Abstract:

Leishmaniasis is a neglected, tropical disease caused by the protozoan parasite *Leishmania* which affects millions of people worldwide. During past many decades, numerous vaccine candidates have been tested against various forms of leishmaniasis, but convincing results are yet to be achieved. Selection of a judicious vaccine candidate from plethora of antigenic proteins is a crucial step in designing an efficacious vaccine. In this study, we identified virulence factors of *Leishmania* based on their expression levels inside peritoneum-derived primary macrophages post-infection. NAD+ synthase, adenylate kinase, LmjF_36_3850, phosphatidic acid phosphatase, thiol-dependent reductase 1, LmjF_33_2620 was observed to be significantly down-regulated in avirulent as compared to virulent strain, and were identified as probable virulence factors and potential vaccine candidates. Hence, we cloned LmjF_36_3850 in pcDNA6/HisA and evaluated its antigenicity and protective efficacy in *L. major* challenge model. DNA vaccination with LmjF_36_3850 elicited IgG2a and IgG1 to similar extent and produced mixed Th response. Upon s.c. challenge of $2x10^6$ *L. major* stationary phase promastigotes, vaccinated mice were unable to control disease progression and had higher parasitic load in draining lymph node. Expression levels of Th2 cytokines such as IL-4 and IL-10, co-inhibitory molecule such as CTLA-4, were found to be higher in lymphocytes of DNA vaccinated mice. Moreover, CSA re-stimulated lymphocytes from vaccinated mice after challenge infection secrete less IFN-γ than controls and comparable levels of IL-10 as compared to non-vaccinated, *L. major*-infected mice. Also, population of pro-parasitic IL-4, IL-10 and IL-17 secreting Th2-, Treg- and Th17-T_{EM} and T_{CM} cells, respectively, were found to be higher; while population of IFN-γ secreting Th1-T_{EM} cells were lower in

LmjF_36_3850 vaccinated mice as compared to control groups. Thus, DNA vaccination with LmjF_36_3850 was unable to protect mice from *L. major* challenge.

Next, to analyze the potential of Heterologous prime-boost (HPB) strategy against *L. major* challenge, we cloned *L. major* adenylate kinase (LmAdeK) in pcDNA6/HisA and pet28a+. We purified rAdeK by Ni-NTA affinity resin and analyzed immunogenicity and protective efficacy of different groups. HPB vaccination with LmAdeK was able to elicit higher levels of Th1-associated IgG2a antibody and lower IgG1 levels than other vaccination strategies and control groups. IgG2a/IgG1 ratio was higher in HPB vaccinated mice than other vaccinated groups and controls and was skewed towards Th1 type. Also, IgG2a levels in HPB vaccinated mice were higher after five weeks of challenge infection. HPB vaccinated mice were able to better control lesion progression in footpad and have lower parasitic load in draining lymph node than other groups. Also, HPB vaccinated mice had higher IFN-γ and lower IL-4, IL-10, CTLA-4 expression in lymphocytes of draining lymph node. Moreover, HPB vaccinated mice had lower population of IL-17 and IL-10 secreting Th17-T_{EM}, T_{CM} and Treg-T_{EM} cells, respectively. HPB vaccinated mice have lower repertoire of IL-4 secreting Th2-T_{EM} and T_{CM} cells, although levels of IFN-γ secreting cells were lower than the rAdeK-vaccinated group. Hence, HPB vaccination strategy was more effective in controlling disease progression and parasitic burden than other vaccination strategies and contains higher IFN-γ expressing lymphocytes and decreased population of pro-leishmanial cytokine secreting Th2-, Th17- and Treg- memory cells. Thus, vaccination strategy plays a vital role in eliciting a host-protective immune response.