INTERACTION OF Leishmania (L.) chagasi WITH THE Vero CELL LINE

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Summary:
The Vero cell line, a non-phagocytic cell, has supported the intracellular mechanism of Leishmania (L.) chagasi. This strain (MHOM/BR/501/MS00) was isolated from a human case of visceral leishmaniasis in Mato Grosso do Sul, Brazil and cultivated in Schneider’s Drosophila medium with 20 % of heat inactivated fetal calf serum. It was allowed to infect the Vero cells at a ratio of 10 to 20 promastigotes per cell. Within six hours of incubation, promastigote forms were found attached to Vero cells without any particular orientation. The number of amastigotes per cell increased during the incubation period. Results showed that promastigotes of L. (L.) chagasi could interact, transform to amastigote forms and multiply in non-phagocytic cells, demonstrating a new model to study the intracellular cycle of this protozoan.

KEY WORDS: Leishmania (L.) chagasi, Vero cells, infection.

Leishmania (Leishmania) chagasi is the causative agent of human and canine leishmaniasis in the New World and has frequently been found in several regions of Brazil. This protozoan develops its life cycle in two forms: in sandfly vectors, where they exist as flagellar promastigote forms and in infected mammalian hosts, where the parasites are found as amastigote forms.

In Leishmania infections, macrophages seem to have a dual role: first, they are the main cell type involved in host resistance to the parasite; second, these cells are the amastigotes main multiplication site. Some authors have reproduced in vitro this mechanism using peritoneal exudate cells from mice and hamsters along with macrophage murine tumor cells (Chang, 1976; Pearson et al., 1981; Aikawa et al., 1982). Leishmania is classically known for not having active means to invade cells, being forced to rely completely on the phagocytic capacity of the host cell for uptaking. However, their infection is not restricted to professional phagocytes and it has already been shown that it may occur in other cell lines (Rittig & Bogdan, 2000). Numerous investigators have reported the infection of various kinds of cell lines of non-professional phagocytes origin by several species of Leishmania, in vitro. Leishmania species have been noted to infect non-professional phagocytic cells such as fibroblast culture (Mattock & Petters, 1975; Chang, 1978; Dedet et al., 1983; Schwartzman & Pearson, 1985; Corté-Real et al., 1995). Furthermore, Hervás Rodrigues et al. (1996) were able to describe, by several methods, the presence of Leishmania amastigotes in fibroblasts of dog skin.

In this study, we observed the interaction between L. (L.) chagasi and Vero cell line (fibroblasts of African green monkey kidney), which had not been done before.
MATERIALS AND METHODS

PARASITES

The strain of *L. (L.) chagasi* (MHOM/BR/501/MS00) was isolated from a human case of visceral leishmaniasis in Mato Grosso do Sul, Brazil, and cultivated in Schneider's Drosophila medium supplemented with 20 % of heat inactivated fetal calf serum (FCS) at 24°C.

VERO CELL LINE

The Vero cells were maintained in RPMI 1640 medium supplemented with 10 % FCS, 2 % L-glutamine, 2.8 % bicarbonate buffer, Penicillin (100 U/ml) and Streptomycin (100 μg/ml) at 37°C in a 5 % CO₂ humidified incubator.

CELLS INFECTION

The cells were infected 24 hours after adhesion in a six well plate containing cover slips, at a ratio of 10 to 20 promastigotes per cell. The cover slips were fixed in Bouin’s solution in a period of 6, 24, 48, 72 and 96 hours after infection and Giemsa stained. The number of infected cells and the average number of intracellular amastigotes per cell were determined by counting 100 cells at least. We have repeated these experiments three times in order to ascertain that the experimental data was reproducible.

ULTRASTRUCTURAL EXAMINATION

Cells infected with *L. (L.) chagasi*, cultivated as described above, were fixed for one hour in a solution containing 2.5 % glutaraldehyde in 0.1 M cacodylate buffer and 3.5 % sucrose. The cells were rinsed in 0.1 M cacodylate buffer with 3.5 % sucrose and post-fixed with 1 % OsO₄ in the same buffer. They were rinsed again, dehydrated in acetone and embedded in Epon. The material was examined in a transmission electron microscope (Zeiss EM10C).

RESULTS

Light microscopical examination showed, six hours after infection, promastigote forms attached to Vero cells without any particular orientation (Fig. 1A, 1B). In the subsequent days, amastigote forms...
could be seen inside parasitophorous vacuole either alone or in groups (Fig. 1C, 1D). The mean number of amastigotes per infected cell increased from 0.15 amastigotes/cell at 24 hours to 0.37 amastigotes/cell 96 hours after infection. At the end of the incubation period many amastigotes could be seen inside the same vacuole.

The ultrastructure of Vero cells infected with *L. (L.) chagasi* was also studied: the cells presented their cytoplasm containing amastigote forms inside the parasitophorous vacuole membrane (Fig. 2A). After 48 hours, amastigote forms were found in initial stages of degeneration presenting vacuoles inside themselves (Fig. 2B).

The electron microscopy showed cells undergoing mitosis with chromosomes inside the nuclei, indicating that Vero cells and their parasites could grow together (Fig. 3).

**DISCUSSION**

Schwartzman & Pearson (1985) showed that *L. (L.) donovani* could interact with human foreskin fibroblasts but the multiplication of amastigotes was not observed, suggesting that the conditions required for promastigote-to-amastigote conversion might be different than those required for amastigote multiplication. Similar to the situation observed in most macrophage populations *in vitro*, a productive infection with a continuous increase in the number of intracellular parasites was rarely described in this non-macrophage host cells, suggesting that non-professional phagocytes do not stand *Leishmania* growth (Rittig & Bogdan, 2000). However, Côrte-Real et al. (1995) showed that *L. (L.) amazonensis* amastigote forms could multiply inside parasitophorous vacuoles of skin fibroblasts. In addition, our experiments also demonstrated that *L. (L.) chagasi* promastigotes could interact with Vero cells, convert to amastigotes and multiply inside the vacuoles, like they do with its main host cells, the macrophages. Bogdan et al. (2000) suggested that fibroblasts could be a less hostile environment for *L. (L.) major* than macrophages and, therefore, allow the persistence of the parasites after chemotherapy. Parasite survival and replication, however,
are subject to control by neighboring macrophages that are effective against *Leishmania* residing in fibroblasts and thereby help to maintain a stable host-parasite relationship.

Dedet et al. (1983) described, by transmission electron microscopy, different stages of degeneration of *L. (L.) amazonensis* amastigotes. In this infection of human fibroblast lines with *L. (L.) amazonensis*, most parasites disappeared within a week. Likewise, we observed *L. (L.) chagasi* amastigotes beginning to degenerate after 48 hours inside parasitophorous vacuoles. This led to the speculation that, in natural infection, promastigotes might convert to the more resistant amastigote forms within fibroblasts and that amastigotes released from fibroblasts might subsequently infect macrophages (Pearson et al., 1983). Furthermore, our results have suggested that the infection does not interfere with the cell lifecycle, just like Chang (1980) demonstrated with J774.G8 macrophages infected by *L. (L.) amazonensis*. Compared with macrophages, cytokine-activated fibroblasts had a reduced ability to express type 2 nitric oxide synthase, responsible for killing intracellular *L. (L.) major*. These data identify fibroblasts as an important host cell for *Leishmania* during the chronic phase of infection and suggest that they might be used as safe targets for the parasites in clinically latent disease (Bogdan, 2000). In our experiments, the Vero cell line could stand intracellular growth of *L. (L.) chagasi*, proving to be a good model to further studies about the necessary conditions for the conversion of promastigotes to amastigotes, considering that this line is an easily cultivated cell line.

**REFERENCES**


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