Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Parasitology 109 (2005) 72–79
Experimental Parasitology
www.elsevier.com/locate/yexpr

Leishmania (*Viannia*) *braziliensis*: human mast cell line activation induced by logarithmic and stationary promastigote derived-lysates

Márcia Pereira de Oliveira[a,d], Marcia Coronha R. Lima[b], Andrea S. Calheiros[b], Marco A. Martins[b], Paulo Renato Z. Antas[c], Paula Melo De Luca[c], Claude Pirmez[a,*]

[a] *Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil*
[b] *Departamento de Fisiologia e Farmacodinâmica, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil*
[c] *Departamento de Imunologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil*
[d] *Curso de Medicina, Universidade Estácio de Sá, Rio de Janeiro, RJ, Brazil*

Received 5 May 2004; received in revised form 16 November 2004; accepted 18 November 2004

Abstract

Herein we investigate the ability of live promastigotes and total lysate of *Leishmania* (*Viannia*) *braziliensis*, derived from parasites in the logarithmic (L-Lb) or stationary phase (S-Lb), to induce human mast cell line (HMC-1) activation. In comparison with medium-treated cells, a significant histamine release was observed in HMC-1 cultures stimulated with S-Lb. Lipophosphoglycan also induced histamine release by HMC-1 cells. In immunocytochemical assays, we found a marked staining for tryptase in medium-treated HMC-1 cells, however, stimulation with L-Lb or S-Lb caused a marked decrease in the color reaction as well as in the number of tryptase-positive cells. L-Lb and S-Lb induced an evident decrease in the intracellular expression of IL-4 but not IL-12. Live stationary promastigotes were able to induce high levels of IL-4 release in HMC-1 cultures. Furthermore, these cells released significant amounts of IL-12 when incubated with both types of live promastigotes. These results indicate that *L.* (*V.*) *braziliensis* promastigotes differ in their ability to induce direct human mast cells activation, according to the growth phase of the parasite. Furthermore, the release of pro-inflammatory mediators and cytokines could represent an important phenomenon that might favor the initial establishment of the infection.

Index Descriptors and Abbreviations: Leishmania braziliensis; Human mast cell line HMC-1; LPG; Histamine; IL-4; IL-12; HMC-1, human mast cell line; *Leishmania* (*Viannia*) *braziliensis* lysate derived from logarithmic (L-Lb) or stationary phase (S-Lb) promastigotes; LPG, lipophosphoglycan; interleukin 4 (IL-4) and 12 (IL-12); FCS, fetal calf serum

1. Introduction

Obligatory intracellular protozoan parasites of the genus *Leishmania* are transmitted as promastigotes to humans and other mammalian hosts by infected female sand flies of the *Phlebotominae* subfamily. *Leishmania* (*Viannia*) *braziliensis* infection leads to a broad spectrum of clinical, histopathological, and immunological manifestations ranging from self-healing cutaneous lesions to severe destructive nasal/oral mucous membrane lesions (Grimaldi and Tesh, 1993; Oliveira-Neto et al., 2000). It has been shown that resistance or susceptibility requires the development of T cell-mediated immune response, characterized by the expansion of functionally opposed Th1 and Th2 subsets (Heinzel et al., 1989). However, a great variety of accessory cells, including mast cells, may be involved in host defense against the parasite (Belkaid et al., 2000; Modlin et al., 1989; Scharton and Scott, 1993; Wershil et al., 1994).

* Corresponding author. Fax: +55 21 2590-3495.
E-mail address: pirmez@fiocruz.br (C. Pirmez).

0014-4894/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2004.11.011

Mast cells have been described as effector cells of allergic reactions, but they also play key roles in innate or acquired immunity (Crivellato et al., 2004; Taylor and Metcalfe, 2001). Indeed, bacterial and parasite antigens modulate secretory and proliferative functions of human and rodent mast cells (Henz et al., 2001). Mast cell activation through IgE-dependent and independent stimuli results in the release of pro-inflammatory and immunoregulatory molecules that are crucial not only in acute allergic reactions but in more persistent and chronic inflammatory responses as well (Boyce, 2003; Mecheri and David, 1997). Recent findings indicate that human mast cells may play important roles in innate and adaptative immunity through expression of a functional Toll-like receptor 4 induced by interferon-γ (Okumura et al., 2003). The HMC-1 cell line was derived from the peripheral blood of a patient with mast cell leukaemia. Previous reports verified that these cells stain metachromatically by toluidine blue, store low levels of histamine, contain tryptase activity, and secrete many cytokines (Butterfield et al., 1988; Grabbe et al., 1994; Nilsson et al., 1994).

It has been demonstrated that resistant and susceptible strains of mice exhibited an extensive dermal mast cell degranulation in the early stages of infection with *Leishmania major*, and that mast cells seem to be associated with an increase of the intensity and maximal size of the cutaneous lesions (Wershil et al., 1994). Furthermore, metacyclic *L. major* and *L. infantum* promastigotes are infective for mouse mast cells (Bidri et al., 1997). Based on these data, it is reasonable to speculate that a direct activation of mast cells by *Leishmania* could be related with immunoregulatory mechanisms of this infection.

Our study reports the ability of human mast cell line HMC-1 to release histamine and cytokines after direct stimulation with live forms or total lysate derived from *L.* (*V.*) *braziliensis* promastigotes as well as purified lipophosphoglycan (LPG).

2. Materials and methods

2.1. Parasites

Leishmania (*Viannia*) *braziliensis* promastigotes (MHOM/BR/75/2903) were grown in Schneider's medium (Sigma) supplemented with 10% heat-inactivated fetal calf serum (FCS) at 28 °C. Promastigotes at logarithmic or stationary phase were harvested and washed in saline three times. The logarithmic (L-Lb) or stationary phase lysates (S-Lb) were obtained by disrupting the parasites in 10 cycles of freezing and thawing, followed by ultrasonication (Lab-Line Instruments, Model 9130, Melrose Park, ILL) at 40 watts for 20 min in ice bath. LPG purified from logarithmic promastigotes of *L.* (*L.*) *donovani* was kindly provided by Dr. S. Turco (University of Kentucky, Medical Center, Lexington, KY, USA). All samples were stored at −20 °C until used. No endotoxin contamination was detected in the parasite preparations when tested by using the Quantitative Chromogenic LAL kit (BioWhittaker, Walkersville, MD) (data not shown).

2.2. Cell culture and stimulation assays

The human mast cell line HMC-1, kindly provided by Dr. Joseph H. Butterfield (Mayo Clinic, Rochester, MN, USA), was maintained at 37 °C and 5% CO_2 in Iscove's modified Dulbecco's medium (IMDM) (Sigma Chemicals, St. Louis, MO) supplemented with 10% FCS (HyClone Laboratories, Logan, UT), 2 mM L-glutamine (Pharmacia Biotech, Piscataway, NJ), 100 UI/mL penicillin, 50 μg/mL streptomycin, and 1.2 mM α thioglycerol (all purchased from Sigma). HMC-1 (1×10^6 cells/mL) was stimulated with the following parasite preparations: (a) live promastigotes at logarithmic or stationary phase (5, 10 or 20 parasites: 1 mast cell); (b) L-Lb or S-Lb (3 and 30 μg of total protein/mL); and (c) LPG (10–200 μg/mL). Saline-treated cultures were used as control. All stimulation assays were performed during 5, 15, and 30 min at 37 °C in a final volume of 200 and 500 μL for cytokine and histamine quantification, respectively. The reaction was stopped by the addition of ice-cold medium (v/v).

2.3. Cell viability

To exclude toxic effects of the experimental procedures on mast cell cultures, the viability of cells was monitored by trypan blue (0.1%) exclusion method. At concentration and time periods tested, almost 100% of the cells remained viable after incubation with the parasite preparations used in this study.

2.4. Histamine quantification

Histamine in supernatant and in pellet fractions was measured using the spectrofluorimetry method (Shore et al., 1959). Samples were collected after parasite stimulation, and both supernatant and cell pellets were obtained by centrifugation for 10 min at 4 °C. The supernatant was diluted in 0.8 N perchloric acid (Merck, Darmstadt) and stored at −20 °C until histamine quantification. Total cellular content of histamine was determined by lysing the non-stimulated cells in 0.4 N perchloric acid at 100 °C. Percentage of specific release of histamine was calculated as: $[(a-b)/c] \times 100$, where (a) is the amount of mediator release from activated cells, (b) is the release from non-activated cells, and (c) is the total cellular content. In all experiments histamine levels were measured in duplicate.