Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 76(2) :213-225, abr./jun. 1981

TRYPANOSOMA CRUZI: ANTIGEN-RECEPTOR MEDIATED ENDOCYTOSIS OF ANTIBODY

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Trypomastigote forms of Trypanosoma cruzi *were derived from tissue culture and incubated with immune and non-immune human sera. All immune sera showed high titers of specific humoral antibodies of the IgM or the IgG type. Agglutination and swelling of parasites were observed after incubation at 37°C, but many trypomastigotes remained free-swimming in the sera for two to three days. The quantity of immune serum capable of lysing a maximum of $10x10^6$ sensitized red cells was not capable of lysing $4 x 10^3$ trypomastigotes. Typically, the parasites underwent cyclical changes with the formation of clumps of amastigotes and the appearance of epimastigote forms. Multiplication of the parasites was observed in immune sera. Further, the infectivity of the parasites to susceptible mice was not lost. All sera used produced similiar general effects on the growth of the parasite. The antibody bound to* T. cruzi *appeared to enter cells by antigen-receptor mediated endocytosis. The ferritin-conjugated antibody was internalized and delivered to phagolysosomes where they might be completely degraded to amino-acids. This seemed to be a coupled process by which the immunoglobulin is first bound to specific parasite surface receptor and then rapidly endocytosed by the cell.*

In the course of isolating trypomastigote forms of *Trypanosoma cruzi* from a man with acute Chagas' disease we observed that the parasite remained free-swimming in the blood after being maintained for 48 hours at room temperature. This isolate is herein named Albuquerque strain of *T. cruzi*. In view of the demonstration that the fresh serum had complement factors and a high titer of specific humoral antibody, this observation suggested that the blood trypomastigotes possess an efficient mechanism to elude the host's humoral immune response.

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For reprints – A.R.L. Teixeira.

This work was supported by Grant PDE-08-1-03 from the Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brasil.

Received for publication on February 17th and accepted on March 30th, 1981.

This paper reports on the results of experiments which showed that *T. cruzi* grows in fresh immune sera, where it undergoes cyclical changes but does lose its infectivity to susceptible mice, after 18 hours of incubation at 37°C. Further, electron microscope studies showed that the ferritin-tagged antibody can be internalized by the parasite and degraded in phagolysosomes.

MATERIAL AND METHODS

Parasite Strains

Two strains of *Trypanosoma cruzi* were used throughout these experiments. The Albuquerque strain of *T. cruzi* was isolated from an adult with the acute infection (Teixeira et al, 1980). The level of trypomastigote forms of *T. cruzi* in the patient's peripheral blood reached 4×10^4 parasites/ml. These parasite forms remained alive and free-swimming in the blood after 48h at room temperature. The Ernestina strain was isolated from a child with acute Chagas' disease in 1964 (Neva & Gam 1977). Both strains of *T. cruzi* have been kept in the laboratory through serial passages in liver infusion tryptose (LIT) medium with 10% bovine serum at 27°C.

Maintenance of parasite in tissue culture and colletion of trypomastigote forms

These strains of *T. cruzi* were maintained at 37°C in Vero cell cultures (green monkey kidney cells) supplied with 15 ml of Eagle's minimum essential medium (MEM) containing 10% fetal calf serum, 100 IU/ml of penicillin and 100 ug/ml of streptomycin. The culture medium was removed twice weekly after which freshly prepared medium was added. The monolayer of growing cells was inoculated with 1 to 2×10^5 trypomastigotes of *T. cruzi*. The parasites multiplying in the cells emerged into the culture medium 7 to 10 days after inoculation. Thus parasite forms were repeatedly harvested from the supernatant nutrient medium removed, and fresh medium was added to cultures. The quantity of $1.3 \pm 0.6 \times 10^6$ parasites/ml were obtained from the tissue cultures. After centrifugation of sediment host cell-debris at 200xg for 10 min the free-swimming trypomastigotes in the supernatant fluids were pelleted at 1,000xg for 20 min, at 4°C (RC5-B refrigerated centrifuge, Ivan Sorvall Inc. Norwalk, Connecticut). The parasites were used after two washings in phosphate buffered saline (PBS), pH 7.2.

Collection of sera

Human sera were obtained from eight patients with acute Chagas' disease and who presented parasitemia in wet preparations, from nine patients with chronic Chagas' infection, and from five normal non-chagasic subjects. Blood samples were allowed to clot for 2h at 4°C. The sera collected were either immediately used or stored at −20°C and used within two weeks.

Detection of specific antibodies in the sera

Antibodies to *T. cruzi* were detected by the direct agglutination of trypsin-treated and formalin-killed epimastigote forms of *T. cruzi* (Vattuone & Yanovsky 1971). A quantitative micromethod (Mendivil et al 1979) was performed with and without previous treatment of sera with a 1 : 100 (v/v) solution of 2-mercaptoethanol. Antibodies to *T. cruzi* were also detected in the sera by the indirect hemagglutination of red cells sensitized to a subcellular *T. cruzi* antigen (Cerisola et al 1969).

Determination of serum complement lytic activity

Human complement lytic activity was assayed by standard methods (Nowotny, 1969). Anti-sheep hemolysin and sheep red blood cells (SRBC) were obtained from the

Instituto Adolfo Lutz, São Paulo, Brazil. A suspension of 5×10^8 sensitized SRBC/ml was used to determine the CH_{50}, that is, the dilution of serum necessary for 50% red cell lysis. Standardization of the procedure was obtained with fresh guinea pig serum, which showed 1250 CH_{50}/ml.

Effect of sera on T. cruzi

The assays of cytotoxicity of sera on trypomastigote forms of the strains of *T. cruzi* were carried out in the wells of Terasaki plates (Falcon plastics, Oxnard, California). Each plate was filled with 10ml of mineral oil and 10 μl of serum was injected with a micro syringe (Unimetrics Corporation, Anaheim, California) through the oil to the bottom of the well. 1 μl of a parasite suspension in PBS, containing 4×10^6 trypomastigotes/ml was then injected into the serum in the wells and mixed thoroughly. Care was taken to perform the procedure under sterile conditions. Each test was run in quintuplicate and the plate was kept at 37°C for five days. The effect of serum factors on the parasite forms was monitored by periodic observation with the aid of an inverted microscope with a 10 x eye-piece and an objective of 2.5 x. The percentage of agglutination of parasites was determined by the differences between the number of free parasites counted at the bottom of the wells, in the presence of each test serum and in equal volume of saline solution. The observers were able to register distinct changes which were recorded every hour during the first 24h and every six hours till the end of the experiment in five days. At the end of experiment the cells at the bottom of the wells were aspirated, smeared on glass slides and stained by Giemsa's method for photomicrograph illustration.

Infectivity of T. cruzi *forms after incubation with antisera*

In these experiments 1×10^6 trypomastigotes were incubated in 10ml conical, stoppered glass tubes with 1 ml of immune or non-immune sera. After 18h of incubation at 37°C the cells at the bottom of the tubes were resuspended, and 0.2ml containing approximately 2×10^5 parasites were injected intraperitoneally in groups of five young swiss mice. Wet preparations of tail blood from the infected mice were examined microscopically two weeks after infection for the presence of trypomastigote forms of *T. cruzi.*

Preparation of pure IgG from a Chagas' patient

The purification of IgG from the serum of a patient with chronic Chagas' disease was obtained by passage in a QAE-Sephadex A-50 column (Sigma Chemical Company, St. Louis, Missouri) according to a standard procedure (Fahey & Terry, 1973). The eluate in ethylene diamine acetate buffer was dialized against PBS and IgG was concentrated to 1 mg/ml by a vacuum operated immersible molecular separator (Millipore Corporation, Bedford, Massachusetts).

Electron microscopy

A suspension of 1×10^6 trypomastigotes of the Albuquerque strain of *T. cruzi* in PBS was used in these experiments. Purified IgG was added to the parasite suspension to a final concentration of 1 mg/ml and incubated at 37°C for 10 min. The parasites were washed twice in PBS and then incubated with 100 μg/ml of ferritin-conjugated F $(ab')_2$ fragment goat anti-human IgG (Cappel Laboratories Inc., Cockranville, Pennsylvania) for 15, 30, 45 and 60 min. This experiment was carried out in Sorvall conical pyrex tubes. Control tube consisted of the parasite suspension mixed with 100 μg/ml of ferritin-conjugated goat anti-human IgG. After centrifugation at 1,000 x g x 20 min the pellets were thoroughly washed and processed for electron microscopy. Fixation was obtained with 2.5% (v/v) glutaraldehyde in PBS pH 7.2. After post-fixation in 1% OsO_4 (w/v) in PBS at 4°C, the pellets were dehydrated in acetone and embedded in araldite. The sections collected on copper grids and stained with uranyl acetate and lead citrate were examined with the aid of a Jeol JEM-100C electron microscope operating at 80 Kv.

RESULTS

Immune serum's agglutination activity

Table I shows the agglutination of formalin-killed epimastigotes of *T. cruzi* grown in LIT medium by host sera. In the group of patients with acute Chagas' disease the agglutinating humoral antibody titers ranged from 1:256 to 1:4096 but these fell to 1:128 to 1:1024 in the group of patients with the chronic infection. Normal control sera agglutinated killed parasites, albeit at much lower dilutions. Incubation of test sera with a 1% solution of 2-mercaptoethanol (1:1 v/v) resulted in changes in antibody titers. In sera collected from patients with acute Chagas' disease the antibody titers decreased considerably. However, sera collected from patients in the chronic phase of infection did not show significant decreases in antibody titers after treatment with 2-mercaptoethanol. Early acute cases of Chagas' disease did not show hemagglutinating antibodies. However, other late cases of acute Chagas' disease showed antibody titers as high as 1:128. In the chronic phase of infection the antibody titers ranged from 1:32 to 1:512. In contrast, normal control sera gave negative results.

Effect of fresh sera on trypomastigote forms of T. cruzi

In these experiments the effect of antibody and other serum factors on trypomastigotes of the Albuquerque and of the Ernestina strain of *T. cruzi* was assayed. The parasites were harvested from infected Vero cell cultures which yielded 90±5 per cent trypomastigotes and 10±5 per cent amastigote forms. Immune and non-immune human sera were used. The antibody titers in these sera are given in Table I. The complement hemolytic activity of human sera used ranged between 200-400 CH_{50}/ml.

TABLE I

Trypanosoma cruzi: agglutination activity of immune host serum

Human serum	*Nº*	*Direct agglutination**		*Hemagglutination***	*CH_{50} units/ml*
		Untreated serum	*2-ME treated serum*		
Acute Chagas' disease	8	1:256 – 1:4096	1:64 – 1:128	Neg – 1:128	200 – 400
Chronic Chagas' disease	9	1:128 – 1:1024	1:64 – 1:1024	1:64 – 1:512	" "
Normal control	5	1:2 – 1:64	Neg – 1:4	Neg	" "

* Dilution of sera vs suspension of formalin-killed epimastigotes of T. cruzi.
** Dilution of sera vs red cells sensitized with *T. cruzi* lysate.

The incubation of 1 μl of a saline suspension of 4 x 10^6 living trypomastigotes with 10 μl of human sera in the wells of Terasaki plates at 37°C resulted in several changes summarized in Table II. Alterations of the parasite forms incubated with undiluted sera were recorded at time intervals with the aid of an inverted microscope. Free-swimming trypomastigotes remained in the sera for periods of two to three days, albeit to much fewer number than those seen at the first half hour of incubation. Usually at late periods of incubation only a few free-swimming trypomastigotes were seen, since the parasites were agglutinated and formed clumps which lay at the bottom of wells. The percentage agglutination of these parasite forms by immune and non-immune sera were recorded after 6h of incubation (Table II). In general, immune sera of chronic Chagas' patients were more capable of agglutinating the living parasite forms than those obtained in the acute phase of the disease. The percentage agglutination reached 30-70%. Further, the agglutination of trypomastigotes of *T. cruzi* was also observed in normal non-immune human sera (5-20%). Similar results were obtained with all sera decomplemented by heating at 56°C for 30 min. In fact, agglutinated and free-swimming trypomastigotes underwent continuous changes to round amastigotes which appeared as swollen, small motile granules at the bottom of the wells (Fig. 1a). In the ensuing 48 hours the amasti-

gotes formed large clumps by binary fission. Single flagellates formed long multinucleated filaments and bizarre syncytial masses. Epimastigote forms emerged from these multinucleated masses. Multiplication of the parasites in immune host sera as well as in non-immune sera was observed after 72 h of incubation at 37°C. In the ensuing hours the parasite stopped growing, probably because of the unfavorable conditions in the nutrient medium. At the end of five days of incubation the parasites were aspirated from the bottom of wells, and the morphologic features were recorded by photomicrographs of Giemsa's stained smears (Fig. 1b and c). In other experiments, 2×10^5 parasites incubated for 18h with immune and non-immune human sera were injected into susceptible mice. After two weeks of infection a search for the parasite was made in wet preparations of a drop of tail blood. In all mice injected with the parasite the search was positive (Table II).

Electron microscope studies on ferritin-labelled T. cruzi

Results from previous experiments showed that trypomastigotes of *T. cruzi* have the ability to survive and to multiply in fresh immune serum. Therefore, experiments were designed to demonstrate the fate of these antibodies after interaction with the parasite *in vitro.* These experiments consisted of incubation of 1×10^6 trypomastigotes of the Albuquerque strain of *T. cruzi* with 1 mg/ml of IgG, for 10 min at 37°C. This IgG was purified from the serum of a patient with chronic Chagas' disease who showed specific antibody titers of 1 : 128 by hemmaglutination and of 1 : 512 by direct agglutination tests. The antibody-coated parasites were throughly washed, incubated with 100 μg/ml of ferritin-conjugated F $(ab')_2$ fragment goat anti-human IgG for 15, 30, 45 and 60', and processed for electron microscopy after fixation with glutaraldehyde.

TABLE II

Effect of immune host serum on living trypomastigote forms of two strains of *Trypanosoma cruzi* grown in tissue culture*

*Human serum***	*Nº*	*% agglutination (range)*	*Cyclical changes*	*Multiplication (72 h)*	*Infectivity**** *to mice*
Acute Chagas' disease	8	20 – 50	+	+	+
Chronic Chagas' disease	9	30 – 70	+	+	+
Normal control	5	5 – 20	+	+	+

*Parasite forms of the Albuquerque and of the Ernestina strains of *T. cruzi* were used (see text). Sera were incubated at 37°C with 4×10^3 parasites in the wells of Terasaki plates and kept under periodic microscopic observation during five days.

**Decomplemented serum resulted in parasite changes similar to those observed with fresh serum.

***Swiss mice became infected with *T. cruzi* incubated with serum for 18 hours at 37°C.

Fig. 2 shows the ferritin-labelled antibody coupled to the parasite envelope and inside the flagellar pocket. Fig. 3 is a section of a parasite examined after 15 min of incubation and shows the ferritin-labelled IgG bound to the parasite outer membrane and inside endocytic vacuoles of its cytoplasm. A great quantity of electron-dense dots, corresponding to the ferritin-containing immunoglobulin, were observed in phagolysosomes of growing parasite forms after long periods of incubation. Fig. 5 shows a parasite with large phagolysosomes filled with ferritin-labeled protein complexes, at 45 min of incubation. Many of these phagolysosomes are filled with an amorphous substance, but the typical ferritin dots can still be seen at the periphery of an electron-transparent matrix and attached to the inner surface of the phagolysosome membrane.

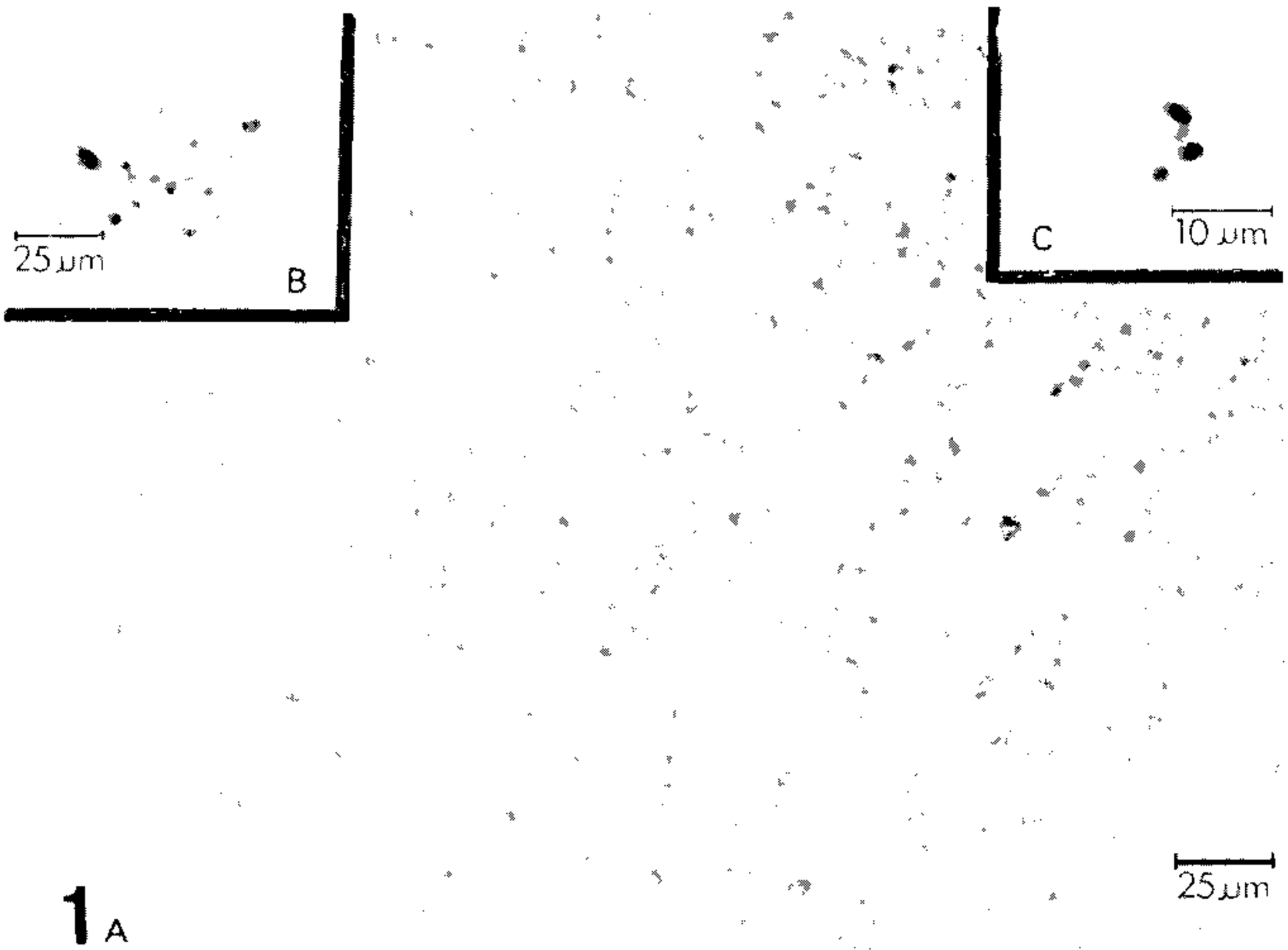


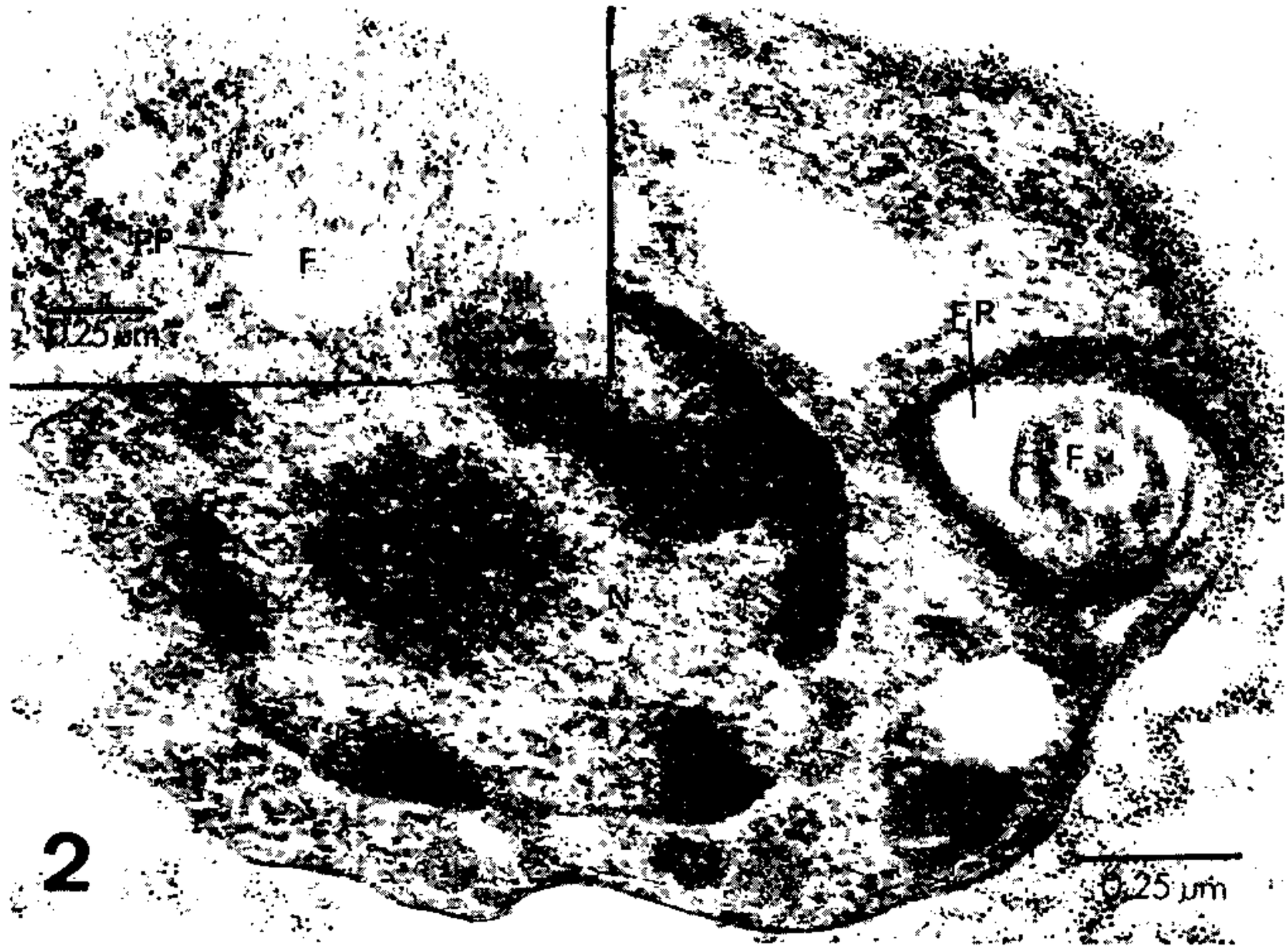
Fig. 1a – *Trypanosoma cruzi* viewed with an inverted microscope at the bottom of the well of a Terasaki plate. 4×10^3 trypomastigotes were incubated with antiserum from a patient with Chagas' disease. Notice agglutination and swelling of many parasite forms after 6 h of incubation. Insert *b* shows a clump of amastigotes after five days of incubation at 37°C, stained by the Giemsa's method. Insert *c* shows a single amastigote form dividing by binary fission.

Control studies consited of incubation of 1×10^6 parasites with 100 µg/ml of ferritin-conjugated goat anti-human IgG. In these experiments ferritin-conjugated IgG bound to the parasite membrane was also seen, albeit to a lesser degree. The ferritin dots were occasionaly found inside phagolysosomes of the parasite cytoplasm (Fig. 4). This observation is consistent with the presence of naturally occurring antibodies in normal sera, which are responsible for the agglutination of trypsin treated parasite forms (our unpublished observations).

Figs. 6 to 8 are sections of parasites treated with specific IgG and then examined during the first 15 min of incubation with ferritin-conjugated goat anti-human IgG. Fig. 6 shows the ferritin-labelled immunoglobulin bound to the parasite's membrane. An empty cytoplasmic vesicle is formed beneath the outer membrane near the nucleus of the cell, at the site of clustering of the ferritin dots. Figs. 7 and 8 show clustering of ferritin-dots bound to the posterior end of the parasite envelope and making their way into cytoplasmic vesicles through openings at the outer membrane. The movements of the vesicles with further interiorization and the appearance of ferritin in phagolysosomes do not favor the possibility that the material is being secreted by the parasite.

DISCUSSION

There is a body of evidence showing that in susceptible hosts the main mechanism of acquired resistance against *T. cruzi* is related to lymphocyte-macrophage



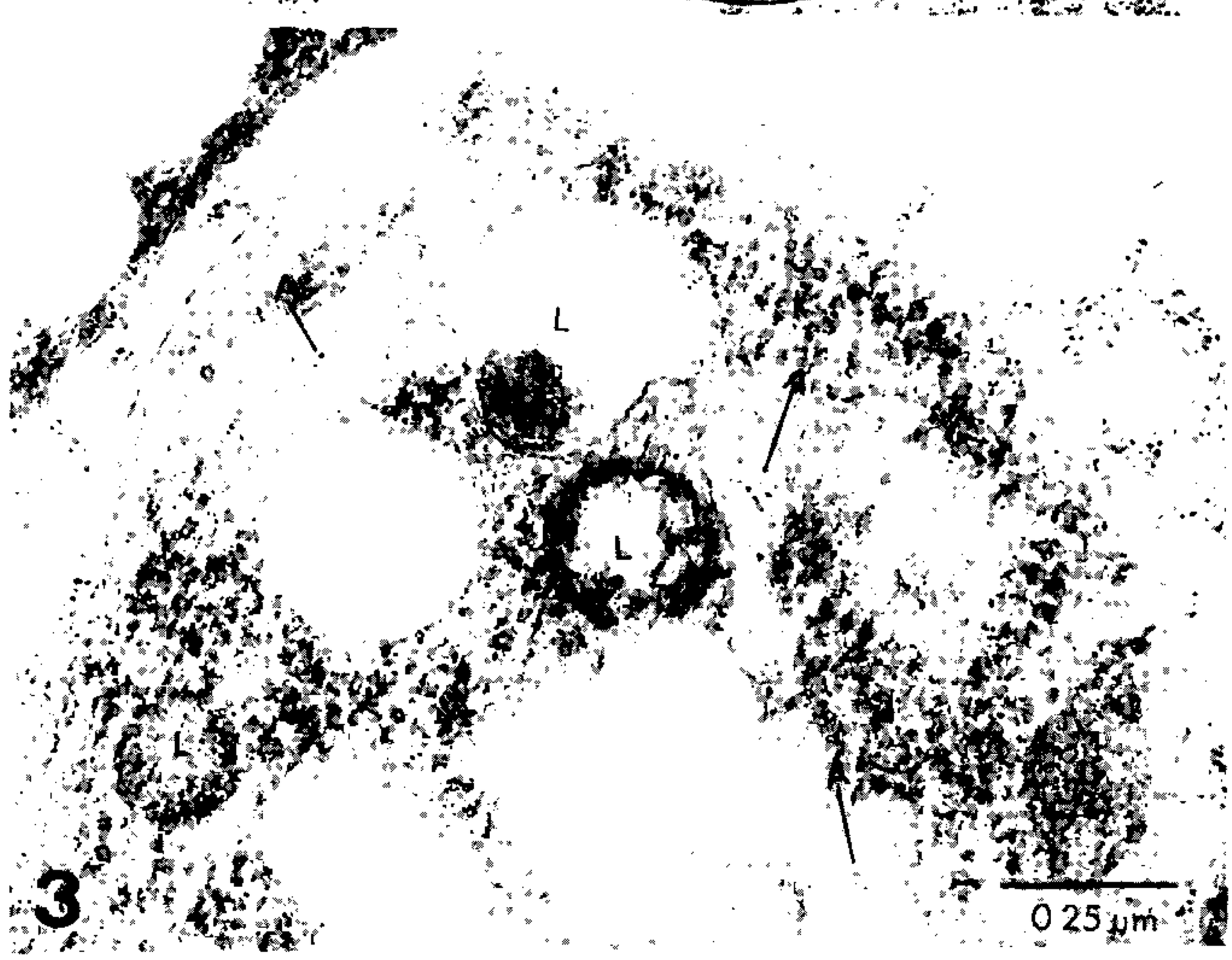


Fig. 2 – Cross-section of a *Trypanosoma cruzi* which was incubated for 10 min with IgG from a patient with Chagas' disease, followed by 15 min incubation with ferritin-labelled goat anti-human IgG. Notice the ferritin dots bound to the parasite's membrane and inside the flagellar pocket (Insert). N: nucleus; F: Flagellum; FP: Flagellar pocket.

Fig. 3 – High-power view of *Trypanosoma cruzi* showing the antibody-ferritin conjugate (A) in endocytic vacuoles inside the cytoplasm. L: Lysosomes.

Fig. 4 – Section of a *Trypanosoma cruzi* after 30 min incubation with ferritin-conjugated goat anti-human IgG. Notice ferritin dots inside many phagolysosomes. N: Nucleus; PL: Phagolysosomes; K: Kinetoplast. Insert shows a detail of ferritin dots inside a phagolysosome at higher magnification.

Fig. 5 – Cross section of a *Trypanosoma cruzi* showing large phagolysosomes filled with ferritin. Some phagolysosomes contain an amorphous matrix of intermediate density but the ferritin dots can be seen at the periphery of the matrix and lining the organelle membrane.

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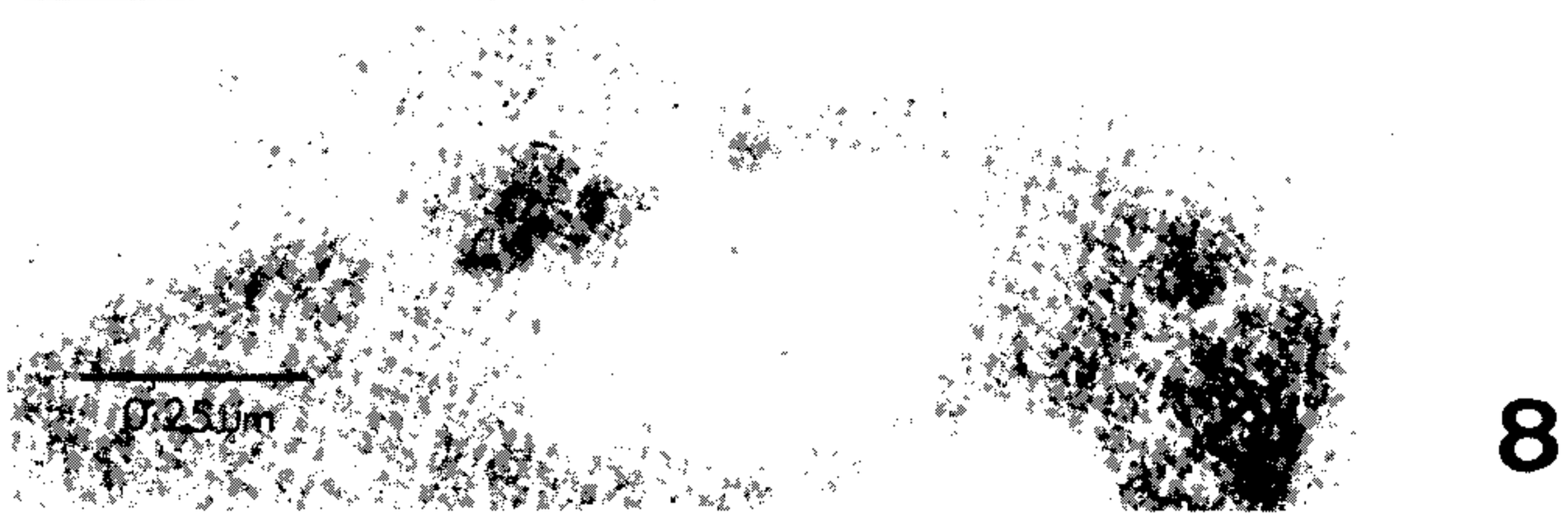

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Fig. 6 – High-power magnification showing clustering of ferritin-immunoglobulin conjugate bound to the *Trypanosoma cruzi* envelope and the presence of a cytoplasmic vesicle beneath the outer membrane.

Fig. 7 – *Trypanosoma cruzi:* the clustering of ferritin granules appears to gain entrance to the cytoplasmic vesicle.

Fig. 8 – *Trypanosoma cruzi:* the ferritin dots are seen in their way through an opening at the membrane connecting the outside with a cytoplasmic vesicle. The dots are seen inside the vesicle.

interactions (Taliaferro & Pizzi 1955, Behbehani 1971). *In vitro* studies demonstrated that macrophages of immune donors destroy phagocytized parasites at a higher ratio than do macrophages of non-immune donors (Hoff 1975, Nogueira & Cohn 1976, Kress et al, 1977). Parasite evasion of this mechanism of resistance is provided by its ability to infect non-phagocytic cells. *T. cruzi* has a marked predilection for heart and skeletal muscle cells. Yet, the lymphocyte-mediated immune lysis of parasitized host cells that has been

demonstrated by Santos-Buch & Teixeira (1974), Kuhn & Murnane (1977), and Teixeira et al (1978) might play some role as a mechanism of defense against the intracellular forms of *T. cruzi*. However, the parasites growing in these host cells burst out in tissue fluids and can circulate transitorily in the peripheral blood before penetration in other cells. Endogenous reinfections of cells by trypomastigotes that escape the host's humoral immune response can explain the persistance of the parasite in the body for life. This work was aimed at examining the role of humoral factors from susceptible immune and non-immune hosts on the infective blood trypomastigotes of *T. cruzi*.

In the experiments reported here we used fresh sera from humans with the acute or the chronic chagasic infections. These sera showed high specific antibody titers detected by agglutination tests. In the acute phase of infection the antibodies were mainly of the IgM type. Antibodies of the IgG class resistant to treatment with 2-mercaptoethanol were present at low concentration in the sera of individuals with chronic Chagas' infection. Also, naturally ocurring antibodies were present at low concentration in all normal control sera. Furthermore, human sera used in these experiments showed 200-400 CH_{50} units/ml.

The incubation of 4×10^3 trypomastigotes of the Albuquerque or the Ernestina strains of *T. cruzi* derived from tissue culture with 10 μl of each serum did not lyse the parasites. Instead, agglutination and differentiation of the parasites were observed, whereas a varying proportion of free-swimming trypomastigotes remained in the serum. Immobilization and swelling of parasite forms that ensued during the first 30 min of incubation could be mistaken for death. However, careful observation in the following hours showed discrete movements. Prolongation of the experiments for several days resulted in cyclical changes which are typical of the parasite's life in *in vitro* culture. All sera produced similar general effects on the growth of the organisms, but there were differences between individual sera with respect to various specific phenomena. Formation of clumps of amastigotes, which underwent cyclical changes with the appearance of epimastigote forms and bizarre masses of parasites, were seen in all sera studied. Further, free-swimming trypomastigotes were seen for a longer time in non-immune sera than immune sera. In general, multiplication of the parasites in immune sera occurred to the same degree observed in non-immune sera.

Multiplication of *T. cruzi* began usually after 24 hours of incubation with serum and persisted for at least 72 hours. Then the parasites stopped growing and tended to form large, non-motile clumps, probably because of unfavourable nutrient and metabolic conditions of the serum. That these parasite forms were still alive was demonstrated by morphologic studies of Giemsa's stained smears (see Figs. 1b and c). Further, the infectivity of the parasites was tested after incubation with non-immune and immune host's sera for 18 hours at 37°C. All swiss mice injected with these parasite forms showed trypomastigotes of *T. cruzi* in wet preparations of peripheral blood. Therefore, the results of these experiments are in agreement with the observation of Muniz & Borriello (1945), Adler (1958), Teixeira & Santos-Buch (1974), Krettly & Brener (1976), Gonzalez-Cappa et al (1977) and Minter-Goedbloed (1978). Adler (1958) suggested that "this relative indifference to immobilizing antibodies is a useful adaptation to life in a vertebrate host, and enables the trypanosome to maintain a vigorous activity and to penetrate cells in spite of antibodies which may be present in the serum or tissue fluids of the host".

However, other *in vitro* studies have described that all stages of *T. cruzi* are susceptible to antibody-mediated complement dependent lysis, suggesting that this immune manifestation of lytic antibodies could contribute to *in vitro* destruction of the parasite (Anziano et al 1972, Budzko et al 1975, Nogueira et al 1975, Kierszenbaum 1976). Krettly & Nussenzweig (1977) found that bloodstream forms of the Y, but not the CL strain, were susceptible to complement dependent immune lysis. In the experiments described here tissue culture derived trypomastigote forms of the Albuquerque and of Ernestina strains of *T. cruzi* survived after incubation with fresh immune sera containing high

titers of antibodies. In fact, 4×10^3 parasites did not undergo lytic changes in the presence of antibodies and 1 to 4 CH_{50} units/10 μl of serum, that is, the quantity of serum complement necessary for lysing 1 to $4 \times 2.5 \times 10^6$ sensitized red cells.

It is reasonable to suggest that the discrepancies among the results reported in the literature mentioned above can be explained by the differences in the characteristics of strains or isolates of *T. cruzi* used, and/or by the different methodologies employed. In this regard, previous observations were limited to one hour of incubation and, therefore, the possibility exists that parasite swelling and immobilization could be interpreted as indications of lysis.

In other experiments we investigated the fate of specific antibody bound to *T. cruzi*. These studies suggest that the immunoglobulin-ferritin conjugate appears to enter cells by antigen-receptor mediated endocytosis. The ferritin-conjugated goat anti-human IgG was seen bound to the parasite membrane, in the flagellar pocket and in phagocytic vacuoles. Similar observations were made by Cherian & Dusanic (1978) in *Trypanosoma lewisi*. We suggest here that the process of internalization follows clustering of selectively bound ferritin-IgG complex at a point of the membrane under which a cytoplasmic vesicle was formed. Internalization then occurred by an orifice at the point of contact of the outer membrane with the vesicle.

It has been recognized that receptor-mediated endocytosis has a fundamental role in the growth, nutrition and differentiation of animal cells (Goldstein et al 1979). The internalized macromolecules are usually delivered to lysosomes where they are completely degraded to amino-acids (Unanue et al 1973, Terris & Steiner 1975, Tolleshaug et al 1977). This appeared to be the mechanism by which *T. cruzi* takes up immunoglobulins from the extracellular fluids and degrades them in the phagolysosomes. In these experiments, many phagolysosomes were seen filled with an amorphous matrix of intermediate density, and in some of them the ferritin dots were still present in the inner surface of their membranes. This observation might be an indication that ferritin-IgG complexes can be hydrolysed by the lysosomal enzymes. The possibility that the phenomenon of interiorization of antibody was actually one of double-ligand induced antigen movement (Unanue et al. 1973) seems to be unlikely, because interiorization was also seen when the labelled, naturally ocurring goat antibody was incubated with the parasite. Therefore we suggest here that at least under these experimental conditions the antibody can ben an alternative source of energy for survival of this protozoan.

While the exact role that humoral factors play in acquired resistance against *T. cruzi* infections is still unknown, it seems clear that this protozoan possesses an efficient escape mechanism. This explains why *Trypanosoma cruzi* can survive in the humoral fluids of patients with Chagas' disease. Fortunately, this demonstration of internalization of antibody by the parasite could raise the possibility of effective chemotherapy, by coupling the IgG molecule to a trypanocidal drug.

RESUMO

Formas tripomastigotas de *Trypanosoma cruzi* derivadas de cultura de tecido foram encubadas com soros humanos imunes e não-imunes. Todos os soros humanos usados tinham títulos elevados de anticorpos das classes IgM ou IgG. Aglutinação e entumescimento dos parasitos eram observados após encubação a 37°C, mas muitos tripomastigotas permaneceram circulando livremente nos soros por dois a três dias. A quantidade de soro imune capaz de lisar um máximo de 10×10^6 hemácias sensibilizadas não foi capaz de lisar 4×10^3 tripomastigotas. Tipicamente, os parasitos apresentavam alterações cíclicas com formação de aglomerados de amastigotas e de epimastigotas. Observou-se multiplicação dos parasitos nos soros imunes, e a infectividade destes parasitos para camundongos não foi perdida. Todos os soros usados produziram efeitos similares no crescimento do pa-

rasito. O anticorpo ligado ao *T. cruzi* parece ser endocitado pela mediação de receptor antigênico. O anticorpo conjugado à ferritina era internalizado e descarregado em fagolisossomas, onde eles podem ser completamente degradados até amino-ácidos livres. Este parece ser um processo de acoplamento pelo qual a imunoglobulina é primeiramente ligada especificamente ao receptor da superfície do parasito e, então, é rapidamente endocitada.

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