

Evaluation of immune prophylactic response of GLP grade *Leishmania major* centrin deleted (*LmCen*^{-/-}) live attenuated parasites as a vaccine against Visceral Leishmaniasis in Hamsters.

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Background: Leishmaniasis is a vector-borne parasitic disease affecting millions of people worldwide. To date, there is no licensed vaccine available against human Leishmaniasis. It has been shown that low dose of dermatotropic wild type *Leishmania major* infection (leishmanization) confers protection against Cutaneous Leishmaniasis (CL) as well as cross-protection against Visceral Leishmaniasis (VL). However, such a method of immunization is not practical because of the great risk of infection in a naïve population. Therefore, genetically modified live attenuated parasites that are non-pathogenic might induce the same protective immunity as leishmanization. We have developed centrin-gene deficient *Leishmania major* (*LmCen*^{-/-}) using CRISPR-Cas methodology and evaluated the safety, immunogenicity as well as protective efficacy against *L. donovani* challenge. Previous studies from our laboratory demonstrated that lab grown *LmCen*^{-/-} induced significantly strong host protective immune response against *L. donovani* infection in hamster model. Six weeks post-immunization hamsters were infected with *L. donovani* by needle injection or by infected sand flies. In both sets of experiments, nine months post-challenge, non-immunized hamsters developed severe pathology of VL, while immunized hamsters showed significantly lower parasite burden in liver and spleen. We also evaluated the cellular immune response between immunized & non-immunized hamsters after challenge with wild type parasites. Spleen cells from *LmCen*^{-/-} immunized and challenged hamsters produced significantly more Th1-associated cytokines including IFN- γ and TNF- α , and significantly reduced expression of the anti-inflammatory cytokines IL-10 and IL-21, compared to non-immunized and challenged animals.

Objective: The goal of the study was to evaluate the safety and efficacy of the *LmCen*^{-/-} parasites generated under GLP (Good Laboratory Practice) condition in Hamster VL model

Results: In this study, we compared the immune response of GLP grade and lab grown *LmCen*^{-/-} parasites. Similar to intradermal immunization of hamsters with lab cultured *LmCen*^{-/-} parasites, GLP grade parasites did not develop any detectable lesion after immunization suggesting these parasites are safe as an immunogen. Spleen and ear cells from either GLP grade *LmCen*^{-/-} immunized or lab grown *LmCen*^{-/-} immunized hamsters produced comparable Th1-associated cytokines including IFN- γ and TNF- α . IgG_{2a} antibodies associated with protection were similar between the groups as well. Studies are underway to evaluate the efficacy of GLP grade parasite against visceral infection.

Conclusions: Our studies demonstrate that the GLP grade *LmCen*^{-/-} mutant parasites are safe and immunogenic as lab grown *LmCen*^{-/-} and have a potential to be an effective vaccine against VL.