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Medical Countermeasures to Address Intracellular Bacterial Pathogens

Immunogenicity and Protective Efficacy of an Outer Membrane Vesicle Vaccine Platform against the Intracellular Bacteria Burkholderia pseudomallei and Burkholderia mallei

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Introduction: Vaccine platforms that are effective against intracellular bacterial pathogens remain a high priority. In addition to the global impact of intracellular infections on human health, the alarming increase in multidrug resistant strains, such as *Mycobacterium tuberculosis*, and the potential threat posed by Tier 1 agents, such as *B. pseudomallei* and *B. mallei*, highlight the urgent need for safe and effective vaccines against this collective group of pathogens. Here, we evaluated the immunogenicity and protective efficacy of an outer membrane vesicle (OMV) vaccine platform designed to target the extracellular and intracellular stages of *B. pseudomallei* and *B. mallei* infection.

Methods: OMVs were purified from the select-agent exempt, biosafety level 2 strain, *B. pseudomallei* Bp82 after 16 hours of culture in nutrient-rich media (LB) or a minimal media (M9) intended to mimic the host macrophage environment. C57Bl/6 mice (n=10 per group) and rhesus macaques (n=6 per group) were immunized subcutaneously with 10 or 100 micrograms of OMVs, respectively, or sham control (saline) twice, 3-4 weeks apart. For rodent studies, a separate group of mice were immunized with the parent live-attenuated Bp82 strain as a positive control. One month after the last immunization, animals were challenged by aerosol with *B. pseudomallei* strain K96243 or *B. mallei* strain 23344. Humoral and cellular immune responses to OMV immunization were assessed by ELISA and flow cytometry, respectively.

Results: Immunization with LB- or M9-derived OMVs provided significant protection against an otherwise lethal aerosol challenge with *B. pseudomallei* and *B. mallei*, however only the M9 OMV vaccine conferred 100% survival and sterilizing immunity in mice. M9 OMVs induced high titers of OMV-, *B. pseudomallei*-, and *B. mallei*-specific serum IgG that was superior to that achieved with the live-attenuated vaccine in mice. OMV vaccines also induced IFN-gamma-producing CD4 and cytotoxic CD8 T cells, indicating that they can elicit all arms of the adaptive immune response. Similar humoral and cellular immune responses were achieved in OMV-immunized macaques without any adverse reactogenicity or toxicity. Protection results in macaques are pending.

Conclusion: Because of their potent immunogenicity and multi-antigenic nature, acellular OMVs represent an effective yet safe cross-protective vaccine platform against the intracellular bacteria, *B. pseudomallei* and *B. mallei*. Interestingly, OMVs derived from bacteria grown in media that mimics the host macrophage elicit better protection than those obtained from a nutrient-rich culture, perhaps due to the confirmed presence of Type 3 and Type 6 secretion system components which could elicit protective immune responses against the intracellular stage of infection. This same approach could be used to develop better vaccines against other intracellular bacteria, such as *Salmonella*, *Shigella*, and *Yersinia* species. Ultimately, demonstration of protection in the (pending) nonhuman primate challenge studies would warrant further development of the OMV vaccine platform as a protective countermeasure for the warfighter.

We thank Herbert Schweizer for providing strain Bp82 and Paul Brett and Mary Burtnick for providing helpful advice and reagents. This work was funded by the Defense Threat Reduction Agency contract number HDTRA1-14-C-0035.