Artigo Original

Persistence of *Leishmania* antigen in C57Bl/6j inbred mice infected with *Leishmania (Leishmania) amazonensis*

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SUMMARY – PURPOSE. To develop an animal model for studying mucocutaneous leishmaniasis.

METHODS. The hind footpad of C57Bl/6j inbred mice was experimentally infected with 10^7 *Leishmania (Leishmania) amazonensis* promastigote and the skin was studied through light and electron transmission microscopy and immunohistochemistry (PAP) techniques.

RESULTS. There were morphological evidences of cellular immune mechanisms and hypersensitivity reaction after eight weeks of infection and metastasis and well shaped parasites at ultrastructural level by fifty-one weeks post infection. Relapse of infection with mucosa lesions occurred around the 50^{th} week after inoculation.

CONCLUSION. The use of this animal model in long term follow up could be an useful experimental model for human mucocutaneous leishmaniasis.

KEY WORDS: Experimental mucocutaneous leishmaniasis. Inbred mice. Transmission electron microscopy. Immunohistochemistry.

INTRODUCTION

Increasing in tourism and labors migration disseminated leishmaniasis throughout the world[1]. In the New World there is a particular expression of the disease called American Tegumentary Leishmaniasis, comprising three clinical features: Localized Leishmaniasis (LCL), Diffuse Cutaneous Leishmaniasis (DCL) and Mucocutaneous Leishmaniasis (MCL)[2].

Some authors[3] suggested that DCL and MCL could be both originating from *Leishmania (Leishmania) amazonensis*[4], but the mechanisms by which *Leishmania* leads to MCL remains obscure, owing mainly to the lack of an appropriate animal model[5-6].

The great majority of papers concerning to animals' models with different susceptibilities to *L. (L.) amazonensis* do so from an immunological point of view. Morphologic studies about the inflammatory response of the inoculation site are scarce and brief in follow up periods of infection. The aim of this study is to propose this model to study the human disease, according to the histopathologic features of the inoculation site and the clinical aspects, in the course of one-year infection.

MATERIAL AND METHOD

Animals. Female 40-day-old inbred mice C57Bl/6j strain from the University of São Paulo Medical School General Colony was used. The animals were maintained in plastic cages and received proper food and water *ad libitum*.

Parasites. *Leishmania (Leishmania) amazonensis* (WHOM/BR/00LTB0016) - G. Grimaldi, Fiocruz - RJ. The strain was sustained in the laboratory through sequential passages in BALB/c mice, culture in NNN / BHI medium and reinoculation at hind footpad in mice[7].

Infection. Experimental groups, each with three animals were inoculated under ether anesthesia with 50 µl of saline solution containing 10^7 *Leishmania (Leishmania) amazonensis* promastigote forms in the stationary phase of growth in the right hind footpad of mice. There was one control animal in each experimental group, inoculated under the same conditions with 50 µl of sterile saline solution. Three inoculated animals and one control were killed 2, 6, 8, 10, 20 and 51 weeks post inoculation (WPI).

Techniques. Fragments from right hind footpad skin were fixed in buffered 10% formaldehyde solution, pH 7.2 and processed by usual histology technique and stained with Hematoxilyn and Eosin. Fragments from the same tissue were processed for immunohistochemistry (PAP) demonstration of *Leishmania* antigen in tissues[18] and for Transmission Electron Microscopy[9].

The experiment was repeated twice.

RESULTS

Clinical features. C57Bl/6j mice put on weight along the experiment. The animals displayed a

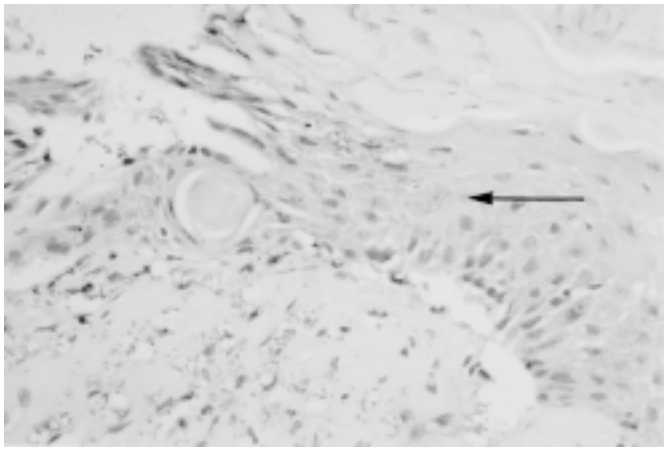

Fig. 1 – *Hind footpad – elimination of cellular debris and parasites (→) through epidermis. Experimental cutaneous leishmaniasis, C57Bl/6j inbred mice, inoculation of 10^7* ***L. (L.) amazonensis*** *promastigote forms at right hind footpad (20^{th} week post infection, PAP, OM 200x).*

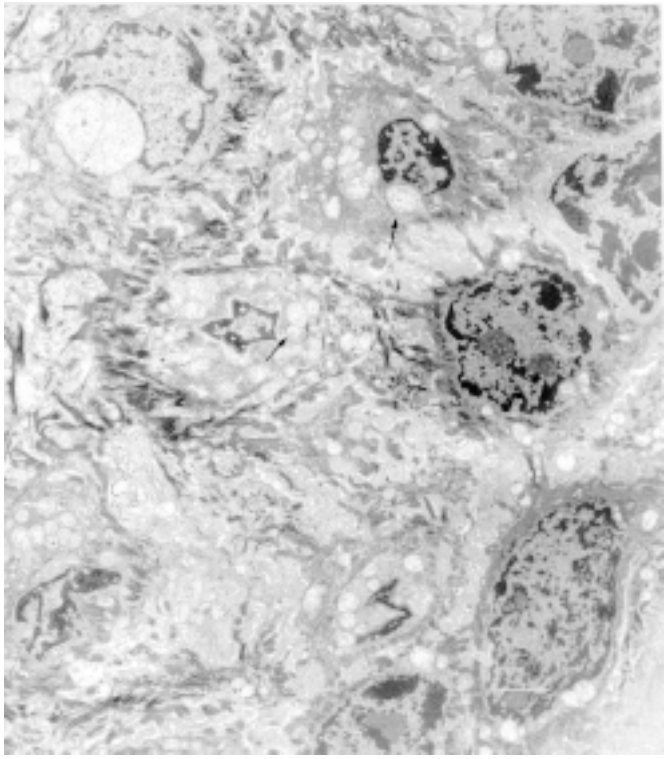

Fig. 2 – *Hind footpad – epidermal keratinocytes mitochondria crystolysis (→). Esperimental cutaneous leishmaniasis, C57Bl/6j inbred mice, inoculation of 10^7* ***L. (L.) amazonensis*** *promastigote forms at right hind footpad (1^{st} week post infection, Transmission Electron Microscopy, Bar = 1µm).*

torpid disease with an apparent healing of hind footpads' cutaneous lesions around 20^{th} WPI. By 50^{th} WPI there was relapse of the lesions at the inoculated footpad and appearance of lesions at the tail and in the nose of all animals.

Histopathology. The C57Bl/6j hind footpad disclosed a rather organized tissue response with granuloma formation after the 8th WPI and fibrosis in the dermis, together with hyperplasia of epidermis, after the 38th WPI. Eosinophils were commonly seen. The nerve endings of the dermis were surrounded by dense macrophage infiltrate very close to the perineurial sheet.

Late in the course of the infection (51st WPI) there were grouped lymphocytes surrounding the vessels of the dermis that showed fibrinoid degeneration. Necrosis was extensive and confluent, coming soon after 2nd WPI and followed by granuloma formation and collagen deposition in the dermis. By the time the dermis of C57Bl/6j mice disclosed granuloma formation and collagen deposition, its epidermis displayed exocytosis of mononuclear cells, acanthosis and transepidermal elimination of parasites (evident by the 20^{th} WPI). The microscopic aspects of control animals were quite normal.

Immunohistochemistry (PAP). This technique displayed well-shaped amastigotes forms of *Leishmania* stained in golden brown in the inoculated hind footpad skin. There was no evidence of amorphous antigenic material inside the cytoplasm of macrophages, as seen in human lesions[8]. By the 20^{th} WPI there was elimination of cellular debris and parasites through the epidermis (Fig. 1).

Transmission electron microscopy. The inoculation site disclosed epidermal keratinocytes mitochondria crystolysis (Fig. 2) throughout the experiment. Some eosinophils showed phagocytised parasites (Fig. 3). There were preserved parasites within macrophages (Fig. 4) and in the extracellular space among collagen fibers of the dermis by 51st WPI.

DISCUSSION

The epidermis of C57Bl/6j mice was clearly involved in the inflammatory process since the beginning of the infection and showed areas of ulceration and hyperplasia. In spite of the presence of parasites in the right hind footpad until the 51^{st} WPI, this animal seems to be able in circumscribing the infection[10,11]. Necrosis was intense with elimination of parasites through the epidermis and the granuloma surrounded necrosis foci, which were partially replaced by collagen.

C57Bl/6j mice showed a well-developed tissue response (granuloma formation) by the 8th week post infection, in spite of macrophage parasitism. This was coincident with the appearing of morphological evidences of cellular immune mechanisms (granuloma) and of hypersensitivity reaction (fibri-

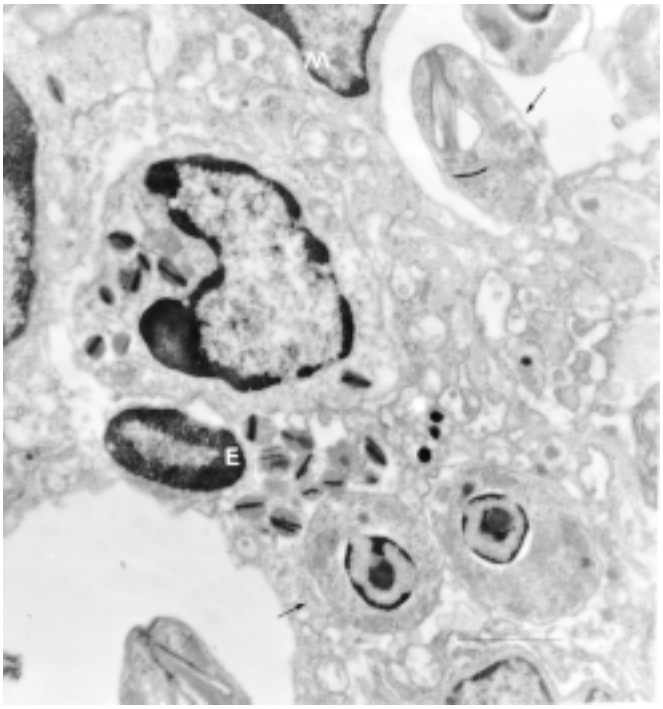

Fig. 3 – *Hind footpad – Leishmania amastigote forms (→) inside eosinophil (E). Experimental cutaneous leismaniasis, C57BL/6j inbred mice, inoculation of 10^7* ***L. (L.) amazonensis*** *promastigote forms at right hind footpad (6^{th} week post infection, Transmission Electron Microscopy, Bar = 1µm)*

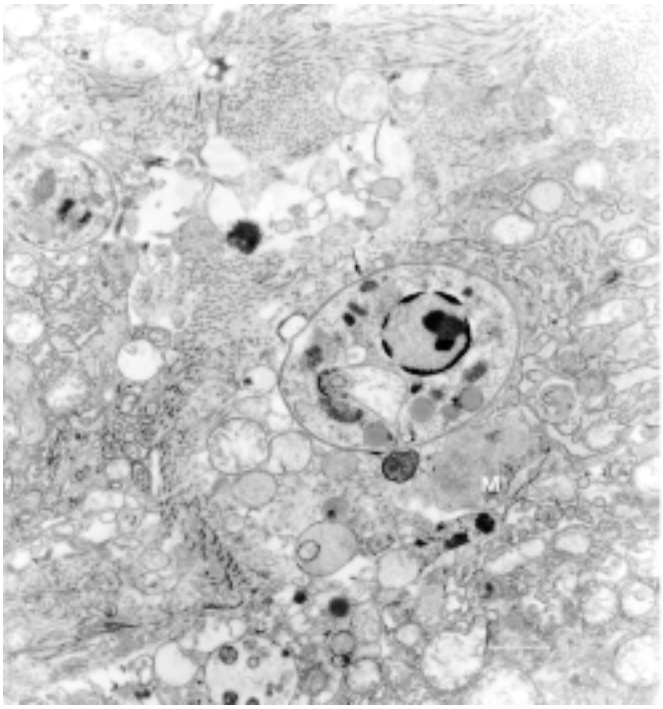

Fig. 4 – *Hind footpad – Upriht amastigote forms (→) inside macrophge (M). Experimental cutaneous leishmaniasis, C57Bl/6j inbred mice, inoculation of 107* ***L.(L.) amazonensis*** *promastigote forms at right hind footpad (51st week post infection, Transmission Electron Microscopy, Bar = 1 µm).*

noid degeneration of vessel's walls and epidermal aggression by the inflammatory infiltrate). This histologic aspect is similar to that of human Mucocutaneous Leishmaniasis[12,13]. Besides, by the final period of the experiment, cutaneous lesions reappeared mucous lesions appeared and well-preserved parasites could be seen at the ultrastructural level. Thus, such an animal model presented here may be used for experimental studies of human mucocutaneous leishmaniasis.

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RESUMO

Persistência do antígeno da *Leishmania* no camundongo isogênico C57Bl/6j infectado com a *Leishmania (Leishmania) amazonensis*.

OBJETIVO. Desenvolver um modelo experimental para o estudo da leishmaniose cutâneo-mucosa.

MÉTODOS. O coxim plantar traseiro de camundongos isogênicos C57Bl/6j foi inoculado com 10^7 formas promastigotas da Leishmania (Leishmania) amazonensis *e a pele foi estudada através da microscopia óptica e eletrônica e de técnica imunohistoquímica (PAP).*

RESULTADOS. Ocorreram evidências morfológicas de mecanismos imunes mediados por células, concomitantemente ao de reação de hipersensibilidade, após a oitava semana de infecção e a presença de parasitas com ultraestrutura preservada na quinquagésima primeira semana após a infecção. Houve recidiva da infecção com surgimento de lesões mucosas por volta da 50ª semana pós inoculação.

CONCLUSÃO. Este modelo animal, com um período de tempo de seguimento prolongado, poderia ser empregado como modelo para o estudo experimental da leishmaniose cutâneo-mucosa. [Rev Ass Med Brasil 1999; 45(3): 225-8.]

UNITERMOS: Leishmaniose cutânea experimental. Camundongos isogênicos. Microscopia eletrônica de transmissão. Imunohistoquímica.

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