Evaluation of the immune response induced by different adjuvants applied to the development of leptospirosis vaccine.

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A potential candidate to antigen in subunit vaccines against leptospirosis is the rLigA protein, which conferred 90-100% protection against lethal challenge in hamsters when formulated with $Al(OH)_3$, but was not able to induce sterile immunity. Thus, this study aimed to evaluate and characterize the immune response induced in mice by $Al(OH)_3$, Nanoparticles $Ca_3(PO_4)_2$, Flagellin and ISCOM (saponin based) adjuvants in association with rLigA protein. The rLigA was expressed in *E. coli*, purified through IMAC and obtained with homogeneity > 90%. Mice immunizations with rLigA (3 and 30µg/dose) and adjuvants were performed in a two doses regime with a 15-day interval. Serum were obtained on days 0, 14, 29,59, 89 and on day 119 followed by euthanasia and splenectomy. The kinetic and profile of the humoral immune response was evaluated by ELISA and antibodies avidity by ELISA, adding urea. The cellular immune response was evaluated by ELISpot for IFN-ɤ, IL-4, IL-2 and IL-17 detection. Statistical analysis was performed using GraphPad Prism®3.5 (p value <0.05). ELISA assays showed that all formulations were able to induce humoral response. The highest titers in ELISA units/mL and avidity index obtained for each adjuvant were: ISCOM (37036; 83.84), Flagellin with $Al(OH)_3$ (14782; 91.92), Flagellin (13933; 86.08), $Al(OH)_3$ (6287; 89.68) and Nanoparticles Adjuvant (3328; 87.11). The IgG1 isotype was the predominant in all formulations. However, formulations with ISCOM and Flagellin with $Al(OH)_3$ revealed a larger equilibrium among the isotypes. ISCOM was the only adjuvant capable of stimulating a significant cellular immune response compared to the control, stimulating mainly IFN-ɤ and IL-2.Overall, this study provided relevant information to understand the immune response triggered by each adjuvant when associated with rLigA protein, helping in the development of leptospirosis vaccines. Furthermore, the obtained data can be used in other immunization models with bacterial antigens.

Key-words: Adjuvants; leptospirosis vaccines; Leptospiral immunoglobulin-like protein.

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