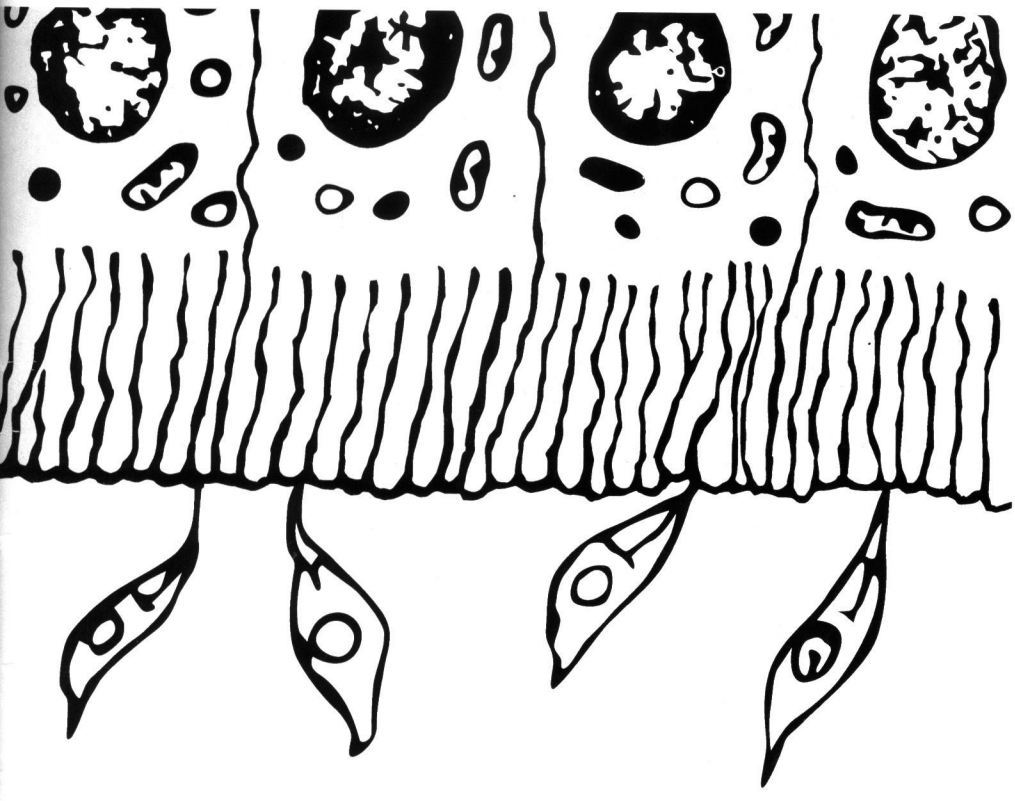
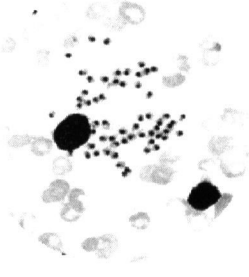


CYTED-RIMLEV



WORKSHOP ON CANINE VISCERAL LEISHMANIASIS

CPqGM-FIOCRUZ (SALVADOR, BA, BRAZIL)

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In *vitro* and in *vivo* models to study the molecular basis of *Leishmania* pathogenesis.

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In vivo model. Infection by *Leishmania amazonensis*, and not by *Leishmania braziliensis*, induces, in a minority of human patients, a progressive skin disease, with multiple skin nodules and a Th1 anergy to *Leishmania* antigens – the diffuse cutaneous leishmaniasis (DCL). DCL is, therefore, a clearcut parasite-species dependent disease outcome: it may occur in *L. amazonensis*, and not in *L. braziliensis*, infected individuals. BALB/c mice are relatively resistant to *L. braziliensis* infection, and skin lesions at parasite injection sites spontaneously subside in most animals. On the other hand, these mice are highly susceptible to *L. amazonensis* infection. Our research group has recently shown that previous and concomitant intravenous injections of a *L. amazonensis* extract enhance the cutaneous infection by *L. braziliensis*. The enhancement has been found to be dependent on the presence of IL-4. The enhancement of the infection can also be induced by only one intradermic injection of a much lower amount of *L. amazonensis* extract than the amounts that have to be injected intravenously. *L. braziliensis* extracts did not enhance the infection when injected intravenously. However, they enhanced the infection when injected intradermically at low amounts, but, contrasting with *L. amazonensis* extract, not when injected at relatively large amounts. Intravenous or intradermal injections of the extract had no *Leishmania* infection-enhancing effect in C57Bl/6 mice. This *in vivo* model uses very little amount of parasite molecules and can be used to identify the *Leishmania* molecules responsible for the infection enhancement effect in BALB/c mice. *In vitro* model. The effect of *L. amazonensis* extract on macrophage functions *in vitro* was investigated as an effort to study a possible mechanism implicated on the aggravation of the experimental cutaneous leishmaniasis induced by *L. amazonensis* extract treatment. The *L. amazonensis* extract suppressed the production of inflammatory cytokines (IL-12p70, IL-6 and TNF-α) by LPS-activated resident peritoneal murine macrophages. Moreover, *L. amazonensis* extract treatment was able to downmodulate the nitric oxide production by activated macrophages. On the other hand, *L. amazonensis* extract increased the production of IL-10 by LPS-activated macrophages, but was not able to upregulate the production of IL-10 in resting macrophages. LPS activates macrophages through the engagement of toll-like receptor 4 (TLR-4), which can also be the target of host-derived ligands, such as neutrophil elastase or products of its enzymatic activity. The *in vitro* treatment of LPS-activated BALB/c macrophages by *L. amazonensis* extract or

purified molecules may constitute a useful model to study the effect of *Leishmania*-derived factors on the main cellular host of the parasite. In the present study, the model was also used to show that fractionation of the extract by liquid chromatography abrogates its enhancing effect on IL-10 and its inhibiting effect on IL-6 and NO productions by macrophages, indicating a synergistic effect of at least two different factors. The identification of the factors and mechanisms that modulate macrophage responses against *Leishmania* may be important for the development of new strategies to combat leishmaniasis.