Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 39 (1982)
Heft: 3

Artikel: Interaction of bloodstream, tissue culture-derived and axenic culture-derived trypomastigotes of "Trypanosoma cruzi" with macrophages

Autor: Meirelles, M.N.L. / Chiari, E. / Souza, W. de

DOI: https://doi.org/10.5169/seals-312977

Acta Tropica *39*, 195–203 (1982)

[1] Fundação Oswaldo Cruz, Av. Brasil 4365, 21040 Rio de Janeiro, RJ, Brasil
[2] Departamento de Parasitologia, I.C.B., Universidade Federal de Minas Gerais, 30000 Belo Horizonte, MG, Brasil
[3] Instituto de Biofísica, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941 Rio de Janeiro, RJ, Brasil

Interaction of bloodstream, tissue culture-derived and axenic culture-derived trypomastigotes of *Trypanosoma cruzi* with macrophages

M. N. L. Meirelles[1], E. Chiari[2], W. de Souza[3]

Summary

Mouse macrophages were infected with bloodstream, tissue culture-derived and axenic culture-derived trypomastigotes of the Y and the CL strains of *Trypanosoma cruzi*. The percentage of infected cells, the mean number of parasites per cell and the incorporation index were determined after 2 h of interaction. Longer periods of interaction were used to evaluate the fate of the different trypomastigotes inside the macrophage. It was observed that the incorporation of *T. cruzi* by the macrophages was high for tissue culture-derived trypomastigotes, intermediate for axenic culture-derived trypomastigotes and low for bloodstream trypomastigotes. For the three types of trypomastigotes, a larger number of macrophages were infected with parasites from the Y than the CL strain. These results suggest that the ability to infect macrophages is a basic characteristic of each strain which is maintained when the parasites are transferred from the vertebrate of the invertebrate host to in vitro systems.

Key words: *Trypanosoma cruzi;* trypomastigotes; macrophage.

Introduction

Three developmental forms, designated as spheromastigote (amastigote), epimastigote and trypomastigote, are found in the life cycle of *Trypanosoma*

Correspondence: Dr. Wanderly de Souza, Instituto de Biofísica – UFRJ, Ilha do Fundão, 21941 Rio de Janeiro, RJ, Brasil

cruzi. The spheromastigotes are multiplicative forms observed in the cytoplasm of vertebrate cells both in vivo, in infected animals and in vitro, in cell cultures. Epimastigotes are multiplicative forms found either in insect vector or when *T. cruzi* is placed in acellular cultures. Trypomastigotes are highly differentiated forms which are unable to divide and are specialized in infecting vertebrate cells.

These are two processes by which trypomastigotes of *T. cruzi* are formed: a) by transformation of spheromastigotes into trypomastigotes via epimastigotes, a process which occurs at the end of the cycle of *T. cruzi* inside vertebrate cells. Such trypomastigotes can be obtained either from the blood of animals or from the supernatant of cell cultures previously infected with the parasite; b) by direct transformation of epimastigotes into trypomastigotes, a process which occurs either at the posterior portions of the intestine of the insect vector or at the stationary phase of growth of *T. cruzi* in axenic culture medium (for a review see Brener, 1973). In the latter case, special conditions of cultivation using selected clones of *T. cruzi* have been recently developed aiming at the regular obtention of populations in which more than 95% of the parasites are metacyclic trypomastigotes (Chiari, 1981).

One important question is to know whether the trypomastigotes of *T. cruzi* obtained from the different sources mentioned above present the same behaviour when in contact with vertebrate cells. Previous studies indicate that macrophages are a suitable system to study interaction of *T. cruzi* with host cells since they are readily infected with the parasite. Using this system it has been shown that different strains of *T. cruzi* present a different behaviour when in contact with the macrophages (Alcântara and Brener, 1978; Kipnis et al., 1979; Meirelles et al., 1980).

In this paper we investigated the interaction of trypomastigotes of the Y and CL strains of *T. cruzi*, obtained from the bloodstream of mice, from cellular and from axenic cultures, with macrophages. The two strains of *T. cruzi* were selected because they are different in several biological characteristics.

Material and Methods

Parasite strains. The Y strain was isolated from a human acute case of Chagas' disease (Silva and Nussenzweig, 1953) and exhibits an in vivo tropism for murine macrophages. The CL strain was isolated from a triatomine bug and has a tropism for muscle cells (Brener and Chiari, 1963). These two strains also present several morphological and immunological differences (Brener, 1965, 1969, 1973; Krettli and Brener, 1976; Krettli, 1978).

Obtention of bloodstream trypomastigotes. The blood was collected with 3.8% sodium citrate as an anticoagulant from heavily infected Swiss Webster mice on the peak of parasitemia (7th day for the Y strain and the 12th day for the CL strain). The blood was centrifuged at 150 *g* for 10 min and the supernatant fluid, which contained the parasites, was collected and centrifuged at 900 *g* for 10 min. The pellet was then washed in Tyrode's solution (compositon in g/l: sodium chloride, 8.0; potassium chloride, 0.2; magnesium chloride, 0.1; sodium bicarbonate, 1.0; glucose, 1.0; sodium monophosphate, 0.05; calcium chloride, 0.2), pH 7.2.

Collection of tissue culture-derived trypomastigotes. The LA9 cell line (tumoral mouse fibroblast) was used. Glass culture flasks (35 cm^2 growth area) were seeded with 1×10^6 cells in 12 ml Eagle's (MacPherson-Stoker) medium supplemented with 5% inactivated fetal calf serum. After 24 h the monolayer was infected with bloodstream trypomastigotes which then penetrate and multiply inside the cells. The medium was changed every 3 days. Ten days after infection the supernatant fluid of the bottles contained a large number of trypomastigotes. The medium was then centrifuged first at 150 *g* for 10 min and the pellet, which contained cells and debris was discarded and the supernatant fluid, which contained the parasites, was collected and centrifuged at 900 *g* for 10 min. The pellet was then washed in Tyrode's solution.

Collection of axenic culture-derived trypomastigotes. Special clones of epimastigotes of the Y and CL strains of *T. cruzi* were cultivated for 6 days at 28° C in M16 medium (Goldberg and Chiari, 1980; Morel et al., 1980; Chiari, 1981).

Macrophages. Cells were collected from the peritoneal cavities of uninfected Swiss Webster mice upon injection of 4 ml of Hanks' balanced solution (HBS). A sample of 0.5 ml of a suspension containing 2×10^6/ml mononuclear cells was placed into Leighton tubes with flying cover-slips. After incubation for 1 h at 37° C the non-adherent cells were removed, the macrophage monolayers were washed twice with Tyrode's solution and 199 medium supplemented with 10% inactivated fetal calf serum was added.

Infection of the cells. Bloodstream trypomastigotes of both the Y and the CL strains were suspended in Tyrode's solution in order to get a parasite concentration such that a ratio of 10 parasites per macrophage was obtained when 0.25 ml of the suspension was added to the macrophage cultures. The number of macrophages in the preparations was estimated by counting 20 microscopic fields. This number varied from 500 to 1000 cells/mm^2. The parasites were maintained in contact with the cells for a period of 2 h, after which time the cultures were rinsed with Tyrode's solution and then fixed with Bouin's fixative and stained with Giemsa. In order to check the viability and the fate of the parasite, some cultures were maintained for more than 24 h.

Evaluation of results. After 2 h of macrophage-parasite interaction the cultures were fixed and stained as described above. The percentage of infected macrophages was determined by randomly examining at least 400 cells with high magnification under a Zeiss Universal Photomicroscope. The mean number of intracellular parasites/infected macrophages was also determined. The phagocytic index was calculated by multiplying the percentage of infected macrophages and the mean number of parasites per infected macrophage.

Results

Light microscopic observations of the population of parasites used in the experiments indicated that when parasites obtained from either cellular or axenic cultures were used, more than 95% of the cells were trypomastigotes. In the cellular cultures the main contaminant form was the spheromastigote (amastigote) and in the axenic cultures the main contaminant stage were epimastigotes.

All types of trypomastigotes were internalized by macrophages (Figs. 1–4). We used 2 h of interaction to compare the ability of the different trypomastigotes to enter macrophages. As indicated in Fig. 1 there was a marked difference in the rate of interiorization of different trypomastigotes of *T. cruzi* by macrophages. The percentage of macrophages containing parasites was higher when trypomastigotes isolated from cell cultures were used. The percentage decreased when trypomastigotes obtained from axenic cultures were used and

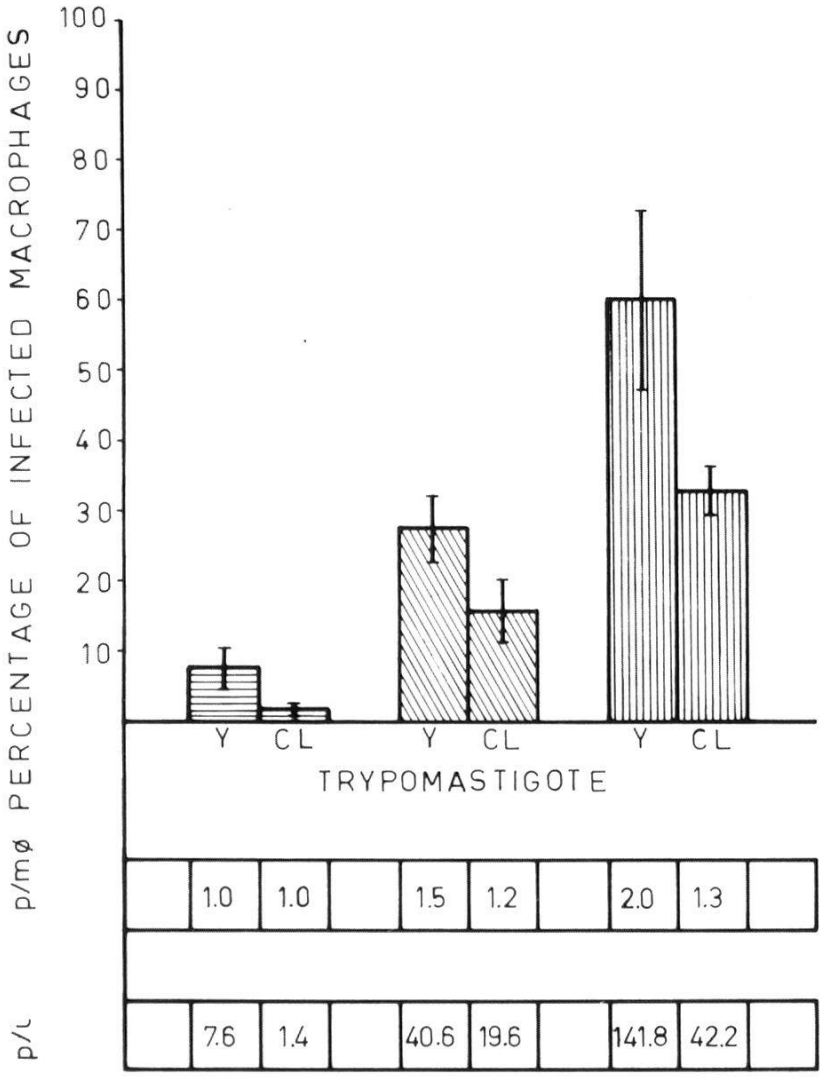
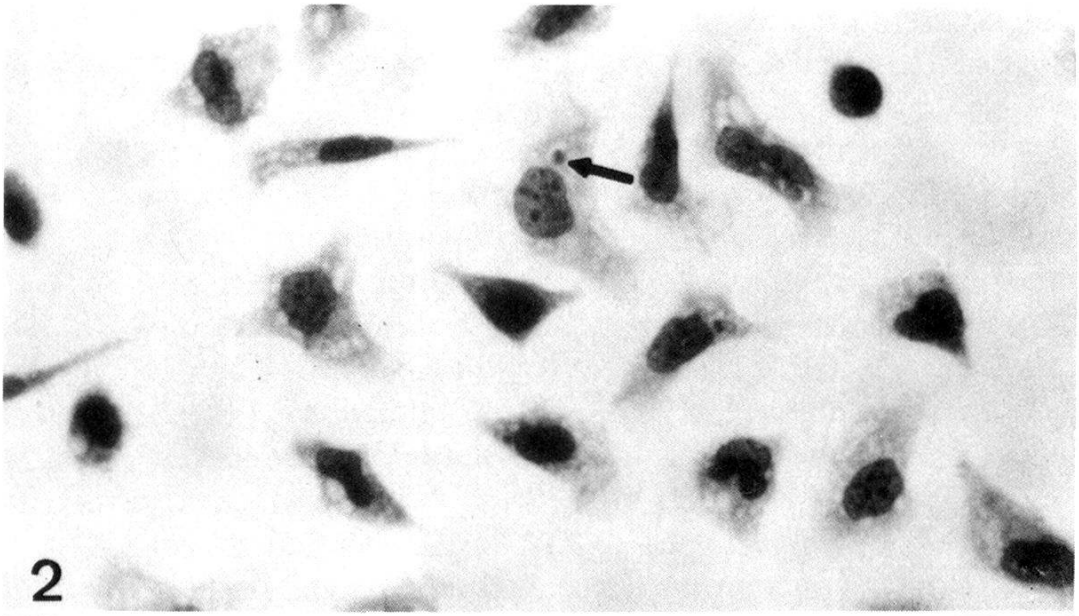


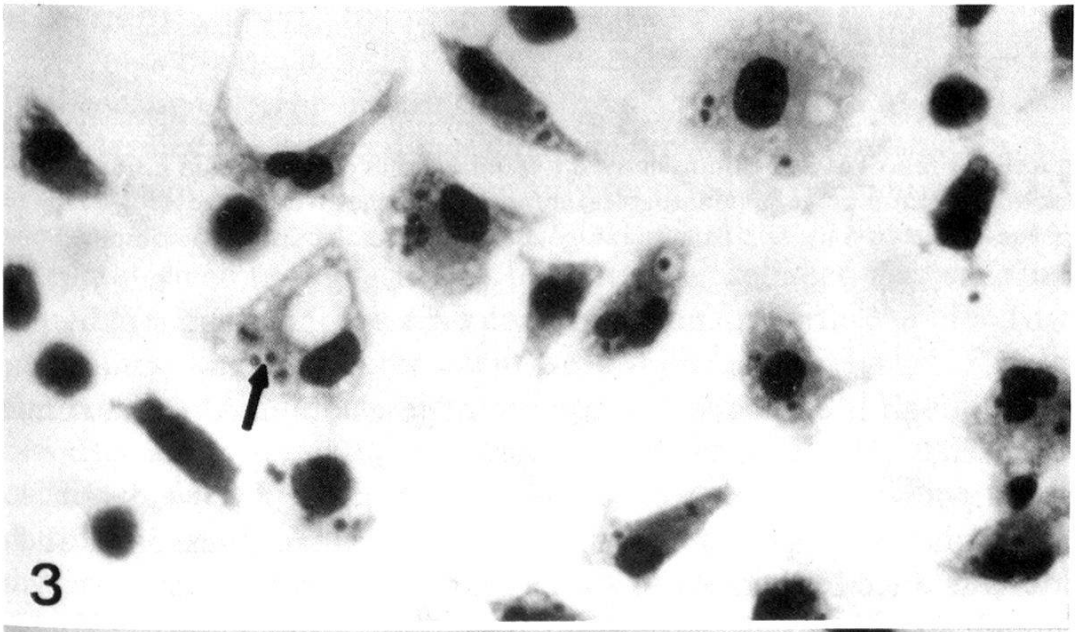
Fig. 1. Infection of macrophages by bloodstream (b), axenic culture-derived (ac) and tissue culture-derived (tc) trypomastigotes of the Y and CL strains of *Trypanosoma cruzi*. The percentage of infected macrophages, the mean number of trypomastigotes per macrophage (p/Mø) and the phagocytic index (p/i) were determined after 2 h of parasite-macrophage interaction.

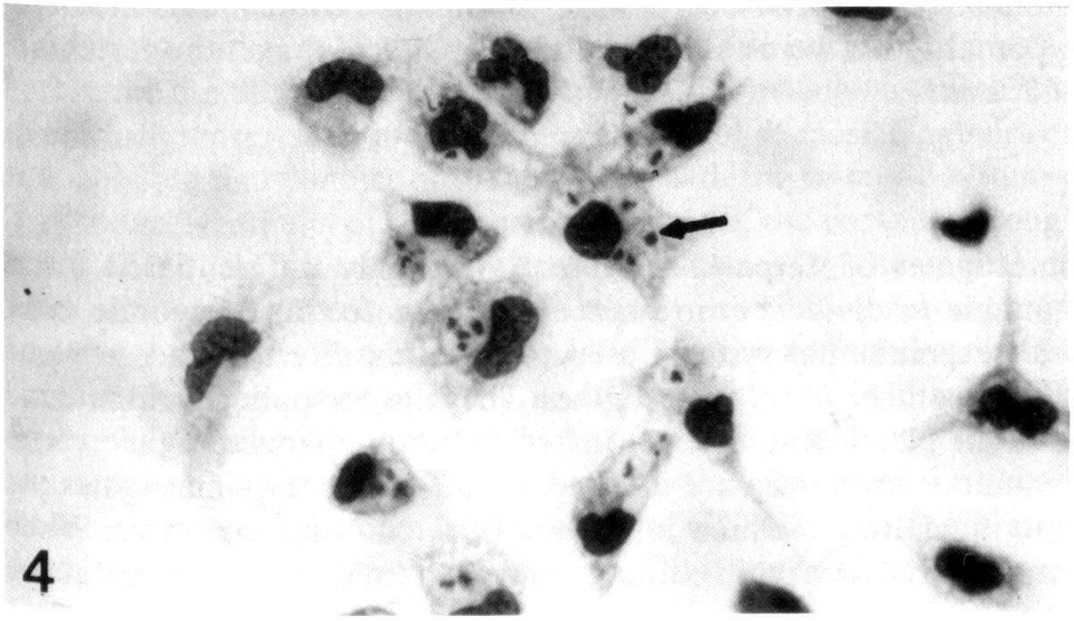
was even lower when bloodstream trypomastigotes were used. Such differences were observed with both the Y and the CL strains of *T. cruzi* used in the experiments. We did not observe morphological signs of digestion of the trypomastigotes interiorized by the macrophages.

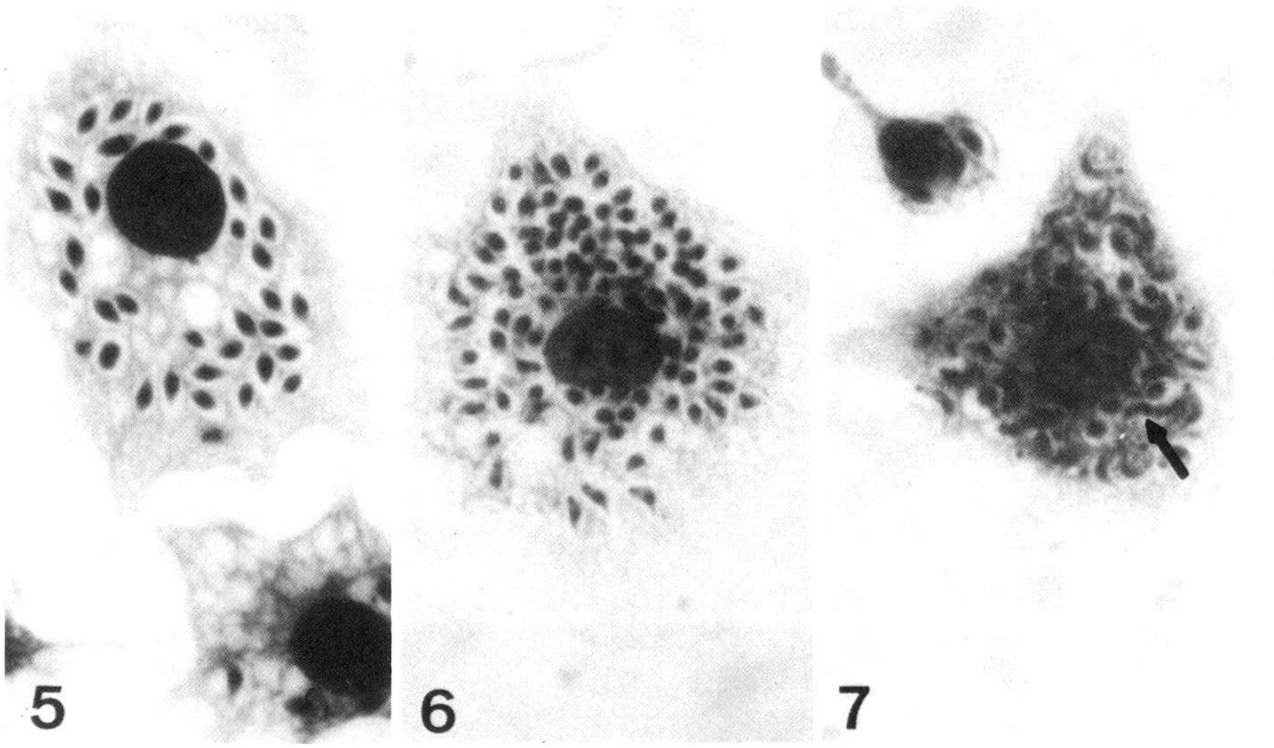
A marked difference, particularly with trypomastigotes obtained from the bloodstream and from cell cultures, was observed in the rate of incorporation of parasites from the Y and CL strains (Fig. 1). Those from the Y strain are more able to enter macrophages than those from the CL strain. This difference was

Figs. 2–4. Macrophages which were incubated for 2 h in the presence of bloodstream (Fig. 2), tissue culture-derived (Fig. 3) and axenic culture-derived (Fig. 4) trypomastigotes of the Y strain of *T. cruzi*. Few macrophages were infected by bloodstream trypomastigotes whereas a large number of macrophages were infected by culture-derived trypomastigotes. Arrows indicate intracellular parasites. 630×.


2


3


4



Figs. 5–7. Intracellular fate of axenic culture-derived trypomastigotes of the Y strain inside macrophages. Intracellular spheromastigotes (amastigotes) are seen inside the macrophages (Figs. 5–6). In other macrophages forms in transition from amastigotes to trypomastigotes can be observed (arrows) (Fig. 7). 1200×.

less evident, although it existed, when trypomastigotes obtained from axenic cultures were used.

Trypomastigotes from the three sources and both strains were able to transform into spheromastigote once inside the macrophage (Figs. 5–7). Such forms divide and after few days transform into new trypomastigotes which could be found both inside the macrophages and in the culture medium. Therefore, the trypomastigotes were able to reproduce in macrophages the vertebrate life cycle of *T. cruzi.*

Discussion

Trypomastigotes of *Trypanosoma cruzi* are highly differentiated forms which are unable to divide but are specialized in infecting vertebrate cells. Practically all experimental systems used to maintain *T. cruzi* lead, at some point, to the appearance of trypomastigotes. They are the only developmental form found in the blood of vertebrates infected with the parasite. When vertebrate cells maintained in vitro are infected with *T. cruzi,* trypomastigotes are liberated into the culture medium at the end of the intracellular cycle. When *T. cruzi* is maintained in axenic culture, some trypomastigotes appear at the stationary phase of growth. Under some experimental conditions, mainly using

special clones and culture medium, it is now possible to obtain acellular cultures in which more than 95% of the cells are metacyclic trypomastigotes (Chiari, 1981).

The results we obtained in the present study confirm previous observations that trypomastigotes of *T. cruzi*, independent their origin, are infective for vertebrate cells. This observation is important in view of the fact that it is very difficult to obtain large amounts of trypomastigotes of *T. cruzi* from the bloodstream of infected mice or from the insect vector. The clones of the Y and the CL strain recently obtained (Goldberg and Chiari, 1980; Chiari, 1981) open the possibility for reproducible harvesting of large numbers of trypomastigotes which are, as shown here, able to infect and to reproduce the intracellular cycle of *T. cruzi* in macrophages.

Our results indicate that although trypomastigotes of *T. cruzi* obtained from different origins are able to infect macrophages in vitro, there are differences in their uptake by macrophages. Macrophages incorporate more trypomastigotes obtained from the supernatant of cell cultures than bloodstream trypomastigotes. The uptake of trypomastigotes obtained from axenic cultures was intermediate between the other two types of trypomastigotes. These results are in concordance with those recently reported by Nogueira et al. (1980) which showed the existence of differences in the ability of bloodstream and axenic culture-derived trypomastigotes to infect macrophages. Evidence has been presented to suggest that there is a component on the surface of bloodstream trypomastigotes which may represent an antiphagocytic factor. Removal of this component by mild trypsin digestion enhances considerably the uptake of bloodstream trypomastigotes, reaching values near that observed with axenic culture-derived trypomastigotes (Nogueira et al., 1980; Kipnis et al., 1981; De Araujo Jorge and De Souza, 1981). It is important to mention here that the antiphagocytic component is not derived from the vertebrate host since experiments utilizing an inhibitor of protein synthesis indicate that it is synthesized by the parasite (Nogueira et al., 1981). We do not have at present an explanation for the fact that only bloodstream trypomastigotes synthesize this protein, which represents a major surface protein of *T. cruzi* (Nogueira et al., 1981). It is possible that contact of the parasites with the immune system of the host may induce synthesis of this protein. Passage of the parasite through the cytoplasm of the host cell apparently does not induce its synthesis, since trypomastigotes obtained from cell cultures are readily ingested by macrophages.

For each type of trypomastigotes of *T. cruzi* we found a significant difference between parasites of the Y and the CL strains in their ability to infect macrophages after 2 h of macrophage-parasite interaction. Previous studies, in which only bloodstream trypomastigotes were used, show the existence of differences between parasites from the Y and CL strain. a) The Y strain shows a predominance of slender forms while the CL strain has a predominance of stout forms (Brener and Chiari, 1963; Brener, 1973). b) The Y strain, when inoculated

into mice shows a marked tropism for cells of the mononuclear phagocytic system while the CL strain has a tropism for muscle cells (Melo and Brener, 1978). c) Trypomastigotes of the Y strain are agglutinated by immune serum and are lysed by human complement while those from the CL strain are not affected by the antibodies and are not lysed by complement unless they are pre-incubated with specific immune serum (Krettli and Brener, 1976; Krettli, 1978). d) Trypomastigotes of the Y strain, contrary to what occurs with those from the CL strain, cap easily when incubated in the presence of specific antibodies (Schmuñis et al., 1980). e) With respect to the interaction with macrophages, marked differences have been observed in the ability of trypomastigotes of the two strains to infect either mouse or chicken macrophages (Alcântara and Brener, 1978; Kipnis et al., 1979; Meirelles et al., 1980). Those from the Y strain were more infective than those from the CL strain. This difference, however, was not observed in a recent study reported by Nogueira et al. (1980). However, these authors used trypomastigotes of the CL strain obtained from mice at the 7th day of infection. In our laboratory we do not obtain a high parasitemia in mice infected for only 7 days with the CL strain of *T. cruzi*. Therefore, we always use CL parasites after 11 days of infection.

Our results suggest that the difference found in the infection of macrophages by parasites of the Y and CL strains is a characteristic of the strain since it is also observed when trypomastigotes obtained from either cellular or axenic cultures were used. These results also suggest that the difference in the ability of the two strains to infect macrophages is not a consequence of the relative percentage of slender or stout forms in the parasite population since differences were not observed in the morphology of the parasites of the Y and the CL strains when tissue culture or axenic culture-derived trypomastigotes were used. It is possible that such differences reflect a basic characteristic of the strains, which are maintained in clones, as is the case of the parasites obtained from acellular cultures, and is independent of the morphology of the parasites. However, the available data for bloodstream trypomastigotes, which show more clearly the existence of slender and stout forms, indicate a positive correlation between the percentage of slender forms in the parasite population and their ability to infect macrophages (Alcântara and Brener, 1978; Kipnis et al., 1979; Meirelles et al., 1980) and non-professional phagocytic cells (Bertelli and Brener, 1980).

Acknowledgments

This work has been supported by UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases, CNPq, FINEP and CEPG-UFRJ.

Alcântara A., Brener Z.: The in vitro interaction of *Trypanosoma cruzi* bloodstream forms and mouse peritoneal macrophages. Acta trop. (Basel) *35*, 209–219 (1978).

Bertelli M. S., Brener Z.: Infection of tissue culture cells with bloodstream trypomastigotes of *Trypanosoma cruzi*. J. Parasit. *66*, 992–997 (1980).

Brener Z.: Comparative studies of different strains of *Trypanosoma cruzi*. Ann. trop. Med. Parasit. *59*, 19–26 (1965).

Brener Z.: The behaviour of slender and stout forms of *Trypanosoma cruzi* in the bloodstream of normal and immune mice. Ann. trop. Med. Parasit. *63*, 215–220 (1969).

Brener Z.: Biology of *Trypanosoma cruzi*. Ann. Rev. Microbiol. *27*, 347–382 (1973).

Brener Z., Chiari E.: Variações morfológicas observadas em diferentes amostras de *Trypanosoma cruzi*. Rev. Inst. Med. trop. S. Paulo *5*, 220–224 (1963).

Chiari E.: Diferenciação do *Trypanosoma cruzi* em cultura, 91 p. PhD Thesis, University of Minas Gerais 1981.

De Araujo-Jorge T. C., De Souza W.: Interação entre *Trypanosoma cruzi* e macrófagos: envolvimento dos resíduos de ácido siálico e galactose na modulação da endocitose. Reun. Presq. Bás. Doença de Chagas, Caxambu, Brasil, p. 107 (1981).

Goldberg S. S., Chiari E.: Growth and isolation of single colonies of *Trypanosoma cruzi* on solid medium. J. Parasit. *66*, 677–679 (1980).

Kipnis T. L., Calich V. L. G., Dias da Silva W.: Active entry of bloodstream forms of *Trypanosoma cruzi* into macrophages. Parasitology *78*, 89–98 (1979).

Kipnis T. L., David J. R., Alper C. A., Sher A., Dias da Silva W.: Enzymatic treatment transforms trypomastigotes of *Trypanosoma cruzi* into activators of alternative complement pathway and potentiates their uptake by macrophages. Proc. nat. Acad. Sci. (Wash.) *78*, 602–605 (1981).

Krettli A. U.: Efeito de anticorpos e do complemento sobre tripomastigotas sanguíneos de camundongos infectados com *Trypanosoma cruzi*. Thesis, Universidade Federal de Minas Gerais 1978.

Krettli A. U., Brener Z.: Tissue tropism of different *Trypanosoma cruzi* strains. J. Immunol. *116*, 755–760 (1976).

Meirelles M. N. L., De Araujo-Jorge T. C., De Souza W.: Interaction of epimastigote and trypomastigote forms of *Trypanosoma cruzi* with chicken macrophages in vitro. Parasitology *81*, 373–381 (1980).

Melo R. C., Brener Z.: Tissue tropism of different *Trypanosoma cruzi* strains. J. Parasit. *64*, 478–482 (1978).

Morel C., Chiari E., Camargo E. P., Mattei D. M., Romanha A. J., Simpson L.: Strains and clones of *Trypanosoma cruzi* can be characterized by pattern of restriction endonuclease products of kinetoplast DNA minicircles. Proc. nat. Acad. Sci. (Wash.) *77*, 6810–6814 (1980).

Nogueira N., Chaplan S., Cohn Z.: *Trypanosoma cruzi:* factors modifying ingestion and fate of blood form trypomastigotes. J. exp. Med. *152*, 447–451 (1980).

Nogueira N., Chaplan S., Tydings J. D., Unkeless J., Cohn Z.: *Trypanosoma cruzi:* surface antigens of blood and culture forms. J. exp. Med. *153*, 629–639 (1981).

Schmuñis G. A., Szarfman A., De Souza W., Langembach T.: *Trypanosoma cruzi:* antibody-induced mobility of surface antigens. Exp. Parasit. *50*, 90–102 (1980).

Silva L. H. P., Nussenzweig V.: Sobre uma cepa de *Trypanosoma cruzi* altamente virulenta para o camundongo branco. Folia clin. biol. (S. Paulo) *20*, 191–207 (1953).