DESTRUCTION OF *LEISHMANIA MEXICANA AMAZONENSIS* PROMASTIGOTES BY NORMAL HUMAN SERUM

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Abstract. Fresh normal human serum was observed to have a lethal effect on *Leishmania mexicana amazonensis* promastigotes obtained from laboratory-bred *Lutzomyia longipalpis* or on promastigotes grown in liquid culture medium, inoculated with the same isolates. Heat inactivation abolished the *Leishmania* lytic activity from the sera.

Resistance of culture promastigotes to lysis by normal human serum was investigated in three isolates of *L. m. amazonensis*. Development of resistance (up to 7%) was found in only one isolate, obtained from the bone marrow in a human case of visceral leishmaniasis.

Survival of *Leishmania* in mammalian hosts depends on their entry into macrophages. Outside the macrophage, promastigotes are exposed to the destructive effects of serum. Complement (C) may be an important factor in mediating the entry of *Leishmania* into the macrophage. On the other hand, it has been shown that normal human serum can destroy *Leishmania* promastigotes, and that this is due to C activation. The development of different C-resistant forms of various species has been described. We compared the effect of normal human serum on *Leishmania mexicana amazonensis* promastigotes from the sand fly *Lutzomyia longipalpis* and to the effect on parasites grown in liquid culture medium. We also compared the development of C-resistant forms in *L. m. amazonensis*.

MATERIALS AND METHODS

Parasites

Isolates of *L. m. amazonensis* tested were: Josefa strain (MHOM/BR/76/Josefa), Maria strain (MHOM/BR/79/Maria), and Dilma strain (MHOM/BR/82/Dilma). All three isolates have been described previously. Characterization was made by isoenzyme analysis and by a monoclonal antibody panel (courtesy of Gabriel Grijaldi, Fundação Oswaldo Cruz, Rio de Janeiro).

Promastigotes were cultivated in LIT medium supplemented with 10% fetal bovine serum. All experiments were performed with promastigotes before the 10th passage in culture.

Sand flies

Three- to five-day-old *Lu. longipalpis* (n = 250) bred in the laboratory as described were fed for 1 hr on the lesions of *Leishmania*-infected BALB/c mice. The sand flies were killed with ether 4 or 7 days after the infective meals, and their alimentary tracts were dissected in saline and microscopically examined. For each assay, contents of 55 infected alimentary tracts were dispersed with Hank's balanced salt solution (HBSS) and collected in a glass tube.

Human sera

Sera from 5 healthy adult donors (without history or clinical symptoms of leishmaniasis, and with ELISA anti-*Leishmania* antibody titers <1:10 against all of the 3 isolates) were used fresh or frozen (−70°C). Aliquots were heat-inactivated (30 min at 56°C).

Parasite-serum interaction

Promastigotes from culture medium (obtained at 2, 4, 5, 7, 9, or 11 days of culture) or from phlebotomine dissection (4 or 7 days after infective meal) were washed in HBSS and concent-
trated to $8 \times 10^5$ parasites/ml. Aliquots of 95, 90, or 80 μl of parasite suspension were dispensed into 12 × 75 mm glass tubes, to which 5, 10, or 20 μl of sera were added, respectively. Capped tubes were incubated for 30 to 60 min in a water bath at 37°C. Ten microliter samples were taken at indicated times, and examined in a hemacytometer under a compound microscope. Flagellar motility was taken to indicate promastigote viability. In some experiments, the uptake of neutral red supervital strain was used.

**RESULTS AND DISCUSSION**

Both phlebotomine-obtained and culture-grown *L. m. amazonensis* promastigotes were extremely motile and were characterized by a spiral-shaped body in the beginning of the experiments. Following incubation with 20% fresh or frozen normal human sera, 92.8% to 99.9% promastigotes from either source were nonmotile and rounded. Incubation of promastigotes (Josefa strain) with 20% of the same serum after heat inactivation did not alter their flagellar motility or shape (Table 1). It was also evident that the lytic effect of fresh sera was virtually identical on promastigotes regardless of the stage of culture or time after the infective meal that they were obtained (Table 1).

Table 2 shows that both 10% or 20% fresh normal human sera were fully effective in lysing phlebotomine-grown promastigotes, whereas the same sera at a concentration of 5% did not cause significant lysis.

This report confirms and extends previous observations of a lethal effect of fresh normal human serum upon *Leishmania* promastigotes. Studies with promastigotes obtained from infected sand flies have investigated their infectivity for BALB/c mice, after treatment with normal serum.

We compared *L. m. amazonensis* promastigotes obtained from the gut of infected laboratory-born *Lu. longipalpis* and culture forms of the same isolate in relation to survival in the presence of normal human serum. Parasites from both sources were susceptible to killing by fresh human serum, and the lethal effect was abrogated by heat inactivation. Although *Lu. longipalpis* is not a vector for *L. m. amazonensis* in nature, it has been shown that Maria strain promastigotes obtained from these sand flies are able to infect BALB/c mice. In contrast, we have used Josefa strain promastigotes obtained 4 to 7 days after insect feeding, and the development of serum-resistant forms was not observed.

Hindle et al. were the first to report that fresh sera from patients with kala-azar and from normal individuals had the capacity to kill *L. donovani* promastigotes. Several other reports have shown a similar effect of human or animal sera upon different species of *Leishmania*. Recently, Pearson and Steigbigel provided evidence that such lethal effect was dependent on C activation through the classical pathway. On the other hand, Mosser and Edelson using *L. tropica* or *L. enriettii* promastigotes, reported the direct activation of C via the alternative pathway. These authors demonstrated that the lytic effect of serum was not observed at a low serum concentration (4%), which was confirmed by us in this report using *L. m. amazonensis*.

It has been shown that promastigotes from

**Table 1**

<table>
<thead>
<tr>
<th>Source of parasites</th>
<th>n†</th>
<th>% Lysis‡ (± SD)</th>
<th>% Lysis‡ (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phlebotomine</td>
<td></td>
<td>20% Fresh normal human serum</td>
<td>20% Heat-inactivated normal human serum</td>
</tr>
<tr>
<td>4 day</td>
<td>5</td>
<td>92.8 ± 10.5</td>
<td>6.8 ± 4.8</td>
</tr>
<tr>
<td>7 day</td>
<td>3</td>
<td>98.7 ± 2.3</td>
<td>9.5 ± 8.2</td>
</tr>
<tr>
<td>Culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 day</td>
<td>5</td>
<td>99.9 ± 0.2</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>7 day</td>
<td>5</td>
<td>94.2 ± 3.8</td>
<td>8.2 ± 4.1</td>
</tr>
<tr>
<td>11 day</td>
<td>5</td>
<td>98.3 ± 0.4</td>
<td>7.2 ± 3.9</td>
</tr>
</tbody>
</table>

* Promastigotes were used 4 or 7 days after phlebotomine feeding, or 4, 7, or 11 days after culture.
† n = number of different sera used; triplicate samples were counted.
‡ Phlebotomine promastigotes vs. culture promastigotes, not significant at any point.

**Table 2**

<table>
<thead>
<tr>
<th>Sera</th>
<th>% Serum</th>
<th>n*</th>
<th>Percent lysis (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>5</td>
<td>3</td>
<td>10.3 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3</td>
<td>98.0 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3</td>
<td>98.7 ± 2.3</td>
</tr>
<tr>
<td>Heat-inactivated</td>
<td>20</td>
<td>3</td>
<td>9.5 ± 8.2</td>
</tr>
</tbody>
</table>

* n = number of different sera used; triplicate samples were counted.
stationary phase cultures of *L. donovani* and *L. tropica* were more infective to hamsters or BALB/c mice.\(^{11-19}\) The resistance to lysis in the presence of serum depends on the species of *Leishmania*.\(^4\) We compared two other isolates of *L. m. amazonensis*, one (Maria) from the nasal lesion of a patient with espundia and the other (Dilma) was from the bone marrow of a child with visceral leishmaniasis. Table 3 shows that up to 7% of the promastigotes from Dilma strain were resistant to lysis by normal human serum, at stationary phase. In contrast, resistance was not greater than 2.5% with the Maria strain. Franke et al.\(^4\) also found no development of resistance with the Maria strain; these results were confirmed by us with the same strain and also with the Josefa strain.

An effective lethal potential of normal serum represents an important host defense mechanism against *Leishmania* infection. We were unable to detect the appearance of parasite resistance to serum lytic effect with *L. m. amazonensis*, except for an isolate which caused visceral disease. In this case, the appearance of almost 10% of forms resistant to lysis was similar to that found with *L. donovani*.\(^4\) The development of forms resistant to lysis by serum in a visceralizing *L. m. amazonensis* isolate suggests that this characteristic may be important in pathogenesis and may have some use in parasite classification.

**ACKNOWLEDGMENTS**

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### Table 3

<table>
<thead>
<tr>
<th>Strain</th>
<th>Days in culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Dilma</td>
<td>150 (1)*</td>
</tr>
<tr>
<td>Maria</td>
<td>110 (0)</td>
</tr>
</tbody>
</table>

* Number of parasites (×10^5) per ml of LIT medium; ( ) percentage alive after incubation with 10% normal human serum.

### REFERENCES


acteristics of so-called natural antibodies in various normal sera against culture forms of *Leishmania*. *J. Parasitol.*, 56: 889–896.

