Leishmania (Viannia) lainsoni: occurrence of intracellular promastigote forms in vivo and in vitro

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Experimental chronic (45-day-old) skin lesion in hamster hind foot induced by Leishmania (Viannia) lainsoni *infection showed the presence of promastigote forms in the tissue, inside parasitophorous vacuoles, as assessed by transmission electron microscopy. Experimental in vitro interaction (24 and 48 h) between* Leishmania (V.) lainsoni *and J774-G8 macrophage cells also demonstrated the same profile. This morphological aspect is unusual, since in this parasite genus only amastigote forms have been described as the resistant and obligate intracellular forms.*

Key words: *Leishmania* - lesion - macrophage - ultrastructure

Parasitic protozoa belonging to the *Leishmania* genus are pathogens that alternate between two evolutive stages: a flagellate promastigote form, which lives as an extracellular parasite in the digestive tract of phlebotomine sand flies, and a non-motile amastigote form, which lives as an obligatory intracellular parasite of mammals. Macrophages are the preferred mammalian host cells, where amastigotes survive and multiply within membrane-bound compartments called parasitophorous vacuoles (Antoine et al. 1999). *Leishmania (Viannia) lainsoni* was first isolated from human single skin lesions in the state of Pará, at the Brazilian Amazon region (Silveira et al. 1987). Studies on this *Leishmania* species are of interest, as biological, biochemical, and molecular analysis revealed its divergent taxonomic position (reviewed in Corrêa et al. 2005a), as well as its atypical biological behavior in axenic culture (Corrêa et al. 2005b). Here we present an unusual characteristic of this parasite, which is the presence of the promastigote form in a 45-day-old hamster skin lesion. Similar promastigote forms could be also found inside parasitophorous vacuoles of J774-G8 macrophages in vitro, after 24 and 48 h of interaction. This record is remarkable within the *Leishmania* genus.

L. (V.) lainsoni (strain MHOM/BR/1981/6425) promastigotes were maintained axenically at 25ºC by serial passages in LIT medium supplemented with 10% fetal calf serum (FCS). Stationary phase promastigotes (1.0×10^6 cells/ml) were rinsed three times in phosphate buffered saline, pH 7.2 (PBS) and then about 100 µl of this cell suspension were inoculated subcutaneously into the hind foot of a hamster (*Mesocricetus auratus*) and as a negative control another animal was inoculated with the same volume of PBS. After 45 days the animals were sacrificed and a skin sample was obtained from the inoculation site and processed for conventional transmission electron microscopy (TEM). Briefly, the samples were fixed for 2 h with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), washed in buffer and then post-fixed for 1 h with 1% osmium tetroxide/0.8% potassium ferricyanide/5 mM calcium chloride in 0.1 M cacodylate buffer (pH 7.2). After dehydratation in acetone series, the samples were embedded in PolyBed-812 resin (Polysciences, Warrington, PA, US) and polymerized for 72 H at 60ºC. Ultrathin sections were stained with uranyl acetate and lead citrate and then observed in a Zeiss (Oberkochen, Germany) EM10C transmission electron microscope.

Furthermore, a cell line of J774-G8 macrophage was maintained by plating in 25 cm^3 culture flasks containing D-MEM medium (Sigma Chemical Co., St. Louis, MI, US) supplemented with 10% FCS and incubated at 37ºC in a 5% CO_2 atmosphere. Stationary phase promastigotes were resuspended in fresh D-MEM medium and then incubated at 34ºC with about 3×10^6 macrophages, at a 5:1 (parasites:macrophage) ratio. After 2 h of interaction the infected cultures were rinsed with PBS to remove non-adhered parasites, and then further incubated at 37ºC and 5% CO_2 atmosphere. The infected macrophages were maintained in cultures for 24 or 48 h and then harvested, fixed and processed as the biopsy material.

Analysis of the hamster 45-day-old skin lesion by TEM showed amastigote forms with the characteristic round shape, a centrally located nucleus, a bar-shaped kinetoplast, and large lipid inclusions (Fig. 1). However, unexpected intracellular promastigote forms were also found in the chronic lesion site, with the typical elongated body shape and a free flagellum (Fig. 1). Similarly, after 24 h of interaction between *L. (V.) lainsoni* and J774-G8 macrophages, promastigote forms could be found inside parasitophorous vacuoles located close to the host cell nucleus (Fig. 2). Occasionally, the promastigote form was observed inside parasitophorous vacuoles in cells that had been incubated for 48 h (Fig. 3).

Henriques and De Souza (2000), using *L. (L.) amazonensis*, have reported intravacuolar promastigote

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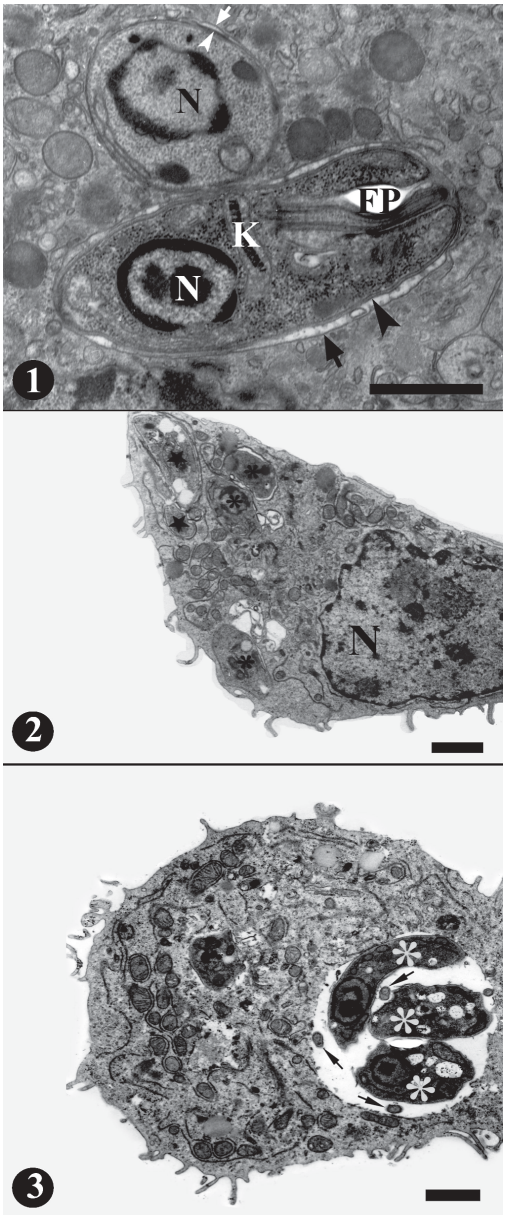

Fig. 1: transmission electron microscopy of a 45-day-old hamster skin lesion, showing amastigote and promastigote forms inside host cell vacuoles. Arrowheads: parasite membrane; arrows: parasitophorous vacuole membrane. N: nucleus; K: kinetoplast; FP: flagellar pocket. Fig. 2: infected J774-G8 macrophage after 24 h of interaction, containing intracellular amastigotes (*) and promastigotes (★). N: nucleus. Fig. 3: infected J774-G8 macrophage after 48 h of interaction. Note the vacuole containing promastigotes (*) and flagella (black arrow). Bars = 1 µm.

forms as a result of assays with short interaction periods (60 min). Unlike the former parasite, *L. (V.) lainsoni* exhibited promastigote forms in the hostile environment of macrophages both in vivo and in vitro after considerable longer periods.

We can speculate that such intracellular promastigote forms may arise several times during the mammal infection with *L. (V.) lainsoni*. The differentiation to promastigotes is usually associated to amastigotes released in the insect host after blood meal. While in the vertebrate host, amastigotes in the parasitophorous vacuole give rise to other amastigote forms. The interesting point in our results is that intravacuolar amastigotes may be giving rise to promastigote forms. At the moment we have no explanation for such phenomenon.

These unexpected characteristics of *L. (V.) lainsoni* deserve further investigation. Therefore, further biochemical and molecular analysis are ongoing with this *L. (V.) lainsoni* strain, in an attempt to obtain more knowledge on this unusual parasite differentiation.

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REFERENCES

Antoine JC, Lang T, Prina E, Courret N, Hellio R 1999. H-2M molecules, like MHC class II molecules, are targeted to parasitophorous vacuoles of *Leishmania*-infected macrophages and internalized by amastigotes of *L. amazonensis* and *L. mexicana*. *J Cell Sci 112*: 2559-2570.

Corrêa JR, Brazil RP, Soares MJ 2005a. *Leishmania (Viannia) lainsoni* (Kinetoplastida: Trypanosomatidae), a divergent *Leishmania* of the *Viannia* subgenus. *Mem Inst Oswaldo Cruz 100*: 587-592.

Corrêa JR, Santos SG, Araújo MS, Baptista C, Soares MJ, Brazil RP 2005b. Axenic promastigote forms of *Leishmania (Viannia) lainsoni* as an alternative source for *Leishmania* antigen production. *J Parasitol 91*: 551-556.

Henriques C, De Souza W 2000. Redistribution of plasma-membrane surface molecules during formation of the *L. amazonensis*-containing parasitophorous vacuole. *Parasitol Res 86*: 215-225.

Silveira FT, Shaw JJ, Braga RR, Ishikawa E 1987. Dermal leishmaniasis in the Amazon region of Brazil: *Leishmania (Viannia) lainsoni* sp.n., a new parasite from the state of Pará. *Mem Inst Oswaldo Cruz 82*: 289-292.