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TRYPANOSOMA CRUZI RECOGNITION BY MACROPHAGES AND MUSCLE CELLS: PERSPECTIVES AFTER A 15-YEAR STUDY

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Macrophages and muscle cells are the main targets for invasion of Trypanosoma cruzi. *Ultrastructural studies of this phenomenon* in vitro *showed that invasion occurs by endocytosis, with attachment and internalization being mediated by different components capable of recognizing epi- or trypomastigotes (TRY). A parasitophorus vacuole was formed in both cell types, thereafter fusing with lysosomes. Then, the mechanism of* T. cruzi *invasion of host cells (HC) is essentially similar (during a primary infection in the absence of a specific immune response), regardless of whether the target cell is a professional or a non-professional phagocytic cell.*

Using sugars, lectins, glycosidases, proteinases and proteinase inhibitors, we observed that the relative balance between exposed sialic acid and galactose/ N-acetyl galactosamine (GAL) residues on the TRY surface, determines the parasite's capacity to invade HC, and that lectin-mediated phagocytosis with GAL specificity is important for internalization of T. cruzi *into macrophages. On the other hand, GAL on the surface of heart muscle cells participate on TRY adhesion. TRY need to process proteolytically both the HC and their own surface, to expose the necessary ligands and receptors that allow binding to, and internalization in the host cell. The diverse range of molecular mechanisms which the parasite could use to invade the host cell may correspond to differences in the available "receptors" on the surface of each specific cell type. Acute phase components, with lectin or proteinase inhibitory activities (α-macroglobulins), may also be involved in* T. cruzi*-host cell interaction.*

Key words: *Trypanosoma cruzi* – macrophage – heart muscle cell – lectins – ligand-receptor interaction – ultrastructure – endocytosis – proteinases – alpha-2-macroglobulin

PRELIMINARY REMARKS

The development of human Chagas' disease commences with an acute phase of active *Trypanosoma cruzi* infection, in which the parasite's ability to invade host tissues play a crucial role. A proper understanding of the molecular events and the regulatory mechanisms involved in this key phenomenom is essential for the design of possible vaccines and chemotherapeutic agents. For these reasons, this field has received considerable attention in the last ten years. In this paper, we review our research on this matter in the context of other data recently made available in the literature, and we highlight possible future lines of inquiry.

SIMILARITIES BETWEEN THE OVERALL PROCESS OF T. CRUZI-HOST CELL INTERACTION IN PROFESSIONAL AND NON-PROFESSIONAL PHAGOCYTIC CELLS

Macrophages and muscle cells are the main targets for *in vivo* invasion of *T. cruzi* during a primary infection, before the establishment of a specific immune response (Brener, 1980). At the time of starting our study on *T. cruzi*-host cell interaction, there was a controversy in the literature about the mechanisms governing parasite invasion of macrophages and non-professional phagocytic cells. Some authors postulated that the parasite was able to make a furrow in the cell membrane and invade the cell "actively" (Dvorak & Schmunis, 1972; Kipnis et al., 1979). The literature also began to provide preliminary insights into the molecular mechanisms of *T. cruzi* adhesion to, and interiorization into host cells (Nogueira & Cohn, 1976; Alcantara & Brener, 1978, 1980).

By that time, the following questions attracted our attention: (1) How does *T. cruzi* invade host cells? (2) Is there a common prop-

erty shared by macrophages and muscle cells that enables recognition of *T. cruzi*?

To study non-professional phagocytic cells, we established two types of primary muscle cell culture, in the laboratory. The first procedure used heart myocytes (isolated from mouse embryo) that were capable of coupling *in vitro* to form a contractile myofiber (Meirelles et al., 1986). Second, skeletal muscle cells were cultured *in vitro* (after isolation of myoblasts), following the normal myogenic procedure for differentiation into multinucleated myotubes (Araujo-Jorge et al., 1986b).

The following experimental approaches were adopted: (a) an investigation in the morphological aspects of *T. cruzi*-host cell interaction; (b) comparisons of the *in vitro* behaviour displayed by different *T. cruzi* strains, at different stages of development and in interactions with different cell types, with regard to levels and kinetics of infection; (c) experimental interference with parasite-cell interaction, using proteinases, sugars, lectins and other agents, under different experimental conditions.

Our results on the morphological aspects of *T. cruzi*-host cell interaction show that trypomastigotes and epimastigotes bind to and are internalized by macrophages in a typical phagocytic process (reviewed in Araujo-Jorge, 1989). The mechanisms of attachment and internalization can be disassociated, either by treating macrophages with cytochalasin B, in which case only adhesion occurs, or by lowering the temperature, with increased adhesion at 4 °C and invasion at 37 °C (Meirelles et al., 1982; Ebert & Barbosa, 1982). In these studies it also became clear that the mechanisms of adhesion and internalization are different in trypomastigotes and epimastigotes, since different levels of adhesion and internalization were observed with these two developmental stages. These differences were further confirmed using other experimental approaches (reviewed in Araujo-Jorge, 1989).

In muscle cell cultures, trypomastigotes are able to bind (Figs 1a, 1b; Barbosa et al., 1991) and induce a typical phagocytic process (Figs 1a, 1c) that can be visualized using transmission electron microscopy. A vacuole containing the parasite can be observed within less than 18h of parasite-cell contact (Fig. 1c) (Meirelles et al., 1986; Barbosa & Meirelles 1993a). The vacuole fuses with lysosomes (Meirelles et al., 1987), in the same manner as during parasite interaction with macrophages (reviewed in Araujo-Jorge, 1989). Our group was the first to show vacuole formation and lysosomal fusion during *T. cruzi* invasion of non-professional phagocytic cells.

In this experimental system, it is also possible to observe the process of vacuolar membrane disruption (Fig. 1d) (Barbosa et al., 1989). This process allows the parasite to escape from the vacuole and develop freely in the cytoplasm in the form of an amastigote, as already described in detail for macrophages (Nogueira & Cohn, 1976; Carvalho & De Souza, 1989). Recently other authors have examined the possible biochemical mechanisms of vacuole membrane disruption, showing that the acidic pH found in this pre-lysosomal compartment is optimal for *T. cruzi* neuraminidase activity (Andrews et al., 1991). These authors have suggested that desialylated vacuolar membranes have a relatively high susceptibility to a *T. cruzi* "pore-forming protein" (Andrews et al., 1990) that could be responsible for the small holes that develop in these membranes, allowing parasites to escape into the host cell's cytoplasm.

The non-infective form of the parasite (epimastigote) is also able to bind to muscle cells (Figs 2a, 2b), but is not able to induce internalization. In addition, antisera to infective metacyclic forms of *T. cruzi* can inhibit trypomastigote invasion of muscle cells and fibroblasts (Fig. 2c), but do not interfere with epimastigote adhesion to the same cells (Fig. 2d). These findings suggest that muscle cells may have many surface components to which *T. cruzi* can bind, but that only a few (or even one) of these components are able to couple to the endocytic machinery of these non-professional phagocytic cells, thereby inducing invasion.

In the light of these findings, our initial conclusions were: (1) that invasion occurs by endocytosis, both in professional and non-professional phagocytic cells, with attachment and internalization being mediated by different components capable of recognizing epi- or trypomastigotes; and (2) that a parasitophorus vacuole was formed in both types of cell, thereafter fusing with lysosomes. In our view, findings such as the recently reported active invasion of fixed epithelial cell lineages by *T. cruzi*

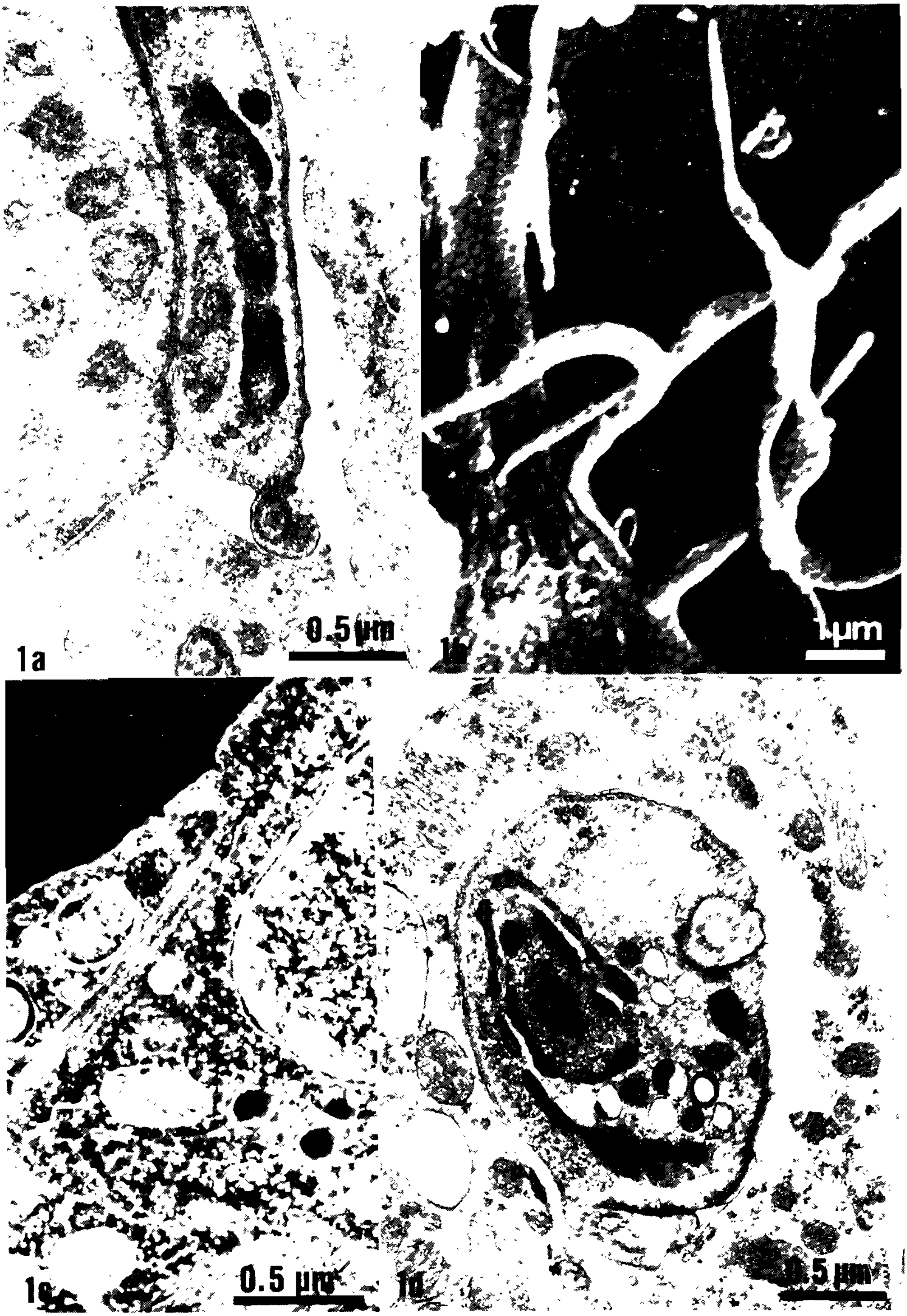


Fig. 1: cultured heart muscle cells exposed to metacyclic trypomastigote forms of *Trypanosoma cruzi* clone Dm28c for 1 (Fig. 1a, b); 18 (Fig. 1c) or 24 (Fig. 1d) hours, observed using conventional transmission electron microscopy (Figs 1a, d), scanning electron microscopy (Fig. 1b) and spectroscopic electron microscopy (Fig. 1c). Note adhesion (Figs 1a, 1b, arrows), internalization (Fig. 1b, arrowhead), the presence of the parasite inside an endocytic vacuole (Fig. 1c arrows) and vacuole membrane rupture (Fig. 1d, arrows).

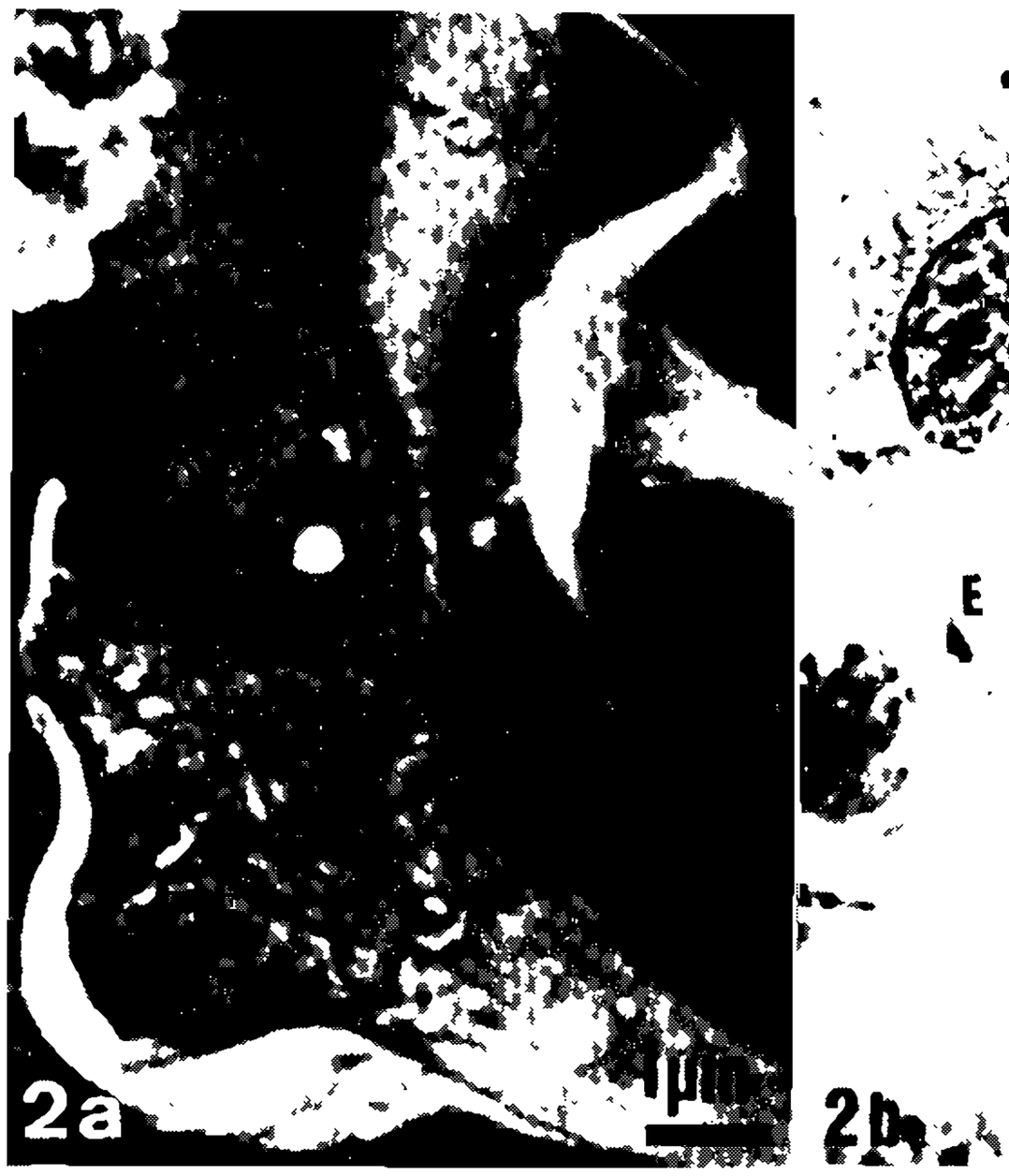


Fig. 2: cultured heart muscle cells exposed for 2 hours to mixed cultures of epimastigotes (E) and metacyclic trypomastigotes (T) of *Trypanosoma cruzi* clone Dm28c, observed using scanning electron microscopy (Fig. 2a) and optical microscopy. Note that both parasite forms bind to muscle cells (Figs 2a, b). The same assay performed in the presence of rabbit sera (1:100) on metacyclic forms (Figs 2c, d) with inhibition of trypomastigote adhesion and internalization (Fig. 2c), but without interference in epimastigote adhesion to muscle cells (Fig. 2d, e).

trypomastigotes (Schenkman et al., 1991a) do not appear to make physiological significance, given our observation that *T. cruzi* penetration of primary muscle cell cultures is endocytic in nature.

INVOLVEMENT OF LECTIN-LIKE RECOGNITION PROCESSES IN T. CRUZI-HOST CELL INTERACTION

It is known that, in order to invade their hosts, many viruses, bacteria and protozoa take

advantage of the physiological processes of receptor-mediated endocytosis of mammalian cells. For example: viruses opsonized with immunoglobulin or complement may use these host ligands to gain access to an intracellular environment and replicate (Maramorosch et al., 1986; Takeda et al., 1988); the uptake of complement-opsonized *Leishmania* parasites by macrophages may be mediated by complement receptors to C3b (CR1/Cr2) or C3bi (CR3), and synergistically by lectin-receptors for mannosyl residues, that conduct microorganisms to different intracellular fates (Mosser & Edelson, 1987; Blackwell et al., 1985). Different receptors are also involved in the endocytosis of formalin-fixed, IgG-coated or unopsonized *Toxoplasma gondii,* and the type of ligand-receptor interaction defines the subsequent intracellular pathway and fate of the parasite, exposing it or not to oxygen metabolites and/or lysosomal enzymes (Joiner et al., 1990).

In mammalian cells, four different genomic families/superfamilies of adhesion molecules are known to mediate heterotypic (different cells) cell-cell interactions with heterophilic molecules (different molecules), leading to binding and endocytosis (Fig. 3): the LECTIN family (Drickamer, 1988; Sharon & Lis, 1989; Lasky, 1991), the short-consensus repeat family of COMPLEMENT binding molecules (Law 1988; Ahaern & Fearon 1989), the IMMUNOGLOBULIN superfamily (Ravetch & Kinet, 1991; Daeron, 1991), and the INTEGRIN family (Larson & Springer, 1990; Hemler et al., 1990). The first two families present variable domains called "short consensus repeats", while the latter two families are structurally unrelated but share complementarity of ligands or receptors (Dustin et al., 1988).

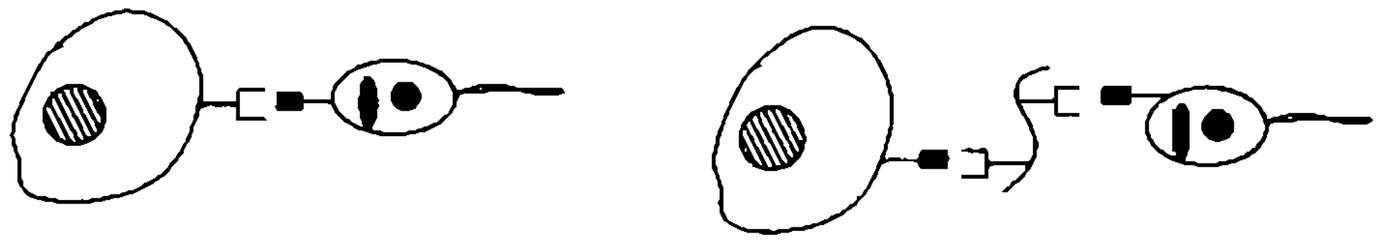
In Fig. 3, we attempted to summarize some of the possible interactions between *T. cruzi* and host cells, taking the macrophage as an example and lectin-phagocytosis (Sharon & Lis, 1989) as a model (Fig. 3A). The surface of macrophages have at least four main receptor systems from the genomic families previously cited (Fig. 3A, B), that mediate adhesion and phagocytosis, any of which could be predicted to participate in the recognition of *T. cruzi* (Araujo-Jorge, 1989).

From a panel of macrophage lectin receptors (Nagamura & Kolb 1980; Kolb-Bachofen et al., 1984; Cherayil et al., 1989; Oda et al., 1989), some members from the family of lectin-cell adhesion molecules (LEC-CAM's) are represented in Fig. 3A, with specificity for galactose, such as the surface antigens Mac 2 (CR2), the low affinity receptor for IgE, FcεRII (Daeron, 1991) and the asialoglicoprotein receptor (GalR). Neutrophils also express a LEC-CAM involved in adhesion processes (Lasky, 1991), ELAM-1 (endothelial lymphocyte adhesion molecule 1) that could hypothetically participate in parasite binding. The acute phase component C-reactive protein, the soluble form of the GalR (Kolb-Bachofen, 1991), may also hypothetically interfere in this process.

In Fig. 3B, are shown possible interactions between molecules belonging to the three other genomic families and superfamilies of adhesion molecules, all of them involving a soluble mediator bridging ligands in the host cell and in the parasite surface. In the case of Immunoglobulin superfamily, parasite antigenic sites bind to soluble immunoglobulins that are recognized by cell surface Fc receptors (Mellman et al., 1988), for different Ig isotypes. Other hypothetical receptor-ligand interaction involving members of this superfamily, could be the binding of soluble I-CAM (intercellular-cell adhesion molecule), a member of the Ig superfamily (Rothlein et al., 1991), to the parasite surface, followed by recognition of this complex by a cell surface integrin receptor, such as LFA-1 (leukocyte function-antigen 1).

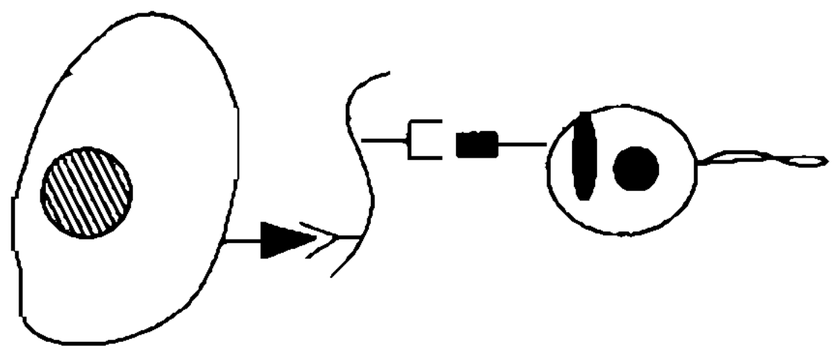
For the different complement receptors - CR- (Law, 1988; Ahaern & Fearon, 1989), molecules of the short consensus repeat (SCR) family, the scheme shows opsonization of parasites by soluble C3b or C4b, that may be recognized by complement receptors type 1 (CR1) and 2 (CR2). C3b preferentially binds to epimastigotes, while trypomastigotes bind iC3b (Rimoldi et al., 1988). Opsonization of the parasite by the inactivacted C3b component (iC3b) may lead to its recognition by receptors CR3 and p150.95, the leukocyte-cell adhesion molecules (Leu-CAM's) of the integrin superfamily (Wright & Detmers, 1988; Larson & Springer, 1990). Another complement component, C1q, is important for *T. cruzi* interaction with host cells (Rimoldi et al., 1989), but the structure of its receptor (s) is not yet known (Guan et al., 1991). Finally, the scheme also shows a possible mechanism of parasite-host cell recognition mediated by integrins that interact with extracellular matrix

A



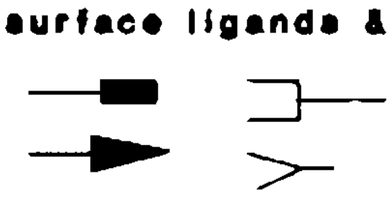
GENOMIC FAMILY		host cell receptor	soluble ligand (brigde)	*parasite ligand*	EFFECT binding	internalization	
LECTIN	Lec-CAM C-type	GalR/CR2 FcεRII		*Gal*	+	+	a
		Gal	CRP	*Gal*	+		b

B



GENOMIC FAMILY		host cell receptor	soluble ligand (brigde)	*parasite ligand*	EFFECT binding	internalization	
IMMUNOGLOBULIN		FcR	Ig	*Ag*	+	+	c
		LFA-1	I-CAM	*I-CAM acceptor*	+		d
INTEGRIN	LeuCAM	CR3/ p150.95	IC3b	*IC3b acceptor*	+	+	e
	VLA	VLA4/5 VLA6	FN/LM	*FN/LM receptor*	+		f
COMPLEMENT (SCR)		CR1/CR2	C3b/C4b	*C3b/C4b acceptor*	+	+	g

surface ligands & receptors



Multivalent soluble mediators bridging surface ligands and receptors

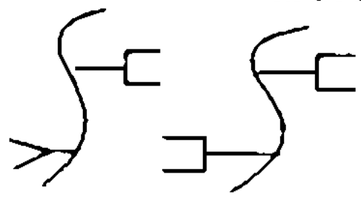


Fig. 3: possible interactions between receptors and ligands for *Trypanosoma cruzi*-cell recognition. Members of known genomic families and superfamilies of adhesion molecules are shown in the column of "host cell receptor". "Soluble ligands" of these same families are proposed to bridge parasite and host cell surface molecules. In Fig. 3A are depicted two possibilities of lectin-phagocytosis, mediated by lectins from the family of lectin-cell adhesion molecules (LEC-CAM's). In Fig. 3B, it is shown possible interactions between molecules belonging to the genomic families and superfamilies of immunoglobulin, integrin, and complement (SCR) adhesion molecules. Indirect evidence in the literature support the possible ligand-receptor interaction system represented in the models in rows a, c, f and g. Rows b, d and e are still hypothetical. See text for details and references.

glycoproteins fibronectin (FN) and laminin (LM), to which *T. cruzi* was shown to bind (reviewed in Ouassi, 1988) and which can be recognized by different integrins of the so-called subfamily of "very late antigens" (VLA). Such interactions could mediate adhesion and cooperate in phagocytosis. Putative ligands to all these receptors may be present on (or may bind to) the parasite surface. Possible multivalent soluble components may also be present in host sera, mediating indirectly in interactions between the parasite and its host cell, or interfering with direct interactions between parasite ligands and host cell receptors. However, the expression of such adhesion molecules is poorly studied in muscle cells.

The FcR and CR systems are typical of professional phagocytes, and have been shown by indirect evidence to participate in, but not to be essential for, *T. cruzi* uptake by macrophages (Nogueira & Cohn, 1976; Alcantara & Brener, 1980). In view of the presence of decay-accelerating factor on trypomastigotes (Rimoldi et al., 1988), opsonization by C3 are circunvented by the parasite, that avoids endocytosis by integrins. FcR are specially important when specific antibodies are produced (Brener 1980; Araujo-Jorge et al., 1992), never before the third week following *T. cruzi* infection. The integrin system is being intensively studied by other groups (reviewed by Ouassi, 1988). We decided to focus on lectin-phagocytosis, to ascertain its role in *T. cruzi*-host cell interaction, exploring the possibility of identifying receptors on the host cell surface and ligands on the parasite, that mediate parasite adhesion to, and internalization into host cells.

Initially using resident peritoneal macrophages as host cells for *T. cruzi*, we showed that by treating trypomastigotes with exogenous neuraminidase, to remove sialic acid, we could increase phagocytic levels (Araujo-Jorge & De Souza, 1984; Fig. 4). Similar results were observed in muscle cells (Soeiro et al., 1991). Blocking sialic acid with a specific lectin produced a similar effect (Araujo-Jorge & De Souza, 1986; Fig. 4), as did treating the parasites with cationized ferritin (Meirelles et al., 1984; Fig. 4), an agent that neutralizes the high negative surface charge of trypomastigotes. In muscle cells, however, cationized ferritin only increased adhesion, without affecting internalisation (Soeiro et al., 1990).

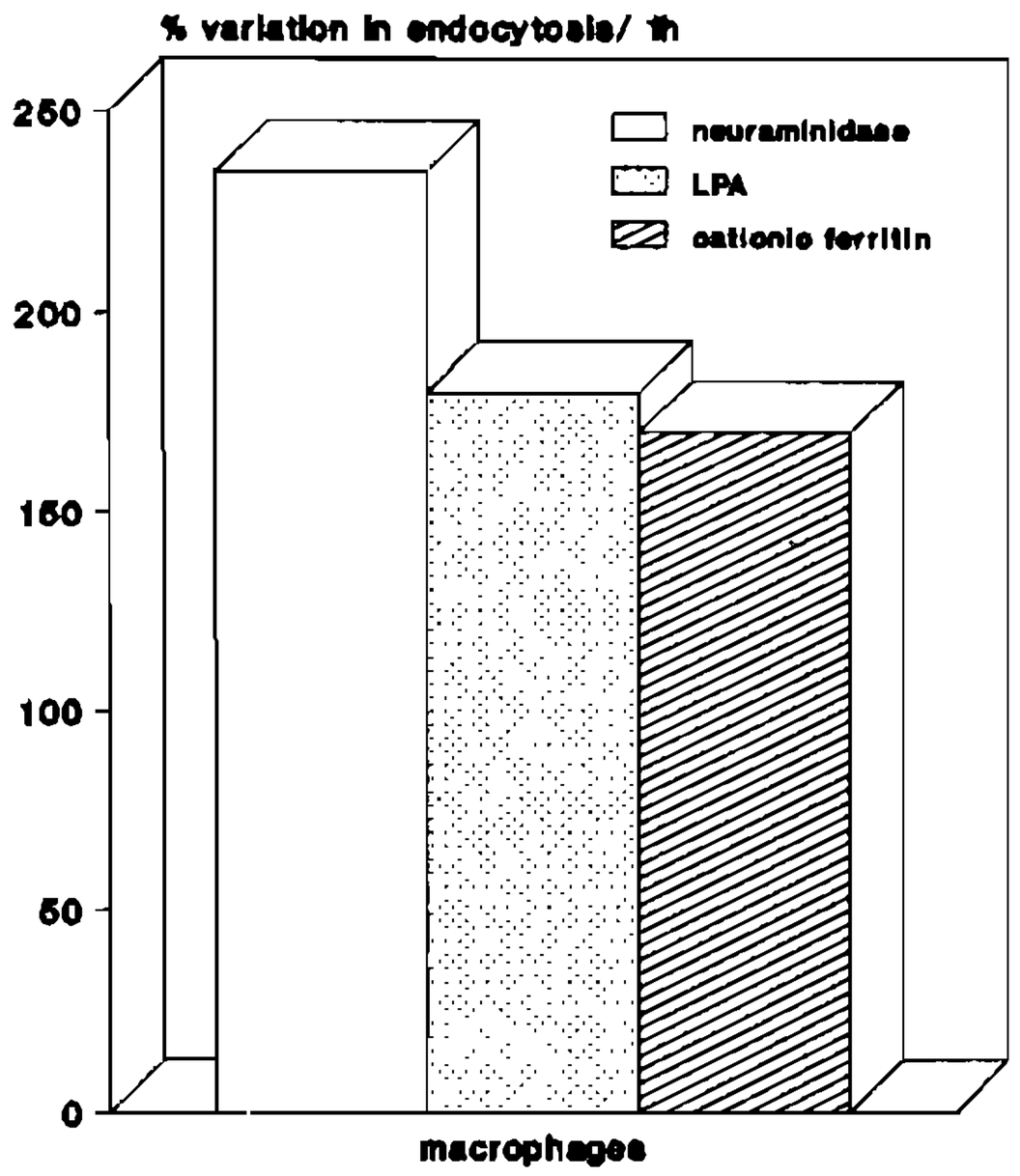


Fig. 4: increases in the endocytosis of bloodstream trypomastigotes of *Trypanosoma cruzi* by resident peritoneal mouse macrophages after treatment of the parasites with neuraminidase from *Clostridium perfringens* (0.25 μ/ml – 60 min), with lectin from *Limulus polyphemus* (LPA – 10 μg/ml – 30 min) or with cationized ferritin (100 μg/ml – 30 min).

Since galactose and N-acetyl galactosamine residues are normally subterminal to sialic acid residues in glycoconjugates, we were able to observe trypomastigote sensitivity to the addition of galactose and N-acetyl galactosamine during interaction with macrophages, even when using two parasite strains, such as Y and Cl (Araujo-Jorge & De Souza, 1984; Fig. 5). We also observed that lectins which block these galactosyl residues on the parasite surface inhibit trypomastigote invasion of macrophages (Araujo-Jorge & De Souza, 1986), as well as partially reversing the effect of desialylation (Araujo-Jorge & De Souza, 1988).

Given that different lectin-like molecules which recognize galactosyl residues had already been detected on the surface of macrophages (Nagamura & Kolb, 1980; Cherayil et al., 1989; Oda et al., 1989), these results suggested that galactose residues on the parasite surface were being recognized by galactose-specific, lectin-like molecules on the macrophage surface (Fig. 3). Accordingly, it appeared that oligosacarides containing galactose (e.g. rafinose) are able to inhibit *T. cruzi* invasion of macrophages and partially reverse the effect of desialylation (e.g. beta-lactose and

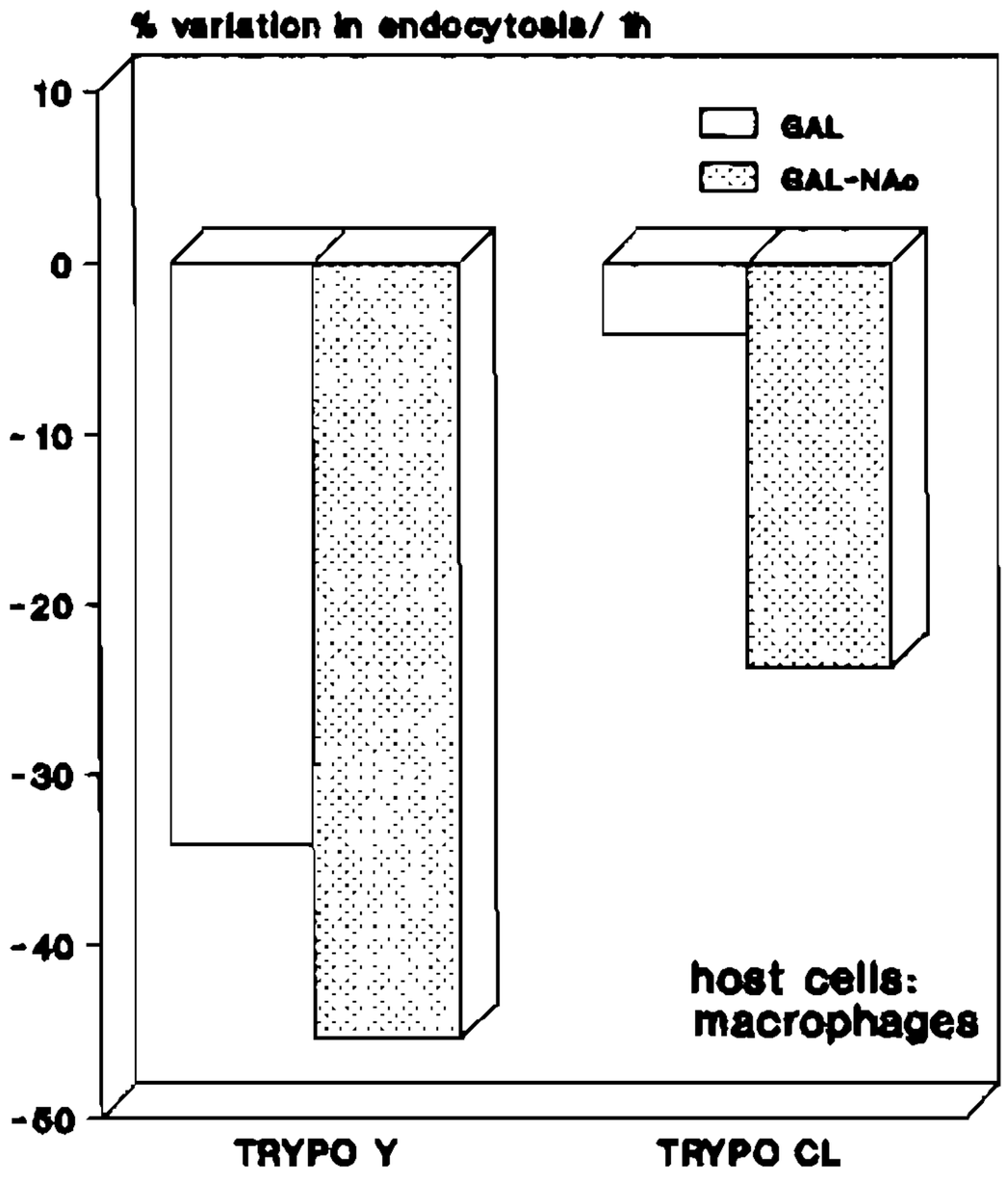


Fig. 5: reductions in the endocytosis of bloodstream trypomastigotes of *Trypanosoma cruzi* (Y and Cl strains) by resident peritoneal mouse macrophages following addition of D-galactose or N-acetyl-D-galactosamine during 1 hour of parasite-cell contact.

rafinose) (Araujo-Jorge & De Souza, 1988). It was also found that the addition of asialoerythrocytes strongly inhibits trypomastigote invasion of resident macrophages, competing for galactose-specific lectin (Araujo-Jorge & De Souza, 1988; Araujo-Jorge et al., 1989).

We concluded that the relative balance between exposed sialic acid and galactose/N-Acetyl galactosamine residues on the trypomastigote surface, determines the parasite's capacity to invade macrophages, and that lectin-mediated phagocytosis (with galactosyl specificity) is important for internalization of *T. cruzi* into these cells. It remains to be determined: (a) whether or not all the already described galactose specific macrophage lectins can mediate attachment and internalization, (b) whether or not galactosyl-lectins are modulated during the acute and chronic phases of a *T. cruzi* infection, (c) whether or not other surface lectins, which are induced in inflammatory macrophages, can also mediate *T. cruzi* recognition (Ezekowitz et al., 1988), (d) whether or not soluble physiological lectins, which are induced in the acute phase of inflammatory responses (Kolb-Bachofen, 1991; Kawasaki et al., 1989), can also interfere with these processes. The relative role of each different receptor-mediated endocytic process in these events also needs to be determined.

Clearly, the field is open for futher studies aimed at achieving a more comprehensive understanding of these complex processes. In this context, mention should be made of the study on the possible roles of the neuraminidase/ transialidase activities that are reported to occur on the surface of *T. cruzi* (Pereira, 1983; Previato et al., 1985; Pereira & Hoff, 1986; Zingales et al., 1989; Schenkman et al., 1991a, b). It is possible that these activities modulate sialylation levels on the surface of both the host cell (Libby et al., 1986) and the parasite, and that they influence the relative balance between exposed sialic acid and galactose/N-Acetyl galactosamine residues, again on the surface of both the host cell and the parasite.

In attempts to identify comparable process in muscle cells, we observed that the membrane of the parasitophorus vacuole reacted positively in the Thiéry's cytochemical staining of glycoconjugates (Barbosa & Meirelles, 1992). Using an ultrastructural technique, we found that galactosyl residues from the muscle cell surface (which could be traced using ferritin-labeled RCAI, the latter being a galactose-binding lectin), accumulated in the region of parasite adhesion, and were interiorized during vacuole formation (Barbosa & Meirelles, 1992, 1993b). When the muscle cells were previously incubated with RCAI, adhesion of trypomastigotes was increased (Barbosa, unpublished results).

Following the discovery of galactose-binding molecules on the surface of trypomastigotes (Deget et al., 1990), we hypothesize that galactosyl residues from the muscle cell surface are important components of a putative "receptor", to which *T. cruzi* lectin-like molecule (s), and/or soluble multivalent lectin with galactosyl specificity, binds before inducing phagocytosis in the host cell. The molecular characterization of the elements involved in this process of recognition is also an open field for further study.

Our overall conclusion, and our present working hypothesis, is that (1) in cells with lectin-cellular adhesion molecules, especially those that bind to galactose/ N-Acetyl galactosamine (e.g. macrophages and neutrophils), these molecules may be important receptors for *T. cruzi* invasion, and that (2) in cells where

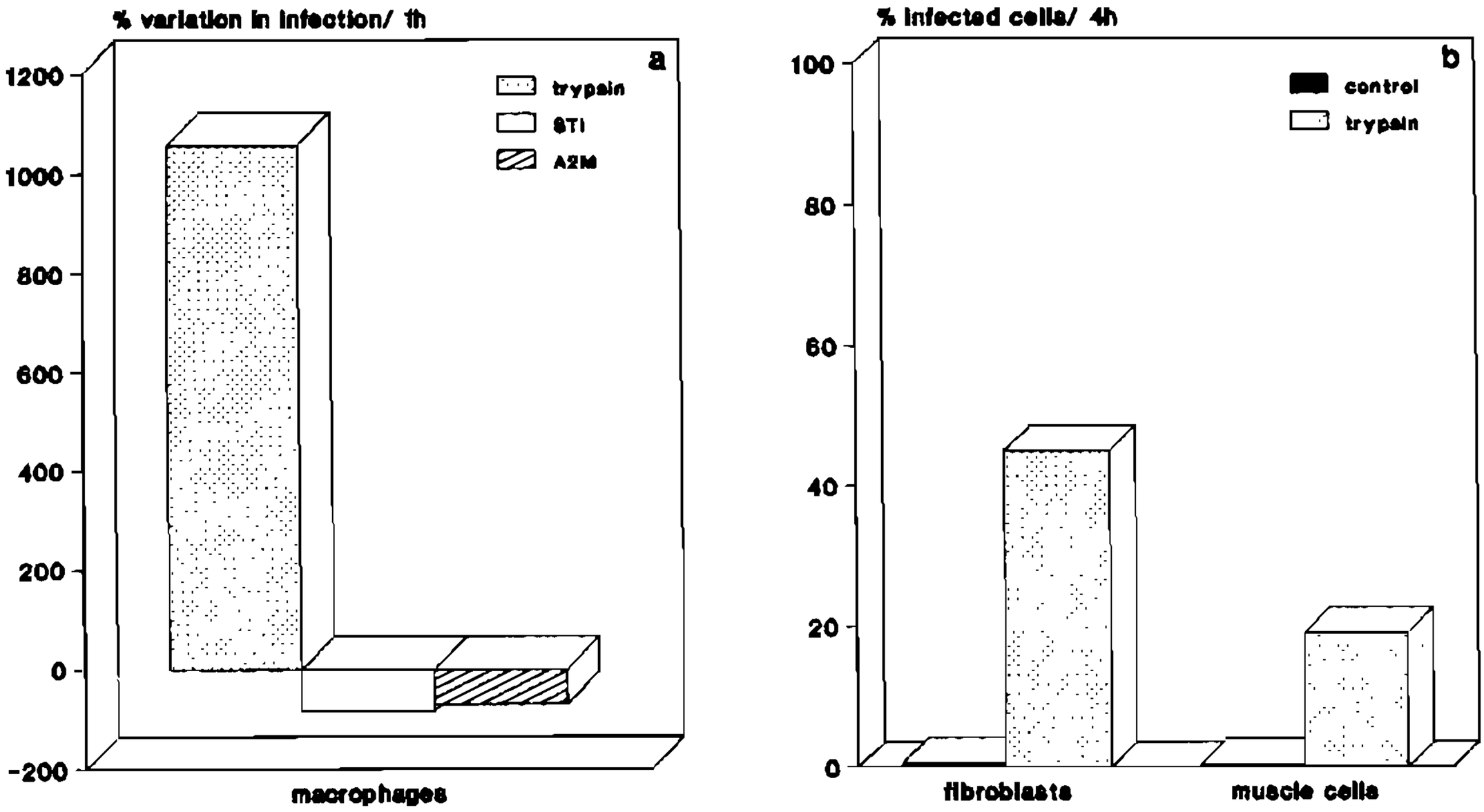


Fig. 6: effects of treating bloodstream trypomastigotes of *Trypanosoma cruzi* (Y strain) with trypsin (500 µg/ml, 10 min) or proteinase inhibitors (alpha-2-macroglobulin 500 µg/ml, 30 min and soybean trypsin inhibitor -STI-, 500 µg/ml, 30 min) on parasite endocytosis by macrophages (Fig. 6a), heart muscle cells and fibroblasts (Fig. 6b) respectively for 1, and 4 hours of parasite-cell interaction).

galactose/N-Acetyl galactosamine residues are exposed, naturally or as a result of *T. cruzi* neuraminidase activity, it is possible that parasite attachment is mediated by soluble multivalent galactosyl-lectins recognizing galactosyl molecules on the surface of both the parasite and the cell, or directly by lectin-like proteins of the parasite.

In this context, it is interesting to remember that, during *T. cruzi* infection, high levels of antibodies against galactosyl-epitopes are produced (Milani & Travassos, 1988; Avila et al., 1989); that anti-galactose antibodies impair *T. cruzi* invasion of other cell lineages *in vitro* (Arruda et al., 1989); and finally that, a protein with galactose lectin activity, namely serum amyloid protein (Pepys & Baltz, 1982; Kolb-Bachofen, 1991), is highly increased in the sera of mice in the acute phase of *T. cruzi* infection (Scharfstein et al., 1982).

Macrophages possess a much larger panel of available "receptors" than muscle cells: they are specialist cells, designed for phagocytosis, and they employ many different Fc, complement and lectin receptors, as well as other endocytic pathways. The endocytic pathways of muscle cells, on the other hand, are very poorly understood, although we believe that a similar mechanism underlies the common pattern of *T. cruzi* invasion observed in these cell types.

In our view, it is now necessary to: 1) press ahead with further *in vitro* and *in vivo* studies on the expression of certain adhesion receptors, lectin-like molecules and integrins, in particularly in cells that are natural targets for *T. cruzi* invasion; 2) further study the molecular basis of the parasite's preference for myofibers; and 3) improve our comprehension of the changes induced in the cytoskeleton of muscle cells by the attachment of trypomastigotes, finally leading to induction of phagocytosis.

THE PROTEOLYTIC SENSITIVITY OF PARASITE SURFACE LIGANDS, THAT ARE NECESSARY FOR HOST CELL INVASION

We observed that treatment of bloodstream trypomastigotes with trypsin increases their ability to infect both professional (Fig. 6a) and non-professional phagocytic cells (Fig. 6b), and that treatment with proteinase inhibitors, such as soybean trypsin inhibitor or alpha-2-macroglobulin, impairs this ability (Araujo-Jorge & De Souza, 1984; Araujo-Jorge et al., 1986a, Fig. 6a). Treatment of bloodstream trypomastigotes with trypsin does not, however, affect their ability to complete their intracellu-

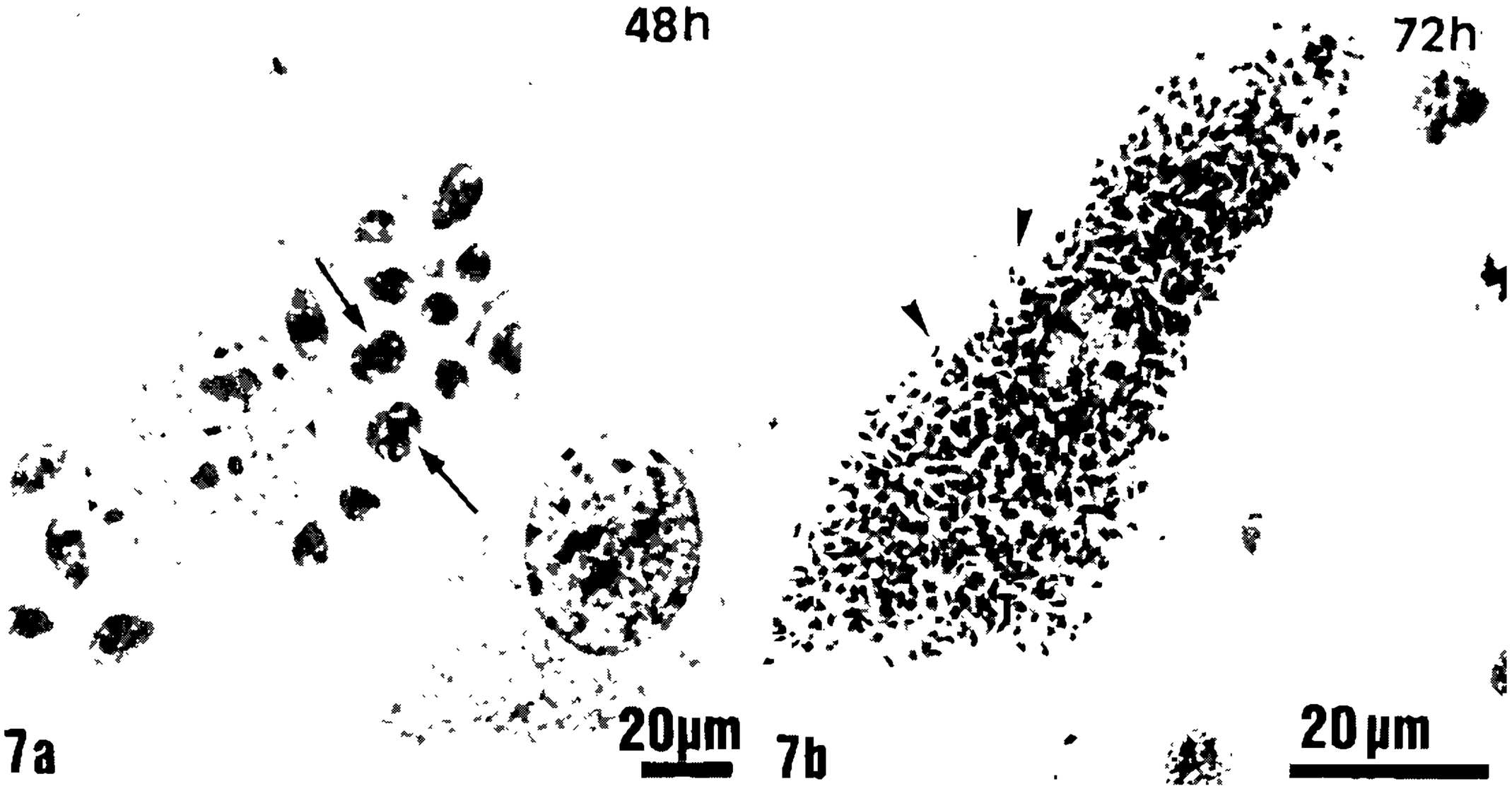


Fig. 7: intracellular development of trypsinized trypomastigotes of *Trypanosoma cruzi* (Y strain) for 48 and 72 hours inside heart muscle cells. Note normal division of amastigotes (Fig. 7a) and differentiation into trypomastigotes (Fig. 7b) respectively on the second and third days after *in vitro* infection.

lar cycle, dividing as amastigotes (Fig. 7a) and differentiating into trypomastigotes (Fig. 7b).

Following tests using a variety of proteinase inhibitors, it seems that cysteine-, serine- and metallo-proteinases are involved in processes discussed above (Meirelles et al., 1990, 1992; Bonaldo et al., 1991). Parasites treated with peptidyl diazomethane (PDAM) – an inhibitor that is specific to cysteino-proteinases, a major proteinase of *T. cruzi* – display an impaired capacity for invasion of, and intracellular proliferation in, heart muscle cells. These studies led us to the conclusion that the ability of *T. cruzi* to infect, and to develop intracellularly in mammalian cells is critically dependent on the activity of a major cathepsin L-like enzyme that is probably identical to GP 57/51 (cruzipain) (Meirelles et al., 1992). Accordingly, when parasites are treated with F(ab')2 fragments of antibody against the cisteine proteinases, their ability to invade macrophages is inhibited (Souto-Padrón et al., 1990).

The effect of alpha-2-macroglobulin (A2M) – a physiological plasma protease inhibitor (Van Leuven, 1984) – was especially interesting and merited further investigation. As mentioned above, treatment of parasites with A2M, or addition of A2M to the culture medium during the period of interaction, inhibits trypomastigotes invasion of macrophages and fibroblasts (Araujo-Jorge et al., 1986b). A2M is an irreversible proteinase inhibitor, and we observed that washing it out and further incubating the parasites in medium led to a progressive recovery of their ability to invade macrophages.

To test if proteolytic "processing" of trypomastigote surface ligands could enhance the parasite's ability to invade host cells, we incubated trypomastigotes for 20 h in axenic culture medium at 37 or 4 °C, observed that they became more infective to macrophages as a result, and A2M was able to inhibit this process (Araujo-Jorge et al., 1986a; Fig. 8). After such incubation, the "processed" parasites became insensitive to the inhibitory effect of A2M during the interaction assay.

Since it is known that protease treatment of trypomastigotes reduces their surface negativity (Souto-Padron & De Souza, 1985, 1986), we also used another approach, which involved measuring the net surface charge of parasites. We collected the supernatant containing parasites that had not been interiorized after 20 h of contact with muscle cells, and, interestingly, found that they showed a reduction in their surface negativity (Soeiro et al., 1991). However, when incubation of parasites with muscle cells was performed in the presence of A2M

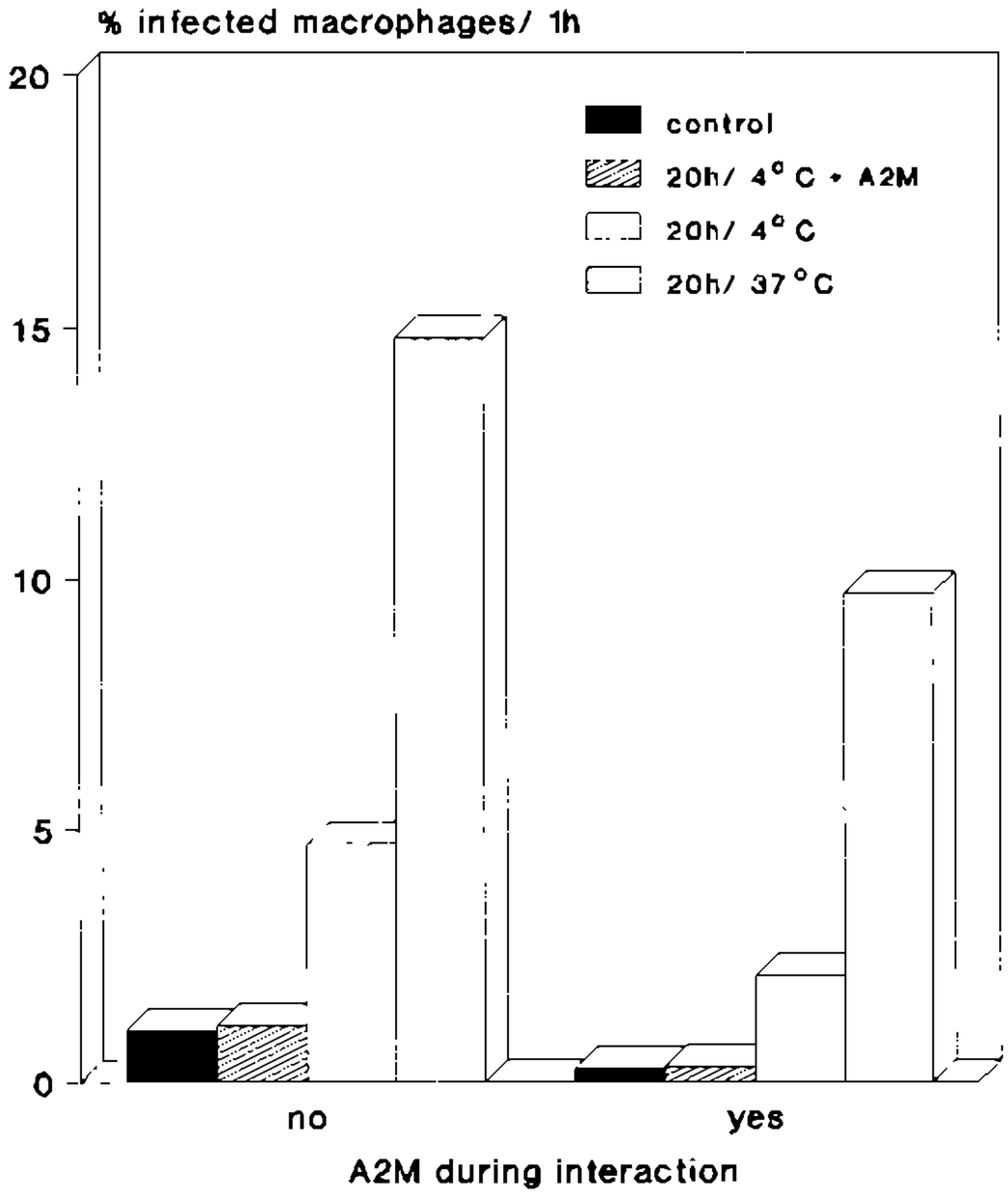


Fig. 8: effect of extracellular proteolytic processing of the surface of trypomastigotes on the ability of parasites to invade resident peritoneal mouse macrophages. Bloodstream trypomastigotes were incubated for 20 hours in axenic culture medium at 4 or 37 °C, in the presence or absence of alpha-2-macroglobulin (A2M), washed, and then exposed to macrophages in the presence or absence of A2M.

or soybean trypsin inhibitor, this reduction of surface negativity was considerably less pronounced.

We therefore concluded that trypomastigotes need to process their own surface proteolytically, in order expose the necessary ligands that allow binding to, and internalization in host cells. We also believe that this process occurs extracellularly, and is time and temperature-dependent. Similar results were reported by Piras' group in Venezuela, using tissue culture-derived trypomastigotes, as well as other experimental approaches (Piras et al., 1985).

We considered these effects of A2M to be highly interesting, since high quantities of this molecule are present in the plasma of mammals, and we speculated that it could play some role during *in vitro T. cruzi* infection. We started investigating the possibility that *in vivo* infection of mice with *T. cruzi* leads to detectable changes in the levels of alpha-2-macroglobulin in these animals. Using different mice strains, such as outbred Swiss or inbred BALB/c mice, we found that an increase in plasma levels of alpha-2-macroglobulin could be detected with rocket immunoelectrophoresis (Isaac et al., 1990), as well as with ELISA (Araujo-Jorge et al., 1991), even when using different *T. cruzi* strains, such as Y, Colombiana, or Tehuantepec. However, the kinetics of increases in A2M levels were different for each mouse model.

In the BALB/c model, we found a significant correlation between the ability of mice to survive the acute phase of infection and increase in macroglobulin levels (Araujo-Jorge et al., 1991), suggesting that the plasma protease inhibitor was in some way participating in host protection.

Since alpha-macroglobulins are elements of the acute phase response in many animals (Koj et al., 1988), and may play an important role both in the clearance of proteinases and as carriers and modulators of cytokines released during inflammation (James 1990; LaMarre et al., 1991), we believe that they may also be involved in *T. cruzi*-host interaction in the acute phase of infection.

CONCLUSIONS

The main conclusions of our studies are that: (1) the biological mechanism of *T. cruzi* invasion of target host cells is essentially the same (during a primary infection in the absence of a specific immune response) regardless of whether the target cell is a professional phagocytic cell, such as a macrophage, or a non-professional phagocytic cell, such as a muscle cell or fibroblast; (2) the diverse range of molecular mechanisms which the parasite may use to invade the host cell may correspond to differences in the available "receptors" and "ligands" on the surface of each specific cell type; and (3) acute phase components, with lectin (galactose) or proteinase inhibitory activities (macroglobulins), may also be involved in *T. cruzi*-host cell interaction.

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