virulence but elicited an immune response leading to a high level of resistance against challenge by the wild-type parasite (106).

Several cysteine proteinases (CPs) of *L. mexicana* knockouts were constructed lacking CPa, CPb or both (107,108). These constructs had reduced pathogenicity and induced partial protection in BALB/c mice against challenge with the wild-type *L. mexicana* (109).

Suicidal Cassettes

A double drug sensitive strain of *L. major* was constructed (110) by introducing HSV-1 thymidine kinase gene (to confers increased sensitivity to ganciclovir) and a *Saccharomyces cerevisiae* cytosine deaminase gene (for sensitivity to 5-fluorocutosine). Progressively growing lesions in BALB/c mice, with this construct, were completely cured by two weeks of treatment with either drug alone or in combination. Treated animals showed no signs of recurrence of infection for at least four months when the experiments were terminated (111). None of these constructs has reached clinical development yet, however the approach provides possibilities for induction of protection with a self-limiting infection, possibly without any pathology.

FIRST-GENERATION VACCINES (KILLED LEISHMANIA WITH OR WITHOUT ADJUVANTS)

Whole killed parasites if given by appropriate route with adjuvant can protect many experimental animals and hence have been used as a golden standard to evaluate different vaccine candidates. Since most Leishmania species can easily be grown in cell free cultures, killed parasite has been tried as a vaccine much in the same way as early bacterial vaccines. The history of vaccine trials using killed *Leishmania* goes back to 1920s and 1930s first for immunotherapy and then in 1940s for prophylaxis [reviewed by Genaro et al. (112)]. In the 1970s and 1980s, Mayrink and his group in Brazil following the earlier studies of Pessoa (113) and Convit and colleagues using BCG as adjuvant in Venezuela initiated vaccine trials with killed *Leishmania* in prophylactic as well as therapeutic trials (see below). The outcome of all these trials have revealed that whole killed parasite alone or mixed with BCG (as adjuvant) are safe but only weakly immunogenic, hence not useful as prophylactic vaccine. However, they may be very good as an adjunct to chemotherapy either to reduce the dose of drugs, duration of treatment or both (see therapeutic vaccines, below).

New World, Multiple Strains (Mayrink's Vaccine)

The initial vaccine of Mayrink consisted of 5 different *Leishmania* and several trials were conducted (114–116). Three injections were given IM one week apart to volunteers with a negative leishmanin skin test (LST) (also called a Montenegro skin test). The antigen is a low concentration (5–10 μg) phenol killed parasite. Vaccination induced LST conversion (>5 mm induration after 48–72 hours) in 35% to 70% of volunteers in different trials ranging from 480 to 2500 volunteers in each trial. The vaccine was well tolerated; acute adverse reactions were rare (mild pain) and long-time follow-up showed no untoward responses, including presence of autoantibodies. Collectively the trials showed the safety of this approach and revealed that skin test conversion as a result of vaccination is a useful tool in field studies to monitor responsiveness of the population. Antunes et al. (114) demonstrated that LST converters have a lower incidence of disease, which has been repeatedly seen in subsequent trials (see ALM trials). A three-species autoclave-killed vaccine was produced in the laboratory of Armijos et al. (117) and the safety, immunogenicity, and efficacy of two injections against cutaneous leishmaniasis was tested in rural Ecuadorian children in a randomized, BCG-controlled, double-blinded study. Live BCG was used as adjuvant. Within the one-year follow-up, the incidence of CL was significantly reduced in the vaccine group compared with the control group (2.1% vs. 7.6%, $p < 0.003$). The protective efficacy of the vaccine was 72.9% (95% confidence interval = 36.1–88.5%). This is the only trial in which a significant difference was observed between killed *Leishmania* + BCG versus BCG alone.

New World (Single Strain)

Brazil

A single-strain *L. amazonensis* vaccine was produced by Biobras, after careful comparison of different strains (112). This vaccine was tested in a dose escalating trial for safety and skin test conversion (118) and further trials were conducted to analyze the immune responses (119–121). The vaccine induced primarily a Th-1 type response with demonstrable IFN-γ but mostly from $CD8^+$ cells—a pattern associated with the healing process in mice (122) and humans (123). This vaccine was effective (96% cure) when added to low-dose antimonial for treatment of CL in Brazil (see Therapeutic Vaccines below).

Colombia

Mayrink's vaccine was formulated by Biobras at higher concentrations for use in combination with BCG, which is given ID. This formulation was compared for safety and immunogenicity with the IM formulation in a double-blind, randomized placebo-controlled trial in Medellin, Colombia (124). Because of side effects of BCG (active lesion for about three weeks, followed by scar formation) volunteers refused to receive the third injection; hence a comparative study could not be completed. Nevertheless, the three injections of Mayrink's vaccine (killed parasite without BCG) were shown to be well accepted with minor side effects. There were 86% and 90% LST conversion on day 80 post vaccination and a year later, respectively. No antibody production to the vaccine antigens was seen and the cytokine pattern was that of a Th-1 response. On the basis of these results, a double-blind randomized, placebo-controlled efficacy trial was conducted in Colombia on a total of 2597 healthy volunteers with negative LST. The participants were selected from rural Colombian soldiers who were going to patrol endemic areas. Safety and efficacy of the vaccine were determined by comparing local and systemic adverse reactions after each dose and the incidence of parasitologically confirmed CL. The vaccine was shown to be safe but offered no protection against CL caused by *L. panamensis*. Unfortunately, there was no LST performed after vaccination to evaluate the immunogenicity of the vaccine and determine if the converted LST subpopulation had a lower incidence of disease as was reported in previous trials (125).

Venezuela

Convit and colleagues were the first to use BCG as an adjuvant and autoclaved *Leishmania* as the immunogen for immunoprophylaxis as well as immunotherapy (126,127). Without the addition of antimonials, three injections of the vaccine