Evaluation of the long-term immune responses following leishmanization using a live- Lizard Leishmania mixed with chitin

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Abstract

Background: Leishmanization using non-pathogenic to human *Leishmania* spp. is considered a reliable approach to immunize subjects against *Leishmania* infection. **Objectives**: Here, we evaluated the long-term immune responses (14 weeks) after immunization with either live- or killed-Iranian Lizard Leishmania (ILL) mixed with chitin microparticles (CMPs) against *L. major* infection in BALB/c mice. **Methods:** In total, nine groups of mice were included in the study. To evaluate short-term immunity, mice were immunized with live-ILL and three weeks later were challenged with *L. major*^{EGFP}. To evaluate the long-term immunity, mice were immunized with either live- or killed-ILL, and 14 weeks after immunization were challenged with *L. major*^{EGFP}. A group of healthy mice who received no injection was also included in the study. Eight weeks after the challenge with *L. major*^{EGFP} all subjects were sacrificed and the parasite burden (quantitative real-time PCR), cytokines levels (IFN-γ, IL-4, and IL-10), *Leishmania*-specific antibody concentration, and total levels of IgG1 and IgG2a were measured. In addition, nitric oxide concentration, and arginase activity were evaluated. **Results:** In mice that were immunized using live-ILL+CMP, the induced proactive immune response lasted at least 14 weeks since, when they were challenged with *L. major* $^{\text{EGFP}}$ at the 14 $^{\text{th}}$-week post-immunization, no open lesion was formed during 8 weeks follow-up, and the footpad swelling was significantly lower than controls. As well, they showed a significant reduction in the parasite burden in splenocytes, in comparison to the control groups including the group that received killed-ILL+CMP. The observed protection was associated with a higher IFN-γ and a lower IL-10 production by splenocytes. Additionally, the results demonstrated that arginase activity was decreased in the ILL+CpG group compared to other groups. **Conclusion:** The long-term response against *L. major* infection induced by Live-ILL+CMP was more competent than the response elicited by killed-ILL+CMP to protect mice against infection with *L. major* $^{\text{EGFP}}$.

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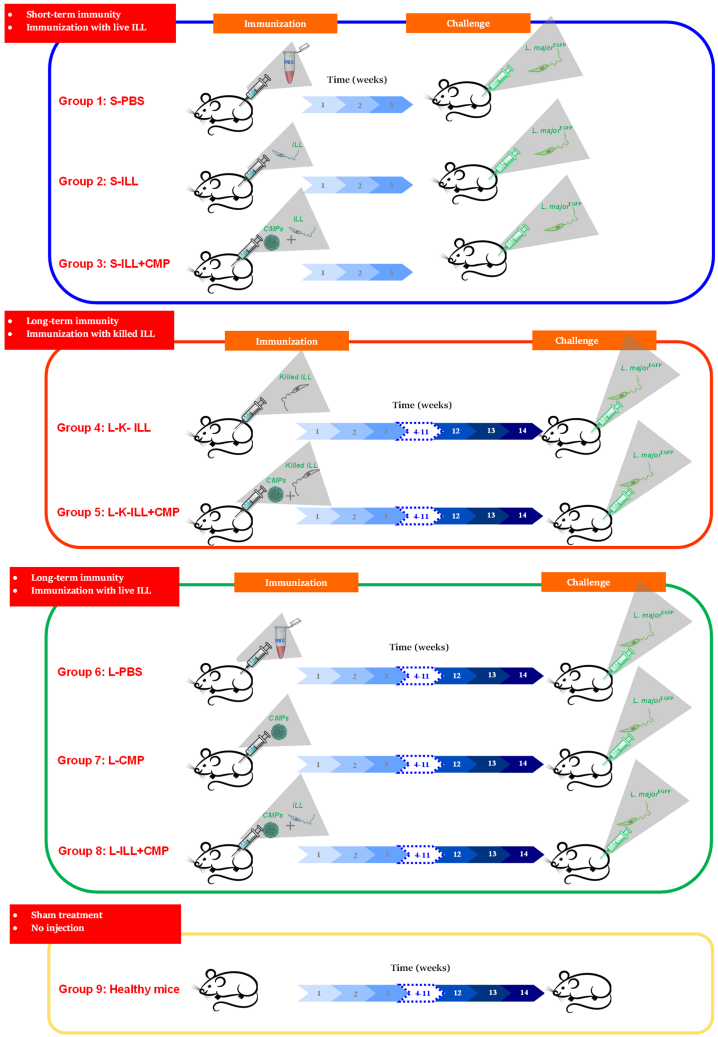

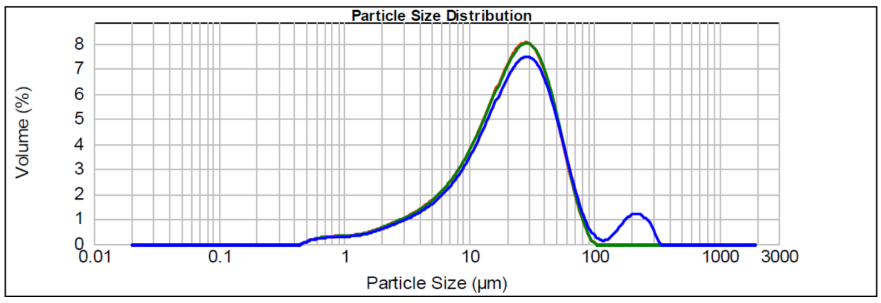

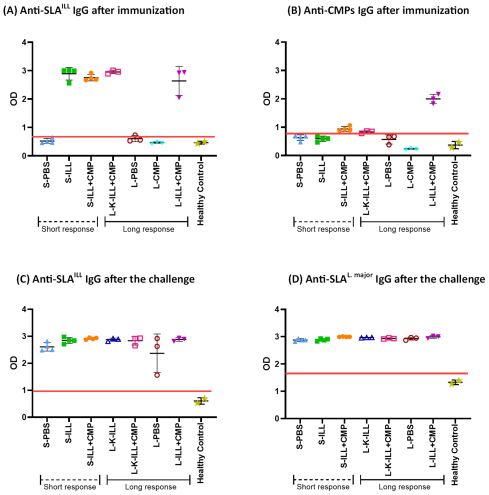

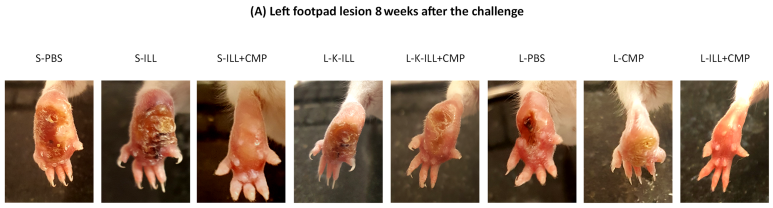

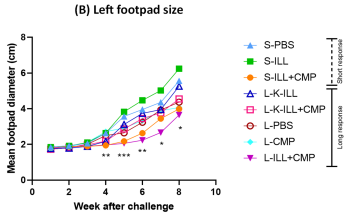

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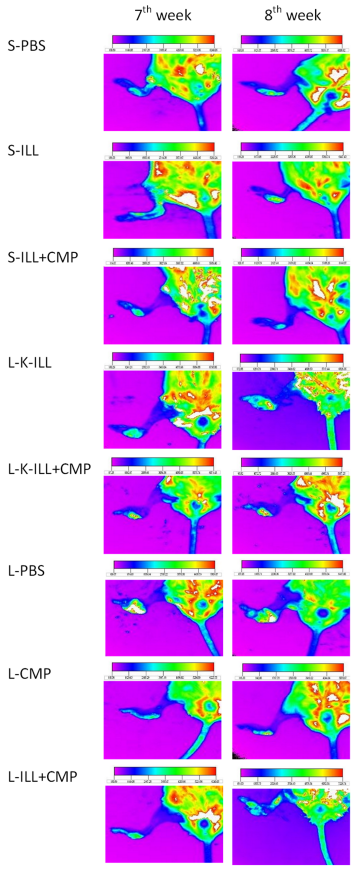

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