Anti-\textit{Leishmania} titers and positive skin tests in patients cured of kala-azar

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Abstract

Visceral leishmaniasis (VL), also known as kala-azar, is an important public health problem. If not treated, virtually all clinically symptomatic patients die within months. The diagnosis is based on the Montenegro skin test (MST) and anti-Leishmania titers. Nevertheless, the time required for cured individuals living in a leishmaniasis-endemic area to present a positive skin test and negative anti-Leishmania serology is known. To determine the cellular and humoral immune response profile in relation to different times post-VL cure, a cross-sectional study was conducted on subjects from a kala-azar endemic area in Paço do Lumiar, MA, Brazil, on the basis of 1995-2005 notifications reported by the National Health Foundation/Regional Coordination of Maranhão. We visited cured individuals with a history of VL within the last 10 years. Seventy-four subjects (30 females) ranging in age from 1 to 44 years were included, all of them symptom free at the time of the study. A cellular immune response was observed in 73 (98.6%) subjects, whereas no significant antibody titers were detected by indirect immunofluorescence (IIF) in the sera of 69 (93.2%) cases. Ten years post-cure, 39 (52%) subjects had a positive MST and negative IIF reaction, while in one subject the skin and anti-Leishmania serology tests were negative. Two other subjects were positive in both tests 1 year after cure. These data suggest that a cellular immune response may still be present in subjects cured of VL regardless of post-cure time, and that the parasite persists in the host after clinical cure of the disease. This would explain the persistence of significant Leishmania sp antibody titers in some subjects after treatment.

Key words: Human visceral leishmaniasis; Montenegro’s intradermal reaction; Indirect immunofluorescence

Introduction

Visceral leishmaniasis (VL), also known as kala-azar, is an important public health problem that affects poor populations in 70 countries in Asia, East Africa, South America, and the Mediterranean region. More than 90% of new cases occur in the 6 most affected countries, namely Bangladesh, Brazil, India, Ethiopia, Kenya, and Sudan (1). The number of patients with VL has increased over the past decade, affecting mainly children younger than 10 years and HIV-infected patients. Leishmaniasis has emerged as an opportunistic infection, representing an important co-infection in immunodepressed patients and a major sanitary problem (2).

If not treated, virtually all clinically symptomatic patients die within months. Diagnosis of VL is based on epidemiological data and clinical and laboratory findings, but the definitive diagnosis can only be made by detecting the parasite in infected tissues (3).

Various immunological abnormalities have been documented in VL patients, including unresponsive skin tests (Montenegro skin test, MST) and high Leishmania antibody titers, which have been used as a laboratory diagnostic criterion of the disease (4,5). Immunocompetent individuals show negative results in skin tests when they are in the acute phase of VL. Serological tests may be particularly useful for the diagnosis of visceral leishmaniasis, as they have a high predictive value in the diagnosis of immunocompetent individuals.

Despite the laboratory findings, the time required for cured individuals living in a leishmaniasis endemic area to show a positive reaction of cellular hypersensitivity (MST) and negative serology (anti-Leishmania) is not known. In the present study, we determined the cellular and humoral...
immune response profile in relation to different post-cure times of VL, and discussed future perspectives that may contribute to intervention and control of this protozoosis.

**Material and Methods**

A cross-sectional study was conducted on subjects from a kala-azar endemic area (Vila São José, a municipality of Paço do Lumiar, Island of São Luís do Maranhão, Brazil). We visited every house that had individuals with a history of VL over a range of up to 10 years (fever, anemia, and splenomegaly), a positive indirect immunofluorescence (IIF) reaction (titer ≥1:80) test, and demonstration of amastigote forms of *Leishmania* in bone marrow aspirates (myelogram). The addresses were obtained from the notifications between 1995 and 2005 reported by the National Health Foundation/Regional Coordination of Maranhão (FUNASA/COREMA). All individuals had been treated with N-methylglucamine antimoniate at a dose of 20 mg Sb<sup>5+</sup>·kg<sup>-1</sup>·day<sup>-1</sup> for 20 days at the time of the disease. These individuals were submitted to clinical and immunological evaluation in 2005, corresponding to an interval of 1 to 10 years of treatment. The subjects showed no signs or symptoms suggestive of the disease at the time of the present study. The study was approved by the Ethics Committee of the Universidade Federal do Maranhão, and written informed consent was obtained from all subjects.

IIF testing was performed for the evaluation of the humoral immune response, and the MST was used to determine the cellular immune response.

For the MST, antigen from *Leishmania (L.) amazonensis* promastigotes (5 x 10<sup>7</sup> parasites/mL), produced at Bio-Manguinhos/FIOCRUZ (Ministry of Health, Rio de Janeiro, Brazil), was used at a standard concentration of 40 µg/mL protein nitrogen diluted 1:10,000 in saline merthiolate. Antigen (0.1 mL) was injected intradermally in the anterior side of the left forearm with a tuberculin syringe and readings were taken 48 to 72 h after injection. The size of the papule was delimited with a ball-point pen and measured with a millimeter ruler. The reaction was considered to be positive when both the cross-sectional and longitudinal diameter of the induration was ≥5 mm. The same researcher applied the antigen and read the result.

The IIF test was performed as previously described (6). For the test, a 5-mL blood sample was collected from each individual and processed at the state Laboratory of Endemic Diseases. Antibody titers ≥1:80 were taken to be positive. Descriptive presentation of the data consisted of the tabulation of frequency and proportions using the Epi-Info software, version 6.

**Results**

Forty-four of the 74 subjects with a history of VL were males and 30 were females, with no significant difference in terms of gender. Age ranged from 1 to 44 years.

All subjects were symptom free at the time of this study. None of the subjects presented hepato- or splenomegaly upon physical examination or any other anomaly. The time after cure of the disease and the frequency of a positive MST and IIF reaction are shown in Table 1. Ten years post-cure, 39 (52%) subjects had a positive MST and negative IIF reaction; only one subject presented negative MST and IIF reactions, whereas three other subjects presented positive serology and a positive cellular response. At 1 year post-cure, two subjects were still serologically positive, but already presented a positive MST. Two to 9 years post-cure, all subjects presented negative serology and a positive cellular response. In summary, over the 1 to 10 years of follow-up, 69 (93.2%) subjects presented

<table>
<thead>
<tr>
<th>Time (years) elapsed between the disease and current assessment</th>
<th>Number of subjects</th>
<th>Montenegro skin test</th>
<th>Anti-<em>Leishmania</em> antibodies (indirect immunofluorescence test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>39</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>(+)</td>
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</tr>
<tr>
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<td>1</td>
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<td>(-)</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>(+)</td>
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</tr>
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<td>8</td>
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<td>(+)</td>
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</tr>
<tr>
<td>7</td>
<td>1</td>
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negative serology and 73 (98.6%) showed a positive re-
sponse to the *Leishmania* skin test.

**Discussion**

In the present study, we found that 98.6% of the subjects were MST positive 10 years post-cure, which contrasts with findings obtained in the endemic area of Jacobina, BA, where only 15.5% of the individuals presented a cellular response at 4 to 9 years post-cure of the disease (7), and with Mayrink et al. (8) who reported a 4.1% positivity rate in individuals with post-cure periods ranging from 8 to 720 days (8). Also, a lower percentage of positive MST was found in another endemic region in Teresina (Piauí State), with 36.9% positivity in 1105 subjects (9).

Years of post-cure are expected to lead to a positive cellular response (MST positive) and loss of specific antibod-
ies (IIF negative), as was the case with 39 of our subjects (52%) after 10 years. A positive MST has been observed after 1 year post-cure.

In areas under strict epidemiological control where pro-
phylactic measures are routinely performed, exposure to the parasitic agent becomes less frequent. As a consequence, individuals become non-responders to the *Leishmania* skin test, as cellular immunity is antigen dependent and requires constant stimulation for its maintenance (10-13).

The observation of high anti-*Leishmania* antibody titers even at 10 years post-cure raises the question of re-infection. Alternatively, parasites may persist in the host for long

**References**


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blot analysis of the humoral immune response to *Leishmania*
