Visceral leishmaniasis (VL) is a serious public health problem in many regions of the world. Apart from its high incidence, VL is spreading across new places and showing worrying signs of urbanization. Furthermore, severe cases of VL resulting in death have been described.\(^1,2\)

The main strategies for controlling VL include the early diagnosis and treatment of human cases and measures directed at the vector and the canine reservoir and health education activities. However, these measures are insufficient for proper control of the epidemic. In Brazil, the epidemic is still spreading; there were a total of 24,977 new cases from 1999 to 2005.\(^2\) Therefore, the search for new measures to curb the epidemic is extremely important.\(^2,3\)

Mounting evidence suggests that the sand fly saliva may influence the course of VL infection. Studies performed in murine models have shown that salivary components of sand flies may exacerbate infection when injected together with *Leishmania*.\(^4–9\) Conversely, pre-exposure to sand fly saliva may even protect against *Leishmania* infection, leading to the development and testing of new vaccines on the basis of inoculation with sand flies’ salivary proteins.\(^8,10–12\)

On exposure to uninfected *Lutzomyia longipalpis* bites, normal human volunteers develop anti-sand fly saliva antibodies and cell-mediated immune response.\(^10\) Host responses to anti-*Lu. longipalpis* saliva antibodies may be related to the development of delayed-type hypersensitivity to *Leishmania* antigen. In a prospective cohort study, development of anti-*L. longipalpis* saliva antibodies was associated with increased delayed-type hypersensitivity to *Leishmania* antigen. In a prospective cohort study, we evaluated 1,080 children from two endemic areas for visceral leishmaniasis (VL) by means of Kaplan-Meier analysis. The incidence rate of delayed-type hypersensitivity to *Leishmania* antigen, measured at the 24th follow-up month, was higher among those reactive to *Lu. longipalpis* saliva antibodies at the beginning of the study (0.0217 cases per person-month) than among those previously negative (0.0131 cases per person-month) (\(P\) value for the log-rank test = 0.0006). It seems that mounting an anti-saliva immune response helps the development of a cell-mediated anti-*Leishmania* response.

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\* Address correspondence to Manoel Barral-Netto, Rua Waldemar Falcão, 121, Candeal, 40296710, Salvador, Bahia, Brazil. E-mail: mbarral@ bahia.fiocruz.br.
To evaluate if the presence of anti-*Lutzomyia longipalpis* saliva antibodies was associated with subsequent mounting of delayed-type hypersensitivity to *Leishmania* antigen, Kaplan-Meier and the log-rank test were used. Significance level was set at 0.05. Prevalence proportion of DTH positivity at time zero was 31.8%. At 12 months, the DTH incidence proportion was 24.6% and at 24 months, it was 7.2% (Figure 1). The prevalence proportion of anti-saliva IgG antibodies was 16.1% at time zero of the study. Incidence proportions of anti-saliva IgG antibodies at the 12th and 24th months of observation were 3.8% and 2.7%, respectively (data not shown).

The total observation time of children with negative anti-saliva antibodies summed to 10,176 months, whereas for those with positive antibodies it totaled 2,448 months. Incidence rate of delayed-type hypersensitivity to *Leishmania* antigen, measured at to the 24th follow-up month, was 0.0131 cases per person-month for those negative for sandflies’ anti-saliva antibodies at the beginning of the study (time zero) and 0.0217 cases per person-month for those with positive anti-saliva antibodies (Table 1).

We have previously shown that positive antibody response to sandfly saliva correlates to delayed-type hypersensitivity against *Leishmania* antigen and that the development of both types of immunity is time-coincident. Furthermore, DTH responses to sandfly bites in humans, increases blood flow at the site of the bite. It is possible that such changes in previously saliva-sensitized individuals help them to mount a cell-mediated immune response against *Leishmania* after exposure to infected sand flies. In this report, the positive association between anti-sand fly saliva antibodies and anti-*Leishmania* DTH was confirmed in a longitudinal prospective design. These findings add strong support to this association as they come from a large cohort study.

**Table 1**

Association between presence of anti-*Lutzomyia longipalpis* saliva antibodies at the beginning of the study and the development of delayed-type hypersensitivity to *Leishmania* antigen up to the 24th follow-up month among children residing in two visceral leishmaniasis (VL) endemic areas, in Raposa County, São Luis, State of Maranhão, Brazil

<table>
<thead>
<tr>
<th>Anti-<em>Lu. longipalpis</em> saliva antibodies at the beginning of the study</th>
<th>Events (DTH-positive to <em>Leishmania</em>)</th>
<th>Sum of time under observation (months)</th>
<th>Incidence rate (cases per person-month)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 months</td>
<td>24 months</td>
<td>Total</td>
</tr>
<tr>
<td>Negative</td>
<td>116</td>
<td>17</td>
<td>133</td>
</tr>
<tr>
<td>Positive</td>
<td>40</td>
<td>13</td>
<td>53</td>
</tr>
<tr>
<td>Total</td>
<td>156</td>
<td>30</td>
<td>186</td>
</tr>
</tbody>
</table>

*P* value of the log-rank test < 0.0006.
Considering that measurable anti-sand fly antibodies develop in only a fraction of exposed children and that a positive anti-Leishmania DTH is associated with resistance, it is tempting to speculate that mounting an anti-saliva immune response helps in the development of a protective anti-Leishmania response. However, increased anti-Leishmania DTH response in persons reactive to *Lu. longipalpis* saliva antibodies at the beginning of the study could quite well reflect increased parasite challenge in individuals who have been bitten more intensively by the sand fly vector. Thus, caution is necessary in extending laboratory results to field situations. Further testing of this hypothesis will probably rely on the use of recombinant salivary proteins to overcome the limitations of using crude sand fly salivary gland sonicates. We have recently showed the feasibility of using recombinant proteins from *Lu. longipalpis* saliva for large epidemiological studies, which opens up the possibility of performing larger studies in endemic areas with a high incidence of VL to address the question of the relationship between anti-saliva response and protection against VL.

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Authors’ addresses: Dorlene M. C. Aquino and Arlene J. M. Caldas, Departamento de Enfermagem, Universidade Federal do Maranhão, São Luís, Maranhão, Brazil; José Carlos Miranda, Laboratório de Imunoparasitologia (LIP), Centro de Pesquisas Gonçalo Moniz (CPqGM), Fundação Oswaldo Cruz – FIOCRUZ - Bahia, Salvador, Bahia, Brazil; Antonio A. M. Silva, Departamento de Saúde Pública, Universidade Federal do Maranhão, São Luís, Maranhão, Brazil; Manoel Barral-Netto, Laboratório de Imuno-regulação (LIMI), Centro de Pesquisas Gonçalo Moniz (CPqGM), Fundação Oswaldo Cruz – FIOCRUZ - Bahia, Salvador, Bahia, Brazil; Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Bahia, Brazil; and Instituto Nacional de Ciência e Tecnologia de Investigação em Imunologia - iii - INCT, Salvador, Bahia, Brazil. Aldina Barral, Laboratório de Imunoparasitologia (LIP), Centro de Pesquisas Gonçalo Moniz (CPqGM), Fundação Oswaldo Cruz – FIOCRUZ - Bahia, Salvador, Bahia, Brazil; Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Bahia, Brazil; and Instituto Nacional de Ciência e Tecnologia de Investigação em Imunologia - iii - INCT, Salvador, Bahia, Brazil.

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