. **Immunology,** Faculty of Veterinary Medicine, Urmia 26004 Summary of the Ph.D thesis No. University.

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Title: Effects of combined alum adjuvant and polymyxin B on cellular and humoral immune responses in BALB/c mice immunized with killed preparation of Salmonella typhimurium

Abstract

Adjuvants are generally defined as compounds that enhance the immune system's response to an antigen and are commonly used to improve vaccine efficacy. Although their exact mechanisms of action remain largely unknown, it is believed that their presence in vaccines facilitates antigen presentation, improves antigen stability, increases cytokine expression, and promotes slow antigen release, among other benefits. To date, numerous adjuvants have been evaluated for use in vaccines; however, only a few have received the necessary approvals. Among these, alum adjuvant has demonstrated the ability to enhance humoral immunity. Notably, adjuvants must induce both humoral and cellular immunity to achieve adequate and effective immune responses. Polymyxin B, an antibiotic effective against resistant Gram-negative bacteria, disrupts the bacterial membrane, leading to bacterial death. Additionally, it acts as a potent agonist for the purinergic receptor P2X7, which plays a critical role in immune modulation and the release of inflammatory mediators. Activation of this receptor by extracellular adenosine triphosphate (ATP) leads to the release of pro-inflammatory cytokines, reactive oxygen species (ROS), TNFs, and other immune-boosting factors. Given that existing adjuvants are often insufficient to induce the required immunity against certain pathogens, including intracellular bacteria such as Salmonella typhimurium, this study aimed to evaluate the immunogenic potential of novel adjuvant combinations. In this study, the immunostimulatory effects of heat-killed Salmonella typhimurium (HKST) and adjuvants such as alum and polymyxin were investigated in a murine model using BALB/c mice immunized with the wild-type strain of Salmonella typhimurium. The mice were randomly divided into five groups: control, HKST, HKST/A, HKST/P, and HKST/A/P. Various immune responses, including delayed-type hypersensitivity, lymphocyte proliferation, antibody titers, cytokine gene expression (IL-4 and IFN-γ), the IFN-γ/IL-4 ratio, and survival rates, were assessed. The results demonstrated that the HKST/A/P group showed significantly enhanced performance in all parameters, including increased antibody production, elevated cytokine gene expression, and improved survival rates, compared to the other groups. In contrast, the control group exhibited the lowest immune response. The HKST/A and HKST/P groups also showed significant improvement in immune indices compared to the control group, but the combined effect of adjuvants in the HKST/A/P group was more effective. These findings highlight the strong potential of the HKST formulation combined with alum and polymyxin as a potent immunogenic and immune-stimulatory formulation. This combination could serve as a foundation for developing more effective vaccines. Further studies are recommended to evaluate the efficacy of this formulation in clinical models.

Keywords: Adjuvant, Alum, Polymyxin B, Vaccine, Salmonella typhimurium