Field Trial of a Vaccine against New World Cutaneous Leishmaniasis in an At-Risk Child Population: Safety, Immunogenicity, and Efficacy during the First 12 Months of Follow-Up

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The safety, immunogenicity, and efficacy of a vaccine against cutaneous leishmaniasis in rural Ecuadorian children was assessed in a randomized, controlled, double-blinded study. Vaccine group subjects received 2 intradermal doses of a whole, killed promastigote vaccine cocktail plus bacille Calmette-Guérin (BCG) adjuvant. Control subjects got 2 doses of BCG only. The subjects who received both vaccination doses, 438 in the vaccine group (79.3%) and 406 in the control group (83.4%), were followed for 12 months. No serious adverse side effects were identified in either group. Significantly more vaccine group subjects than controls converted to a positive Montenegro skin test (85.1% vs. 20.1%; \( \chi^2 = 279; P < .001 \)). The incidence of cutaneous leishmaniasis was significantly reduced in the vaccine compared with the control group (2.1% vs. 7.6%; \( \chi^2 = 8.95; P < .003 \)). The protective efficacy of the vaccine was 72.9% (95% confidence interval = 36.1%–88.5%).

Cutaneous leishmaniasis (CL) is a globally distributed parasitic disease that causes unsightly topical ulcers and often results in permanent disfigurement. The disease is endemic throughout most of the Americas [1–3]; recent estimates place the annual number of new CL cases in the region at 59,300, with an additional 39.3 million persons reported to be at risk [3]. Cutaneous leishmaniasis is endemic in >80% of provinces in Ecuador [4–6], with young children reported to be at particular risk for contracting the disease in many populations [5–8]. Conventional disease control efforts in Ecuador [6], as in most other Latin American nations, have had very limited success [2]. Thus, the development of a vaccine effective against New World CL would represent an important public health advance.

The body of data from experimental [9–14], clinical [15–17], and field studies [18–20] suggest that vaccines based on killed whole, fractionated, or recombinant *Leishmania* promastigotes are nontoxic and possess immunogenic properties. However, the results of prior field studies have been difficult to interpret for various reasons that have been discussed recently [2, 17, 21]. For this reason, it remains unclear whether such vaccines can confer significant protection against the development of New World CL in humans.

This evidence constituted the basis for the development of an antileishmaniasis vaccine by our research group in 1989. The development of the phenol-killed triple-strain parasite vaccine plus bacille Calmette-Guérin (BCG) adjuvant proceeded through a series of laboratory studies and small randomized, controlled, double-blinded clinical trials in nonsensitized human volunteers. These investigated various questions related to the safety, immunogenicity, dosage level, and administration of the vaccine antigen [22, 23]. Subsequently, the results of a small randomized, controlled, and double-blinded pilot field study conducted in a population in which leishmaniasis is endemic suggested that the vaccine possessed an acceptable level of safety and immunogenicity. The disease incidence was 0 in the vaccine group compared with 6% in controls during 1 year of follow-up [23, 24]. These favorable results led to the undertaking of a larger phase III field study of the vaccine candidate. This article reports on the results obtained in the randomized, double-blind, placebo-controlled field trial of the vaccine in a high-risk child population during the first 12 months of follow-up.

Materials and Methods

*Vaccine.* The vaccine antigen was prepared under sterile conditions in the Immunology Research Laboratory, School of Medical Sciences, Central University of Ecuador in Quito. It consisted of 3 different strains of phenol-killed whole *Leishmania* promastigotes obtained from the lesions of patients living in the study area. The strains used were *Leishmania (V) braziliensis* [MHOM/EC/
Leishmania (V) guayanensis [MHOM/EQ/90/Juberly], and Leishmania (L) amazonensis [MHOM/EC, WR910]. To prepare the vaccine, the strains were grown in modified NNN (Merck, Rahway, NJ) at 24°C for 2–3 subcultures and then expanded. The L. (L) amazonensis strain was expanded by use of RPMI 1640 (Sigma, St. Louis) plus 15% fetal calf serum (Sigma), while the other 2 strains were expanded with RPMI (Sigma), 15% fetal calf serum (Sigma), and 30 mg/L hemin (Sigma). The L. (L) amazonensis strain was harvested after 5 days of growth and the L. (V) braziliensis and L. (V) guayanensis after 6 days. Each strain was washed five times with PBS, counted, and added to a sterile solution containing PBS plus 0.25% phenol. These were kept under refrigeration at 4°C. The strains were checked for viability by microscopy and culture. They also were each reviewed for sterility, nontoxicity, and innocuity. After passing these tests, the 3 strains were combined to produce the final vaccine product. To accomplish this, 2.4 × 10^9 each L. (L) amazonensis, L. (V) braziliensis, and L. (V) guayanensis promastigotes were combined to produce a vaccine containing 7.2 × 10^6 promastigotes/mL. Subsequently, the final product was again checked for parasite viability and sterility before being bottled into 10-dose sterile vials. The vaccine was kept under refrigeration until used, and any unused portions were discarded after 30 days.

BCG. The BCG used as the vaccine adjuvant (BCG, Tokyo) was reconstituted in its diluent and added to the vaccine just before use. In addition, BCG was used as the control solution. Both of these preparations contained 5 × 10^9 viable organisms per injection dose (100 µL), corresponding to the 1:2 dilution of normal dose of BCG used for vaccination against tuberculosis.

Montenegro skin test (MNST). The MNST antigen was produced under sterile conditions at the Immunology Research Laboratory in Quito from the same Leishmania strains that were used in vaccine production. Equal parts of the 3 strains were combined to produce a solution containing 100 µL of 6 × 10^6 phenol-killed promastigotes/mL. The MNST was administered intradermally. The results were measured and recorded 48 h later, and reactions having an induration of ≥5 mm were classified as positive.

Laboratory diagnosis of leishmaniasis. Direct smear and aspirate culture techniques were used to confirm the presence of Leishmania amastigotes or promastigotes in subjects with suspicious lesions [5].

ELISA. The L. (V) guayanensis [MHOM/EQ/90/Juberly] antigen used in vaccine preparation also was used to measure the specific anti-Leishmania serum antibody levels 1 month after the second vaccination dose. Specific anti-Leishmania IgG and IgM serum antibody levels were quantified by use of an ELISA and were read with a Multiscan reader (Labsystems, Helsinki) set at 492 nm [5].

Nutritional status. Weight, height, mid–upper arm circumference, head circumference (in children aged <24 months), and triceps skin fold measurements were taken from all subjects [25]. Weight-for-height, height-for-age, and weight-for-age were compared with international growth reference standards by use of the Epi Info 6.02 nutritional anthropometry program [26].

Study area description. The study was done on a population living in a rural subtropical rainforest area in which leishmaniasis is endemic in the northwest of Ecuador (Pichincha Province). The population incidence of CL in children during the 4 years before the study was estimated at ~14% [5, 25, 27] (Artnojis RX, unpublished data). The Leishmania species identified as responsible for most human disease in the study area include L. (V) guayanensis, Leishmania (V) panamensis, L. (V) braziliensis, and L. (L) amazonensis [5, 27–29].

Subject selection procedure. The MNST was used to initially screen the study population. Children who tested positive with the MNST were excluded from further consideration. Those with a negative MNST then underwent a clinical history and comprehensive physical examination. Children with evidence of current or prior CL were excluded from further consideration, as were those with allergies, autoimmune diseases, other serious chronic conditions, tuberculosis and other acute infections, or severe malnutrition defined as <–3 SD below the mean for weight-for-height. Children without evidence of present or past CL were defined as eligible for study participation.

Vaccination procedure. The subjects were randomized to either the vaccine or control groups. The BCG adjuvant was reconstituted in its diluent and added to each 10-dose aliquot of vaccine antigen within a 10-min period immediately before administration. Ten 100-µL doses of this solution were withdrawn into coded disposable syringes, which were wrapped in an opaque material. Likewise, 10 doses of the BCG control solution were withdrawn into individual coded syringes wrapped with the same opaque material. Twenty subjects were vaccinated intradermally on the upper right deltoid with either the vaccine or placebo solutions (n = 10 each) according to the randomization code. Depending on group assignment, 1 month after the first vaccination dose, the subjects received a booster dose of either the vaccine or control solution administered to the upper left deltoid. The subjects and the study field personnel were blind to the subject coding scheme.

Subject follow-up. The subjects were observed for 24 h after each vaccination dose to identify and treat any adverse side effects. Clinical evaluations were conducted 1 month after each vaccination for the purpose of detecting lymphadenopathy or other deleterious conditions and to examine the vaccination site for scarring, redness, edema, induration, and ulceration. In addition, data were collected by interview from subjects and their parents regarding any pain, itching, fever, malaise, anorexia, or other side effects that may have occurred during the prior month. One month after the second vaccination dose, the subjects were administered another MNST and donated an 8-mL blood sample that was used in the determination of specific anti-Leishmania IgG and IgM serum antibodies.

Subjects were followed by passive and active surveillance methods for 12 months after the second vaccination. Subjects and their parents were instructed to report immediately to a local Ministry of Public Health health center that was affiliated with the study if they developed any dermal lesions that failed to heal promptly. Those reporting to the affiliated centers underwent a parasitologic examination to determine whether they had CL. In addition, community surveillance was done by the study team on a regular basis, beginning 1 month after the first and second vaccination doses and every 6 months thereafter. During these visits, all subjects received a comprehensive physical examination that concentrated on the skin and mucous membranes. The size, placement, and characteristics of all dermal lesions and healed scars were inspected, measured, and recorded. Tissue samples were taken from subjects with suspicious lesions and evaluated parasitologically. Subjects diagnosed with CL received antimonial drug treatment.

Outcome measures. The three major outcome measures of interest were vaccine safety, immunogenicity, and efficacy. Safety
was measured as the absence of adverse (greater than grade 2) toxic side effects. Immunogenicity was defined as a conversion to a positive MNST (≥5 mm) at 1 month after the second vaccination dose. A CL case was defined as isolation of Leishmania parasites from the suspicious lesions of subjects by direct smear or aspirate culture. Vaccine efficacy was determined by comparing the proportion of CL cases that occurred in the vaccine versus the control group during the 12-month follow-up period [30].

**Sample size and power.** The final sample size obtained in the 2 experimental groups was sufficient to detect a vaccine effectiveness of 70%, assuming an 7.5% annual disease incidence, and a 90% chance (1 − β) of finding a 5% (α) significant difference in a two-tailed test.

**Data analysis.** The descriptive data were analyzed by use of frequencies, means ± SDs, percentages, and other standard techniques. Student’s t test, contingency table analysis with corrected chi squared, and McNemar’s χ² were used where appropriate. Vaccine efficacy for the 12-month follow-up period was determined with the formula for case-control studies [30].

**Results**

A total of 1042 subjects was randomized to either the vaccine (n = 552; 53.1%) or control group (n = 487; 46.9%). No significant differences were detected between the 2 experimental groups regarding compliance with the vaccination schedule. The 2-dose vaccine schedule was completed by 438 (79.3%) of the subjects in the vaccine group and 406 (83.4%) in the control group (χ² = 2.48; P > .05). The characteristics of the subjects who received only 1 dose of the vaccine did not differ significantly at baseline from those who received the complete 2-dose course with respect to age, sex, ethnicity, nutritional status indicators, and mean MNST induration size. The prevaccination characteristics of subjects in the 2 experimental groups who completed both vaccination doses were comparable (table 1).

**Safety.** No severe systemic reactions were recorded in either experimental group. The most frequent side effects that occurred after the first vaccination were local pain, swelling, and redness at the injection site, mostly confined to the initial 3 days. The most common side effects reported during the 1-month period after vaccination were ulceration, secretions, and itching at the inoculation site (table 2). No significant differences were found between the vaccine and control groups with respect to the proportion of subjects who experienced these side effects after the first and second vaccination. However, vaccine group subjects were more likely to report low-grade fever after the second vaccination (14.7%) than after the first (5.6%), a difference that was significant (χ² = 16.4; P = .00005).

**Immunogenicity.** Significantly more subjects in the vaccine group than control group converted to a positive MNST 1 month after the second vaccination (85.1% vs. 20.1%; χ² = 279; P < .000001). Likewise, the average MNST induration size was measurably higher in the vaccine group 1 month after the second dose (mean = 6.7 ± 3.0 vs. 3.4 ± 2.5 mm; t = 15.8; P < .0001), and there was a significant average increase of 5.9 mm in MNST size between baseline and 1 month after the final vaccination dose (mean = 1.1 ± 1.3 vs. 6.7 ± 3 mm; t = 32; P < .0001). In contrast, the mean difference in pre-and post-MNST values of the control group was only 2.2 mm. Although this change was statistically significant (1.3 ± 4 vs. 3.4 ± 3.4 mm; t = 14.5; P = .0001), it appeared to lack clinical importance, since 80% of the subjects remained MNST-negative. The characteristics of the vaccine group subjects who converted to a positive MNST, or responders, were compared with those who continued to have a negative MNST, or nonresponders. However, MNST responders were not significantly different from nonresponders with respect to average age (mean = 5.9 ± 4.0 vs. 4.9 ± 4.4 years), proportion of females (16.8% vs. 13.1%), persons of mestizo ethnicity (97.5% vs. 96%), or nutritional status indicators, including mean weight (19.5 ± 10.0 vs. 18.0 ± 11.6 kg), height (105.2 ± 27.1 vs. 96.9 ± 29.6 cm), mid–upper arm circumference (16.8 ± 2.0 vs. 17.5 ± 8.3 cm), triceps skin fold (9.0 ± 3.5 vs. 7.6 ± 2.1 mm), head circumference (44.3 ± 3.1 vs. 44.6 ± 3.0 mm), and proportion with height-for-age (25.2% vs. 37.8%), weight-for-age (18.3% vs. 29.2%), and weight-for-height (11.2% vs. 15.8%) that were <2SD below the mean according to international reference standards.

**Mean specific anti-Leishmania IgG optical densities (ODs)** measured 1 month after the second vaccination were significantly elevated in the vaccine group compared with the control group (0.701 ± 0.403 vs. 0.430 ± 0.269; t = 4.8; P = .0001). In contrast, the mean IgM ODs of subjects in the vaccine and control groups were comparable (0.508 ± 0.323 vs. 0.481 ± 0.35; t = 0.44; P > .05).

**Table 1.** Baseline characteristics of subjects who completed 2 vaccination doses: vaccine group (n = 438) versus placebo group (n = 406).

<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>Vaccine group</th>
<th>Placebo group</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>5.4 ± 4.0</td>
<td>5.7 ± 3.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>54.3</td>
<td>57.5</td>
<td>&lt;1.0∗</td>
</tr>
<tr>
<td>Ethnicity (% mestizo)</td>
<td>96.2</td>
<td>95.4</td>
<td>&lt;1.0∗</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>18.7 ± 10.7</td>
<td>19.9 ± 11.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>103.9 ± 27.5</td>
<td>105.4 ± 26.2</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Mid–upper arm circumference (cm)</td>
<td>17.4 ± 3.3</td>
<td>17.1 ± 2.7</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Triceps skin fold (mm)</td>
<td>8.7 ± 3.5</td>
<td>8.8 ± 3.5</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Head circumference (mm)</td>
<td>44.3 ± 3.0</td>
<td>45.2 ± 2.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Low height-for-age (%)</td>
<td>29.7</td>
<td>29.3</td>
<td>&lt;1.0∗</td>
</tr>
<tr>
<td>Low weight-for-age (%)</td>
<td>23.9</td>
<td>21.4</td>
<td>2.6∗</td>
</tr>
<tr>
<td>Low weight-for-height (%)</td>
<td>15.4</td>
<td>10.0</td>
<td>3.0*</td>
</tr>
</tbody>
</table>

NOTE. Data are mean ± SD. All subjects had negative Montenegro skin tests at baseline. P > .05 for each comparison between vaccine and placebo.

— Measurement was made in children ≤24 months of age only.

* Data were analyzed by 2×2 contingency table analysis with corrected χ².

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Vaccine efficacy. The incidence of CL in the vaccine group after the first 12 months was significantly lower than that in the control group (2.1% vs. 7.6%; \( \chi^2 = 8.95; P = .0028 \)). Children who received the complete 2-dose vaccine had about only one-fourth the risk of developing the disease during the first 12 months of follow-up compared with that in controls (odds ratio = 0.27; 95% confidence interval = 0.12–0.64). The protective efficacy of the vaccine was calculated at 72.9% (95% confidence interval = 36.1%–88.5%).

Discussion

To our knowledge, this study is the first to report on the efficacy of a vaccine against New World CL consisting of killed whole promastigotes plus a BCG adjuvant. The results of the randomized, double-blinded, controlled field trial suggested that the vaccine was safe, immunogenic, and efficacious in preventing CL in Ecuadorian children for a 12-month period. The randomization process appeared to be successful, since the sociodemographic, nutritional, and MNST characteristics of the vaccine and control groups were not significantly different. The vaccine was well-received by the population, and there was a high level of compliance, \( \sim 80\% \), in both experimental groups. This magnitude of compliance was exceptional, since many subjects and their families had to travel under adverse conditions to reach the study sites. The high observed compliance reinforces the findings of recent KAP (knowledge, attitudes, and practices) studies conducted in the population in which many adults expressed a desire for a vaccine to prevent CL in children [27, 31]. Most subject loss during the study was caused by the out-migration of farmworker families to the Amazon or other areas of the country in search of improved economic prospects.

No severe adverse side effects were recorded during the course of the study. Most of the vaccination-associated reactions were of a minor, local nature, the type, severity, and timing of which were not significantly different between the 2 experimental groups. In both groups, subjects typically developed small painless ulcers that usually disappeared 6–8 weeks after vaccination. The only type of systemic side effects that could be linked to vaccination were low-grade fever and micromymphadenopathy. However, these reactions affected only a small minority of subjects, and the proportions of these were comparable between groups. The low-grade fever was more common after the second dose of the vaccine but responded readily to treatment with pediatric acetaminophen. Fever was not unexpected, since most vaccine booster doses in children typically induce such a response. Likewise, transient micromymphadenopathy that disappeared without treatment was detected in 11% of vaccine group and 14% of control group subjects. This also was anticipated, since all but a handful of children in the study already had received one routine BCG vaccination at birth, and those aged \( \geq 6 \) years had gotten an additional booster dose on their sixth birthday. However, despite repeated exposure to BCG, no cases of gross lymphadenopathy occurred in either experimental group. In addition, the proportion of children who developed this mild form of “BCG-itis” was not greater after the second vaccination dose than the first in either group. These side effects were comparable to those identified during our previous studies with the vaccine [22–24]. Furthermore, the type and severity of reactions were similar to those reported recently by Dowlati et al. [32], who used various dosages of a Leishmania major–based vaccine plus BCG adjuvant. In fact, the reaction pattern seen in our current and prior studies was more characteristic of a response to BCG than to the Leishmania antigen itself. This hypothesis is supported indirectly by the evidence from studies of the L. amazonensis–containing Leishvacin [33, 34] and other similar vaccines [18–20] composed of killed whole-parasite promastigotes but that do not contain an adjuvant, all of which have fewer reported side effects.

In the vaccine group, average MNST size increased 6 mm over prevaccination values. This was significantly higher than that observed for control subjects. In addition, only 15% of the children in the vaccine group did not convert to a positive MNST, compared with 80% of controls. These data are in agreement with observations in prior clinical studies [22, 23].

### Table 2. Comparison of frequency of adverse side effects between experimental groups in 1 month after vaccination dose 1 and 1 month after vaccination dose 2.

<table>
<thead>
<tr>
<th></th>
<th>First vaccination dose</th>
<th></th>
<th>Second vaccination dose</th>
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<tbody>
<tr>
<td></td>
<td>Vaccine</td>
<td>Placebo</td>
<td>Vaccine</td>
<td>Placebo</td>
</tr>
<tr>
<td>Local pain</td>
<td>207 (52.4)</td>
<td>191 (51.3)</td>
<td>159 (45.0)</td>
<td>146 (44.8)</td>
</tr>
<tr>
<td>Local itching</td>
<td>213 (53.9)</td>
<td>219 (58.9)</td>
<td>164 (46.5)</td>
<td>141 (43.3)</td>
</tr>
<tr>
<td>Local edema</td>
<td>74 (18.7)</td>
<td>65 (17.5)</td>
<td>54 (15.3)</td>
<td>58 (17.8)</td>
</tr>
<tr>
<td>Microlymphadenopathy</td>
<td>40 (10.1)</td>
<td>46 (12.4)</td>
<td>51 (14.4)</td>
<td>47 (14.4)</td>
</tr>
<tr>
<td>Local bleeding (injection site)</td>
<td>2 (0.5)</td>
<td>2 (0.5)</td>
<td>13 (3.7)</td>
<td>6 (1.8)</td>
</tr>
<tr>
<td>Abscess</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (0.03)</td>
</tr>
<tr>
<td>Low-grade fever</td>
<td>22 (5.6)</td>
<td>34 (9.1)</td>
<td>52 (14.7)</td>
<td>43 (13.2)</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>0</td>
<td>3 (0.8)</td>
<td>7 (2.1)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4 (1.0)</td>
<td>6 (1.6)</td>
<td>7 (2.0)</td>
<td>4 (1.2)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are frequency (%). \( \chi^2 < 1, P > .05 \) for all comparisons between vaccine and placebo groups.
and the pilot field trial [23, 24]. The postvaccination MNST behavior of our vaccine was comparable to [21, 33] or improved over that reported for similar New World whole-parasite vaccines that did not contain any adjuvant [15, 18–20]. Mean specific anti-Leishmania IgG serum antibody concentrations measured 1 month after the second vaccination were significantly higher in the vaccine than in the control group, although those of IgM did not differ. This behavior of the latter, however, was not unexpected, since it is characterized by a rapid response within days and it was measured after 1 month already had elapsed. In contrast, IgG responds more slowly. The antibody response finding is of interest, since even though increased humoral response to vaccination has been demonstrated in mice [12], the evidence from humans vaccinated with killed Leishmania promastigotes [15, 21] is not clear. Anti-Leishmania antibodies have been linked with the presence of active disease in humans [5, 35] as well as with clinical severity indicators, such as lesion evolution time, lesion number [5, 35], and lesion size [5, 36]. However, it is unknown whether antibody response to vaccination is associated with protection. It has been suggested that even if elicited, antibody response may be irrelevant or that antibody levels may only be paralleling the activation of specific effector mechanisms required for a protective response. More detailed studies of vaccination-induced cellular and humoral immune response are required to better understand the mechanism(s) by which the vaccine may confer protection.

The efficacy of the vaccine in the Ecuadorian child population measured at the end of the 12-month follow-up period was estimated at 73%. The results of previously published phase III studies conducted in other New World populations investigating the prophylactic immunization of humans with killed whole-promastigote vaccines without adjuvant are difficult to interpret regarding efficacy for various methodologic problems outlined recently [2, 21]. However, the performance of the vaccine investigated in this study appears to be improved compared with that reported for a series of polyvalent vaccine field trials done in Brazil during the previous 2 decades [18–20]. On the other hand, the observed efficacy is similar to the 82% reported by Pessoa and Pestana [37] >50 years ago for a killed L. braziliensis vaccine.

It is unclear why the incidence of CL was lower than expected in the control group. Two studies conducted in the population during the 4 years before the start of the vaccine trial reported that the rate in children aged <15 years ranged between 13% [25] and 15% [5]. Likewise, the estimated incidence among the same age group of unvaccinated children in the population after the 12-month follow-up was similar, ~12% (unpublished data). Moreover, post hoc analysis of the data available for children who received only 1 dose of the vaccine plus BCG (n = 51) or 1 dose of BCG only (n = 47) but who were still observed over the entire follow-up period revealed that the proportion in both experimental groups who developed CL was similar: 11.9% and 12.8%, respectively. One possible explanation for the lower observed incidence in the control group may be that BCG is not a true placebo, since it may have boosted cellular defenses through immunomodulatory action. There exists some evidence for BCG’s immunomodulatory role. For example, inoculation with BCG has been reported to appear to confer protection against leprosy [38–40], an effect that may be enhanced by multiple doses [38, 39]. In addition, BCG treatment of BALB/c mice has been cited as reducing disease severity in animals infected with Leishmania tropica, Leishmania donovani, and L. major [41, 42]. Furthermore, it was found that animals given BCG 2 weeks before immunization with L. mexicana amazonensis resulted in protection against infection that was associated with a strong delayed hypersensitivity reaction [43]. Thus, if it is true that control subjects who were given BCG did not receive an inert placebo, then it is possible that the true efficacy of the vaccine is higher. The possible immunomodulatory role of BCG in protection against CL should be investigated further in future studies.

In conclusion, the results indicate that the polyvalent vaccine plus BCG adjuvant tested in this field trial was safe, immunogenic, and efficacious in children living in an area of Ecuador in which CL is endemic. The 2-dose vaccine appeared to invoke a similar level of immune response and confer protection against CL in children with mild to moderate malnutrition and in their better-nourished counterparts. These results suggest that malnourished children should not be automatically excluded from participation in future trials of this and other anti-Leishmaniasis vaccines. It remains to be answered how long the apparent protection conferred by the vaccine lasts. This important issue will be answered in the near future, since after the data for the initial 12 months reported here were retrieved, the study was reblinded, and follow-up has continued for an additional 3 years.

Acknowledgments

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