COMBATTING FUTURE BIOLOGICAL THREATS – HOST-DIRECTED INTERVENTIONS TO EMERGING THREATS FOR RAPID RESPONSE

Membrane Engineering Of Outer Membrane Vesicles Allows Tunable Interactions With The Innate Immune System

Michael Norris PATH Group, University of Hawai'i at Mānoa **Thi Hai Au** PATH Group, University of Hawai'i at Mānoa **Prashant Dahal** PATH Group, University of Hawai'i at Mānoa **Ian McMillan** School of Life Sciences, University of Hawai'i at Mānoa **Brad Borlee** Department of Microbiology, Immunology, and Pathology, Colorado State University

Background information.
Outer membrane vesicles (OMVs) have emerged as a promising vaccine platform for several infections. OMVs have natural adjuvinating properties because of the membrane they are composed of. The lipid A in the outer membrane of gram-negative bacteria is endotoxic and activates the human innate immune response through activation of TLR4, efficiently linking innate and adaptive immune responses. One such OMV is from Burkholderia pseudomallei. The B. pseudomallei lipid A is variably phosphorylated and has reduced acylation compared to Escherichia coli, rendering it less toxic and still immunogenic. The lipid A pool produced in B. pseudomallei is heterogeneous and can be optimized for peak performance.

Purpose.
Support the burgeoning OMV vaccine platform by generating the most immunogenic, least toxic lipid A profiles. This strategy will strengthen the innate to adaptive transition during immunogen presentation of vaccine components. Alternatively, stable, engineered non-toxic OMV can be given prophylactically to non-specifically boost warfighter immunity.

Objective.
Produce B. pseudomallei OMVs with defined profiles of lipid A modifications to facilitate reliable, tunable OMV TLR4 responses. Rationale of the research.
Monophosphorylated lipid A (MPLA) is already an FDA approved adjuvant because of its low endotoxic/strong immunologic response. The outer membrane of B. pseudomallei contains inconsistent lipid A profiles. By engineering the genome of the attenuated B. pseudomallei Bp82 coupled with controlled expression of a suite of lipid A modification genes, a consistent desirable profile of lipid A can be achieved.

Relationship to other areas of study.
OMVs can be used as host-targeted therapies by boosting the immune system and combined with antigen material to defend against many biothreats.

Methods.
Allelic replacement was used to knockout lipid A modification genes in Bp82, an adenine requiring B. pseudomallei auxotroph. Specific genes were stably inserted in the genome under control of the rhamnose inducible promoter to tune modifications. Consistent panels of lipid A were prepared and structurally verified by MALDI-TOF. Their ability to induce innate immunity were measured in cell lines.
Preliminary results.
Genes were knocked out and their effect on lipid A structures was verified. Rhamnose inducible promoter-driven genes that modify lipid A by regulating deacylations, hydroxylations, dephosphorylations, and arabinosylations were stably inserted in the genome and verified. Engineered OMVs showed induction of the inserted genes and evidence of tunable membrane profiles.

Preliminary conclusions.
The tunable, defined OMV lipid A are another way to define and control the OMV immune inductive functionality in vaccine formulations.
Impact to the DTRA JSTO mission and the Joint Force.
Prevention of melioidosis or glanders is a direct application when using this platform. A host-targeted innate immune booster would prepare the warfighter for exposure to biothreats from a general defensive posture. Combining antigens to protect against other bacterial and viral infections. Innate immune boosting and specific adaptive protection can provide broad health benefits to maintain Joint Force optimal readiness.