native promastigote PSA-2 protected mice against *L. major* (144), but vaccination with a recombinant *E. coli*–derived promastigote or amastigote protein showed lack of protective efficacy despite the ability to induce T_h1-type immune responses (157). Protective vaccination was also achieved against *L. amazonensis* (174).

The kinetoplastid membrane protein 11 (KMP-11), a highly conserved surface membrane protein present in all members of the family Kinetoplastidae, is differentially expressed both in amastigote and promastigote forms of *Leishmania* is a vaccine candidate antigen (225). KMP-11, unlike gp63, for example, induced significant production of IFN-γ from lymphocytes of patients cured of VL (226). A DNA vaccine encoding KMP-11 was evaluated for it is protective ability in genetically immunized hamsters. The protective effect was thought to be linked to the generation of functionally active IL-2-producing T cells along with specific anti-KMP-11 CTL-like response and other leishmanicidal effector mechanisms.

Sterol 24-cmethyltransferase (SMT), was recently identified by serological screening using sera from *L. infantum*–infected hamsters (227). SMT is an enzyme involved in biosynthesis of ergosterol, which is a target molecule of leishmanicidal and fungicidal amphotericin B. The antigenicity, immunogenicity, and protective efficacy of SMT were recently evaluated (210). SMT formulated in MPL®-SE was found to be protective against *L. infantum* challenge perhaps through the induction of a T_h1-type immune response with antigen-specific $CD4^+$ and $CD8^+$ T cells. The rSMT plus MPL-SE vaccine induced SMT-specific T cells which were capable of producing multiple T_h1-type cytokines (TNF, IL-2, and IFN-γ) in response to Antigen recall. TNF, IL-2 and IFN-γ are involved in protection against VL (228–230). As TNF synergizes with IFN-γ in killing *Leishmania* parasites, induction of antigen-specific T cells capable of producing multiple cytokines upon antigen recall might be more beneficial for control of *Leishmania* infection than those producing a single cytokine.

Antigens expressed in the amastigote stage may be the most important vaccine candidates since this form of the parasite is both the main inducer and the target of the immune response. The cathepsin L-like CPs are thought to be good vaccine candidates because of their high immunogenicity and important role in host-parasite interaction (231,232). Three classes of CPs have been identified: Type I (CPB), Type II (CPA) and Type III (CPC) (233). Immunization of mice with recombinant CP induced partial protection against *L. major* challenge (156) (Table 3). Recently, a hybrid fusion protein composed of CPA and CPB was used to immunize mice and dogs and partial protection against *L. major* infection was obtained (189,234).

Few studies have been directed at the potential for a vaccine derived from one *Leishmania* species to provide cross-protection against another species. Initial results using sequential infections with distinct species have suggested complex cross-protection relationships. For example, immunization of mice with heat-killed *L. donovani* can induce protection against a subsequent infection with *L. major* (235). Few antigens, including LACK (146,172,176), dp72 (236), and P4 nuclease (203), have been tested for cross-protection in mice with varied success. In contrast to the strictly *L. major* species-specific protection with LACK, cross-species protective efficacy has been demonstrated for dp72. This protein, purified from *L. donovani* promastigotes was able to protect mice against *L. major* challenge (236). Cross-species protection is also a feature of the acidic ribosomal P0 protein from *L. infantum* that was able to protect C57BL/6 from *L. major* infection. In this case, however, protection could not be induced in BALB/c mice, reinforcing the importance of host genetics (237).

Soon after the great success in producing these recombinant antigens it became evident that a critical step in vaccine development was missing, that is, an acceptable adjuvant/delivery system capable of promoting the induction of an immune response biased toward T_h1. Perhaps more critical than the choice of antigens for future vaccine development is the selection of an appropriate adjuvant or delivery system. Successful protection in mouse models has been achieved by vaccination with antigens delivered as DNA, or as proteins delivered with a variety of adjuvants. DNA encoding the leishmanial proteins LACK, LmSTI1, and TSA could effectively immunize susceptible BALB/c mice against *L. major* by inducing protective T-cell responses (213,238,239). However, DNA as a means to deliver prophylactic vaccines has lost its momentum, as studies in monkeys and man have yielded disappointing results.

Similarly, cytokine adjuvants are not a practical alternative for vaccine development, but have nonetheless provided early proof of concept data illustrating the ability of crude protein preparations or of defined recombinant proteins to induce solid protection against disease in the mouse model. Both IFN-γ and IL-12 have been used as adjuvants to induce antigen-specific protective T_h1 responses (36,240). IL-12 injected subcutaneously with leishmanial soluble antigens (SLA), induces a strong anti-SLA T_h1 response and no detectable T_h2 response to this antigen. Importantly, this protocol of immunization confers excellent protection in BALB/c mice challenged with *L. major* (241). IL-12 has been successfully used as a T_h1 adjuvant for a variety of antigens in both the murine and in the nonhuman primate models of several infectious diseases including leishmaniasis (135,140,241–243). However, in contrast with conventional adjuvants and with DNA immunization, it seems that the immunological memory to the immunizing antigen is not stimulated appropriately when IL-12 is used as adjuvant. Thus, vaccination of BALB/c mice with the leishmanial antigen LACK mixed with IL-12 as adjuvant resulted in short term protection against challenge with *L. major*.

The two adjuvants approved for human use, alum and squalene, induce potent antibody responses but are poor inducers of antigen-specific T_h1 responses. Protection against leishmania parasites appears to require the induction of IL-12 by antigen-presenting cells, which can be achieved via stimulation of toll-like receptor 4 (TLR-4), TLR-9, or TLR 7, or TLR 7/8. One recent study has reported that protection against leishmania is dependent on TLR-4 (244,245). Thus, monophosphoryl lipid A (MPL) (or related molecules with similar properties) is a logical choice as a vaccine adjuvant, as it stimulates TLR-4 and is a component of several experimental and two approved vaccines (Fendrix hepatitis B vaccine and cervarix, HPV vaccine, GSK). Building on the experience with MPL, a new synthetic adjuvant molecule, glucopyranosyl lipid A, or GLA, has been developed for next-generation *Leishmania* Vaccines.

CpG ODN alone has been shown to induce a state of partial resistance in BALB/c mice for up to five weeks against challenge with *L. major* (246). If the CpG ODN is injected in conjunction with SLA significant protection is obtained in these animals that is maintained for as long as six months. In these experiments, the immunostimulatory properties of the CpG ODN were associated with production of IL-12 and the emergence of strong T_h1 response to SLA (247,248). However further studies are required to optimize CpG ODN as an adjuvant for humans.