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LETTER TO THE EDITOR

Schwann cells as putative safe host cells for *Leishmania amazonensis*

A hallmark of Leishmania infection is the invasion of tissue macrophages by the parasite as an essential step for the establishment of parasitism. In fact, macrophages are considered the most important host cell for Leishmania parasites,[1] although several other cell types are able to endocytose Leishmania in vitro or in vivo.[2–4] Previous data published by our and other groups support the notion that Schwann cells (SCs) act as a putative host cell for Leishmania infection.[5,6] In this context, we hypothesized that SCs, abundant in highly innervated skin, might form a less hostile environment for Leishmania than macrophages and, thus, enable the persistence of the parasite. We evaluated the infection of a human SC line (ST88-14) by promastigotes of *Leishmania amazonensis* (LA) and also the potential effect of infection on nitric oxide (NO) production by these cells. The ST88-14 SC line represents a good model for our study because these cells express some phenotypical markers of normal SCs.[7,8]

ST88-14 cells (generously donated by Jonathan Fletcher, Dana Farber Cancer Institute, Boston, USA) were isolated from a patient with neurofibromatosis type 1. LA (MHOM/BR/77/LTB0016), isolated from a human case of cutaneous leishmaniasis, was kindly supplied by Dr. Gabriel Grimaldi Jr. (Fundação Oswaldo Cruz, Rio de Janeiro, Brazil). An in vitro protocol for infection of ST88-14 by LA promastigote forms was performed at skin temperature (34 °C), as previously described.[6] The percentage of ST88-14 cells containing internalized parasites was determined after Giemsa staining and examination in bright-field optics. In order to investigate NO production, culture supernatants were analyzed for their content of nitrite using the Griess reaction.[9] Highly purified bovine serum albumin (BSA) at 2% concentration was tested in place of fetal bovine serum (FBS) during the NO measurement and infection protocols. The supernatants from infected and non-infected ST88-14 cells were collected at different times and afterwards mixed with 100 μl of the Griess reagent. After 10 min incubation at room temperature, the optical density was measured in a microtiter plate reader (Flow, Meckenheim, Germany) at 546 nm against a medium without phenol red as blank. A sodium nitrite standard curve (0—100 μM) was generated in parallel.

As interferon-γ (IFN-γ) plays a major role in NO production, we investigated whether treatment with recombinant IFN-γ (rIFN-γ) could alter the base levels of NO in ST88-14

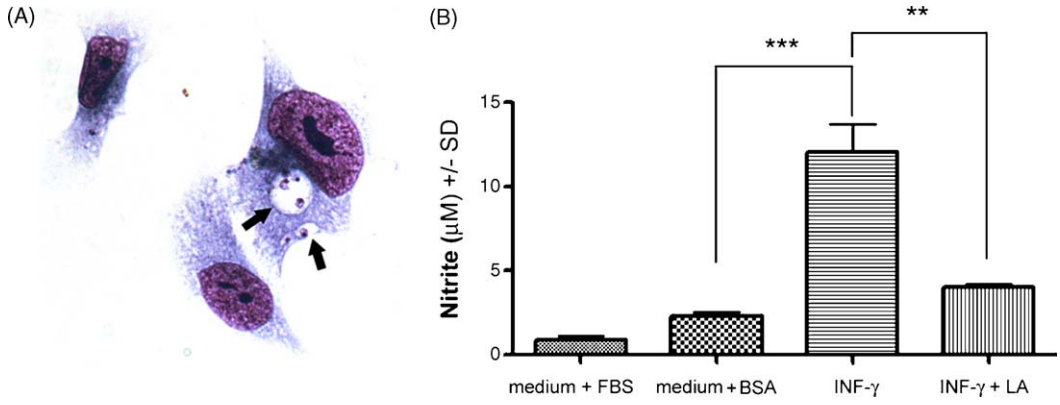

Figure 1 Infection of ST88-14 cells by *L. amazonensis* (LA) and the effect of this infection on INF-γ stimulated nitrite production. ST88-14 cells either treated with INF-γ or untreated were infected for 24 hr at 34 °C with LA (ten parasites per cell), washed and incubated at 37 °C. (A) After 24 hr at 34 °C, some parasitophorous vacuoles (arrows) were observed in the cytoplasm of the cells. (B) Nitrite concentration was measured in the supernatants for 48 hr. Note the substantial increase in NO release by ST88-14 cells after 48 hr of treatment with INF-γ; this was impaired during stimulation of infected cells. *** $P < 0.001$; ST88-14 cells treated with INF-γ versus ST88-14 cells maintained in medium plus BSA. ** $P < 0.002$; ST88-14 cells treated with INF-γ versus ST88-14 cells infected by LA and treated with INF-γ. Each bar represents the mean ± SD of two experiments, each of which was done in triplicate.

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cells both infected and not infected by LA. Treatment of the cultures with rIFN-γ (100 U/ml) resulted in a substantial increase in NO release by ST88-14 cells after 48 hr of stimulation at 37 °C. On the other hand, this increase was not observed in cultures previously infected by LA at 34 °C for 24 hr and maintained for an additional period for 48 hr at 37 °C (Figure 1). Neither control nor infected cells augmented their (low) production of NO by incubation with lipopolysaccharide (LPS). No synergic action was observed after LPS combination with rIFN-γ not shown).

Our results show that rINF-γ treatment drives SCs into a potentially lethal function, increasing NO levels, which in turn can be suppressed by LA infection. These data contrast markedly with previous work carried out in a murine macrophage cell line, in which it was observed that addition of rIFN-γ restores NO levels in cultures that were infected with LA before LPS stimulation.[10] Thus, we suggest that, in contrast to macrophages, SCs might serve as safe targets for the parasite, providing a more stable environment for evasion of the immune system. Collectively, the preliminary results presented here, as well as our previous work,[6] identify SCs as a prospectively important host cell for Leishmania. SCs might exert immunoregulatory functions determining susceptibility or resistance to this intracellular parasite.

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Conflict of interest: No conflict of interest to declare.

The authors have no commercial association in any of the products, drugs or instruments described in the article. This study was approved by our local ethics committee.

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