



Figure 1 Lesions induced by leishmanization and by natural infection.

above, everyone entering in a leishmaniasis vaccine trial that includes leishmanization as live challenge will be protected against the disease, either by the experimental vaccine or by leishmanization. Considering the benign nature of the lesions developed by leishmanization and the site of the lesion, in usually covered part of the body (upper deltoid), compared with unsightly lesions on the face by natural infections (Fig. 1), there is a high willingness in certain hyperendemic areas to participate in a vaccine trial with leishmanization.

Live *Leishmania* Vaccines

Live *Leishmania* vaccines have general drawbacks and none have reached the stage of preclinical development. Since live *Leishmania* cannot be lyophilized to maintain viability and they transform in vitro with differential expression of genes, standardization and delivery to many leishmaniasis endemic foci would be a major obstacle. *Leishmania* can be kept viable frozen in liquid nitrogen, but delivery to the population at risk would require much infrastructure beyond the means of many endemic countries. In addition, with the danger of present expansion of HIV infection, live *Leishmania* vaccines are unlikely to find wide acceptance, since nonpathogenic parasites can coinfect and have been isolated from HIV-infected patients.

Using wild-type virulent *L. major* for leishmanization (LZ), a lesion must develop before protection is induced. However, there are examples of protective vaccination in mice with genetically modified parasites (either by mutagenesis (101) or by genetic modification (102) that do not produce the pathology, yet induce protection against wild-type parasites under certain conditions. Another approach for genetic modification is to add external gene(s) "suicidal cassettes" (103) to render the parasite more responsive to drugs. In all these models, the idea is to induce protection but not the pathology associated with LZ.

Knockout Parasites

The first construct generated by gene targeting, was a dihydrofolate reductase-thymidylate synthase (DHFR-TS) *L. major* knockout (102). Injected mice showed significant protection (short term) against challenge with wild type (104). Although the knockout parasite showed a type-1 cytokine response by human cells in vitro, further studies in monkeys were disappointing (105) hence the DHFR-TS knockout construct has not been further developed as a vaccine.

Using a similar technique of homologous recombination with *L. donovani*, the bipterin transporter (BT1) was inactivated. The BT1 knockout construct had much-reduced