inefficiently invade target cells because they establish more frequently an interaction mediated by gp90 or gp35/50, which results in Ca^{2+} signalling of low intensity, which is unfavourable for invasion.

Although there is the possibility that structural differences in G and CL strain surface glycoproteins, which could influence cell-binding capacity and Ca^{2+} signalling activity, may contribute to the differential infectivity of these parasites, the available data do not favour that hypothesis. The G strain gp82 differs from the

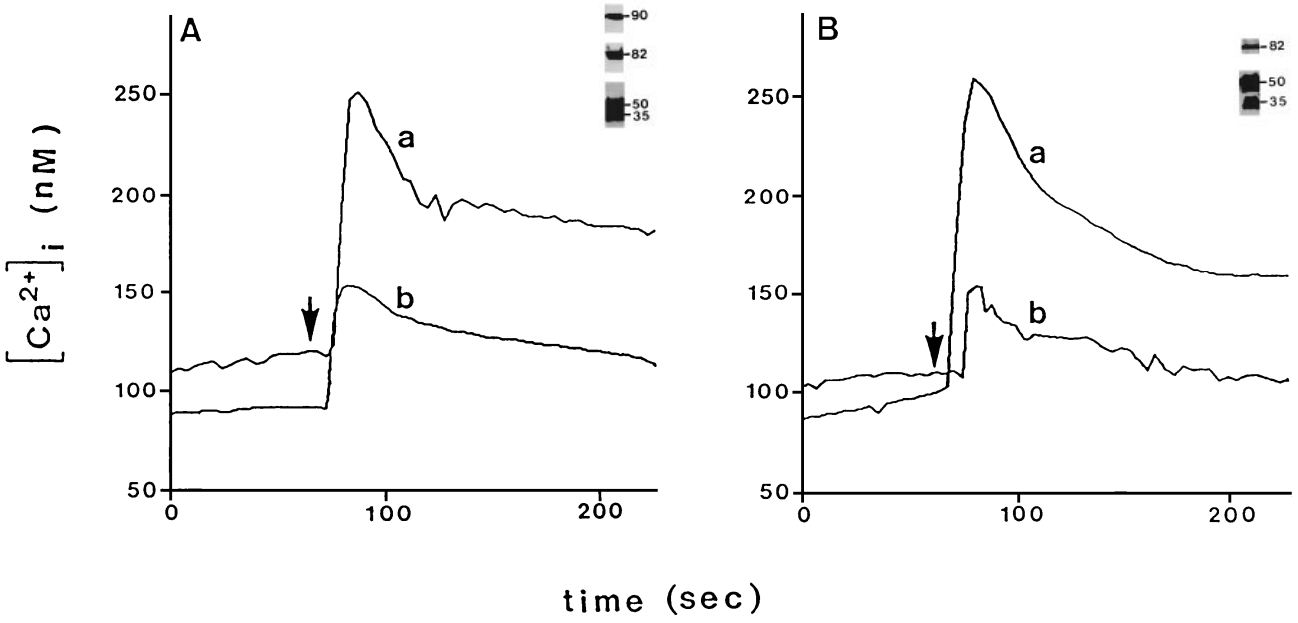

Figure 5 Inhibitory effect of mAb 3F6 on the Ca^{2+} signalling activity of metacyclic trypomastigote extract towards HeLa cells

Sonicated extract of metacyclic forms of the G strain (**A**) or the CL strain (**B**), containing an equivalent of 5×10^7 cells in 50 μl, either untreated (trace a) or treated with mAb 3F6 (trace b), was added to fura 2-loaded HeLa cells at the indicated times (arrow). Results representative of three experiments are presented. On the upper right side of the Figure the immunoblot profiles of the main surface glycoproteins contained in the metacyclic trypomastigote extract are shown.

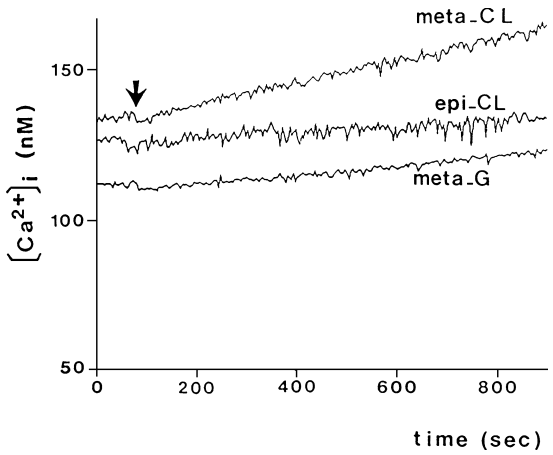

Figure 6 Ca^{2+} mobilization triggered in *T. cruzi* by host-cell components

Sonicated extract of HeLa cells, containing an equivalent of 25 μg of protein in 50 μl, was added to live epimastigotes (epi-CL) or to metacyclic trypomastigote of the CL strain (meta-CL) or the G strain (meta-G) preloaded with fura 2. Representative results from four experiments are shown. Note the lack of Ca^{2+} response in epimastigotes.

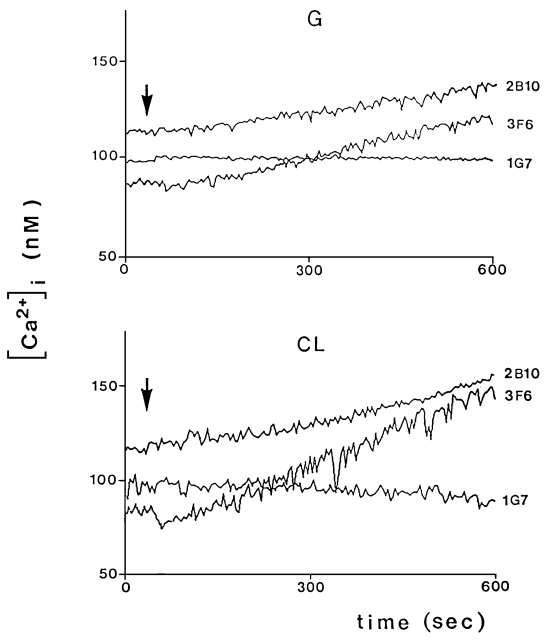

Figure 7 Effect of mAbs directed to metacyclic trypomastigote surface molecules on *T. cruzi* Ca^{2+} mobilization

mAbs 1G7, 3F6 and 2B10, directed respectively to gp90, gp82 and gp35/50, were added to fura 2-loaded G strain or CL strain metacyclic trypomastigotes at the indicated times (arrow). Note the lack of signalling activity of mAb 1G7.

CL strain gp82, in that it is always detected as a doublet. However, functionally, it is indistinguishable from the CL strain gp82. Both glycoproteins bind to HeLa cells and induce a Ca^{2+} signal in a similar manner. This is in accord with the finding that the peptide sequence of G strain gp82 [31] has 97.9 % identity at the amino acid level with the CL strain gp82 sequence (R. C. Ruiz, J. Araya, J. Franco da Silveira and N. Yoshida, unpublished work), and is compatible with the possibility that the two bands of G strain gp82 are closely related, as they share several characteristics, such as similarity of isoeletric points, in the 4.5–5.0 range, and reactivity with monoclonal and polyclonal antibodies. As regards gp35/50, it differs considerably in G and

Table 2 Inverse correlation between infectivity and expression of gp90 and gp35/50 in metacyclic forms of different *T. cruzi* strains

Parasites were incubated with HeLa cells at 37 °C for 3 h and the number of intracellular parasites was counted in a total of 500 Giemsa-stained cells.

T. cruzi strain	Expression of glycoprotein			Cell invading parasites/100 cells*
	gp90	gp82	gp35/50	
Y	−	+	+†	100.8 ± 15.1
CL	−	+	+†	120.0 ± 19.9
F	+	+	+	29.6 ± 8.0
Tulahuen	+	+	+	40.6 ± 9.4
G	+	+	+	15.8 ± 2.5
M226	+	+	+	24.6 ± 4.8
Costalimai	+	+	+	14.8 ± 4.9
Dm28	+†	+	+	59.0 ± 5.6
Dm30	+	+	+	26.2 ± 7.3
Guafitas	+	+	+	35.0 ± 9.2

* Values are the means ± S.D. of five assays.
† The glycoprotein is expressed at lower levels, as compared with the G strain.

CL strains [11]. If the invasiveness of G and CL strain metacyclic forms correlated with the molecular species of gp35/50 rather than the levels on the parasite surface, we would expect the CL strain gp35/50 to bind less to target cells and/or to induce a Ca^{2+} signal of lower intensity as compared with the G strain molecule. What we have found is that CL strain gp35/50 binds to target cells and induces a Ca^{2+} signal in the same manner as the G strain gp35/50.

The difference in the Ca^{2+} signalling effects of gp90, gp35/50 and gp82 presumably results from recognition of these molecules by distinct host-cell receptors. These receptors have not been defined. What is presently known is that gp35/50, which are highly glycosylated mucin-like molecules [32] resistant to proteases [11], interact with target cells via their carbohydrate moiety [8]. On the other hand, gp82 and gp90 are both glycoproteins containing N-linked oligosaccharides of the high-mannose type [8,9], which bind to host cells through a peptide sequence. The receptor-binding site of gp82 is localized in the central domain of the molecule, as shown in experiments using recombinant gp82 generated in bacteria [28], whereas that of gp90 seems to be a sequence at the C-terminus. Results of preliminary experiments suggest that the receptors for gp82 and gp90 are different molecules.

During *T. cruzi*–mammalian cell interaction, an elevation in $[Ca^{2+}]_i$ is induced not only in host cells but in the parasites as well, and this also contributes to parasite invasion [2,12]. Our study has shown that a Ca^{2+} signal can be triggered in metacyclic trypomastigotes by a HeLa cell extract and that the intensity of the signal is invariably higher in the CL strain than in the G strain (Figure 6). We have indications that gp82 may be the principal cell-surface mediator of the Ca^{2+} response in *T. cruzi* metacyclic forms. Firstly, HeLa cell extract mixed with recombinant gp82 had a greatly reduced Ca^{2+} signalling effect towards CL strain metacyclic forms (results not shown). Second, mAb 3F6 directed to gp82, presumably by mimicking the host-cell receptor for gp82, induced Ca^{2+} mobilization in metacyclic trypomastigotes of both strains (Figure 7). On the other hand, the lower Ca^{2+} signalling effect of mAb 2B10 directed to gp35/50 and the lack of activity gp90-specific mAb 1G7 (Figure 7), plus the finding that gp90 or gp35/50 does not inhibit the Ca^{2+} signalling activity of HeLa cell extract (results not shown), suggest that gp90 is not involved and that gp35/50 plays only a minor role, if any, in Ca^{2+} mobilization in *T. cruzi* metacyclic trypomastigotes.

The mechanism by which gp82 transduces external signals to the parasite interior is not known. gp82 is anchored to the plasma membrane via a glycosylphosphatidylinositol (GPI) moiety [33] and therefore associates only with the outer leaflet of the lipid bilayer. In mammalian cells, interaction of GPI-anchored proteins with downstream components of signalling pathways, such as tyrosine kinases, has been demonstrated [34–36]. As gp35/50 and gp90 are also GPI-anchored molecules [32,37], one interesting question to be investigated is whether the nature of the lipid moiety of the GPI anchor influences its ability to interact with plasma membrane components associated with downstream intracellular molecules. In this regard, it is of note that the lipid portion of gp90 GPI is composed essentially of 1-*O*-hexadecyl-2-*O*-hexadecanoyl-phosphatidylinositol and of 1-*O*-hexadecyl-2-*O*-octadecanoyl-phosphatidylinositol [38], whereas that of gp35/50 is mainly ceramide-phosphatidylinositol [39]. The type of lipid present in the gp82 GPI anchor remains to be determined.

Taken together, all our results give us an idea of why metacyclic forms of *T. cruzi* strains may differ in their ability to invade mammalian cells. We propose that expression of high levels of gp90 and gp35/50 impairs the binding of metacyclic trypomastigotes to host cells through the most productive gp82-mediated interaction, which leads to Ca^{2+} mobilization in both cells and ultimately to parasite internalization.

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