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

Development Of Scalable Processes To Facilitate Gmp-compliant Production Of A Melioidosis Subunit Vaccine Candidate

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Burkholderia pseudomallei, the etiologic agent of melioidosis, is a bacterial pathogen that causes severe disease in humans and animals. Diagnosis and treatment of melioidosis can be challenging and no licensed vaccines currently exist. Several studies have shown that this Tier 1 select agent expresses a variety of structurally conserved protective antigens that include cell-surface polysaccharides, cell-associated and secreted proteins. Based on this, such antigens have become important components of the subunit vaccine candidates that we are currently developing in our laboratory. In the present study, the 6-deoxyheptan capsular polysaccharide (CPS) from Burkholderia thailandensis E555 was purified using a phenol-less extraction procedure, chemically activated and covalently linked to recombinant CRM197 diphtheria toxin mutant (CRM197) to produce CPS-CRM197. Additionally, affinity chromatography techniques were used to prepare highly purified, recombinant, tag-less B. pseudomallei hemolysin co-regulated protein 1 (Hcp1). Immunization of C57BL/6 mice with nanogram (low dose) or microgram (high-dose) amounts of CPS-CRM197 plus Hcp1 resulted high-titer IgG and opsonizing antibody responses against the CPS component of the glycoconjugate as well as high-titer IgG and robust IFN-γ secreting T cell responses against Hcp1. Following an inhalational challenge with a high-dose (~40 LD50) of B. pseudomallei K96243, 75% of the mice immunized with the low-dose formulation and 67% of the mice immunized with the high-dose formulation were still alive upon termination of the study at day 61. Importantly, the majority of the tissues (lungs, livers and spleens) from the survivors in both groups were found to be sterile when assessed for bacterial loads. Collectively, these studies have enabled us to develop scalable processes to facilitate GMP-compliant production of a melioidosis subunit vaccine candidate for use in an upcoming Phase I human clinical trial. Furthermore, they help to better establish correlates of antigen-induced immunity against B. pseudomallei and provide valuable insights towards the development of a safe, affordable and effective melioidosis vaccine to protect the warfighter as well as civilians living in regions where the disease is endemic.

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