Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 38 (1981)
Heft: 4

Artikel: Phagocytosis of the three developmental forms of "Trypanosoma cruzi" : effect of specific sera

Autor: Villalta, F. / Oda, L.M. / Angluster, J.

DOI: https://doi.org/10.5169/seals-312839

Acta Tropica *38*, 375–381 (1980)

Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Cidade Universitária, Rio de Janeiro, Brasil

Phagocytosis of the three developmental forms of *Trypanosoma cruzi:* effect of specific sera

F. Villalta, L. M. Oda, J. Angluster, C. S. Alviano, W. Leon

Summary

Studies were carried out aiming at comparing the uptake of the three evolutionary stages of *Trypanosoma cruzi* by mouse peritoneal macrophages, influenced by specific immunosera. Incorporation of *T. cruzi* by macrophages was time dependent. In absence of antibody, trypomastigotes are forms more effectively incorporated by macrophages. Pre-incubation of macrophages with specific sera against each of the *T. cruzi* morphological stages was followed by an increase in the uptake of amastigotes and trypomastigotes but not of epimastigotes. Our results show that amastigotes, in comparison with the other *T. cruzi* forms, are more actively phagocytized in presence of specific serum.

Key words: macrophages; *Trypanosoma cruzi;* phagocytosis; specific sera; differences in the evolutionary stages of parasite.

Introduction

Cell-mediated immunity plays an important role in resistance to infection by *Trypanosoma cruzi*, the causative organism of Chagas' disease or American trypanosomiasis (Goble, 1970). The participation of macrophages is not yet fully understood. In vitro studies on the interaction of macrophages and *T. cruzi* showed that slender forms of the Y strain were 20 to 30 fold more infective than the stout forms of CL strain (Alcantara and Brener, 1978).

The ability of *T. cruzi* to enter, survive and multiply inside macrophages is still unclear. Experiments in this area made use of epimastigote and trypomastigote developmental forms (Dvorak and Schmuñis, 1972; Kress et al., 1975; Nogueira and Cohn, 1976, 1977; Williams et al., 1976; Williams and Reming-

Correspondence: Dr. Wilson Leon, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Cidade Universitária, C.P. 68.040, 21.941 – Rio de Janeiro, RJ–Brasil

ton, 1977). To date, no studies have been reported describing the interaction c macrophages with amastigotes, the intracellular stage of *T. cruzi*. This is proba bly due to the difficulty in obtaining a sufficient number of tissue-free amasti gotes (Leon et al., 1979).

The present work aims at the comparative study of the uptake of thre evolutionary stages of *T. cruzi* by mouse peritoneal macrophages as influence by specific antisera.

Materials and methods

Organism. Trypanosoma cruzi (Y strain) cells were obtained as follows: epimastigotes were grown in Roux bottles containing 100 ml of Warren liquid medium (Warren, 1960); amastigotes were isolated on day 7 from liver and spleen of heavily infected mice (Leon et al., 1979); trypomastigotes were isolated from mice bled on the 7th day of infection (Villalta and Leon, 1979). Cells were washed three times in Hanks solution and resuspended in the same solution to a concentration of 1×10^6 cells/ml.

Specific serum. Nine rabbits were inoculated (i.v.) with 1×10^8 cells of each *T. cruzi* developmental stage suspended in phosphate saline buffer (pH 7.2). After 30 days the group inoculated with epimastigotes, was reinoculated with the same suspension by the same route. Antibody titers were evaluated as described previously (Schmuñis et al., 1972) using the Direct Agglutination test (DA), with or without previous treatment with 2-mercaptoethanol (2-ME) (DA and 2-MEDA, respectively), and by the Indirect Immunofluorescence (IIF) (Alvarez et al., 1968) using fluorescent rabbit anti-immunoglobulin (Cappel Laboratories). For serum titrations, the corresponding *T. cruzi* developmental stage was used as antigen fixed with 1.0% formaldehyde. Rabbits inoculated with trypomastigotes and amastigotes were bled by heart puncture after 30 days, those inoculated with epimastigotes were bled after 30 days from the second reinoculation.

Specific sera were prepared as follows: anti-amastigote serum (1 ml) with titers of 1:2048 (DA) and 1:512 (2-MEDA) was incubated at 37° C for 2 h and at 4° C for 12 h with 10^8 formaldehyde(10%)-fixed trypomastigotes and epimastigotes. Agglutinated parasites were removed by centrifugation (10,000 rpm/30 min). The adsorptions of the anti-amastigote serum were repeated until the serum presented no reaction by indirect immunofluorescence using as antigen the trypo- and epimastigote forms of the parasite. Anti-trypomastigote serum with titers of 1:4096 (DA) and 1:2098 (2-MEDA) was absorbed with amastigotes and epimastigotes, and anti-epimastigote serum 1:2098 (DA) and 1:256 (2-MEDA) was absorbed with amastigotes and trypomastigotes, at the same proportion as described above. The evaluation of the specificity of these sera was similarly done by indirect immunofluorescence using as antigens the two other developmental forms of the parasite. Normal sera was adsorbed with the three developmental forms of the parasite in the same way as above described.

Final specific sera had the following titers as determined by immunofluorescence: 1:512, 1:32, and 1:32 for the antisera against trypomastigote, amastigote and epimastigote, respectively.

Macrophages. Peritoneal macrophages were collected from inbred C3H mice weighing 20–25 g, which had been injected with 1 ml of 4.05% thioglycollate medium without carbonate (Difco), 4 days before the experiment.

Immediately after sacrificing the animals with ether, their peritoneal cavities were washed with 5 ml of Hanks solution containing glucose (1 g/l) and heparin (10 units/ml). Approximately 0.3 ml of this exudate containing 5×10^4 macrophages/ml was dispensed on microscope slides (Lay and Nussenzweig, 1968). Slides were incubated for 45 min at 37° C to allow adherence of cells onto the glass surface. After pouring off the supernatant, slides were washed with 0.01 M phosphate buffer +0.15 M sodium chloride (PBS), pH 7.2.

Phagocytosis. Macrophages were incubated for 20 min at 37° C with 0.1 ml of specific serum

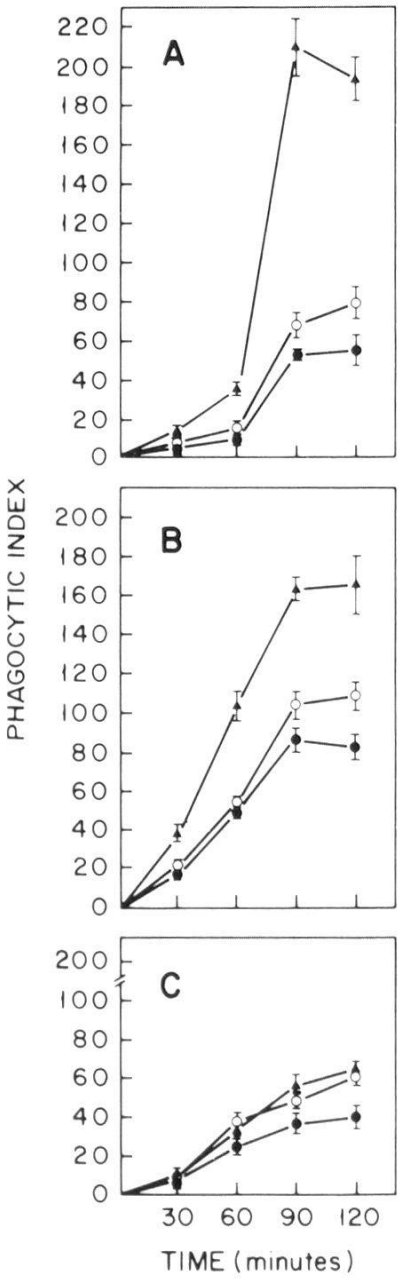

Fig. 1. Kinetics of phagocytosis of the three evolutive stages of *Trypanosoma cruzi* by mouse peritoneal macrophages. Phagocytosis of amastigotes (A), trypomastigotes (B) and epimastigotes (C). ●—● = macrophages + *T. cruzi;* ○—○ = macrophages pre-incubated with normal serum + *T. cruzi;* ▲—▲ = macrophages pre-incubated with specific serum + *T. cruzi.* Specific sera were diluted in Hanks solution to 1:32 (A), 1:512 (B); and 1:32 (C). Normal serum was not diluted.

or normal serum diluted with Hanks solution to 1:512 (α-Tryp), 1:32 (α-Am), 1:32 (α-Epi). After incubation, macrophages were rinsed with PBS and incubated with 0.1 ml of *T. cruzi* suspension at a ratio of about 5 cells per macrophage. Parasites were kept in contact with macrophages at 37° C for 30, 60, 90 and 120 min. Slides were then rinsed with PBS, fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 for 30 min, and Giemsa-stained.

Evaluation of results. The percentage of macrophages containing *T. cruzi* was determined by random microscopical examination of at least 200 cells. The average number of intracellular parasites per infected macrophage was also determined.

Table 1. Kinetics of phagocytosis of *Trypanosoma cruzi* by macrophages in presence of specific antibodies

Serum	Macrophages with amastigotes (%)			
	30 min	60 min	90 min	120 min
NA[a]	5.3 ± 0.8	7.3 ± 0.8	29.2 ± 1.3	30.8 ± 2.2
NRS[b]	7.0 ± 0.5	10.7 ± 1.2	32.2 ± 1.7	35.8 ± 2.5
α-AM[c]	10.3 ± 1.0	15.2 ± 0.8	61.5 ± 2.3	61.0 ± 1.5
Serum	Macrophages with trypomastigotes (%)			
	30 min	60 min	90 min	120 min
NA	9.5 ± 1.0	25.6 ± 1.3	42.2 ± 1.3	42.5 ± 2.0
NRS	12.2 ± 1.5	29.6 ± 1.3	46.6 ± 0.8	47.6 ± 1.9
α-TR[d]	16.3 ± 1.0	46.5 ± 1.0	55.5 ± 1.0	55.0 ± 1.5
Serum	Macrophages with epimastigotes (%)			
	30 min	60 min	90 min	120 min
NA	7.3 ± 0.8	24.0 ± 1.3	28.5 ± 2.0	29.6 ± 2.5
NRS	8.6 ± 1.0	26.1 ± 1.5	35.0 ± 1.5	36.2 ± 2.5
α-EP[e]	10.5 ± 1.0	27.0 ± 1.3	35.1 ± 1.5	39.3 ± 1.0

[a] No addition
[b] Normal rabbit serum not diluted
[c] Specific anti-amastigote serum diluted to 1:32
[d] Specific anti-trypomastigote serum diluted to 1:512
[e] Specific anti-epimastigote serum diluted to 1:32

Amastigotes: The effect of NRS[b] compared with NA[a] at 30′, 60′ and 120′, $p < 0.001$; at 90′, $p < 0.005$. The effect of α-AM[c] compared with NRS[b] at 30′, 60′, 90′ and 120′; $p < 0.001$.

Trypomastigotes: The effect of NRS compared with NA at 30′, 60′, 90′ and 120′; $p < 0.001$. The effect of α-TR[d] compared with NRS at 30′, 60′, 90′ and 120′; $p < 0.001$.

Epimastigotes: The effect of NRS compared with NA at 30′, $p < 0.01$; at 60′, 90′ and 120′, $p < 0.001$. No significant difference between α-EP[e] and NRS at 60′, 90′, and 120′.

All analysis by Student's t-test.

The phagocytic index was calculated according to Bianco et al. (1975) by multiplying the percentage of infected macrophages by the mean number of parasites per infected macrophage.

Results were evaluated according to the Student's t-test (Snedecor and Cochran, 1967) and chi-square analysis (Dunn, 1964).

Results

Incorporation of three developmental stages of *T. cruzi* by macrophages was time dependent as shown in Fig. 1 and Table 1. When macrophages were not pre-incubated with specific antibodies the maximum uptake of the *T. cruzi*

Table 2. Effect of serum concentration on the phagocytosis* of *Trypanosoma cruzi*

Forms of *T. cruzi*	Serum dilutions	Parasites per macrophage	Percentage of infected macrophages	Phagocytic index
Amastigote	Anti-serum not diluted	3.2 ± 0.2**	62.7 ± 2.4	200.2 ± 5.8[a]
	1:100	3.3 ± 0.2	59.7 ± 0.8	196.8 ± 9.5[a]
	1:320	2.8 ± 0.2	46.2 ± 2.0	127.7 ± 8.8[a]
	NRS	2.2 ± 0.1	35.8 ± 2.5	78.9 ± 8.4
Trypomastigote	Anti-serum not diluted	3.1 ± 0.3	56.0 ± 1.5	173.8 ± 18.4[b]
	1:5200	2.9 ± 0.2	46.6 ± 1.9	136.7 ± 9.9[b]
	NRS	2.2 ± 0.1	47.6 ± 1.7	107.9 ± 7.3
Epimastigote	Anti-serum not diluted	1.6 ± 0.1	39.5 ± 1.7	65.6 ± 3.0[c]
	1:320	1.6 ± 0.2	37.3 ± 0.8	60.9 ± 5.7[c]
	1:3200	1.7 ± 0.1	36.3 ± 0.8	61.8 ± 4.9[c]
	NRS	1.6 ± 0.1	38.5 ± 1.6	61.6 ± 2.5

* Data obtained after 120 min incubation

** Mean ± SD (three independent determinations)

NRS = Normal rabbit serum (not diluted)

[a] $p < 0.005$ compared with their respective control (NRS) by chi-square analysis

[b] $p < 0.005$ compared with their respective control (NRS) by chi-square analysis

There are no significant differences between *c* and their respective control (NRS) by chi-square analysis.

There are no significant differences in the percentage of infected macrophages with their respective control (NRS) in the epimastigote form, as determined by chi-square analysis.

forms occurred after 90 min (Fig. 1). After 30 min of interaction, few macrophages contained parasites. After 90 min, 30% of macrophages contained amastigotes and epimastigotes and 42% contained trypomastigotes (Table 1). Macrophages pre-incubated with normal rabbit serum were equally ineffective.

Pre-incubation of macrophages with specific sera was followed by an increase in the uptake of amastigotes and trypomastigotes. At high antibody concentration the percentage of infected macrophages increased although the mean number of amastigotes and trypomastigotes per macrophage did not change (3 per cell): epimastigotes were phagocytized at a rate of 2 cells per macrophage (Table 2). Amastigotes, in comparison with other *T. cruzi* forms, are more effectively taken by macrophages in presence of the corresponding specific serum.

The effect of specific anti-epimastigote and anti-trypomastigote sera on the phagocytosis of the amastigote form was the same as that observed with normal serum. Similarly phagocytosis of both trypomastigotes and epimastigotes, was not altered in presence of the specific sera against each one of the other forms of the parasite.

Discussion

Previous studies (Alcantara and Brener, 1978; Milder et al., 1973; Nogueira and Cohn, 1977; Sanderson and De Souza, 1979) and the present data have demonstrated that epimastigotes and trypomastigotes are phagocytized by macrophages. Additionally, our results show for the first time that amastigotes, the intracellular stage of *T. cruzi* are also ingested by macrophages.

The kinetics of phagocytosis varied according to the morphological stage of *T. cruzi*. A somewhat linear uptake of trypomastigotes by macrophages was observed. Amastigotes and epimastigotes were not so promptly interiorized. These data suggest possible differences in the *T. cruzi* surface constituents which are important in phagocytosis (Rabinovitch, 1970). Previous studies concerning binding of cationized ferritin and measurement of cellular electrophoretic mobility (De Souza et al., 1977) as well as involving cytochemistry and freeze-fracture (De Souza et al., 1978) have shown differences in the cell surface of epimastigotes and trypomastigotes.

As expected, antibodies, which bind to macrophage receptors (Griffin et al., 1976), enhance the uptake of the parasites in general. We observed, however, that the phagocytosis of epimastigotes was not stimulated by the specific antiserum, as compared to that of trypomastigotes and amastigotes. This could be related to the lateral mobility of membrane components which is well known as an important factor for phagocytosis (Bar-Shavit and Goldman, 1976). In fact, recent work showed that amastigotes and trypomastigotes but not epimastigotes, capped their surface antigens in the presence of antibodies (Kloetzel and Deane, 1977; Leon et al., 1979; Schmuñis et al., 1978) or lectins (Szarfman et al., 1980; Villalta et al., 1980).

Acknowledgments. Funds from the Financiadora de Estudos e Projetos (FINEP), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) helped to support this work. We are indebted to Prof. L. R. Travassos for his editorial assistance. We are grateful to M. A. Loures for technical assistance. Dr. F. Villalta is a Visiting Professor from the Peruvian University Cayetano Heredia, Lima, Perú, A.P. 5045.

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

Alvarez M., Cerisola J. A., Rohweder R. W.: Test de inmunofluorescencia para el diagnóstico de la enfermedad de Chagas. Bol. chil. Parasit. *23*, 4–9 (1968).

Bar-Shavit Z., Goldman R.: Concanavalin A-media ted attachment and ingestion of yeast cells by macrophages. Exp. Cell Res. *99*, 221–236 (1976).

Bianco C., Griffin F. M., Silverstein S. C.: Studies of macrophage complement receptor. J. exp. Med. *141*, 1278–1290 (1975).

De Souza W., Arguello C., Martinez-Palomo A., Trissl D., Gonzáles-Robles A., Chiari E.: Surface charge of *Trypanosoma cruzi*. Binding of cationized ferritin and measurement of cellular electrophoretic mobility. J. Protozool. *24*, 411–415 (1977).

De Souza W., Martinez-Palomo A., Gonzáles-Robles A.: The cell surface of *Trypanosoma cruzi*: cytochemistry and freeze-fracture. J. Cell Sci. *33*, 285–299 (1978).

Dunn O. J.: Basic statistics: a primer for the biomedical sciences, ed. by W. J. Dixon, p. 112. John Wiley & Sons, New York 1964.
Dvorak J. A., Schmuñis G. A.: *Trypanosoma cruzi:* interaction with mouse peritoneal macrophages. Exp. Parasit. *32*, 289–300 (1972).
Goble F. C.: South American trypanosomes. In: Immunity to parasitic animals, ed. by G. J. Jackson, R. Herman and I. Singer, vol. 2, p. 597. Appleton-Century Crofts, New York 1970.
Griffin F. M. jr., Griffin J. A., Silverstein S. C.: Studies on the mechanism of phagocytosis. 2. The interaction of macrophages with anti-immunoglobulin IgG-coated bone marrow-derived lymphocytes. J. exp. Med. *144*, 788–807 (1976).
Kloetzel J., Deane M. P.: Presence of immunoglobulins on the surface of bloodstream *Trypanosoma cruzi.* Capping during differentiation in culture. Rev. Inst. Med. trop. S. Paulo *19*, 397–402 (1977).
Kress Y., Bloom B. R., Wittner M., Rowen A., Tanowitz H.: Resistance of *Trypanosoma cruzi* to killing by macrophages. Nature (Lond.) *257*, 394–395 (1975).
Lay W. H., Nussenzweig V.: Receptors for complement on leukocytes. J. exp. Med. *128*, 991–1007 (1968).
Leon W., Villalta F., Queiroz T., Szarfman A.: Antibody-induced capping of the intracellular stage of *Trypanosoma cruzi.* Infect. Immun. *26*, 1218–1220 (1979).
Milder R., Kloetzel J., Deane M. P.: Observation on the interaction of peritoneal macrophages with *Trypanosoma cruzi.* I. Initial phase of the relationship with bloodstream and culture forms in vitro. Rev. Inst. Med. trop. S. Paulo *15*, 386–392 (1973).
Nogueira N., Cohn Z.: *Trypanosoma cruzi:* mechanism of entry and intracellular fate in mammalian cell. J. exp. Med. *143*, 1402–1420 (1976).
Nogueira N., Cohn Z.: *Trypanosoma cruzi:* uptake and intracellular fate in normal and activated cell. Amer. J. trop. Med. Hyg. *26*, 194–202 (1977).
Rabinovitch N.: Phagocytic recognition. In: Mononuclear phagocytes, ed. by R. van Further, p. 299–313. Blackwell Scientific Publication, Oxford 1970.
Sanderson C. J., De Souza W.: Morphological study of the interaction between *Trypanosoma cruzi* and rat eosinophils, neutrophils and macrophages in vitro. J. Cell Sci. *37*, 275–286 (1979).
Schmuñis G. A., Szarfman A., Vattuone N.: Direct agglutination test in the detection of anti-*Trypanosoma cruzi* antibodies in mice. J. Parasit. *58*, 1006–1007 (1972).
Schmuñis G. A., Szarfman A., Langenbach T., De Souza W.: Induction of capping in blood-stage trypomastigotes of *Trypanosoma cruzi* by human anti-*Trypanosoma cruzi* antibodies. Infect. Immun. *20*, 567–569 (1978).
Snedecor G. W., Cochran W. G.: Statistical methods, 6th ed. Iowa State University Press, Ames 1967.
Szarfman A., Queiroz T., De Souza W.: Mobility of Concanavalin A receptor in *Trypanosoma cruzi.* J. Parasit. *66*, 1055–1057 (1980).
Villalta F., Leon W.: Effect of purification by DEAE-cellulose column on infectivity of *Trypanosoma cruzi* blood forms. J. Parasit. *65*, 188–189 (1979).
Villalta F., Katzin A. M., Leon W., Gonzales-Cappa S. M.: Concanavalin A binding receptors on *Trypanosoma cruzi* amastigotes. J. Parasit. *66*, 1053–1055 (1980).
Warren L.: Metabolism of *Schyzotrypanum cruzi* Chagas. I. Effect of culture age and substract concentration on respiratory rate. J. Parasit. *46*, 529–539 (1960).
Williams D. M., Sawyer S., Remington J. S.: Role of activated macrophages in resistance of mice to infection with *Trypanosoma cruzi.* J. infect. Dis. *134*, 610–614 (1976).
Williams D. M., Remington J. S.: Effect of human monocytes and macrophages on *Trypanosoma cruzi.* Immunology *32*, 19–23 (1977).