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## Identification of novel *Leishmania donovani* antigens for development of second generation vaccine against Leishmaniasis

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Visceral leishmaniasis (VL), caused by the intracellular parasite *Leishmania donovani* is a major public health problem in the developing world. But there is no effective and safe vaccine approved for clinical use against any form of leishmaniasis. Through reactivity with kala-azar patient and cured sera, polypeptides ranging from 91 to 31-kDa from *L. donovani* promastigotes were previously identified as potential protective vaccine candidates. In this study four polypeptides 91(LD91), 72 (LD72), 51(LD51) and 31 (LD31)-kDa were purified from leishmanial antigens (LAg) using sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by electroelution. Another peptide of 36-kDa (LD36) was purified in its native form using soluble leishmanial antigens (SLA) as a source. We compared the vaccine efficacy of these antigens encapsulated in cationic liposomes in BALB/c mice against challenge infection with *L. donovani*. Our results demonstrated that liposomal LD31 (74%-77%), LD36 (79%-81%) and LD51(72%-75%) vaccination reduced parasite burden to the greatest degree followed by liposomal LD72 (65%-67%) and LD91 (46%-49%). Analysis of cytokines in protected mice revealed induction of Th1 cytokines IFN-  $\gamma$  with IL-12 with a down-regulation of Th2 cytokines IL-4 along with immunosuppressive IL-10, hinted toward a Th1 polarized immune response instrumental for protection. The 31, 36, 51 and 72-kDa bands were identified as ATP synthase chain, Elongation factor-1 $\alpha$ ,  $\beta$ -tubulin and heat shock 70-related protein 1 precursor of *Leishmania*, respectively using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF/TOF) mass spectrometry. These four leishmanial antigens have not been described before as successful vaccine candidates examined against in vivo VL model. Thus, these antigens can be potential components of a future second generation antileishmanial vaccine.