Field Trial of a Vaccine against New World Cutaneous Leishmaniasis in an At-Risk Child Population: How Long Does Protection Last?

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During 12 months of follow-up in a randomized double-blind controlled field study, a killed whole-promastigote vaccine cocktail plus bacille Calmette-Guérin (BCG) adjuvant significantly reduced the incidence of cutaneous leishmaniasis (CL) in Ecuadorian children, compared with BCG alone. To determine how much longer protection might continue, the study was reblinded to permit 48 additional months of follow-up. During months 13–18, CL incidence remained lower in the vaccine group, compared with that in the control group (5.9% vs. 13.8%; \( \chi^2 = 8.8; P = .003 \)), with vaccine efficacy calculated at 56.5% (95% confidence interval, 18.7%–76.7%); however, during months 24–60, no significant between-group differences were detected. Periodic administration of boosters may be necessary to maintain whole-parasite–vaccine protection against New World CL.

We recently reported the results of a randomized double-blind controlled field study that evaluated the efficacy, immunogenicity, and safety of a killed whole-promastigote vaccine cocktail [1] against cutaneous leishmaniasis (CL), in children living in Ecuador in an endemic area in which human disease is caused by multiple parasite species [2, 3]. At the end of the 12-month study period, CL incidence was significantly lower in the vaccine group (2.1%), compared with that in the placebo group (7.6%), with the protective efficacy of the vaccine calculated at 72.9% (95% confidence interval [CI], 36.1%–88.5%). The present study was undertaken to determine how long after the initial, 12-month follow-up period the protection is conferred by the vaccine. To answer that question, the study was subsequently reblinded to permit an additional 48 months of follow-up.

Subjects, materials, and methods. The original study methodology has been described in detail elsewhere [1]. In brief, children with a negative leishmanin skin test (LST) who were without physical evidence of prior or current CL and were free of allergies, autoimmune disease, tuberculosis, other acute conditions, or severe malnutrition were randomized to either the vaccine group or the control group. The LST antigen was produced by use of the same 3 Leishmania-isolate strains that were included in the vaccine. The LST was administered intradermally (id), and the results were read and recorded 48 h later. Test results with an induration \( \geq 5 \) mm were classified as positive.

Each vaccine-group subject received 2 id doses of the vaccine antigen plus bacille Calmette-Guérin (BCG) adjuvant; each control-group subject received 2 id doses of BCG alone. In both groups, the 2 vaccinations were administered in an identical fashion, with a 1-month interval between vaccinations. The vaccine was composed of phenol-killed whole promastigotes from 3 different stocks of Leishmania obtained from the lesions of patients living in the study area [1]. During the initial, 12-month follow-up period after the second vaccination, active surveillance supplemented by passive surveillance was used to monitor subjects; during the subsequent, 48-month follow-up period, both active and passive surveillance were used. As described elsewhere [1], tissue samples obtained from subjects with suspect lesions were evaluated by use of direct smear and aspirate culture techniques, to confirm the presence of Leishmania amastigotes or promastigotes. Subjects diagnosed with CL received, at no cost, a complete course of meglumine antimonialate. The LST was administered at month 1 after the second vaccination supplemented by passive surveillance was used to monitor subjects; during the subsequent, 48-month follow-up period, both active and passive surveillance were used. As described elsewhere [1], tissue samples obtained from subjects with suspect lesions were evaluated by use of direct smear and aspirate culture techniques, to confirm the presence of Leishmania amastigotes or promastigotes. Subjects diagnosed with CL received, at no cost, a complete course of meglumine antimonialate. The LST was administered at month 1 after the second vaccination and again at months 18 and 60.

The major outcome of interest in the follow-up study is the protection provided by the vaccine during months 13–60. Vaccine efficacy during the follow-up period was determined by comparison of the proportion of CL cases that occurred in the vaccine group versus that which occurred in the control group;
CL was diagnosed when *Leishmania* parasites were isolated from suspect lesions of a subject, by direct-smear and/or aspirate-culture techniques. A secondary outcome of interest was the proportion of subjects in each of the 2 groups who exhibited a positive LST result at months 13 and 60.

The descriptive data were analyzed by standard techniques, including analyses of frequencies, means ± SDs, and percentages. Differences between proportions were analyzed by use of 2 × 2 contingency table analysis with corrected χ². These data were used to calculate odds ratios (ORs) and their respective 95% CIs, where appropriate. Student’s *t* test (2-tailed) for independent samples was used to analyze mean differences between groups. The EpiTable program was used to calculate vaccine efficacy [4].

**Results.** Table 1 compares the characteristics of the vaccine-group and control-group subjects, at the beginning of the second follow-up period (month 13). The 2 groups were not significantly different with regard to mean age, sex, ethnicity, or indicators of nutritional status.

Table 2 shows the incidence of parasitologically confirmed CL incidence during the second, 48-month follow-up period. During months 13–18, CL incidence continued to be lower in the vaccine group, compared with that in the control group, and the risk of CL in the vaccine group was only 40% of that in the control group (OR, 0.40; 95% CI, 0.21–0.49). The protective efficacy of the vaccine during this 6-month period was calculated as being 56.5% (95% CI, 18.7%–76.7%); in contrast, thereafter, until the study ended at month 60, the observed incidence of CL was not significantly different between the 2 groups.

In addition to the LST that they had received at month 1, the study subjects were administered an LST at months 18 and 60. As reported elsewhere [1], the proportion of vaccine-group subjects who converted to a positive LST result was significantly increased, compared with that of control-group subjects (85.1% vs. 20.1%; χ² = 279; *P* < .000001). By month 18, the proportion of vaccine-group subjects with a positive LST result—whereas reduced, compared with that at month 2—was still higher than that of control-group subjects (67% vs. 36%; χ² = 34.8; *P* = .001); however, by month 60, no significant between-group differences were found with regard to the proportion of subjects with a positive LST result (40.8% vs. 32.5%; χ² = 1.9; *P* > .05).

**Discussion.** The present study has investigated how much longer after the initial, 12-month follow-up the protection conferred by the whole-promastigote vaccine cocktail continues. The results indicate that the vaccine continues to confer significant protection for an additional 6 months after the initial, 12-month period, even though the apparent level of protection is reduced. These results suggest that the administration of a vaccine booster at month 12 may be necessary to both enhance the immune response against *Leishmania* and prolong the period of protection.

The LST data collected at months 1, 18, and 60 reveal a progressive decrease, over time, in the proportion of subjects in the vaccine group who have a positive LST result. These data appear to correspond with the time-dependent decrease in vaccine protection. At months 1 and 18, the proportion of vaccine-group subjects with a positive LST result was significantly lower, compared with that of the control-group subjects, but, by month 60, this difference was no longer evident. In the absence of boosting by natural infection, it was expected a priori that the LST in this group would decrease.

In contrast to the observed decrease, the proportion of control-group subjects with a positive LST result unexpectedly increased between months 1 and 18. The reason for this is not immediately evident. Although it is possible that this increase may have been due to increased exposure to the sand-fly vector, one would also expect to see, over time, an increase in LST, rather than the consistent decrease that was observed in the vaccine-group subjects. It does not seem unreasonable to suggest that the increase in the proportion of control-group sub-

<table>
<thead>
<tr>
<th>Follow-up months</th>
<th>No. of subjects (odds ratio)</th>
<th>Vaccine group</th>
<th>Placebo group</th>
<th>Total</th>
<th>χ²</th>
<th><em>P</em></th>
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</thead>
<tbody>
<tr>
<td>0–12</td>
<td>7 (2.1)</td>
<td>24 (7.6)</td>
<td>31 (4.8)</td>
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<tr>
<td>13–18</td>
<td>14 (5.6)</td>
<td>26 (13.8)</td>
<td>40 (9.1)</td>
<td>8.8</td>
<td>.003</td>
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<td>19–25</td>
<td>7 (3.1)</td>
<td>7 (4.1)</td>
<td>14 (3.5)</td>
<td>&lt;1.0</td>
<td>&gt;.05</td>
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<td>26–30</td>
<td>11 (6.0)</td>
<td>4 (2.9)</td>
<td>15 (4.7)</td>
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<td>31–36</td>
<td>2 (1.2)</td>
<td>2 (1.7)</td>
<td>4 (1.4)</td>
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<td>1 (0.8)</td>
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<td>43–48</td>
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<td>56–60</td>
<td>2 (1.2)</td>
<td>3 (2.3)</td>
<td>5 (1.7)</td>
<td>&lt;1.0</td>
<td>&gt;.05</td>
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* Months 0–12 are part of the initial study (data published elsewhere); months >12 are part of the present study.
jects with a positive LST result during this period may be the result of sampling error, since the proportion decreased during the remainder of the observation period.

We were unable to find any significant differences, in CL risk, between LST responders and LST nonresponders, during either the initial, 12-month follow-up period or the subsequent 48-month follow-up period. Even though it is recognized that conversion to a positive LST result may not necessarily reflect protection against CL, the test still may be a useful parameter for monitoring the immune status of vaccinated populations. Mayrink et al. [5] have reported that a similar LST pattern was observed during the 2 years after administration of a promastigote-based vaccine in a Brazilian population, although, because of technical problems, they were unable to find any correlation between it and vaccine efficacy.

In both experimental groups, the incidence of CL cases after month 31 was lower than what had been anticipated. This unexpected finding appears to be due to the occurrence of the “El Niño” and the subsequent “La Niña” meteorological phenomena in the study area, where events were similar to those which took place in the rest of subtropical and tropical Ecuador. These events resulted in a change in normal patterns of precipitation, a change that appears to have negatively influenced vector reproduction. A similarly reduced incidence of CL was detected during the same period in the general nonvaccinated study population (authors’ unpublished data) and in an adjacent tropical area [6].

In summary, the vaccine cocktail appears to continue to confer significant protection against CL for 6 months after the initial 12 months of follow-up, even though the level of protection is lower during months 13–18. Future studies should investigate whether administering a vaccine booster at 1 year after the initial vaccination will improve immune response against Leishmania and prolong the period during which at-risk children are protected.

References