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Autor(en): **Reis, M.G. / Roters, S.B. / Barral-Netto, M.**

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[1] Centro de Pesquisas Gonçalo Moniz (FIOCRUZ),
[2] Hospital Prof. Edgar Santos, Universidade Federal da Bahia, Salvador, Bahia, Brazil

Immune serum from both susceptible and resistant strains of mice increases phagocytosis of *Leishmania mexicana amazonensis* by macrophages

M. G. Reis[1], S. B. Roters[2], M. Barral-Netto[2]

Summary

Immune sera obtained from either BALB/c mice (susceptible) at 7 weeks, or C57BL/6 mice (resistant), at 7 weeks after infection with *L. m. amazonensis,* were effective in increasing internalization of homologous promastigotes into starch-induced peritoneal macrophages (from both mouse strains). Both the internalization enhancing effect and the levels of anti-leishmanial antibody (ELISA) were removed from sera by absorption with heat-killed promastigotes. Sera at 1/200 dilution obtained from either mouse strain at 2 weeks after infection did not enhance parasite internalization into macrophages. The factors leading to susceptibility or resistance during leishmaniasis do not appear to be related to differences in antibody-mediated opsonic activity.

Key words: cutaneous leishmaniasis; murine leishmaniasis; *Leishmania; L. mexicana.*

Introduction

Although immune serum is not effective in protection against leishmaniasis it may contribute to the protection afforded by transfer of immune cells, as suggested by some observations (Mauel and Behin, 1974; Preston and Dumonde, 1976; Poulter, 1980). In addition, evaluation of immunoglobulins may yield evidence of T cell function by identifying IgG subclasses which are regulated by T cells (Mongini et al., 1981). Opsonic activity has a special role because it provides an interface between serum and cellular functions.

Correspondence: Dr. Manoel Barral-Netto, Hospital Prof. Edgard Santos, R. João das Botas s/n Canela, 40,000 Salvador, Bahia, Brazil

There are very few observations on the effect of serum upon the interiorization of *Leishmania* by macrophages. Herman (1980) demonstrated that anti-leishmanial hyperimmune serum from C57BL/6 mice contained cytophilic and opsonic antibodies, which increased phagocytosis of *L. donovani* by murine macrophages. Recently, it has been suggested that Fab fragments of monoclonal antibodies directed at certain *Leishmania* antigens may decrease attachment of *L. major* to macrophages (Handman and Goding, 1985).

Although serum from mice immunized with *L. mexicana* increases the phagocytosis of the parasite by macrophages (Bray, 1983) nothing is known about the opsonic effect of serum from susceptible and resistant strains of mice infected by *Leishmania* from the New World. In order to evaluate this effect we tested sera obtained from BALB/c (susceptible) or C57BL/6 (resistant) mice, at different periods after infection by *L. mexicana amazonensis.*

Material and Methods

Heat-inactivated (56 °C – 30 min) sera were pooled from five BALB/c or five C57BL/6 mice at 2 weeks and 7 weeks after infection by 5×10^6 promastigotes of *L. m. amazonensis* (MHOM/BR/76/Josefa). This strain has been previously characterized (Andrade et al., 1984). One milliliter aliquots of immune serum were incubated at 37 °C for 30 min with 10^9 heat-killed (56 °C – 60 min) promastigotes of the "Josefa" strain 3 or 6 times. Anti-leishmanial antibody titers before and after absorption were performed as described elsewhere (Barral-Netto et al., 1987).

Starch-induced peritoneal macrophages were obtained from BALB/c or C57BL/6 mice, collected in Dulbecco's Minimal Essential Medium (M. A. Bioproducts, Walkersville, MD) containing heparin (10 U/ml), 10 mM HEPES, 2×10^5 M 2-Mercaptoethanol, penicillin (100 U/ml) and streptomycin (100 μg/ml). After adjusting to 10^6 cells/ml in DMEM supplemented with 5% fetal calf serum (FCS), cells were cultured in 0.3 ml in 8-chambered slides (Lab-Tek Products, Div. Miles Lab., Westmont, Ill.), for 2 h at 37 °C in a humid atmosphere of 5% CO_2. Non-adherent cells were removed by repeated washing with supplemented medium. Macrophage cultures were infected with LIT-grown promastigotes ("Josefa" strain) at a ratio of 5:1. Tested serum or normal mouse serum, were added to give a final concentration of 0.5%. Promastigotes were incubated for 3 h at 35 °C in 5% CO_2 and 95% humidified air. Subsequently free parasites were removed by extensive washings with PBS. Cells were fixed in methanol and stained with Giemsa stain. Infection was evaluated calculating the percent of infected macrophages, as well as the number of parasites per 100 cells, in triplicate cultures. Comparisons were made by non-paired or paired samples Student's t test, as well as one-way analysis of variance where appropriate.

Results and Discussion

Table 1 shows that sera obtained from BALB/c or C57BL/6 mice at different periods of infection by *L. mexicana amazonensis,* increase interiorization of homologous promastigotes, when compared to normal mouse sera of each strain. Comparison between immune and normal serum, for each time point, have shown that differences reached statistically significant levels ($p < 0.05$), for both strains at 7 weeks, but not at 2 weeks after infection. Results are presented on homologous macrophages, but similar enhancing effect was obtained with heterologous combinations of macrophages and sera. Differences

Table 1. Comparison of the effect of serum from BALB/c and C57BL/6 mice, obtained at different periods of infection by *L. mexicana amazonensis*, upon internalization of promastigotes in homologous macrophages

Time post-infection	Parasites/100 mø		% infected mø	
	BALB/c*	C57BL/6*	BALB/c*	C57BL/6*
0	95±5.5	97±5.6	64.9±1.0	65.0±1.1
2 w	119±5.6	107±6	65.5±1.3	64.5±2.0
7 w	147±4.5**	157±5.5**	69.5±1.3**	70.5±1.1**

* Mean±S.D. of values obtained with serum minus values of cultures without serum.
** Statistically different ($p < 0.05$) from values of the same column at time point zero.

Table 2. Effect of partial or extensive absorption of immune serum (7 weeks after infection) by heat killed promastigotes as compared to unabsorbed serum, upon internalization of promastigotes in homologous macrophages

Serum conditions	Parasites/100 mø		% infected mø	
	BALB/c*	C57BL/6*	BALB/c*	C57BL/6*
Unabsorbed	175±8.8	188±5.7	63±1.2	68±1.1
Partial (3×) absorption	113±3.4	145±4.5	54±1.1	57±2.0
Extensive (6×) absorption	86±2.9**	75±2.4**	45±1.0**	45±0.9**

* Mean of S.D. of values obtained with serum minus values of cultures without serum.
** Statistically different ($p < 0.05$) from values of unabsorbed serum of the same column.

between the titers of sera from BALB/c or C56BL/6 mice, at any time point, were not statistically significant.

Depletion of anti-leishmanial antibodies from the sera obtained 7 weeks after infection caused a marked and dose-related reduction in the ability of the infected sera to enhance the internalization of promastigotes (Table 2). Extensive absorption (6×) significantly ($p < 0.05$) reduced the enhancing effect of sera from both strains. Sera obtained 7 weeks after infection had ELISA anti-leishmanial IgG antibody titers of 1,280 for BALB/c and 320 for C57BL/6; extensive absorption resulted in titers of 40 and below 10, respectively.

Previous studies have shown that sera from mice immunized with *L. donovani* (Herman, 1980), or with *L. mexicana* (Bray, 1983) increased parasite phagocytosis by murine macrophages. Our findings have showed similar effect with sera from susceptible and resistant strains of mice infected by *L. mexicana amazonensis*. In contrast, Chang (1981) observed inhibition of phagocytosis of *L. donovani* amastigotes by human macrophages in the presence of immune serum.

Specific antibodies in the heat inactivated sera are most likely responsible for the enhancing effect on promastigote interiorization since absorption with promastigotes markedly decreased parasite entry into macrophages. Previous reports have implicated antibodies as agents of the internalization enhancing effect of immune sera based on observations of opsonization of promastigotes and the presence of cytophilic antibodies (Herman, 1980: Bray, 1983) but specific antibody depletion was not attempted.

Although sera from infected animals may contain a mixture of antibodies favoring or opposing parasite entry into macrophages, our study shows that the overall effect upon internalization of *L. mexicana amazonensis* is not different between a resistant and a susceptible strain of mice.

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