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The effects of the combination of alum adjuvant and chlorogenic acid on cellular and humoral immune responses against the killed form of *Salmonella typhimurium*

Summary:

Aluminum-based adjuvants are insufficient to induce Th1-type immune responses when administered with inactivated microorganisms, whereas Th1 responses play a critical role in host defense against intracellular pathogens. Chlorogenic acid (CGA), a naturally occurring polyphenolic compound, has the capacity to modulate immune responses from a Th2-biased profile toward Th1 polarization, thereby enhancing protective immunity. The present study aimed to investigate the immunomodulatory effects of combining CGA with aluminum adjuvant on both cellular and humoral immune responses following immunization with heat-killed *Salmonella typhimurium* (HKST). Male Balb/c mice received two doses of HKST at a two-week interval, either alone or in combination with aluminum, CGA, or both. Immune responses were evaluated two weeks after the final immunization, including lymphocyte proliferation, delayed-type hypersensitivity (DTH) reactions, antibody titers, IgG subclass distribution (IgG1 and IgG2a), and IgG2a/IgG1 ratio as an indicator of Th1 polarization. Protective efficacy against acute and sublethal *S. typhimurium* challenge was also assessed. The results demonstrated that the combination of aluminum and CGA significantly enhanced HKST-induced lymphocyte proliferation, DTH responses, and humoral immunity, particularly by increasing the IgG2a/IgG1 ratio, indicative of a shift toward Th1-biased immunity. This immunological modulation correlated with improved host protection, as evidenced by prolonged survival following acute challenge and reduced hepatic bacterial burden upon sublethal exposure. These findings highlight the synergistic effect of CGA and aluminum in potentiating both cellular and humoral immune responses and promoting protective immunity in a murine model. Overall, this study underscores the potential of combining aluminum adjuvant with CGA as a novel strategy for the development of vaccines targeting intracellular pathogens and emphasizes the utility of natural immunomodulatory compounds in enhancing the quality and directionality of immune responses.

Key Words: *Salmonella typhimurium*, Adjuvant, Alum, Chlorogenic acid.