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THE BIOLOGY OF *TRYPANOSOMA CRUZI*-MACROPHAGE INTERACTION

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1. INTRODUCTORY REMARKS

Trypanosoma cruzi is the aetiological agent of Chagas' disease, which causes a considerable mortality and morbidity in Latin America, without cure up to the present time. The life cycle of the protozoa takes place in vertebrate and in invertebrate hosts, with three developmental stages: amastigotes/trypomastigotes in the vertebrates and epimastigotes/trypomastigotes in the insect vectors, with the central stage trypomastigote making the infective linkage between them (reviewed by Brener, 1973; by De Souza, 1984). The reproduction of *T. cruzi* in the vertebrate host occurs mostly, if not exclusively, inside host cells, and despite the ability of the parasite to invade almost any vertebrate cells *in vitro*, it shows *in vivo* a tropism for some cells, especially for macrophages, muscle and nerve cells.

The interaction of *T. cruzi* with host cells has been studied *in vitro* since 1935 (Kofoid et al., 1935), and has received special efforts in the last 12 years, because it is the basis for the *in vivo* pathogenic and immunological responses to the infection. The more recent reviews on this matter (Nogueira, 1983; Colli, 1984; Zingales & Colli, 1985; Snary, 1985) do not deal with the bulk of results published in the last five years, especially on the interaction of *T. cruzi* with the professional phagocytic cells. We intend to present here a more consolidated and complete view only of the data existing on the interaction of this parasite with the macrophage, a cell that is probably one of the first to fight the infection (Kierszenbaum et al., 1974), regardless serving as initial host for this parasite (Deutschlander et al., 1978), a central cell for the host in architecturing its specific immune defense response, and also a cell which has as its main function the recognition and the phagocytosis of particles and microorganisms, and has to display rapid physiological abilities to destroy them (reviewed by Adams & Hamilton, 1984; Gordon, 1986; Unanue, 1984).

2. PHASES OF *TRYPANOSOMA CRUZI*-MACROPHAGE INTERACTION

The recognition and the fate of *T. cruzi* in macrophages follows three basic phases (Fig. 1), which can be studied independently: a) *Adhesion:* with the stages of attraction, contact, recognition and binding of the parasite to the macrophage surface; b) *Internalization:* with the signalling mechanisms to activate the intracellular endocytic machinery, the formation of pseudopodial processes and an endocytic vacuole; c) *Intracellular fate:* with intravacuolar digestion of the parasite following phagolysosomal fusion, or disruption of the vacuolar membrane and proliferation of the parasite free and in contact with the cytoplasm of the macrophages.

Depending on the developmental stage, on the sources or methods applied for the isolation of the parasite, on the physiological state of the

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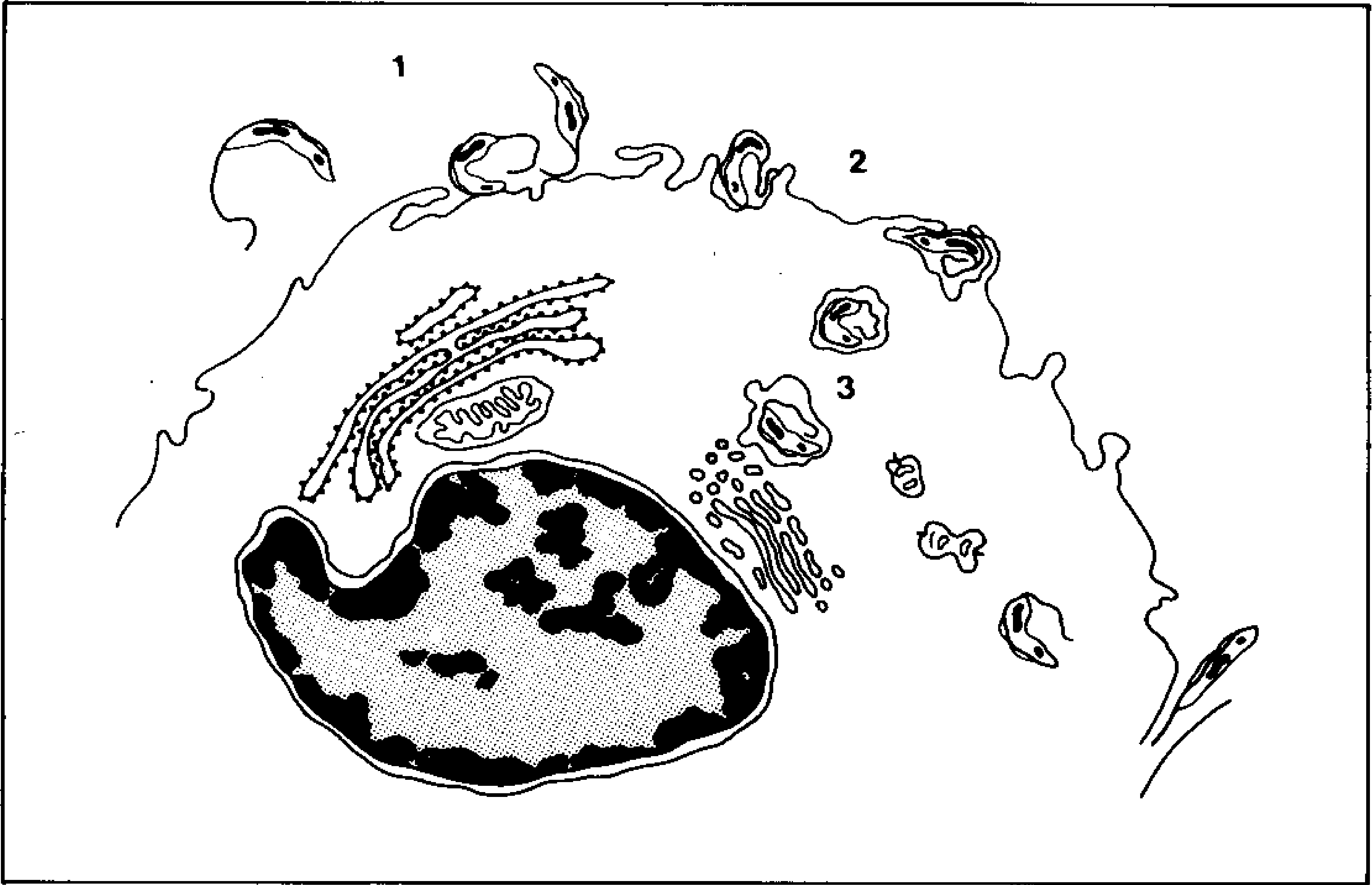

Fig. 1: schematic representation of the different phases of *T. cruzi*-macrophage interaction. (1) = ADHESION with the stages of attraction, contact, recognition and binding of the parasite to the macrophage surface; (2) = INTERNALIZATION, with activation of the intracellular endocytic machinery; and (3) = INTRACELLULAR FATE, with phagolysosomal fusion and further digestion of the parasite or disruption of the vacuolar membrane and proliferation of the parasite free in the cytoplasm.

macrophages or of macrophagic cell clones, or even on the class of vertebrate from which the macrophages were obtained, different aspects emerged in the studies of these phases.

Adhesion (Fig. 2), internalization (Fig. 3) and vacuole formation (Fig. 4), occur in all the mentioned situations (Nogueira & Cohn, 1976; Milder et al., 1973, 1977; Kress et al., 1977; Maria et al., 1982), in the typical phagocytic mechanism. But probably they occur by very different pathways of molecular recognition, and at different quantitative levels, for the *T. cruzi* developmental stages, or functional states of the macrophages, as will be discussed in section 3. Phago-lysosomal fusion (Fig. 5) is also observed for the three developmental stages (Milder & Kloetzel, 1979, 1980; Meirelles & De Souza, 1985; Kress et al., 1975; Ley et al., 1988), but occurring later for trypomastigotes than for epimastigotes (Osuna et al., 1986).

The next step in the parasite's intracellular fate, however, is different for the three forms. As a rule, normal resident macrophages of mammals serve for the reproduction of *T. cruzi* when infected with trypomastigote forms, but reject the infection when epimastigotes are used (Kress et al., 1975; Nogueira & Cohn, 1976), or amastigotes obtained from the spleen of infected animals (by enzyme-free disruption) (Carvalho et al., 1981) or cell cultures (Villalta & Kierszenbaum, 1984a). There is some controversy in the literature, on the infectivity of the amastigote forms of *T. cruzi* (reviewed by Carvalho & De Souza, 1986), but macrophage-infective amastigotes were described, as depending on the method used for the obtention of these forms (McCabe et al., 1984, Carvalho & De Souza, 1986; Andrews et al., 1987; Ley et al., 1988). This is especially relevant because amastigotes liberated after premature cell rupture in sites of tissue lesion may remain extracellular and interact with other cells. Amastigotes may also be obtained as a result of extracellular differentiation of trypomastigotes, both *in vitro* (Villalta & Kierszenbaum, 1982; Lima & Kierszenbaum, 1984; Rondinelli et al., 1988) and possibly also in *in vivo* conditions (Andrews et al., 1987; De Castro, personnal communication).

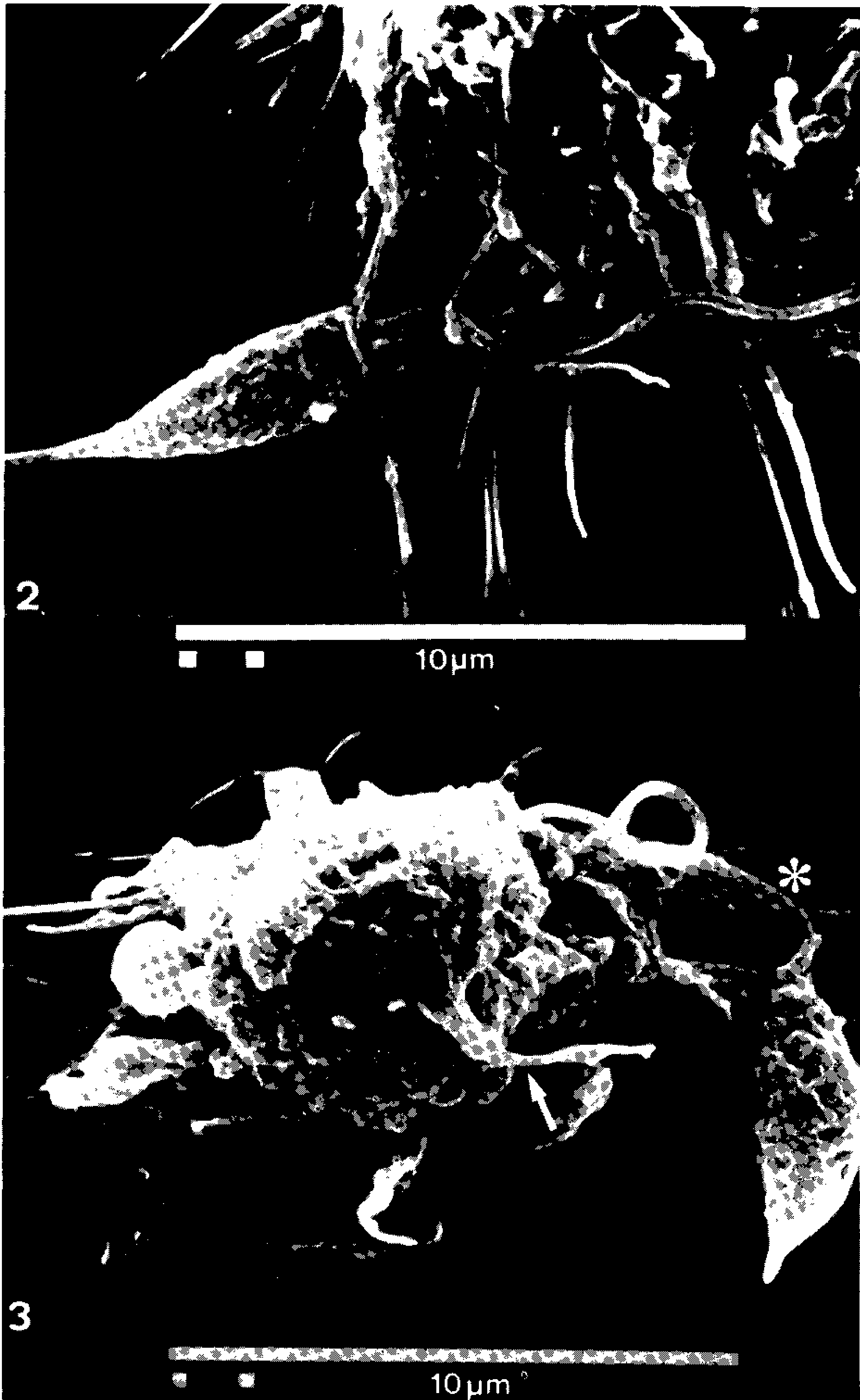

Fig. 2: adhesion of *T. cruzi* epimastigotes to cytochalasin B-treated chicken macrophages, as described by Meirelles et al., 1982a. Note the strong adherence of macrophages' phylopodium processes both to body and flagellum of the parasite. X 78,000.

Fig. 3: internalization of epimastigotes into normal chicken macrophages, as described by Meirelles et al., 1982a. In this case, the entry of the parasite proceeded by its posterior end firstly, while the flagellum remained outside the cells (arrow). Another parasite can be seen adherent to the macrophage (*). X 78,000.

Fig. 4: endocytic vacuole formation in a *T. cruzi*-infected macrophage, which was previously incubated with cationized ferritin, as described by Meirelles et al., 1984. Note that the vacuole which contains a parasite (P) is free of cationized ferritin particles which, by its turn, can be seen in other intracellular vacuoles (arrows). X 72,800.

Fig. 5: phagolysosomal fusion in macrophages previously labelled with peroxidase (secondary lysosomes are formed full of peroxidase), as described by Meirelles & De Souza, 1985. Notice the cytochemically detected dense deposits inside the vacuoles (arrows) that contains the parasite (P). X 20,790.

Different from normal resident peritoneal macrophages, trypomastigotes are killed within mammal macrophages that were stimulated *in vivo* by inflammatory agents or activated by specific *T. cruzi*-induced immune response (Kress et al., 1975; Nogueira & Cohn, 1976, 1978; Nogueira et al., 1977). In these cells the parasites are killed by intermediate oxygen

metabolites as O_2^-, OH^- and H_2O_2 (Nathan et al., 1979; Nogueira et al., 1982; Nogueira, 1983). NADH and NADPH oxidase activities were citochemically localized focally in the regions where the parasites are attached to the cell surface (Carvalho & De Souza, 1987a), as well as in the parasitophorus vacuole. This O_2^- generator enzyme is only scarcely seen in the plasma membrane of macrophages which did not have parasites attached to their surface. Nevertheless, in the avian model, monocyte-derived macrophages can also reject the trypomastigote infection (Meirelles & De Souza, 1985), probably by a different mechanism, as may be judged by the kinetics of the parasite's cell cycle interruption and the type of the lesion. In the same marcophages in which trypomastigotes are destroyed, another intracellular parasite, *Toxoplasma gondii* can survive in a different vacuole, thus supporting the idea of a possible involvement of lysosomal products and/or other 02-independent systems in *T. cruzi* destruction. Recently other chemical agents have been described to "activate" the macrophage microbicidal activity upon *T. cruzi*, which will be discussed in section 4, some of them involving possibly 02-independent cytotoxic mechanism(s).

3. MOLECULAR BASIS OF RECOGNITION

The experimental approaches to study *T. cruzi*-macrophage recognition at the molecular level imply always quantitative assays of the adhesion and internalization steps, or of the total parasite-cell association. As described recently (Araújo-Jorge et al., 1988), many experimental variables interfere strongly with the phagocytic indices. This is the case of the medium or the pH in which *T. cruzi*-macrophage interaction takes place, the number of macrophages offered in the assay despite the parasite-cell ratio, or the washing procedures during isolation of trypomastigotes. The number of macrophages plated and offered to parasite invasion were considered as the main limitant factor, a result which suggests that the macrophage surface components that recognize *T. cruzi* are a saturating factor in the measurement of the parasite-cell interaction. These observations led the authors to suggest a reference protocol to perform the *T. cruzi*-macrophages assays, in order to allow a more direct comparison of the data published by different research groups.

For the adhesion step, very little information is available on the attraction and contact phases, in which take part, undoubted, the motility of the parasites, their sensitivity to chemotaxic factors and the physicochemical interactions driven by the surface charges and hydrophobicity of both the parasites and the macrophages. The net negative surface charge of both *T. cruzi* and macrophages plays a role. With treatment of the parasites or the cells with cationic ferritin, a probe to anionic sites, there is enhancement of phagocytosis. On the contrary, there is an inhibition when the interaction is assayed in a condition in which both the macrophages and the parasites are positively charged by the probe (Meirelles et al., 1984). For the contact phase, there seems to be no preferential site in the parasites for the adhesion to macrophages, since *T. cruzi* can be observed adherent to these cells both by the posterior or the anterior (flagellar) regions.

Adhesion (Fig. 2) and internalization (Fig. 3) are dissociated steps in *T. cruzi* uptake by macrophages. The treatment of the macrophages by low temperature or with drugs which interfere with the phagocytic machinery allows adhesion to takes place while internalization is blocked. Extracellular adherent parasites can be quantified and/or osmotically lysed to evaluate the indices of adhesion, internalization or total association (Henriquez et al., 1981a; Andrews & Colli, 1982; Meirelles et al., 1982a). Drugs as cytochalasin B or D, actin-repolimerizing interruptors (Alexander, 1975; Nogueira & Cohn, 1976; Kipnis et al., 1979; Meirelles et al., 1982a; Ebert & Barbosa, 1982; Zenian & Kierszenbaum, 1983) or colchicine, microtubule-repolimerizing interruptor (Zenian & Kierszenbaum, 1983), inhibit the internalization step. Similar results are obtained when low temperature (Meirelles et al., 1982a; Ayala & Kierszenbaum, 1987), energy-blocking agents as 2-deoxy-glucose (Zenian & Kierszenbaum, 1983) or irreversible protein synthesis inhibitors as pactamicin (Lima & Kierszenbaum, 1982) are used, impairing the phagocytic activity and thus facilitating the study of the adhesion phase. In non-professional phagocytic cells the study of adhesion yielded similar results (Henriquez et al., 1981a; Andrews & Colli, 1982). Some apparent controversy emerged from studies in which only light microscopy was used (Dvorak & Schmunis, 1972), or in which the reversibility of the effect of cytochalasin B difficulted the interpretation of the results (Kipnis

et al., 1979). Today the phagocytic nature of the *T. cruzi*-macrophage association is well stablished and ultrastructurally documented (Maria et al., 1982; Meirelles & De Souza, 1985, 1986; Meirelles et al., 1983, 1984; Milder et al., 1973).

The very different rates of interiorization of epimastigotes and trypomastigotes in chicken macrophages (Meirelles et al., 1980), as well as the differences in the adhesion rates of these stages when the process of phagocytosis is inhibited (Meirelles et al., 1982a; Zenian & Kierszenbaum, 1983) led to the suggestion of an involvement of different elements in the binding of these two forms of *T. cruzi,* which was emphasized also by other experimental approaches, as the effect of treatment with enzymes, lectins or inhibitors of glycosylation and sorting of proteins upon the endocytosis of epi- and trypomastigotes (Araújo-Jorge & De Souza, 1984, 1986; Souto-Padrón & De Souza, 1989). These differences are possibly related to the surface differences between these forms (reviewed by De Souza, 1984; Zingales & Colli, 1985). However it still has to be elucidated which are the precise components that mediate the differential binding of these two developmental stages of *T. cruzi* to the macrophages.

Recognition, the last step of the adhesion phase, implies a thight adhesion, mediated by specific ligand(s) and receptor(s), that may be located on the surface of both the parasite and the macrophages. Among the most suggestive evidences of participation of ligand-receptor interaction in this step are the results which show that the simple increase in the macrophage: parasite initial ratio gives a strong enhancement of endocytic indices, regardless the correspondent decrease in parasite: macrophage ratio (Araújo-Jorge et al., 1989).

Up to the present time, the lack of purified elements from the *T. cruzi* surface that could be tested as putative ligands or receptors, allowed the studies on the molecular basis of *T. cruzi*-macrophage recognition to be based only on indirect evidence, as for example, on the effect of the treatment of host cells and/or the protozoa with enzymes as proteases (Nogueira & Cohn, 1976; Alcântara & Brener, 1980; Nogueira et al., 1980; Araújo-Jorge & De Souza, 1984), glycosidases (Araújo-Jorge & De Souza, 1984; Villalta & Kierszembaun, 1983, 1984b, 1985a), and lipases (Connely & Kierszenbaum, 1984, 1985), lectins (Zenian & Kierszenbaum, 1982; Meirelles et al., 1983; Araújo-Jorge & De Souza, 1986; Stiles & Kierszenbaum, 1986), inhibitors of glycosylation (Souto-Padrón & De Souza, 1989) or several other agents to be reviewed later on. The results obtained by these approaches suggest the involvement of surface components of lipidic, proteic and glycidic nature. The main conclusions of these studies are that recognition is not a passive phenomenon, and that both, the surface of the macrophage and that of the parasite, should have recognition sites, probably ligand(s) and receptor(s). Most of the times the results vary according to the drug treatment whether it is performed on the macrophages or on the parasites, before or during the interaction assay and with epimastigotes or trypomastigotes. We will examine these effects separately, focusing first on the macrophage surface components that may mediate *T. cruzi* binding and/or uptake, and later, on the parasite's components.

3.A. RECEPTORS OF THE MACROPHAGES THAT RECOGNIZE *TRYPANOSOMA CRUZI*

The infective stage of *T. cruzi,* the trypomastigote, circulates in the blood of the mammal host. This way, parasite surface glyconjugates (reviewed by De Souza, 1984) and/or plasma components bound or adsorbed to its membrane may interact with receptors on the macrophage's surface. These cells express many surface receptors which mediate phagocytosis (Fig. 6 A-C). There are the receptors for the different isotypes of the Fc portion of immunoglobulins (Fc-R) (reviewed by Unkeless et al., 1988), the three receptors for complement components (respectivelly C3b/C4b, C3d and iC3b for CR1, CR2 and CR3 – reviewed by Griffin, 1982; Inada et al., 1983), receptors for fibronectin (FN-R) (Doran et al., 1980; Pommier et al., 1984) and for protease complexed-alpha-2-macroglobulin (A2M-R) (Kaplan & Nielsen, 1979a, b), and lectin-like receptors that recognize sugar residues (Sharon, 1984; Stahl et al., 1984; Neufeld & Ashwell, 1979; Kolb-Bachofen et al., 1984; Croker & Gordon, 1986). As has been observed, trypsinization removes many surface receptors of the macrophages and inhibits the *in vitro* infection of both epi- and trypomastigotes (Nogueira & Cohn, 1976; Alcântara & Brener, 1980; Zenian & Kierszenbaum, 1983; Souto-Padrón & De Souza, 1989).

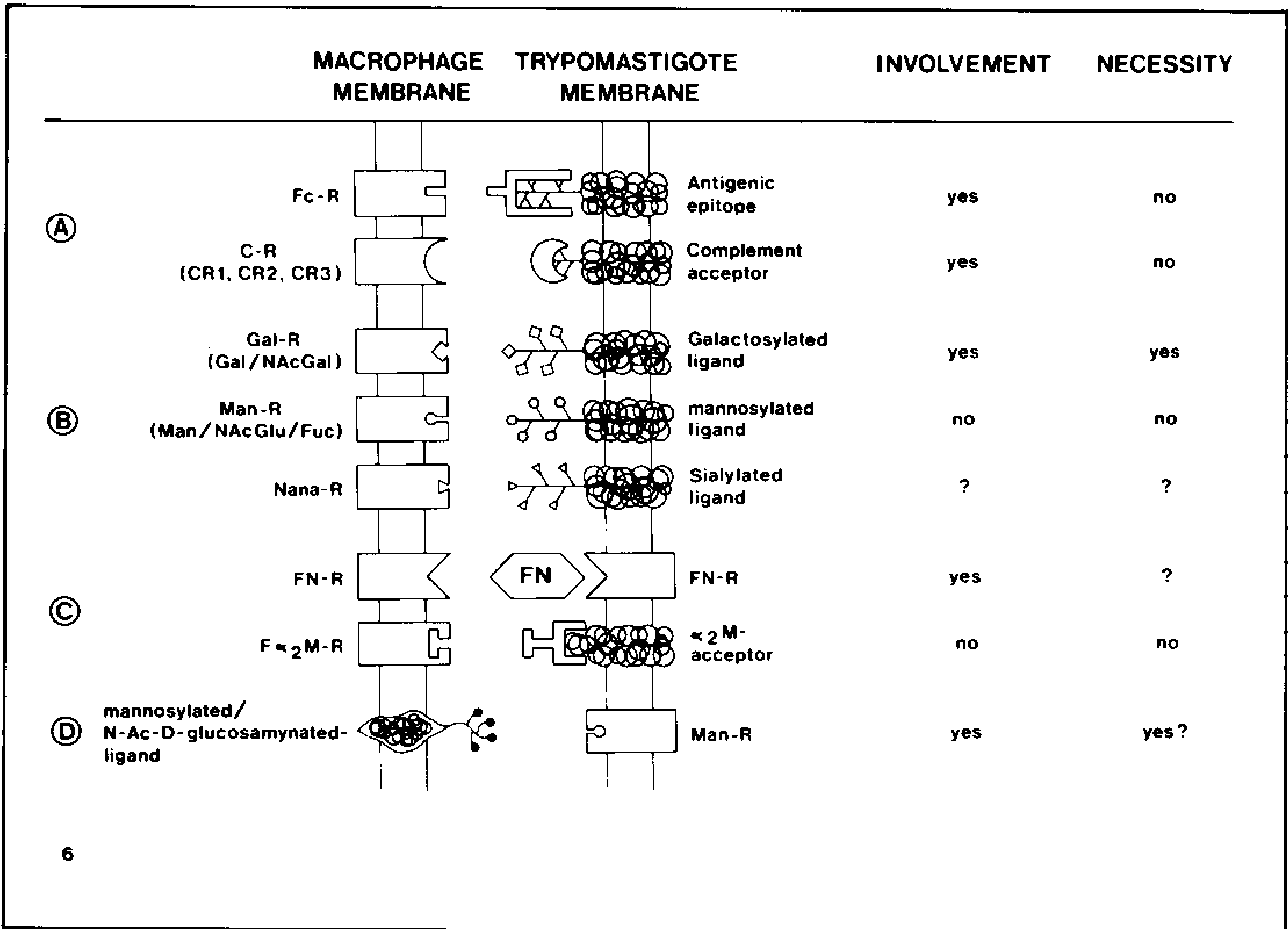

Fig. 6: schematic representation of the components from the surface of macrophages and *Trypanosoma cruzi* (trypomastigotes) involved in the parasite-cell recognition. A = Immunological receptors for Fc portion of immunoglobulins (FcR) and components of the complement system (CR); B = Lectin-like receptors for galactosylated (Gal-R), mannosylated (Man-R) or N-acetyl-neuraminylated (Nana-R) ligands; C = Receptors for mammal the plasma components fibronectin (FN-R) and protease-complexed alpha-2-macroglobulin (F- 2M-R); D = Lectin-like receptors in the surface of the trypomastigotes for mannosylated/N-acetyl-D-glucosaminylated ligands in the macrophage surface.

Trypomastigotes bind unspecific or specific immunoglobulins and complement components by antigenic and complement acceptor sites respectively (Krettli, 1982; Sanchez et al., 1983; Hudson, 1983), as well as by specific Fc and C receptors (Miranda Santos & Campos Neto, 1981; Krettli & Pontes de Carvalho, 1986) on its own surface. Nevertheless, unless the antisera bear protective (or "lytic") antibodies, the opsonizing effect is low (Lages-Silva et al., 1987). Furthermore, the opsonic effect of these immunoglobulins, even the antibodies that can be detected by conventional sorology, are not related to the host protection (Lages-Silva et al., 1987), at least for some strains of *T. cruzi,* because the parasite has evolved mechanisms to escape from their action, as capping and shedding of antigen-antibody complexes (Schmunis et al., 1978, 1980; Leon et al., 1979; Szarfman et al., 1980; Katzin et al., 1984; De Gasparini et al., 1985). *T. cruzi* can also inactivate the binding of iC3b by DAF (Kipnis et al., 1986; Rimoldi et al., 1988a). On the other hand, *in vitro* experiments indicate that trypomastigotes may or may not get an intracellular access via FcR or CR1 (Fig. 6-A) (Nogueira & Cohn, 1976; Alcântara & Brener, 1978, 1980; Zenian & Kierszenbaum, 1983). Incubation of the parasites with specific immune sera enhance its ingestion via FcR, and incubation with a complement source gives a similar result via CR (in this case using activated macrophages). The treatment of the macrophage with antibodies against FcR, on the other hand, inhibits the ingestion of IgG-opsonized trypomastigotes (Nogueira & Cohn, 1976). In this context, the results of Lages-Silva et al. (1987) showed that only the immune sera of active *T. cruzi* infections (chronic chagasic animals or chagasic patients untreated or treated but not cured), bearing lytic antibodies of the IgG2 isotype, significantly enhance the uptake of bloodstream trypomastigotes by normal peritoneal macrophages, whereas sera

that bear only the antibodies involved in the conventional sorology (from cured chagasic patients or from animals immunized with glutaraldehyde-fixed trypomastigotes, with a purified 90 Kd mol wt antigen from epimastigote surface glycoprotein or with freeze-thawed epimastigotes) did not efficiently opsonized the parasites.

Otherwise, the FcR and the CR are not essential for the *T. cruzi* recognition (Alcântara & Brener, 1980) since (1) pronase treatment of the macrophages, an assay in which FcR-mediated phagocytosis is retained, abolishes the uptake of both epimastigotes or bloodstream trypomastigotes, and (2) both normal or immunoglobulin-free bloodstream trypomastigotes collected respectively from normal or lethally X-irradiated mice are still phagocytized by normal or trypsin-treated macrophages, a condition in which CR1 is removed. An interesting result, not well explored up to date, was the effect of IgG-coating the so called "myotropic" CL strain of *T. cruzi*, which has its intracellular fate modified by the immune-opsonization (Alcântara & Brener, 1978). In the *Leishmania*-macrophage interaction model, it has recently been shown that the parasite's intracellular fate depends on the type of receptor which mediates the parasite interiorization (Mosser & Edelson, 1987). The penetration through C3b receptors led to intracellular multiplication, while through iC3b receptors the microbicidal activity is stimulated, with the triggering of the respiratory burst.

Another complement component, C1q, was also recently shown to influence the phagocytosis of *T. cruzi* by macrophages (Rimoldi et al., 1988b). C1q, the C1 subcomponent which initiates the activation of the complex, is a collagen-like molecule and bear antigenic similarities with FcR (Heinz et al., 1984). When C1q interacts with a specific C1q receptor (C1qR) on macrophages, it markedly enhances the FcR-mediated phagocytosis (Bobak et al., 1987), an effect which is signalized by the collagen-like tail portion of the C1q molecule. Epi- and trypomastigotes of *T. cruzi* are able to bind human C1q at their surface, and activate it in an unusual way, which results in the exposure of C1q tails. Treatment of trypomastigotes with normal human serum or with C1q, but not with heated or C1q-depleted serum, enhance the parasite internalization by 2-3 fold (Rimoldi et al., 1988b). This effect was not obtained when epimastigotes were assayed. The enhancement could also be observed after plating the macrophages on C1q-coated surfaces, indicating that in this case, the internalization of the parasites might not be mediated by the C1qR, but by other receptor systems that could be up-modulated following the C1q-C1qR interaction (as FcR or the fibronectin receptor). The interaction of C1q with fibronectin may also enhance parasite attachment and entry. It seems necessary therefore, to construct a more complete view of the complex network of interactions between the different macrophage receptor-ligand systems, to a better understanding of the *T. cruzi*-macrophage invasion mechanism. In this parasite-cell system, regardless of the possible involvement of immunoglobulins and complement in the clearance of trypomastigotes from the peripheral circulation, which indeed occurs after the architecturing of the specific immune response, other recognition mechanisms do mediate their binding and internalization into the cells. This feature is possibly useful to the parasite when it is interacting with resident macrophages, allowing to use them as hosts in the early phases of an *in vivo* infection. Recently liberated extracellular trypomastigotes, which do not get immediate contact with serum components, can also use other possible recognition systems to invade cells locally in a tissue lesion. Further studies are necessary for a better knowledge of the receptors from fibroblasts, muscle, nerve, epithelial cells and others which *in vivo* are used by *T. cruzi* to get its intracellular habitat.

Three lectin-like receptors that recognize specific carbohydrate residues in macromolecular or cellular ligands are present and well studied in the macrophages: the Mannose/N-acetyl-D-glucosamine/Fucose (Man-R), the Galactose/N-acetyl-D-galactosamine (Gal-R) and the Mannose-6-phosphate (Man-6P-R) receptors (Stahl et al., 1984; Kolb-Bachofen et al., 1984; Neufeld & Ashwell, 1979, respectivelly). The evidence that some of these sugars, when added to the interaction media, may inhibit the uptake of epimastigote and trypomastigotes (Araújo-Jorge & De Souza, 1984) in a strain-dependent way, suggested that these receptors might also mediate the binding and uptake of *T. cruzi.* It was shown recently that the Man-6P-R participates on the *Leishmania mexicana amazonensis*-macrophage interaction (Saraiva et al., 1987). However, its involvement

in the *T. cruzi*-macrophage interaction was not investigated. A fourth lectin-like receptor, with carbohydrate affinity for sialylated compounds is expressed in resident macrophages from stromal tissues, specially from lymph nodes, liver and spleen (Crocker & Gordon, 1986), and a possible access of *T. cruzi* to the cytoplasm of these cells via this receptor was not studied but is still an undiscarded hypothesis.

The Man-R was recently shown to mediate the recognition of *Leishmania*, acting sinergistically with CR (Blackwell et al., 1985). Nevertheless, for the *T. cruzi* recognition, this system seems not to be used by the macrophage (Fig. 6-B), regardless of the presence of mannose (Man) and/or N-acetyl-D-glucosamine (Glu-Nac) on the parasite surface (Pereira et al., 1980; Gonzáles-Cappa & Katzin, 1985). The remotion or the blockage of Man or Glu-Nac by the treatments of the trypomastigotes with mannosidase (Villalta & Kierszenbaum, 1983), N-acetyl-D-glucosaminidase (Villalta & Kierszenbaum, 1985a), the Man-binding lectins from *Canavalia ensiformis* (Con A) (Meirelles et al., 1983) and *Lens culinaris* (LCA) (Araújo-Jorge & De Souza, 1986), and the Glu-Nac-binding lectin from wheat germ (WGA) (Araújo-Jorge & De Souza, 1986) do not prevent the *T. cruzi* ingestion by the macrophages. These results suggest that the inhibitory effect seen with the addition of Man and Glu-Nac during the interaction assay (Araújo-Jorge & De Souza, 1984) must be mediated by the interference of these sugars with lectin-like elements that may be present on the surface of the parasite (Fig. 6-D) and not on the host cell's surface, as has been suggested by Piras et al. (1983) and Boschetti et al. (1987), and seemed indicated by the success of Bongertz et al. (1983) on the preparation of monoclonal antibodies against Glu-Nac-bearing glycoproteins extracted from some mammal muscle in WGA-affinity columns, which could inhibit *T. cruzi* invasion in BESM cells. To be correct, this hypothesis might be corroborated by inhibition of host-cell recognition when these sugars are removed or blocked on the macrophage's surface. This is true at least for the effect of the two respective glycosidases (Villalta & Kierszenbaum, 1983, 1985a) on the macrophage, and for the effect of LCA (Araújo-Jorge & De Souza, 1986), which inhibit the uptake of trypomastigotes. Con A and WGA enhance it, probably because of its multiple binding sites, that can act as a bridge to the same sugars on the parasite and macrophage's surface (Meirelles et al., 1983; Araújo-Jorge & De Souza, 1986). When the monovalent succinil Con A is used, not any effect can be shown (Zenian & Kierszenbaum, 1982) and when the lectin treatment is performed in the presence of serum, Man-bearing components of the serum bind to Con A thus yielding an inhibitory effect (Stiles & Kierszenbaum, 1986), probably because there are no more Man-binding sites free to attach to the macrophage.

Different from the negative results on the involvement of the Man-R on the *T. cruzi* recognition by macrophages, much indirect evidence exists today that the Gal-R is involved in the phagocytosis of *T. cruzi* by macrophages (Fig. 6-B): (1) Addition of galactose (Gal) and N-acetyl-D-galactosamine (Gal-Nac) during the interaction assay inhibits the interiorization of epimastigotes and trypomastigotes (Araújo-Jorge & De Souza, 1984); (2) remotion of sialic acid residues by neuraminidase (Araújo-Jorge & De Souza, 1984) reducing the surface negativity and exposing new Gal/Gal-Nac sites (Souto-Padrón & De Souza, 1985, 1986), enhances specially the uptake of trypomastigotes, an effect partially reversed by Gal and Gal-Nac blockage by the appropriate lectins (Araújo-Jorge & De Souza, 1988); (3) the ingestion of these asialo-trypomastigotes is competitively inhibited by the addition of Gal, Gal-Nac, and Gal-oligosacharides as beta-lactose and rafinose, and they compete with asialoerythrocytes for the same receptor (Araújo-Jorge & De Souza, 1988; Araújo-Jorge et al., 1989); (4) lectins that blocks Gal and Gal-Nac from the parasite surface inhibit, and lectins that block sialic acid residues (probably exposing galactosyl sites), enhance the uptake of trypomastigotes (Araújo-Jorge & De Souza, 1986, 1988). There are other contradictory results showing no change in the endocytic indices when trypomastigotes of *T. cruzi* are treated with exogenous neuraminidases (Zenian & Kierszenbaum, 1982; De Titto & Araujo, 1987) but we think that this might be explained by the differences in the activity of the sialidases used in these studies, one using a type II neuraminidase from *Clostridium perfringens*, which is known to contain other proteases (Zenian & Kierszenbaum, 1982), and the other using a *Vibrium cholerae* neuraminidase, which is protease-free but has different substrate specificity as compared to the bacterial enzyme (Drzeniek, 1973). Since the parasite expresses a developmentally regulated neuraminidase activity on its surface

(Pereira, 1983; Harth et al., 1987), which can also be demonstrated in sera from mice acutely infected with *T. cruzi* (De Titto & Araujo, 1987), it is possible that the parasite itself could control the exposure of galactosyl residues and thus its own ability to invade macrophages via the Gal-R. This hypothesis is consistent also with data showing that some strains that express high neuraminidase activity (Pereira & Hoff, 1986) also have reduced cationized ferritin – binding sites (mostly sialyl residues as shown by Souto-Padrón & De Souza, 1985) and exposed galactosyl residues reactive to cytochemical revelation by the *Ricinus communis*-ferritin complexed lectin (Barbosa et al., 1987). It is interesting also to note that most of the studies on the effect of *T. cruzi*'s neuraminidase foccus on its possible action upon the host cells, showing indeed that its effect upon non professional phagocytes (myocardial and endothelial cells – Libby et al., 1986 – or L929 fibroblasts – De Titto & Araujo, 1987) is different from the effect upon macrophages (De Titto & Araujo, 1987). In the first case there is decrease and in the second increase of the internalization of the parasites. In this context, some authors proposed that the sialidase activity may play a role in the invasion of cells by *T. cruzi* during the early stages of the infection, while a mechanism to neutralize it (anti-sialidase activity), which has also been demonstrated (De Titto & Araujo, 1987), is not effectively developed. The recently proposed hypothesis (Pereira, 1988) of an up-modulation of infectivity depending on a low neuraminidase activity with high sialylation and vice-versa (down-modulation with high neuraminidase activity and low sialylation) is controversial, and perhaps could only be proposed for cells which do not express receptors for galactose/N-acetyl-D-galactosamine.

Another possible way used by trypomastigotes to enter macrophages may be a fibronectin receptor –FnR– (Fig. 6-C). Both these phagocytes (Doran et al., 1980) and the parasite (Ouassi et al., 1984, 1986a) express fibronectin (Fn) binding sites. Fn attachment to *T. cruzi* was found to be mediated by the arginine-glycine-aspartic acid-serine (RGDS) tetrapeptide on the cell-binding domain of Fn (Ouaissi et al., 1986b). The previous incubation of any of the two interaction partners with fibronectin enhances the cellular infection *in vitro* (Wirth & Kierszenbaum, 1984a; Ouassi et al., 1985) and the presence of antibody against fibronectin impairs the internalization of *T. cruzi* (Ouassi et al., 1985). As already shown, fibronectin presents a synergistic effect upon the endocytosis via the CR1 (Wright et al., 1984; Bohnsack et al., 1986), and it may be possible that the fibronectin bound to the surface of circulating bloodstream trypomastigotes contribute to enhance their uptake by macrophages that have been activated for phagocytosis via the complement receptor. There is also some *in vitro* evidence that alpha-2-macroglobulin, another plasma component, can activate macrophages to ingest particles at greater rates, via IgG, IgM, complement and galactosyl recognition, mechanisms that could be used by the macrophage to enhance *T. cruzi* ingestion (Araújo-Jorge et al., 1989). The use of a different receptor-mediated entry mechanisms could be of advantage to the parasites, at least in an early stage of infection in mammals, when the macrophages are easy accessed and can support their reproduction as hosts instead of destroying them.

Finally, it is improbable that the macrophage receptor for protease complexed-alpha-2-macroglobulin (Kaplan & Nielsen, 1979a, b) mediates the phagocytosis of *T. cruzi* (Fig. 6-C), because the treatment of the parasites with A-2-M does not enhance their interiorization (Araújo-Jorge et al., 1986a). On the contrary, A-2-M effect upon the parasite seem to be that of a protease inhibitor, similar to the soybean trypsin inhibitor, impairing the ability of the parasite to process proteolytically some component(s) of its surface that are necessary to mediate its binding and/or internalization to the host cells, in a process similar to that proposed for Vero cells (Piras et al., 1983, 1985).

Since most of the surface receptors of the macrophage are glycoproteins that reach the plasma membrane via the Golgi-vesicular transport system, it is conceivable that the inhibition of glycosylation at the Golgi level or of the transport of membrane proteins would inhibit infection. This was in fact obtained with the treatment of the macrophages with the antibiotic tunicamycin, an inhibitor of the protein N-glycosilation, and with the monovalent ionophore monensin, which decreases the invasion of trypomastigotes (Souto-Padrón & De Souza, 1989), with swainsonine, an inhibitor of Golgi mannosidase II (Villalta & Kierszenbaum, 1985b) and also by the treatment of

macrophages with N-glycanase (Villalta & Kierszenbaum, 1987). However, the macrophage treatment with tunicamycin and monensin have opposite effects upon the ingestion of epimastigotes, respectivelly enhancing and inhibiting it. Nevertheless, in the tunicamycin-treated macrophages, it is not the overall population of cells which ingest the parasites, but a subpopulation of already infected cells, which supports higher parasitic loads, as indicated by an enhancement in the number of parasites per infected macrophage. This result, associated to the different kinectics of trypsinized macrophages to recover their phagocytic ability for epi- and trypomastigotes, is also additional evidence for the fact that macrophages use different receptors to mediate the phagocytosis of these two developmental stages of *T. cruzi* (Souto-Padrón & De Souza, 1989).

3.B. SURFACE COMPONENTS OF *TRYPANOSOMA CRUZI* INVOLVED IN ITS RECOGNITION BY THE MACROPHAGES

Most of the available data on glycoconjugates of the surface of *T. cruzi* (glycoproteins and glycolipids) that could be involved in host-parasite recognition were performed in non-professional phagocytic cell lineages and were reviewed by Zingales & Colli (1985) and by Piras et al. (1983). A trypomastigote-specific glycoprotein of Kd 85,000 (Tc-85) (Andrews et al., 1984; Alves et al., 1986), as well as the recently described galactofuranosylated peptides of 90-80 kDa and 60-50 kDa (Zingales et al., 1988), both suggested to be involved in this recognition, were not tested in the *T. cruzi*-macrophage interaction system. The same is the case with the protein of 83 kDa, isolated by Boschetti et al. (1987), and supposed to be involved in the parasite adhesion to Vero cells. From the experiments dealing with the interaction with macrophages, some of the conclusions were already commented together with the macrophages' receptors that are involved in *T. cruzi* binding and internalization. The main conclusions one can get from all these studies are as follows (Fig. 6):

(1) The surface of trypomastigotes obtained from different sources may have different binding sites or different binding site-mask elements for the recognition by macrophages, since their infection level *in vitro* vary greatly (Meirelles et al., 1982b; Kloetzel et al., 1982). De Titto et al. (1987) also reported that even parasite populations derived from a single clone can behave differently as "entering" or "non-entering" subpopulations. These latter are those which after more than 18 h over cell culture at 37 ^{o}C had not developed the ability to infect macrophages, but did not lose the differentiation or replicative abilities. One can speculate on the possible correlation of these different binding sites to the developmentally regulated expression of some key molecules, as neuraminidase (Pereira, 1983) or DAF (Rimoldi et al., 1988a), or with a proteolytic processing of the parasite surface, as discussed in 3.B.5.

(2) Probably different ligands and/or receptors are involved in the steps of binding and internalization of *T. cruzi* into macrophages, judging by the ability of impairing phagocytosis by chemical or physical treatments of the cells, thus dissociating these steps, as well as by the findings that sulphydryl groups are required only for the internalization step (Ayala & Kierszenbaum, 1987).

(3) Gal and Gal-Nac residues in the parasite surface are required for its recognition by the macrophages, since their blockage by the specific lectin inhibits the interaction (Araújo-Jorge & De Souza, 1986, 1988).

(4) Man, Gal, Glu-Nac and sialic acid from the trypomastigote surface impair its recognition by macrophages, though the respective specific glycosidase or lectin treatments on the parasite enhance their uptake (Villalta & Kierszenbaum, 1983, 1984b, 1985a; Araújo-Jorge & De Souza, 1984, 1986) as well as the treatment with cationized ferritin does (Meirelles et al., 1983).

(5) Parasite's proteases are necessary for the exposure of surface components that act as ligand(s) to the macrophage's receptors, because during the extracellular incubation of the parasites of 18-24 h they can process their surface in a protease inhibitor-sensitive way, thus enhancing its recognition by the macrophages (Araújo-Jorge et al., 1986a); other evidences are the treatment of parasites with exogenous protease, which enhances their uptake by the macrophages (Nogueira et al., 1980; Kipnis et al., 1981; Araújo-Jorge & De Souza, 1984), and the inhibition of the endocytic indices, when the physiological protease inhibitor alpha-2-macroglobulin or the soybean

trypsin inhibitor are used (Araújo-Jorge et al., 1986a). A time-dependent several-fold increase in the *T. cruzi* ability to bind to RGDS-peptides of fibronectin was also recently shown (Ouaissi et al., 1988), and is also probably related to a "maturation" process of the parasite ability to adhere and infect host cells. Similar results of protease involvement were obtained in the *T. cruzi*-Vero cell system (Piras et al., 1985).

(6) It is possible that a lectin-like protein from the parasite surface may mediate its binding to Man or Glu-Nac-bearing glycoconjugates in the macrophage surface, since the treatment of the phagocytes with the appropriate lectin or glycosidase impair their association to the parasites. In fact, the recent results of Boschetti et al. (1987) indicate that a protein of 83 kDa, extracted from highly adherent trypomastigotes, could act as a lectin to bind mannose residues in Vero Cells or BESM cells, as suggested (Crane & Dvorak, 1982; Dvorak, 1984). This remains to be tested using macrophages as host cells. Recently De Souza & his colleagues (personal communication) were able to demonstrate direct binding of coloidal gold marked-galactosylated and N-acetyl-D-glycosaminylated ligands to lectin-like receptors at the surface of a subpopulation of tissue culture derived trypomastigotes. However this preliminar hypothesis deserves further investigation. A similar mechanism was proposed to the *T. cruzi*-Vero cell interaction system (Piras et al., 1983; Boschetti et al., 1987).

(7) *T. cruzi* polyamines seem to be necessary for full infectivity of trypomastigotes, since pre-treatment of the parasites with inhibitors of polyamine biosynthesis (inhibitors of arginine decarboxylase) can reduce the endocytic levels in a process which can be prevented by the simultaneous presence of agmatine or putrescine, the two metabolites that result sequentially from arginine decarboxylation (Kierszenbaum et al., 1987a). These are interesting results, because arginine decarboxylase is not found in mammalian cells and may have a potential application in chemotherapy against *T. cruzi* infection.

(8) Some parasite phospholipids may impair its recognition, as the treatment with phospholipases A2 and D facilitates the uptake of trypomastigotes by macrophages (Connely & Kierszenbaum, 1984, 1985). The effects of these two enzymes in the recognition process may be indirect, beginning as an enzymatic cascade effect that lead to the production of leukotrienes (Wirth & Kierszenbaum, 1985b) or alternatively, removing glycosylphosphatidylinositol-anchored glycoproteins that mask binding sites (Andrews et al., 1988b; Cardoso de Almeida et al., 1987).

(9) Free sulphydryl groups from proteic ligands and/or receptors in the parasite surface impair the proper interaction of the elements mediating the internalization step of *T. cruzi* invasion into macrophages (Ayala & Kierszenbaum, 1987). This conclusion derives from the noted enhancement of invasion when the parasite is treated with the specific SH-blocker p-chloromercuriphenylsulfonic acid (PCMS), a 2-4 h naturally reversible effect which can also be abolished when cysteine or glutathione are added during the incubation of the parasite with the drug, resulting in an excess of sulphydryl groups. The authors suggest that the effect of PCMS could be explained by (a) a possible PCMS-inducible rearrangement in the parasite components involved in the invasion process, (b) a possible reduction in the sulphydryl-mediated interactions of macromolecules that normally bind to the parasite surface, thus hindering its internalization by the cells, or (c) accelerating a "maturation" process proposed to enhance the infective capacity of trypomastigotes (Piras et al., 1983). It is interesting to note that the sensitivity to PCMS is also a property of *T. cruzi* phospholipase C, which mediates the shedding of phosphatidylinositol-anchored glycoproteins (Andrews et al., 1988b).

(10) It stills to be determined the precise reason for the reported differences in the ability of different *T. cruzi* strains to infect host cells *in vitro*, including macrophages (Alcântara & Brener, 1978, 1980; Meirelles et al., 1980, 1982a, 1982b; Araújo-Jorge & De Souza, 1984). It was recently shown that even cloned populations can display such differences (De Titto et al., 1987), but the molecular basis of the extense natural heterogeneity of *T. cruzi* stills unknown (Dvorak, 1984). Differences in the exposed carbohydrates and antigenic epitopes, fibronectin receptors or key surface enzymatic activities (as neuraminidase, phospholipase or proteases) may affect qualitatively as well as quantitatively the parasites' ligands that can recognize host cell's surface receptors.

So, these "strain" differences, as well as the differences seen when bloodstream-, axenic- or tissue culture-derived trypomastigotes are compared, may reflect different physiological states of parasite subpopulations rather than phenotypic and genotypic differences. In this context, it is interesting to point out the results showing that large differences in the *in vitro* infection levels can also be observed when two well known *in vivo* myotropic strains (CL and Colombiana) interact with muscle cells, as well as when a same strain (CL) interacts with undifferentiated (myoblasts) or differentiated (myotubes) muscle cells (Araújo-Jorge et al., 1986b). Probably the trypomastigotes from the CL strain, which show low infection indices after short periods of interaction time, can not be considered as "non-infective", but delayed in the development of its infectivity, as compared to the Colombiana or Y strains. Since the induction of phagocytosis is not passive but actively done by *T. cruzi*, even in macrophages, we think that it will be the balance of the available parasite ligands and macrophage surface receptors that will finally lead to a high or to a low infection index.

3.C. ON THE MOLECULAR BASIS OF THE INTERIORIZATION MECHANISM AND ON THE COMPOSITION OF THE VACUOLAR MEMBRANE

Little is known about the molecular signals that mediate the internalization of *T. cruzi* in the intracellular region. This subject has been mainly approached by treating macrophages with different general inhibitors or inductors of endocytosis. Two metabolic processes of the macrophages were shown to be involved: the intracellular balance of cyclic nucleotides and the polyamine levels. The rise in cAMP levels inhibits, and the decrease in cGMP levels enhances quantitatively the association of the parasites to the macrophages (Wirth & Kierszenbaum, 1984b, c). For the involvement of the polyamine levels it was shown that the ornithine decarboxilase activity, which by ornithine decarboxylation produces putrescine (which by its time can be converted in spermidine and spermine) seems to be important for the binding and/or internalization of *T. cruzi*, as many drugs that inhibit this activity also inhibit the internalization of trypomastigotes by macrophages (Kierszenbaum et al., 1987b). This effect can be prevented if exogenous putrescine is added during the incubation period. The precise role of this enzyme activity is unknown but it is probably related to the phagocytic function itself, since the uptake of inert particles is not affected.

Using an ultrastructural cytochemical approach to identify and compare the chemical nature of the macrophage plasma membrane with the vacuolar membrane of the formed parasitophorus vacuole, some differences were pointed out (Meirelles et al., 1983, 1984; Meirelles & De Souza, 1985). Mannosyl (Con A-receptors) residues and anionic sites (cationized ferritin-binding sites, majorly sialyl-residues), uniformely distributed in the plasma membrane were revealed with an heterogeneous distribution at the vacuolar membrane, respectively patchs of Con A-receptors and absence of anionic sites. Nevertheless the anionic sites were revealed in other endocytic parasite-free vacuoles (Meirelles et al., 1984, and Fig. 4). Other differences were also noted when typical plasma enzyme markers were studied, as 5'nucleotidase, adenyl-cyclase, Mg^{++} ATPase, Na^{+}/K^{+} ATPase, all of them absent and/or impossible to be revealed by the cytochemical methods, using the appropriate substrate and controls (Meirelles & De Souza, 1986). Also interesting are the findings of similar results obtained with heart muscle cells (Meirelles et al., 1986). In these endocytic vacuoles, cationic proteins that are cytochemically reactive to tannic acid, were localized surrounding the trypomastigotes from the infective *T. cruzi* clone Dm28c (Barbosa et al., 1988). Although the physiological role of all these differences observed in the composition of the plasma and vacuolar membrane are still unknown, it is possible that the parasite modifies the vacuolar membrane in such a way to allow its survival from the destructive mechanisms of the macrophage in the endocytic vacuole, and/or for provoke its rapid lysis to get in direct contact with the cytoplasm.

Recently it was shown that amastigotes and trypomastigotes secrete into the culture medium heat-labile, trypsin-sensitive molecules which lyse erythrocytes from various mammal species, expressing their maximal activity at pH 5.5 and causing lesions larger than 10 nm in target ghost membranes (Andrews et al., 1988a). These authors also showed evidence of an involvement of the *T. cruzi* hemolysin in the disruption of the acidic phagosomes. Treating cells with weak bases they found a decrease in

the number of parasites found outside the vacuoles.

4. MODULATION OF THE *TRYPANOSOMA CRUZI* FATE IN THE MACROPHAGES

As we have already discussed in section 1, the intracellular fate of *T. cruzi* in macrophages depends on the developmental stage of the parasite, as well as on the functional state of the macrophages. More recent results and reviews in the literature (Nogueira, 1983; Zingales & Colli, 1985; Carvalho & De Souza, 1986) point to the trypomastigote and to the amastigote stages as being infective forms to macrophages, due to their abilities to escape the effector mechanisms of killing by resident, unstimulated, unactivated or even thyoglicolate-stimulated macrophages. *In vivo* BCG or *T. cruzi*-activated macrophages, as well as *in vitro* lymphokine-activated macrophages can destroy the parasites (Hoff, 1975; Nogueira et al., 1977; Nogueira & Cohn, 1978; Wirth et al., 1985c). Interferon, (mainly gamma, but possibly also alpha and beta) and not interleukin 2 is the lymphokine involved in this effect (Kiersenbaum & Sonnenfeld, 1982, 1984; Wirth et al., 1985c), a result also obtained with HeLa cells (Plata et al., 1984; Osuna et al., 1985). Using the macrophage-like cell line J774-G8, Alcina & Fresno (1987) showed that the activation of these cells to induce their complete trypanocidal activity depends on the synergistic action of lymphokines (mainly gamma-interferon) and endotoxin (LPS). Interleukin 4 was also recently shown to activate the phagocytic and microbicidal activities of macrophages, but this effect was only assayed using non-infective amastigotes (Wirth et al., 1989). Other cytokines as tumor necrosis factor –TNF– (De Titto et al., 1986) and granulocyte/macrophage colony stimulating factor –GM–CSF– (Reed et al., 1987) do not affect the phagocytic ability of resident peritoneal macrophages to ingest *T. cruzi* but they activate the microbistatic intracellular replication of the parasite. The effect of TNF can not be reproduced with *T. gondii,* possibly because it remains inside the parasitophorus vacuole, while *T. cruzi,* free in the cytoplasm, may be a target for the cytokine effect. The ability of the macrophages to destroy the parasites seems to be associated both to the parasites' ability to evade the 02 intermediate metabolites and to the macrophage's ability to maintain the parasite inside a vacuole and process the phagolysossomal fusion in time (Osuna et al., 1986). Besides, the lysosomal attack may also contribute to the parasite's destruction. It was also shown that a 18S fraction of immune RNA –iRNA– (extracted from the spleens of *T. cruzi*- infected mice) can act over the macrophages *in vitro* to induce their microbicidal activity (Bertolini & DeLucca, 1986). Injected intracutaneously into mice, this iRNA significantly reduces the peaks of parasitemia and increases the survival rate.

The killing of *T. cruzi* by phagocytic cells, as neutrophils and macrophages (Nathan et al., 1979; Villalta & Kierszenbaum, 1984a) has been postulated to involve peroxidase activity and/or production of hydrogen peroxide or both (Nogueira et al., 1982; Wirth et al., 1985c). Another interesting report (Tanaka et al., 1983) on the mechanism of killing related to oxygen intermediates was described with the appropriate stimulation of the oxidizing glucose via hexose monophosphate shunt to produce O_2 and H_2O_2 in *T. cruzi*-infected 0_2-deficient macrophagic cell clone. This cell can be is reconstituted with an H_2O_2-generating system, thus enabling the intracellular killing of the parasites. The authors showed that even when *T. cruzi* infection was sufficient to stimulate the increase of oxygen metabolism, the requirement for epimastigotes killing is lower than that for trypomastigote destruction. Scavengers as catalase eliminated the killing by 90%.

However, in the light of the more recently studied O_2-independent cytotoxic mechanisms (Elsbach & Weiss, 1983; Catteral et al., 1986), there is a bulk of new findings pointing to other mechanisms that may also be involved in the parasite's destruction. The first of them appeared in 1985, with the reported results on the ability of avian macrophages to kill *T. cruzi* and *L. mexicana,* while *T. gondii* survived (Meirelles & De Souza, 1985). As the killing of both *T. cruzi* and *T. gondii* is assumed as a parameter for macrophage activation (Gordon, 1978), also commonly correlated to the macrophage's ability to produce large amounts of reactive oxygen intermediates (Thorne & Blackwell, 1983; Nathan et al., 1979), the fact that in the same macrophage *T. gondii* can survive, while *T. cruzi* is killed, is suggestive for the involvement of another mechanism in the killing of the trypanosome. To further support this hypothesis, the authors showed that *T. gondii* survives within chicken macrophages but not within

activated peritoneal mouse macrophages (Meirelles & De Souza, 1985), suggesting that chicken macrophages are not "activated" in the common sense applied to the concept of peritoneal macrophage activation (Gordon, 1986). The levels of H_2O_2-production by the chicken macrophages and by resident peritoneal mice macrophages do not differ significantly (Meirelles, personal communication). The killing pathway that is mediated via O_2 metabolites has a fast kinectics, while in the case of the cited results, the invading trypomastigotes were able to transform into amastigotes, and just after 24-36 h post-infection the division ability of the latter was impaired within endocytic vacuoles of the avian macrophages, with the subsequent death of the parasite. One possibility is that *T. gondii's* escape of the killing is related to its ability to inhibit phagolysosomal fusion. This does not occur in the case of *T. cruzi,* and indicates a correlation with lysosomal effects. All these observations suggest the existence of different killing mechanisms in the chicken macrophages to digest trypomastigotes. Intriguing are also the results which show that the addition of phorbol-12-myristate-13-acetate (PMA), a trigger of the respiratory burst, when present during a 60 min incubation of *T. cruzi* with resident or activated macrophages, does not interfere with the ingestion of trypomastigotes, and in spite of decreasing, it increases that uptake of epimastigotes, as judged by a 4 or 24 hour observation (Carvalho & De Souza, 1987b). Four metabolites with different activities, other than cytokines, were shown to act *in vitro* upon resident macrophages enabling them to destroy trypomastigotes and amastigotes of *T. cruzi:* Cord factor (Kierszenbaum et al., 1984), a mycotic agent, and the physiological mediators leukotriene B4 and C4 (Wirth & Kierszenbaum, 1985a, b), lactoferrin (Lima & Kierszenbaum, 1985, 1987), and alpha-2-macroglobulin (Araújo-Jorge et al., 1989).

Treatment of the macrophages *in vitro* with cord factor (trehalose 6,6'-dimycolate), a glycolipid product of some microorganisms, known to stimulate some macrophage functions led to the enhancement of the association and the intracellular killing of *T. cruzi* (Kierszenbaum et al., 1984). In these studies, the involvement of peroxidase or hydrogen peroxide in the killing mechanism was discarded by the use of specific inhibitors that showed not any effect on the enhanced microbicidal activity of the macrophages. This was another evidence for the involvement of an O_2-intermediary metabolite-independent mechanism.

Alpha-2-macroglobulin is a physiological component which could modulate the phagocytic and microbicidal activities of resident macrophages upon trypomastigotes of *T. cruzi* (Araújo-Jorge et al., 1989). This mammal plasma glycoprotein and acute phase protein has the well recognized role of complexing with proteases to mediate their plasma clearance, and also some recently described effects upon immunological effector functions of lymphocytes and macrophages, and can be produced and secreted by these cells. The previous treatment of macrophages with alpha-2-macroglobulin turns them able to ingest and kill the three developmental stages of *T. cruzi* at greater levels, without affecting the H_2O_2 production, as measured by an ELISA assay. The same treatment affects other receptor-mediated phagocytic pathways, the IgG-opsonized erytrhocytes via Fc-R, The IgM-C-opsonized erythrocytes via CR and asialo-erythrocytes via Gal-R.

The effect of previous incubation of the macrophages with leukotrienes B4 and C4, metabolites of the lipooxygenase pathway of the arachidonic acid, which can be produced by the membrane enzyme phospholipase A2, is the enhancement in the trypomastigote-macrophage association indices after 1 h of interaction, and also the decrease in the survival of the parasites after a 24 h post-infection period (Wirth & Kierszenbaum, 1985a, b). Nevertheless, the controls of these experiments, as well as the ones of the studies using cord factor, were somewhat ambiguous, as the parasites also failed to develop in medium-treated macrophages, despite a lower parasitic load. The authors have shown indirect results suggestive of the involvement of the cGMP levels as a possible cause of the leukotriene C4 effects. The participation of O_2 metabolites as possible mediators of cytotoxicity for the parasites was not studied.

A similar effect was shown by this same group using lactoferrin (Lima & Kierszenbaum, 1985, 1987), an iron-binding protein found in higher levels in milk, saliva, gastrointestinal fluids and plasma during inflammatory conditions, which can be produced and secreted by neutrophils. These effects were reported using

two protocols: the interaction of amastigotes with previously lactoferrin-treated macrophages and the interaction of both cells in the presence of this iron-protein. The reactive oxygen intermediates were implicated as mediators of the parasite killing, being of special relevance the hydrogen peroxide and the hydroxyl radical, as the effect on killing could be completely abolished by catalase and by thiourea or alpha-ketobutyric acid, their respective scavengers. Superoxide ion and singlet oxygen were also partially involved, because their respective scavengers histidine and superoxide dismutase caused a partial inhibition of the killing activity. The requirement of iron ions at the lactoferrin molecule to enable the cytotoxic activity of the macrophage was shown recently (Lima & Kierszenbaum, 1987) by comparing the lack of a similar effect in the presence of iron chelator agents, as well as by iron-free human lactoferrin (apo-lactoferrin), a result which can be reversed after restoration of ferric ions prior to its use or after a co-incubation of apo-lactoferrin with another iron carrier, transferrin. These results support the recently reported suggestion of this group, that lactoferrin can be a marker for the amastigote form of *T. cruzi*, the unique stage expressing a lactoferrin receptor (Lima et al., 1988).

Summarizing, these results suggest that some physiological components, that may be soon recruted or secreted by the cells in the inflammatory sites of chagasic tissue lesions, can modulate the ability of the macrophages to face the infection, probably early in its acute phase, while a specific and effective immune response is being elicited.

5. PERSPECTIVES

The biochemical basis of the *T. cruzi* recognition will be better understood when isolated surface components of both the parasites and the macrophages could be available to be tested in *in vitro* assays, specially those suspected to act as ligands and receptors. The availability of Fab' fragments from polyclonal or monoclonal antisera against these components, will also help to elucidate the precise mechanisms of interaction. Monoclonal antibodies against macrophage receptors or macrophage-like mutant lineages, deficient in some receptor-mediated phagocytic systems, will also be useful tools in this area of investigation. Recent findings showed that the basic endocytic mechanisms are similar in non-professional phagocytic cells and on macrophages (Meirelles et al., 1986, 1987; Araújo-Jorge et al., 1986b). A similar behavior of macrophages and other non-professional phagocytic cells was noted in experiments of modulation of the parasite-cell interaction, as the treatment of host cells or parasites with different enzymes, lectins or carbohydrates, protease inhibitors or sulphydryl groups blockers (Araújo-Jorge & De Souza, 1984, 1986; Araújo-Jorge et al., 1986b, Henriquez et al., 1981b; Piras et al., 1983; Villalta & Kierszenbaum, 1983, 1984b, 1985a; Connely & Kierszenbaum, 1984, 1985; Ayala & Kierszenbaum, 1987). It is possible therefore, that the system of macrophage cultures, the primary culture system which is the easier to be obtained, will soon be considered a useful model to investigate the mechanism of action of drugs and immunomodulators, as a support for quimiotherapy and vaccination research.

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