IMMUNE RESPONSE AND HISTOLOGICAL ANALYSIS OF *kh1*-DEFFICIENT LIVE-ATTENUATED *Leishmania infantum* IN MURINE MODEL

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Abstract

Live-attenuated vaccines expose the host immune system to a broad antigen repertoire, which is important for protective immunity. Our group selected *Leishmania infantum* strains lacking the *kharon* gene (*LiKh1*$^{-/-}$), essential for parasite virulence. We previously demonstrated their immunogenicity and ability to induce a protective adaptive response against virulent *L. infantum*. In this study, we assess the immunogenicity through innate immune response profiling and histological analysis in murine models. Macrophages, *Leishmania*'s primary host cells, play a key role in immunity. Since their activation profile affects infection outcomes, we assessed nitric oxide (NO) production from peritoneal macrophages through Griess assay. NO levels were undetectable after *L. infantum* stimulation and very low after IFN-γ pre-stimulation, possibly influenced by TLR2. However, post-infection, LPS+IFN-γ co-stimulation induced NO, suggesting innate immune modulation. Live-attenuated strains remained infective but failed to sustain infection *in vitro*. Understanding the local immune mechanisms required for the control of infection is essential for developing effective vaccines. To further evaluate their impact, BALB/c mice were infected with 1×10^7 *L. infantum* (*LiKh1*$^{-/-}$ or WT) via IP and analyzed 30 dpi. Liver and spleen sections were stained with HE for histopathology and anti-mTXNPx mAb for IHC. Splenic architecture was preserved in all groups, though mild white pulp disorganization was observed in WT. WT induced mild focal liver inflammation, while the attenuated strain apparently caused less damage, consistent with antigen labeling, RT-PCR, and LD. However, IHC revealed strong splenic staining in both groups. These findings highlight the attenuated strain as a promising vaccine candidate, inducing an immune response with less tissue damage. Further studies are needed to confirm its efficacy and to assess its impact on parasite differentiation.

Keywords: *Leishmania infantum*, Attenuated Vaccine, Innate Immune Response

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