ABSTRACT

Visceral leishmaniasis is a chronic and severe disease of humans, dogs, and certain rodents caused by protozoan parasites of the genus *Leishmania*. The disease is characterized by lymphadenopathy, weight loss, anaemia, renal failure, and occasionally epistaxis or ocular lesions. If left untreated, VL is nearly always fatal. The global prevalence of VL is around 12 million. Approximately 500,000 new cases of visceral leishmaniasis arise annually worldwide. Over 90% of visceral leishmaniasis cases occur in India, Bangladesh, Sudan, Brazil, and Nepal. Further a concomitant HIV infection has increased the risk of developing active VL. In southern Europe, up to 70% of cases of visceral leishmaniasis in adults are associated with HIV infection. The current control strategies for VL rely on reservoir and vector control, the use of insecticide-impregnated material, active case detection and chemotherapy. The chemotherapy has been associated with toxicity, side effects and resistance. The insecticides used for vector control are also becoming resistant. Therefore, vaccination remains the best hope for control of the disease, and the development of a safe, effective and affordable antileishmanial vaccine is a critical global public-health priority. Extensive evidence from studies in animal models indicates that solid protection can be achieved by immunization with defined subunit vaccines or live-attenuated strains of *Leishmania*. However, to date, no such vaccine is available despite substantial efforts by many laboratories. The immune responses to *Leishmania* infection are highly complex. They may accelerate, cure or exacerbate the disease. The protective Th1 type of immune response is associated with increased levels of IFN-γ, IL-2, IgG2a and DTH responses. The deleterious Th2 type of immune response is associated with increased level of IL-4, IL-10 and IgG1 levels.

Heat shock proteins (Hsps) are highly conserved molecules and are present in subcellular compartments in eukaryotes and prokaryotes. They play a key role in mammalian protective immunity by participating in the aggregation of antibody molecules and stabilization of MHC class I and II molecules. They can potently stimulate innate and antigen-specific pathway through synthesizing various cytokines. In the current study, the protective efficacy of two heat shock proteins, that is, Hsp70 and Hsp83 of *L. donovani* in combination with different adjuvants (MPLA and ALD) was evaluated in BALB/c mice. Both proteins have previously been reported to induce a strong cell mediated and humoral immune response in various infectious diseases.
For determining protective efficacy of various vaccine formulations inbred BALB/c mice were vaccinated with Hsp70+Hsp83+MPLA, Hsp70+Hsp83+ALD and only Hsp70+Hsp83 without any adjuvant. Thereafter, they were challenged with $10^7$ promastigotes of *L. donovani* and were sacrificed on 30, 60 and 90 days post infection/challenge. Further, the vaccine efficacy was evaluated by determining hepatic and splenic parasite load, delayed type hypersensitivity responses (DTH), cytokine responses (IFN-γ, IL-2, IL-4 and IL-10) and antibody responses (IgG1 and IgG2a).

A significant reduction in parasite load was seen in all immunized groups as compared to infected control. Maximum parasite reduction was seen in animals immunized with Hsp70+Hsp83+MPLA followed by Hsp70+Hsp83+ALD and Hsp70+Hsp83. The protective Th1 type of immune response was also most pronounced in Hsps plus MPLA vaccinated groups with maximum DTH responses, cytokine responses (IFN-γ and IL-2) and IgG2a antibody responses. It was followed by Hsps plus ALD vaccinated groups. The lowest Th1 type of immune response was observed in animals vaccinated with the cocktail of both the Hsps without any adjuvant.

In the present work, the diagnostic potential of Hsp70 and Hsp83 was also evaluated. It was found that simultaneous occurrence of both of these heat shock proteins may be used as a diagnostic marker for visceral leishmaniasis.