

# Vaccine for Prophylaxis and Immunotherapy, Brazil

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Over the last 10 years, 153,283 cases of American cutaneous leishmaniasis (ACL) were reported in Brazil. ACL incidence has been estimated to be around 20,000 new cases per year over the last five years,<sup>1</sup> characterizing this disease as highly endemic in many parts of the country. Prevention of ACL is based largely on avoiding contact with the vector, a method not always feasible because of the way the disease is transmitted. Contrary to what has been observed in visceral leishmaniasis, the complex epidemiology combined with the problems associated with drug treatment (prolonged treatment time and numerous side effects, in addition to drug resistance) make prophylaxis against ACL a serious health problem in countries affected by the disease. Due to the peridomestic habits of the only vector of American visceral leishmaniasis known to date (*Lutzomyia longipalpis*) and the fact that the disease is relatively easy to detect in the main reservoir, the domestic dog, effective prophylactic measures such as patient treatment, insecticide spraying, elimination of the reservoirs, and epidemiological surveillance are usually successful in American visceral leishmaniasis.<sup>2,3</sup> Unfortunately, this is not the case in ACL. Due to the sylvatic nature of both the vectors (many sandfly species have been identified as possible vectors) and reservoirs (most of them, still not identified),<sup>4</sup> effective prophylactic measures are rarely effective in this form of leishmaniasis.<sup>5</sup> Since most of the infections are acquired inside the forest, measures such as insecticide spraying and elimination of the reservoirs are virtually unfeasible. In addition, the possibility of development of insecticide resistance<sup>6</sup> in some sandfly species has also to be taken into consideration, not to mention the severe risks of environmental contamination associated with such procedures. ACL is, thus, an

occupational disease among individuals who work in areas such as the Rio Doce Valley, in the Minas Gerais State in Brazil<sup>7</sup> and in the periphery of cities such as Rio de Janeiro and Belo Horizonte. Vaccination remains, then, one of the most acceptable, safe, and practical prophylactic measures against ACL.<sup>5</sup> Many efforts have been made over the years, both in the Old and New Worlds in order to develop a vaccine against the cutaneous forms of leishmaniasis.

According to Modabber,<sup>5</sup> two approaches are currently being taken on this issue: a systematic one and a pragmatic one. The first includes studies on the identification, purification, and production of protein fractions from various *Leishmania* species capable of inducing protection, as well as adjuvant selection and evaluation of immunological responses in animal models. The second approach, which includes most of the vaccines in clinical trials today, uses crude leishmanial extracts or live organisms, which, although standardized in many ways, are still poorly defined. Both approaches have made considerable progress through the years and three vaccination procedures have been used:

1. Leishmanization. This procedure involves the inoculation of live *Leishmania* in a nonexposed area of the body, usually the deltoid area of the arm, of people living in regions of high incidence of cutaneous leishmaniasis in the Old World. The rationale is based on the fact that most of the Old World species of *Leishmania* develop self-healing lesions that are associated with a state of protection against further infections by the parasite. However, reports of many cases where the lesion failed to heal have made this procedure inappropriate for general use and, thus, not recommended by WHO.

2. Vaccination with fractions from *Leishmania* sp. extracts. Although mostly restricted to experimental models, this approach has been recently tested in humans. Based on the results from a study made on one human volunteer,<sup>8</sup> Monjour et al.<sup>9</sup> carried out a small noncontrolled clinical trial in an endemic area of the Pernambuco state in Brazil. In this trial antigen fractions from a local *Leishmania braziliensis* strain were successfully tested, a result that is highly encouraging.

3. Vaccines based on dead promastigotes. In the New World, studies on vaccine development have fo-

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cused on the use of dead promastigotes as an immunizing antigen against ACL. These studies date back to 1939 when Salles-Gomes and Pessôa attempted to immunize the local population in São Paulo State in Brazil with this type of preparation. These studies were, later on, followed by a series of trials performed by Mayrink and colleagues, which culminated in the development of a vaccine for ACL. Production of this vaccine has presently been licensed for use in trials under permit and supervision of the Brazilian Ministry of Health.

### Early Clinical Trials in Brazil

The first attempts to vaccinate a human population with a dead leishmanial promastigote preparation date back to 1939. At this time, Salles-Gomes<sup>10</sup> tested the effects of inoculation of a suspension of phenol-killed dermatropic *Leishmania* sp. on cutaneous leishmaniasis patients. Two effects drew the author's attention: (1) the general reactions, observed when the suspension was inoculated intravenously, decreased in strength as increasing doses of the suspension were administered; and (2) the suspension had an immunotherapeutic effect since a decrease in lesion size was observed as treatment proceeded. Based on these observations, he suggested that the suspension might be able to induce protection, perhaps mediated by antibody production. A vaccination trial was then initiated but, unfortunately, was not concluded.

In the early 1940s, Pessôa and colleagues reported the first vaccination trials against leishmaniasis to be conducted in humans.<sup>11-13</sup> The vaccine consisted of a suspension of  $1.2 \times 10^8$  promastigotes from a pool of 18 dermatropic *Leishmania* sp. strains, prepared in saline-phenol solution. The vaccine was given to a group of 527 individuals, who tested negative for the Montenegro skin reaction, by intramuscular injection in three doses of 1, 2, and 3 ml each with a 7-day interval between doses. A control group of 600 nonvaccinated individuals was also included in the trial. After an observation period of 20 months, it was shown that 18% of the control group had been infected by *Leishmania*, as compared to 3.2% of infection in the vaccinated group, corresponding to a reduction of approximately 80% in the incidence of the disease in the vaccinated group. It is important to mention that no major side effects were observed within the vaccinated group and also that the Montenegro skin test remained negative after the vaccination.

### Polyvalent Mayrink's Vaccine

The studies of Pessôa and colleagues were followed much later by a series of trials conducted by Mayrink and colleagues. Some modifications were introduced in the vaccination protocol regarding not only the number of *Leishmania* strains but also the preparation of the sus-

pension itself. Thus, only five dermatropic *Leishmania* strains were used and sonication of the parasites was also introduced. Furthermore, total nitrogen content was used as a way of standardizing the preparations.

The protocol for vaccine preparation was originally described by Mayrink et al.<sup>6</sup> Basically, five strains of *Leishmania*, isolated from different areas in Brazil, were grown separately in liver infusion tryptose (LIT) culture medium. After a 7-day culture period, the parasites were harvested by centrifugation followed by three washes in sterile saline, the last wash being made in saline containing thimerosal at 1:1000 dilution in order to kill the parasite. Half the concentrate was sonicated to disrupt the parasites while the other half was left intact. The nitrogen content of both preparations was determined, after which the two preparations were mixed and diluted with the appropriate amount of buffered phosphate solution to achieve a final concentration of 120 µg/mL of total nitrogen and 1:10,000 merthiolate.

Mayrink et al.<sup>7</sup> verified that administration of this vaccine by intramuscular injection was able to induce a positive Montenegro skin test in human volunteers, demonstrating for the first time that a dead protozoan preparation generated a cellular immune response in humans. Furthermore, no major side effects were observed with this vaccine preparation.

### First Clinical Trial, Caratinga, Minas Gerais

The first clinical trial to evaluate the ability of the vaccine to induce protection was conducted in the Barracão Valley.<sup>6,14</sup> A total of 1588 volunteers, negative for Montenegro skin test, were randomly assigned to two groups of 614 (vaccinated) and 974 (control). Volunteers were injected IM with 120 µg N/mL according to the protocol described by Pessôa.<sup>12</sup> Three months after vaccination a sample of the volunteers was submitted to a new Montenegro skin test. Analysis of the results showed that 78.4% of the vaccinated group had converted their skin test to positive whereas all controls were negative. A new evaluation after one year showed a 73.2% conversion rate and after two years 54.1% of the vaccinated individuals still showed a positive reaction. Since the trial no cases of cutaneous leishmaniasis have occurred in the area, making it impossible to assess the protection induced by vaccination. This trial, however, confirmed the observations that the vaccine was safe and able to induce in vivo cellular responses as measured by its ability to promote the conversion of the Montenegro skin test. Fifteen years later, 18 cases of cutaneous leishmaniasis occurred in the area. Of these patients, 16 had not been vaccinated and the other two had received the vaccine but did not convert the Montenegro skin test.

## Second Clinical Trial, Viana, Espírito Santo

Concomitant with the first trial, an increase in the number of cases of ACL in the area of Viana, Espírito Santo State<sup>15</sup> opened the opportunity for another clinical trial.<sup>16</sup> Again, after selection for negative Montenegro skin test, a total of 483 volunteers were randomly assigned to two groups of 216 vaccinated and 267 controls. Forty days after the last dose of vaccine, around 80% of the vaccinated individuals showed a positive skin test, while controls remained negative. Both groups were continually monitored for a period of two years after which, mainly due to migration from the area, only 203 individuals from the control group were still living in the area. Of these, 18 (8.9%) contracted ACL as compared to only 3 (1.7%) of the 179 vaccinated individuals that remained in the region, a statistically significant difference ( $p < .01$ ). Although these were not double-blind controlled trials, some conclusions were drawn: (1) vaccine concentration could be raised to 240 µg nitrogen/mL, thus reducing the inoculation volume; (2) an immunization protocol of two injections at an interval of 7 days apart was successful, an important observation for future trials; and (3) the vaccine could be stored at 4°C for a period of 5 years without loss of immunogenicity, as evaluated by the skin test responses obtained with the same vaccine preparation.

## Third and Fourth Clinical Trials, Manaus, Amazonas

Based on the preliminary studies made with the vaccine and stimulated by a report of an epidemic of ACL among paratroopers from the Brazilian Army that remained in the Amazon jungle for a period of 4 days, as well as observations of increased occurrence of the disease among soldiers during training in that area, two double-blind controlled clinical trials were conducted during the years 1981 and 1983. These trials<sup>17,18</sup> were executed in an area of high endemicity for ACL in the Amazon region with volunteers chosen among Brazilian Army conscripts in training in the area. The trials involved a total of 1311 volunteers in 1981 and 1274 in 1983. Vaccine composition was slightly changed with the substitution of stock BH4 for stock M1176 (M1176 is a *Leishmania guyanensis* stock, isolated from the region). The doses contained 240 µg of nitrogen/mL and were administered by slow deep intramuscular injections of 1.5 ml into the arm with a 7 day interval between the two doses. Control groups received merthiolated saline as placebo.

Skin-test conversion was 33% in 1981 and 70% in 1983, giving an overall protection rate of 23% and 60% for each trial, respectively. The low skin-test conversion rate in 1981 was attributed to a possible immunosuppressive effect of yellow-fever, tetanus, and typhus vaccines

administered just before the volunteers had received the experimental vaccine.

Based on the four trials, the following conclusions were drawn: (a) the vaccine does not induce any undesired side effects; (b) it is able to induce cellular immune response as measured by the high skin-test conversion rates observed after vaccination; (c) protection rate among vaccinated individuals that converted the skin test was significantly higher than among those that did not convert the test or nonvaccinated controls; and (d) adequately stored vaccine preparations do not lose immunogenicity, at least for a period of 4 years, as measured by skin-test conversion and immunoprotection.

Histological samples of the skin-test reaction in vaccinated individuals were analyzed and compared to those of patients that suffered from cutaneous leishmaniasis and had a positive skin-test.<sup>19</sup> Results demonstrated a great similarity between the two reactions, the principal pattern being multiple inflammatory perivascular and perianexial foci in the superficial and deep reticular dermis. The exudate consisted mainly of mononuclear cells with scanty interspersed granulocytes and no giant Langerhans' cells. These observations suggest that vaccination induces, in those individuals that converted the skin-test reaction, the same cellular immunological alterations observed in those that naturally acquired ACL. Although these observations do not necessarily imply that a positive skin-test reaction after vaccination is an indicator of protection against the disease, they do demonstrate that the vaccine induces a cellular immunological condition similar to those that have overcome the disease and are, thus, protected against it.

## Fifth Clinical Trial, Belo Horizonte, Minas Gerais

Evaluation of the cellular and humoral responses in another group of army conscripts vaccinated<sup>20</sup> showed that vaccination increases the lymphocyte proliferative responses of peripheral blood mononuclear cells when compared to those from nonvaccinated controls. No differences, however, were observed between vaccinated individuals that received *Corynebacterium parvum* as an adjuvant and those that received the vaccine alone. A correlation of 90% was found between positive skin-test results and positive lymphoproliferative reactions. Immunoprecipitation assays of <sup>125</sup>I-labeled vaccine demonstrated that sera from vaccinated individuals recognized antigens with molecular masses of 13.5, 25, 63, 73, 85, 97, and 160 kDa. IgM was the predominant antibody in the sera of vaccinated subjects.

In another study addressing the immunogenicity of the vaccine<sup>21,22</sup> lymphoproliferative reactions before vaccination were compared to those obtained 40 days after vaccine administration. Results showed that lym-

phoproliferation after the vaccination was significantly higher when cells were stimulated with antigen preparations obtained from that of the *Leishmania* strains present in the vaccine, as well as one *Leishmania braziliensis* preparation (not present in the vaccine composition). Interestingly, however, no differences in cellular proliferation before and after vaccination were observed when cells were stimulated with an antigen preparation from *L. chagasi*, the causative agent of visceral leishmaniasis, a result that suggests some degree of specificity in the response induced by the vaccine. IFN- $\gamma$  has been demonstrated, in several situations, to be involved in resistance to leishmaniasis.<sup>23-25</sup> Analysis of IFN- $\gamma$  production by cells from five vaccinated individuals and stimulated with *L. braziliensis* showed an increased production of this cytokine after vaccination. A positive correlation was found between IFN- $\gamma$  levels and cellular proliferation but not with the intensity of the skin-test reaction. Peripheral blood mononuclear cells obtained from eight individuals one year after vaccination were stimulated in vitro with *L. braziliensis* antigens and analyzed by flow cytometry.<sup>25,26</sup> In all cases a predominance of CD8+ T cells was observed, contrasting with a predominance of CD4+ T cells observed when cells from a control group of cutaneous leishmaniasis patients were stimulated with the same antigen preparation.

### New Directions Proposed by the Vaccine Advisory Group—WHO

In spite of the extraordinary results obtained with the vaccine preparation mentioned above, several criticisms were made as to its composition and characterization. One of the main issues is whether or not there is a need for a multistrain vaccine, not only because of lack of evidence for this but also due to manufacturing difficulties, such as differences in growth characteristics of each strain. In addition, the presence of taxonomically ill-defined strains in the vaccine composition could cause problems in standardization of the vaccine for general use. Furthermore, identification of the antigens responsible for the protective effect would be complicated with such a complex antigenic composition.

Two scientific meetings were organized to analyze this issue, one hosted by the Pan-American Health Organization and UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) in Washington in February 1991 (A Technical Consultation on Vaccine Development in Brazil) and another hosted in September 1991 in Belo Horizonte, Brazil, by TDR and Biobras S.A. (a pharmaceutical company interested in the vaccine production). Based on the suggestions from these meetings, a single-strain vaccine formulation, using a *Leishmania amazonensis* strain, was adopted. Because of insufficient indications of differences in immunogenicity of the different strains, technical rather than immunological criteria were

adopted in choosing the *Leishmania* strain to be used. It was also decided that Biobras S.A. would produce the vaccine under GMP (Good Manufacturing Practices) conditions and supply it for the new trials. Phase I trials were conducted in Rio de Janeiro.<sup>27</sup> According to the clinical and laboratory assessments performed, and despite the high concentration of total protein per inoculum (1440  $\mu$ g), it was concluded that the vaccine is innocuous, whether applied in a single dose or two doses with 7-day or 21-day intervals, either autoclaved or not. Phase II trials are currently being performed.

In parallel to the trials just mentioned, a double-blind controlled clinical trial was designed in order to compare the immunogenicity of some of the *Leishmania* strains present in the original vaccine.<sup>28</sup> In this trial 63 individuals with negative skin test were randomly assigned to four different groups. Group A received a vaccine prepared with *Leishmania amazonensis* strain PH8 alone (IFLA/BR/67/PH8); group B was immunized with a vaccine made of *L. guyanensis* strain M1176 (MHOM/BR/70/M1176); group C received the original vaccine, now prepared by Biobras S.A. (Leishvacin®); and group D was injected with merthiolated saline as a control. The dose used was 240  $\mu$ g nitrogen/mL, and each individual received two doses of 1.5 mL with a 21-day interval. Blood samples were collected before and 21 days after the last vaccine dose for immunological assays. Skin tests were performed 40 days after the second dose using *L. amazonensis*, *L. guyanensis*, classical leishmanin antigen preparation, and merthiolate-saline. Two antigens were administered in each forearm (40  $\mu$ g/test) in a double-blinded protocol. No differences in the size of the skin reaction ( $p > .05$ ) were observed when comparing the different antigen preparations in each vaccine group (data not shown). As shown in Table 1, all vaccine preparations induced a significant skin-test conversion when compared to the control group. No differences were detected among the vaccinated groups. Furthermore, the lymphoproliferative response of vaccinated individuals was significantly higher than those of placebo-injected controls, again with no differences between vaccine preparations (Figure 1). The same pattern was observed when IFN- $\gamma$  production was measured in the supernatants of pe-

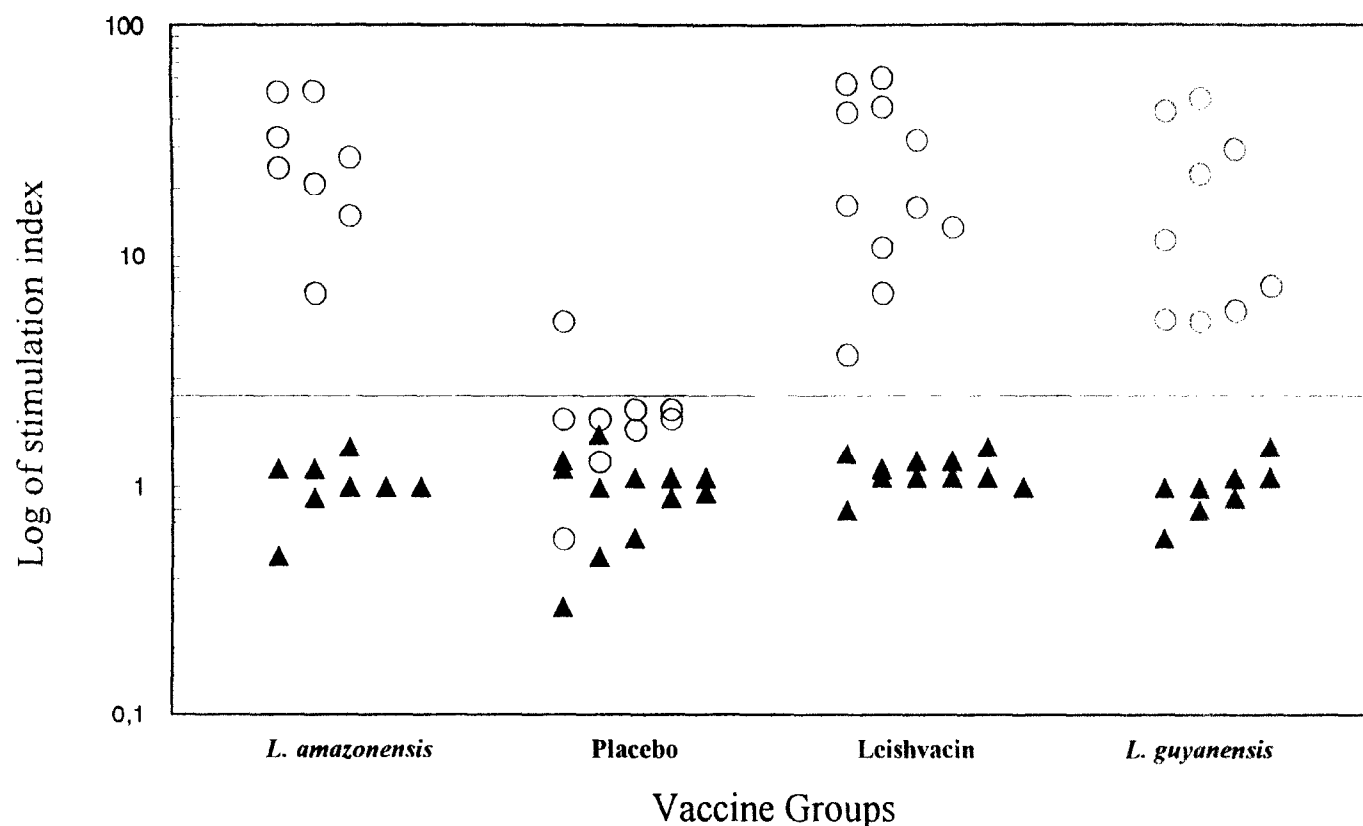
Table 1. Delayed Hypersensitivity Skin-Test Responses in Individuals Immunized with Different Formulations of Killed Promastigotes Vaccine

Vaccine	Number of subjects	Positive skin-tests (%)*
<i>L. amazonensis</i>	15	80% (59.8–100%)
<i>L. guyanensis</i>	14	57% (31–83%)
Leishvacin	18	83% (65.6–100%)
Placebo	19	5.2% (0–15.2%)†

Test was performed 40 days after the second vaccine dose.

\* 95% confidence interval.

† Chi-square test showed significant differences between vaccinated patients and placebo patients ( $\chi^2 = 28.6$ ;  $p < .05$ ).



**Figure 1.** Lymphoproliferative responses of peripheral blood mononuclear cells from subjects immunized with different *Leishmania* vaccine preparations. Cells were stimulated with *Leishmania amazonensis* antigen (20  $\mu$ g protein/mL) for 5 days after which they were pulsed with [ $^3$ H]thymidine for 18 h.<sup>19</sup> Proliferation indexes = cpm stimulated cultures/cpm nonstimulated cultures. Symbols represent data from individuals before (▲) and 20 days after the second vaccine dose (○). A proliferation index  $\geq 2.5$  (horizontal line) was considered significant. All vaccine preparations induced significant proliferation when compared to placebo (covariance analysis,  $p < .05$ ).

peripheral blood mononuclear cells stimulated in vitro with leishmanial antigen preparations (Figure 2).

Taken together, these data show that the original vaccine and the ones prepared with single strains do not differ in their ability to induce cellular and humoral immune responses, suggesting that the protection against *Leishmania* infection induced by these preparations would be similar. This conclusion is important in view of the current effort of TDR and WHO in standardizing the production of leishmaniasis vaccines throughout the world.

### Studies with Animal Models

During the development of the human studies mentioned above, questions regarding vaccine composition, immunization protocol, adjuvant usage, evaluation of immune responses, etc., had to be addressed using the mouse model, not only because of the ethical restrictions imposed by the experiments themselves but also because some of the necessary human reagents were not available yet. Thus, using the multistrain vaccine originally described, a series of experiments were conducted with the C57BL/10 mouse and infection with *L.*

*amazonensis* (PH8 strain) as a model. The vaccine, when associated with *Corynebacterium parvum* as an adjuvant, is able to induce a 50% protection in C57BL/10 mice against a challenge infection by *L. amazonensis*.<sup>29</sup> This protection is associated with the development of a Th1 type response as measured by increased IFN- $\gamma$  and lack of IL-4 production by spleen and lymph node cells from vaccinated animals.<sup>30</sup> Studies with a single-strain vaccine demonstrated that protection levels induced by these preparations were similar to those obtained by the multistrain vaccine.<sup>30,31</sup> Furthermore studies with purified antigens<sup>20,29,32</sup> demonstrated that an increased protection against the challenge infection can be obtained and that the antigens of 46, 63, and 97 kDa purified from the vaccine may be involved in the induction of protective immunity.

### Immunotherapy for Cutaneous and Mucocutaneous Leishmaniasis

Treatment for cutaneous leishmaniasis is based mainly on chemotherapy. Some new drugs have been tested; however, pentavalent antimony salts (Pentostam and Glucantime) are still the drugs of choice, although



ments) during the first 5 days of treatment, after that a volume of 500  $\mu$ L was given until day 10 followed by 10 days without treatment. New series of 10 days of treatment were given until complete cure was observed. From a group of 122 patients, 60 received the vaccine and the remaining were treated with conventional chemotherapy. The results from this trial confirmed the first one in that 95% of the patients treated with the vaccine were cured after a maximum of 20 series (400 days) with an average of  $7.6 \pm 4.0$  series. Patients receiving conventional chemotherapy were clinically cured with a maximum of nine series and an average of  $4.0 \pm 1.4$  series. Of importance in this trial was the fact that some of the patients treated with immunotherapy could not receive the conventional treatment due to heart disorders (including Chagas' disease) or liver and renal dysfunctions. Again, immunotherapy proved to be an effective alternative treatment for cutaneous leishmaniasis, specially when conventional chemotherapy is not recommended.

In trying to improve the efficacy of the immunotherapeutic protocol, a third trial was carried out where BCG was used as an adjuvant to the vaccine. This time, patients were treated with either Leishvacin alone (600  $\mu$ g protein/dose), BCG alone (100  $\mu$ g/dose), or both. All injections were given intradermally once a month for five months (30 patients per group). A control group was treated with conventional chemotherapy with Glucantime. At 150 days after treatment, clinical cure was observed in 28% of patients with BCG, 22% with Leishvacin, and 40% with the combination. No differences were observed among the different protocols ( $p > .05$ ). Ninety-eight percent of the conventionally treated patients were completely cured after eight series of treatment (150 days).

Finally, a fourth trial was conducted where immunotherapeutic intervention was combined with drug administration in order to reduce the time of healing and amount of antimonial given. Thus, a combination of subcutaneously injected Leishvacin (using the serial sequence described above) and intramuscular administration of Glucantime was compared to conventional chemotherapy. One hundred five patients were involved in this trial; 51 received immunochemotherapy and 54 chemotherapy. Patients treated with the combination of vaccine and antimonial were completely cured after a maximum of seven treatment series (140 days) with an average of  $3.2 \pm 1.1$  series. On the other hand, nine series (180 days) were necessary for complete cure of conventionally treated patients where the average was  $4.5 \pm 1.7$  treatment series. It should be noted that about 65% and 90% of the patients treated with immunochemotherapy were completely cured after three and four treatment series, respectively. Cure rates for the conventionally treated patients were 30% and 62% for the same number of series. Figure 3 shows

a patient treated by this scheme. These findings are of great importance since they will permit the establishment of an alternative treatment protocol with reduced time for complete cure and consequently decreased risk of toxicity and also reduced cost. This trial is currently being repeated under double-blinded randomized conditions by using the *L. amazonensis* single strain vaccine.

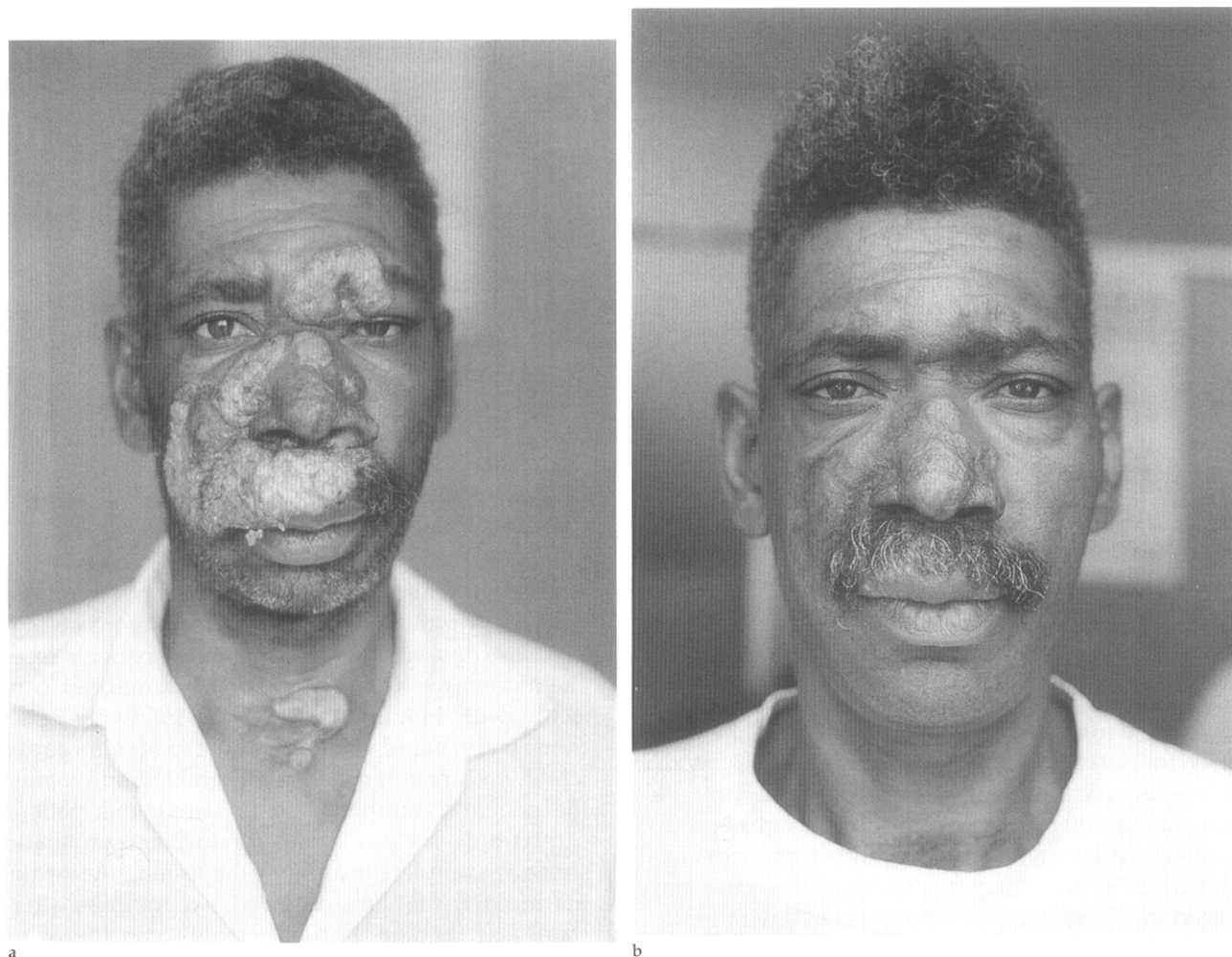
### Immunotherapy for Diffuse Cutaneous Leishmaniasis

In addition to the cases of cutaneous leishmaniasis mentioned above, immunotherapy (using the vaccine alone) was used to treat five patients suffering from diffuse cutaneous leishmaniasis (DCL).<sup>38</sup> The vaccine (600  $\mu$ g protein) was injected by intradermal route in association with BCG (200  $\mu$ g) in three spots of the deltoid region, and three or four doses were administered at 30-day intervals. Three of five patients became Montenegro skin-test positive 30 days after receiving the first dose of vaccine, after which these patients received chemotherapy (Glucantime or pentamidine) concurrently with immunotherapy. The following observations were made on these three patients: patient 1 (child, 6 years old, male): considerable improvement in the patient's condition had occurred; this patient returned only six years later for further examination,<sup>40</sup> showing complete healing of the lesions. According to his mother, the cure occurred two months after the treatment; patient 2 (adult, male): considerable improvement was noted, and the lesions almost completely healed; this condition was maintained while the patient continued to give positive response to Montenegro skin test; three months after the first dose, when this test reverted to negative, lesions began to reappear. The third patient (adult, male) had an improvement in the overall condition, but 98 days after the second dose, he became Montenegro skin-test negative, with concurrent reversion to the pretreatment status. The two other patients did not undergo a change in Montenegro response or any clinical improvement. Although limited to a few cases, it might be suggested that, at least in some cases, progress towards cure can be obtained by proper stimulation of cell-mediated immune mechanisms through vaccine administration. Further investigation in this area is still required.

### Immunotherapy for Cutaneous Leishmaniasis in HIV or Leprosy Patients

The occurrence of concomitant leishmaniasis (both cutaneous and visceral) in HIV patients has increased over the last five years. To add insult to injury, these patients are usually resistant to conventional chemotherapy with bad prognoses in the majority of the cases.





**Figure 3.** (a) Glucantime-resistant American cutaneous leishmaniasis patient. No lesion regression was observed after administration of three series of antimonial (20 ampoules per series). (b) The same patient after 4 series of immunochemotherapy (20 ampoules of Glucantime plus 5 ml of Leishvacin per series).

Four HIV-positive patients with Glucantime-resistant cutaneous leishmaniasis were treated with combined immunochemotherapy.<sup>41-43</sup> In all cases lesion regression was successfully achieved with at least<sup>42</sup> one patient showing an increased proliferative response to leishmanial antigens, with a predominance of CD8+ T cells and IFN- $\gamma$  production by stimulated peripheral blood mononuclear cells. One of these patients (unpublished data) had over 250 lesions spread all over the body surface. All of these lesions successfully healed after the treatment and have remained inactive for the last two years.

Two other patients with ACL-leprosy association (lepromatous and indeterminate forms)<sup>40</sup> received the vaccine combined with BCG by the intradermal route. Three doses of vaccine plus BCG, with a three-month interval, were required to induce complete healing of the ACL lesions of the lepromatous patient, whereas only one dose was needed to induce healing of the ACL lesions of the indeterminate leprosy patient.

## Conclusion

Although complete protection has not been accomplished yet with the current dead promastigote preparations and immunization protocols, it seems that effective prophylaxis against ACL can be achieved by controlled administration of this vaccine. This assumption is based not only on the results of protection against the disease in the various clinical trials described here but also on the results of the *in vivo* and *in vitro* immune responses (Montenegro skin-test reaction, T-cell proliferation, and IFN- $\gamma$  production) observed in vaccinated individuals. Furthermore, the successful use of this vaccine in treatment of established cutaneous leishmaniasis makes this preparation an excellent alternative for therapy not only in cases of single-lesion cutaneous leishmaniasis where conventional chemotherapy is not recommended due to parasite resistance to the drug or patient's incompatibility with the treatment, but also, and perhaps more impor-



tantly, in more severe cases of the disease such as diffuse cutaneous leishmaniasis, or leprosy/HIV and *Leishmania* coinfections.

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