Rev. Inst. Med. trop. São Paulo
26(4):179-185, *julho-agosto*, 1984

UDC 593.161.13
616.937.3-092.9

TRYPANOSOMA CRUZI INTERACTION WITH MACROPHAGES: DIFFERENCES BETWEEN TISSUE CULTURE AND BLOODSTREAM FORMS (*)

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SUMMARY

Mouse peritoneal elicited macrophages cultured on coverslips were infected with **Trypanosoma cruzi** trypomastigotes from both the F strain and Y strain obtained either from tissue culture or from the bloodstream of infected mice. Both the Y strain and F parasites obtained from tissue culture were interiorized by macrophages at a much higher rate than bloodstream trypomastigotes. Tissue culture parasites incubated with normal mouse serum, mouse plasma obtained at the 7th day after infection, or specific hyperimmune serum at sub-agglutinating concentration, behaved essentially as non-opsonized parasites. Ultrastructural differences were seen at the early interaction phase between macrophages and trypimastigotes from both sources. After 30 minutes, tissue culture trypomastigotes were located in clusters at the area of contact with macrophages. While bloodstream trypomastigotes, at 3 hours post-infection were most frequently enclosed in a loose phagocytic vacuole, tissue culture trypomastigotes were enclosed in single tight vacuoles. Both tissue culture and bloodstream trypomastigotes of the Y strain multiplied within macrophages; F strain bloodstream trypomastigotes did not develop within the host cells, while tissue culture trypomastigotes multiplied.

INTRODUCTION

Several papers have been published on the interaction between **T. cruzi** and macrophages with both epimastigotes and trypomastigotes, the latter being the forms able to multiply within mammalian cells [2]. In most of these papers trypomastigotes are either obtained from the bloodstream of infected animals or from acellular cultures. Bloodstream trypomastigotes tend to infect only a small percentage of macrophages in vitro, while the uptake of parasites obtained from acellular cultures is more intense [2].

An alternative source of trypomastigotes are infected tissue cultures; these forms have been rarely employed in studies on the interaction between trypanosomes and macrophages. They are reported as being either destroyed within these cells [5] or surviving and multiplying in their interior [15].

Recently a study comparing macrophage interaction with bloodstream, tissue culture and acellular culture derived trypomastigotes of the Y and CL strains has been published. Tissue culture parasites of both strains are incorporated more intensely than bloodstream forms, and parasites of both sources multiply within these cells. However, CL strain parasi-

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(*) This work was supported by CNPq Grants 2222.8.097/80 and 2222.8.141/80 and FINEP Grant B7680/23/00/00
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tes of both sources infect macrophages to a lesser extent than their Y strain counterparts [10].

In the present paper bloodstream and tissue culture trypomastigotes of the Y and F strains interacting with mouse peritoneal macrophages "in vitro", were compared. An attempt was also made to correlate quantitative differences of parasite uptake with the presence of host serum components on trypanosome's membrane. At the ultrastructural level, comparison between bloodstream and tissue culture trypomastigotes at the initial interaction with macrophages was made for the first time, showing visible differences.

MATERIAL AND METHODS

Parasites — The Y strain [16] and F strain [4] of **T. cruzi** were employed. Y strain is reticulotropic and myotropic, and has characteristically slender forms. The F strain infects mainly skeletal muscle, with a predominance of broad forms.

Bloodstream trypomastigotes (BT) were obtained from Swiss albino mice at the peak of parasitemia (7th day for the Y strain, and 35th day for the F strain). They were separated from defibrinated blood by differential centrifugation, washed, and suspended in M 199 (Medium 199, Flow Lab., U.K.), containing 20% fetal calf serum, at the desired concentration, as previously described [12].

Tissue culture trypomastigotes (TCT) were obtained from the supernatant of LLC-MK_2 monolayers, infected 7 days previously, washed and suspended as above.

Macrophages — Peritoneal macrophages were obtained from Swiss mice on the 4th day after peptone stimulation, pooled, seeded on coverslips in Petri dishes and incubated for 24 to 48 hs in M199,20% fetal calf serum. They were then infected with parasites at a parasite-cell ratio of 1:1. After 30 min to 3 hs contact they were washed and either fixed immediately or reincubated for an additional 48 hs in fresh medium, all procedures being carried out as previously described [12].

Sensitization of parasites with mouse sera. Y strain TCT were suspended in: a) normal mouse serum; b) plasma obtained at the 7th day of infection with Y strain trypanosomes, or c) hyperimmune mouse anti-**T. cruzi** serum at subagglutinating dilution (1:50). At this dilution an anti-mouse immunoglobulin fluorescein isothyocyanide conjugated rabbit immunoglobulin revealed a very slight fluorescence. After incubation for 1 h at 37°C, they were diluted to the desired concentration and put in contact with macrophages at a 1:1 parasite cell ratio, for 3 hs.

Microscopy — For light microscopy coverslips were stained with Giemsa. Percentage of parasitized cells and number of parasites per 100 cells were scored in 500 cells 3 hs and 48 hs after parasite contact. Electron microscopy was made with Y strain parasites and specimens were processed at 30 min and 3 hs post-infection. Cells were washed with Hank's solution, fixed "in situ" with 2% (v/v) glutaraldehyde. After scraping the cells with a rubber policeman they were washed again, fixed in 1% osmium, dehydrated and embedded in Araldite. Sections were examined either in a Philips 301 or Zeiss EM-9 electron microscope.

RESULTS

Figure 1 shows quantitative differences between interiorization of BT and TCT of both the Y and F strain. As can be seen, parasites originating from tissue culture were interiorized by macrophages at a much higher percentage than those originating from the bloodstream of mice. Initial infection rate with TCT of both strains was higher than one (Table I), while usually only one parasites per cell was seen in infections with BT.

Differences at the initial phase of interaction between Y strain BT and TCT with macrophages were also noted at the ultrastructural level. After 30 min TCT were frequently seen in clusters at the area of contact with macrophages (Fig. 2). At this area the macrophages cytoplasm was almost devoid of organelles except for smooth vacuoles, with expanding pseudopodia indicating an active process of phagocytosis, although the macrophage already harbored one or more parasites within large vacuoles. With BT at the same parasite cell ratio (1:1) we were not able to observe the attachment phase.

After 3 hs interaction, BT were localized inside a large phagocytic vacuole. Frequently

TABLE I

Interiorization and multiplication of bloodstream and tissue culture trypomastigotes in macrophages

Strain	Parasites Origin	Time of interaction 3hs % cells infected (A)	3hs No. parasites/ 100 cells (B)	3hs infection rate (B/A)	48hs % cells infected (C)	48hs No. parasites/ 100 cells (D)	48hs multiplication rate (D/B) x (A/C)
Y	Bloodstream	5.7	5.7	1.0	5.3	16.5	3.09
	Tissue culture	30.3	56.0	1.84	40.8	209.0	2.76
F	Bloodstream	9.0	9.0	1.0	0	0	0
	Tissue culture	34.0	68.0	2.0	40.0	198.0	2.47

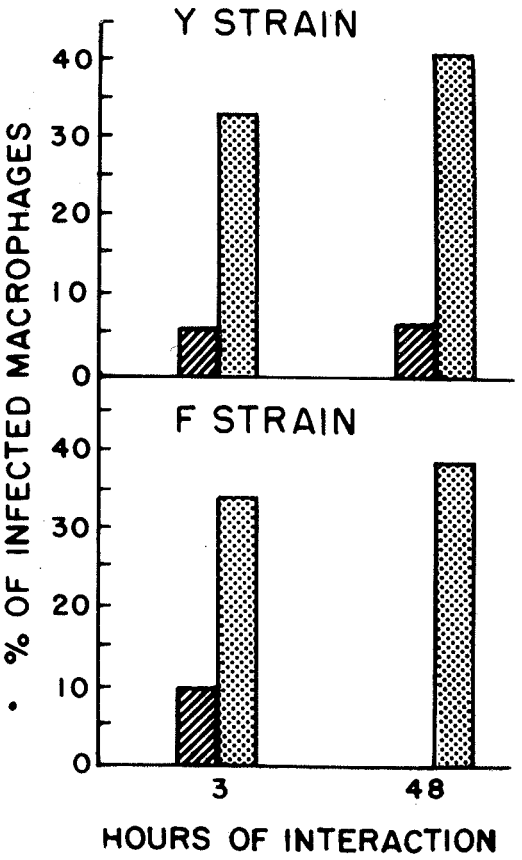

Fig. 1 — Infection of macrophages 3 and 48 hs after exposure to **T. cruzi** bloodstream forms ▨ and tissue culture forms ▩

an amorphous, somewhat granular substance was also seen enclosed in this vacuole together with membrane debris and flagellar fragments (Fig. 3). On the other hand, at this period, TCT were enclosed in a more clear, tight vacuole, with practically no space left between the parasites and the phagocytic membrane (Fig. 4).

Figure 1 and Table I also present results after 48 hs interaction. The percentage of macrophages infected with Y strain BT and TCT was not altered significantly when compared with 3 hr interaction. However, with F strain parasites there was a clearcut difference between BT and TCT: while TCT developed and multiplied within macrophages this did not happen with BT. This can be visualized more clearly in Table I: after 48 hs interaction with F strain, macrophages initially infected with BT no longer harboured parasites while TCT survived and multiplied. Both BT and TCT of the Y strain multiplied within the host cells.

Interaction for 3 hs between macrophages and Y strain TCT which had been previously incubated with normal mouse serum, 7th day infection plasma or hyperimmune specific antiserum, did not present any significant differences in infections rate among these groups.

DISCUSSION

The fact that TCT infect macrophages to a much higher degree than BT may be due to some features of parasites' membrane. Thus, trypsinization of BT results in infection rate comparable to that now observed for TCT [6,14]. One of the possible differences would be the presence of host derived elements on the parasite's membrane, such as immunoglobulins that are found on circulating **T. cruzi** parasites [7,8]. Receptors for the Fc portion of IgG have been described for **T. cruzi** [13] and this may be one of the mechanisms which might inhibit phagocytosis. In our system we were unable to detect any significant influence on macrophage uptake of TCT pre-incubated with normal mouse serum, 7th day infection plasma or hyperimmune serum at sub-agglutinating concentration.

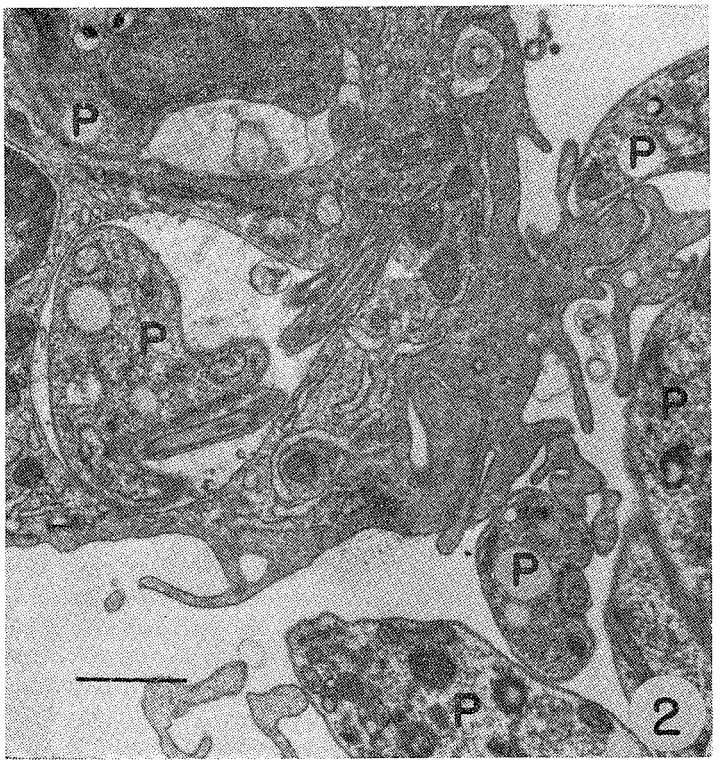

Fig. 2 — 4 — Electron micrographs of **T. cruzi** Y strain. 2. Tissue culture trypomastigotes. 30 min interaction. Parasites (P) are seen inside the macrophage and outside the cell attached to the expanding pseudopodia. X 28,500; Bar = 0,5 μ

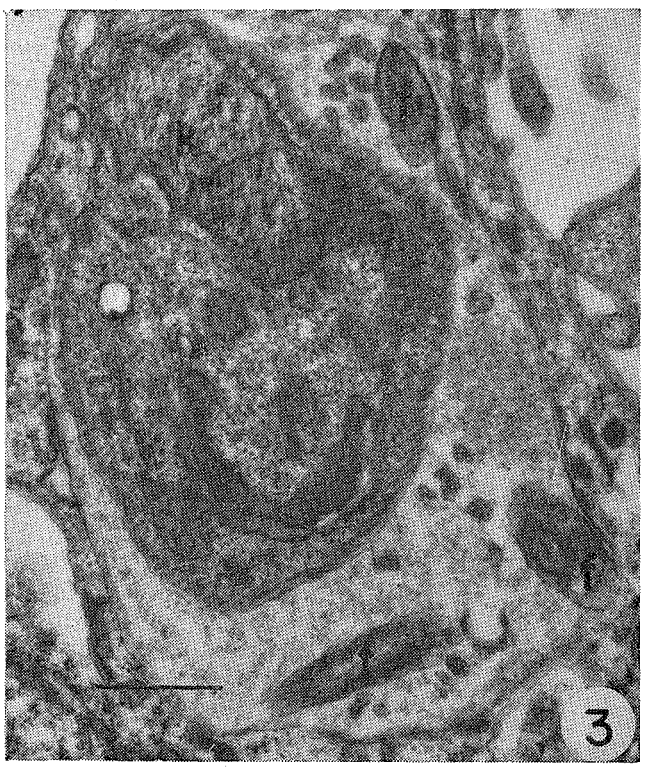

Fig. 3 — Bloodstream trypomastigotes. 3 hs interaction. Trypanosome within a "large" phagocytic vacuole which contains an amorphous material. f — flagellum; K — kinetoplast. X 32,000; Bar = 0,5 μ

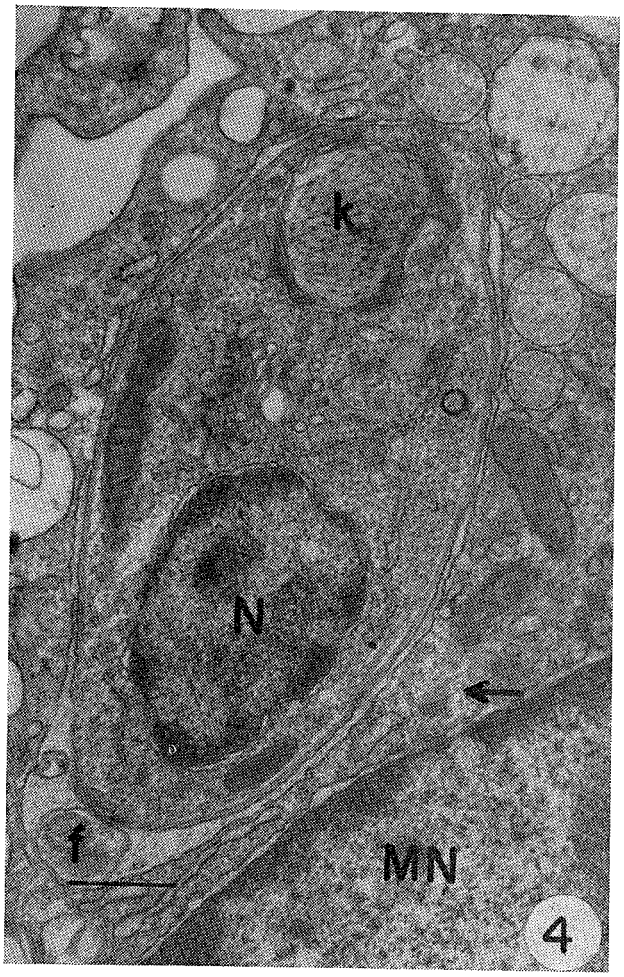

Fig. 4 — Tissue culture trypomastigote. 3 hs interaction. Cross section of the parasite within a "tight" vacuole. F — flagellum; K — kinetoplast; MN — macrophage nucleus; N — parasite nucleus. Microtubules are seen nearby (arrow). X 29,000; Bar = 0,5 μ

Our electron microscopic studies of the early post infection events indicated that TCT penetrated macrophages by phagocytosis, as is described for the interiorization of BT [9,11,12].

The presence of clusters of parasites concentrated at the macrophage surface corroborated the high phagocytic rate of TCT which was also expressed by the simultaneous presence of more than one parasite inside the host cell. This parasite concentration in certain areas of the macrophage membrane suggested the existence of a preferential surface area for the attachment of trypanosomes. Whether this represents specific interaction sites at the host cell and/or at the parasite surface remains unknown. We were not able to study this phase with BT, since their phagocytosis is much lower, thus rendering the opportunity to visualize the event more difficult. Other Authors [9] were able to see the attachment of BT, using a 10:1 parasite-cell ratio, but even at this high concentration only single parasites were attached to the cell surface. In any case, attachment process of TCT seemed to be similar to that of epimastigote culture forms [17] as well as for the culture forms of **Leishmania** [3].

Also the way the parasite is eventually lodged in the phagocytic vacuole was variable depending on the origin of trypomastigotes envolved. While at 3 hs BT were enclosed in a loose vacuole filled with dense material, the TCT was enclosed in a clear, tight vacuole. The meaning of these morphological differences is unknown.

T. cruzi strains differ in their behaviour in many aspects, and several features, such as parasitemia, mortality in mice and the infectivity for macrophages, among others, are so-

metimes ascribed to some morphological characteristics of these strains, i.e., the predominance of broad or slender population [2]. The CL strain, with predominance of broad forms, is reported to have low infectivity for macrophages, even when TCT are employed, suggesting that this may be a characteristic of the strain [10]. The F strain employed in the present paper is also a broad population strain and BT are destroyed in macrophages "in vitro" [12]. However, when comparing BT with TCT of this strain, we verified that the latter were able to multiply within macrophages, and therefore their behaviour is distinct from CL parasites. We may conclude that the capacity to multiply or not within macrophages may be inherent to the trypanosoma strain itself, but depends also on the parasite's origin; thus, while with Y strain, both BT and TCT multiply in macrophages, with F strain BT are rapidly destroyed by macrophages and only TCT are capable of multiplication within these cells.

RESUMO

Interação entre Trypanosoma cruzi **e macrófagos: diferenças entre tripomastigotas sangüícolas e de cultivo de tecidos**

Macrófagos obtidos do peritoneo de camundongos após estímulo, com peptona, foram cultivados em lamínulas, infectados com tripomastigotas das cepas F e Y de **T. cruzi**, obtidos de cultivo de tecidos ou do sangue de camundongos infectados. Os parasitas, obtidos de cultivo de tecidos, tanto da cepa Y como os da cepa F, são interiorizados por macrófagos em proporção muito mais elevada do que os sanguícolas. Parasitas de cultivo de tecidos incubados com soro de camundongos normais, ou soro hiperimune específico em diluição sub-aglutinante, comportam-se essencialmente como parasitas não opsonizados. Foram observadas diferenças a nível ultraestrutural na fase inicial de interação entre macrófagos e tripomastigotas das duas origens. Após 30 minutos, tripomastigotas de cultivo de tecidos localizam-se em agrupamentos na área de contato com os macrófagos. Enquanto os tripomastigotas sanguícolas estão na maioria das vezes no interior de vacúolos fagocíticos largos, após 3 horas de interação os tripomastigotas de cultura situam-se em um único vacúolo estreito. Tanto as formas de cultivo de tecidos quanto os tripomastigotas sanguícolas da cepa Y multiplicam-se em macrófagos; os tripomastigotas sanguícolas da cepa F são destruídos no interior da célula hospedeira, enquanto os tripomastigotas de cultivo de tecidos desta cepa são capazes de multiplicar-se.

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Recebido para publicação em 9/11/1983.