PALADINS: PROTECTIVE APPROACHES LEVERAGING AD-APTIVE AND IN-NATE SYSTEMS

Protective Efficacy And Immune Response Of Subunit Hcp1-based And Live Attenuated Bp 668 Δilvi Candidate Vaccines

Sergei Biryukov USAMRIID **Jennifer Dankmeyer** USAMRIID **Nathaniel Rill** USAMRIID **Christopher Klimko** USAMRIID **Jennifer Shoe** USAMRIID **Melissa Hunter** USAMRIID **Paul Brett** University of Nevada, Reno School of Medicine **Mary Burtnick** University of Nevada, Reno School of Medicine **Christopher Cote** USAMRIID **David Deshazer** USAMRIID

Background & Objective: Melioidosis is caused by Burkholderia pseudomallei (Bp), an environmental gram-negative bacillus that is endemic in Southeast Asia, northern Australia, and other tropical regions around the globe. The disease is difficult to diagnose due to diverse clinical manifestations resulting from various routes of exposures such as abrasion, ingestion, or inhalation, as well as varying incubation periods. Treatment options are limited since Bp is intrinsically resistant to various antibiotics, which results in a high mortality rate, thereby necessitating a safe and effective vaccine. The immune response in C57BL/6 mice was evaluated pre- and post-aerosol challenge with Bp K96243 after vaccination with live attenuated Bp 668 Δilvl strain. In addition, we tested two candidate subunit vaccines, composed of hemolysin co-regulated protein 1 (Hcp1) mixed with the capsule polysaccharide conjugated to the highly immunogenic CRM197 protein carrier (Conjugate) with and without alkyl hydroperoxide reductase (AhpC).

Methods: The C57BL/6 mice were administered three doses (days 0/21/38) of the subunit vaccines (Hcp1 + Conjugate or Hcp1 + Conjugate + AhpC) and two doses (days 0/24) of Bp 668 Δilvl were given. The mice were exposed to approximately 1.35 x 103 CFU (~3.4 LD50) of aerosolized Bp K96243 at 38 days after the last vaccination. Tissue and blood were collected for immunoassays at three intervals: 6 and 27 days after vaccination and 3 days after challenge (day 79) for cytokine analyses.

Results: Six days after vaccination, the number of IFN-γ secreting splenocytes from vaccinated mice were enumerated after stimulation with purified Hcp1 or AhpC antigens. Stimulation with Hcp1 significantly upregulated IFN-γ secreting splenocytes, specifically in the Conjugate + Hcp1 group, whereas the least stimulation relative to the control group was observed for 668 Δilvl splenocytes. Furthermore, splenocytes stimulated with AhpC predominantly induced IFN-γ secreting splenocytes in the Conjugate + Hcp1 + AhpC vaccine group, although the number of IFN-γ secreting splenocytes was much greater relative to Hcp1 stimulation. Twenty-eight days after vaccination, the levels of IL-3 and IL-13 were elevated in Bp 668 Δilvl vaccinated mice relative to Conjugate + Hcp1 +/- AhpC vaccinated mice in the lung homogenates. Three days after challenge with Bp K96243, all vaccine groups generally exhibited a significant reduction in cytokine levels compared to the controls, in the lung homogenates, with the exception of elevated IFN-γ and IL-22 in the Bp 668 Δilvl vaccinated group. Furthermore, the relative protective efficacies based on survival rates were most protective after Conjugate+Hcp1 vaccine followed by Bp 668 Δilvl and Conjugate+Hcp1+AhpC.

Conclusion: The overall suppression (or reduced upregulation) of cytokine/chemokine production, such as IL-1α, IL-1β, IL-6, TNF-α, IL-18, CXCL1, and CCL3 in vaccine compared to control groups, suggests that the vaccines protected the mice against the destructive effects of hypercytokinemia, resulting in improved survival of vaccinated mice. The unique cytokine profiles of these three protective vaccines suggests that each vaccine may promote distinct mechanisms of protection. Heterologous vaccination regiment that combine subunit and live attenuated candidate vaccines may further augment the protective efficacy against melioidosis.