United States Army Medical Research
Institute of Infectious Diseases

Preparation of *Burkholderia pseudomallei* Polysaccharide-CRM_{197} Conjugate, a Potential Vaccine Candidate for Glanders and Melioidosis

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Melioidosis

- Melioidosis is an infectious disease caused by the bacterium *Burkholderia pseudomallei*
- It is most frequently reported in Southeast Asia and Northern Australia
- Melioidosis most commonly involves the lungs where the infection can form a cavity of pus (abscess)
- It can spread from the skin through the blood to affect the heart, brain, liver, kidneys, joints, and eyes
- Patients can have associated headaches, fever, chills, cough, chest pain, and/or loss of appetite

Glanders

- Glanders is an infectious disease that is caused by the bacterium *Burkholderia mallei*
- The types of infection include localized, pus-forming cutaneous infections, pulmonary infections, bloodstream infections, and chronic infections of the skin

Current Status Treatment of Melioidosis and Glanders

- *Burkholderia mallei* and *B. pseudomallei* are the causative agents for glanders and melioidosis, respectively
- Both of these organisms have been considered as potential agents for biological warfare and biological terrorism
- Currently, these infections are treated with antibiotics
- Currently, no vaccines are available for protection against glanders and melioidosis

Strategy for Preparation of Vaccine for Glanders and Melioidosis

- The strategy is to prepare capsular polysaccharides in milligram quantities and employ them as protective antigens
- The next step is to conjugate the polysaccharides to a carrier protein
- Examine the efficiency of conjugation
- Assay the polysaccharide- protein conjugate
- Test the polysaccharide-protein conjugate in a mouse model against aerosol challenge with *B. mallei* and *B. pseudomallei*

Methods

- Phenol-sulfuric acid method:
  - To assay total neutral sugars - used to measure polysaccharide-protein conjugate, monitor purification process of polysaccharide from the bacteria
- Reducing sugars - MBTH/ferric ammonium sulfate method:
  - To prepare polysaccharide-protein conjugate, the polysaccharide will be coupled to the protein via the reducing group of the polysaccharide
  - The reducing sugar estimation method is employed to test the efficiency of the conjugation procedure

Methods (continued)

- Acetolysis- 2% HAc, 100^0 C, 2h:
  - Cleaves the linkage at KDO in LPS to release lipid A, core oligosaccharides, and O-antigens (repeating sugar units)

- Western blot:
  - Polysaccharide-protein conjugate, Antibody produced against the whole cells, capsule, LPS

Rationale for Choosing a Protein Carrier

- To increase the immune response, polysaccharides are usually conjugated to protein carriers
- We chose CRM_{197} as the carrier
- CRM_{197} is diphtheria toxin mutant, commercially available
- CRM_{197} had been conventionally used as a protein carrier for polysaccharide vaccines for example meningococcal polysaccharide vaccine, pneumococcal and Haemophilus influenze b polysaccharide vaccines (Aventis Pasteur, Merck and Weyth)

Polysaccharides

- *Burkholderia mallei* and *B. pseudomallei* produce two types of polysaccharides:
  - Capsular polysaccharide - 2-O-acetyl- 6-deoxy manno heptose homopolymer - <200kDa
  - LPS- Heteropolymer of repeating D-glucose and L-talose

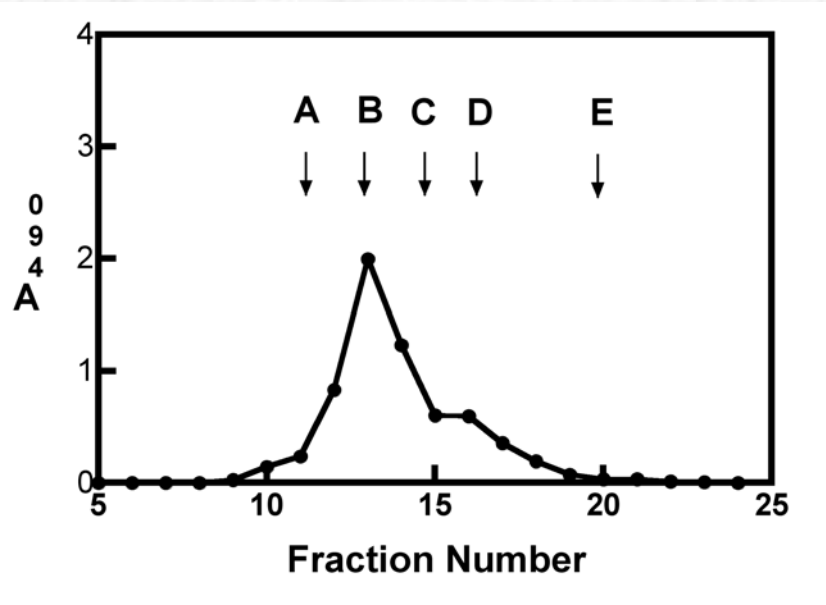
Rationale for choosing capsular polysaccharides as vaccine candidates

- Capsule is the virulence factor:
  - Dave DeShazer prepared a capsule mutant (DD3008) and demonstrated that the mouse aerosol LD_{50} was at least 10^3 times greater than the wild type (China7)

- *B. mallei* and *B. pseudomallei,* the pathogenic bacteria produce capsule, while *B. thailandensis* (non pathogenic) does not produce capsule

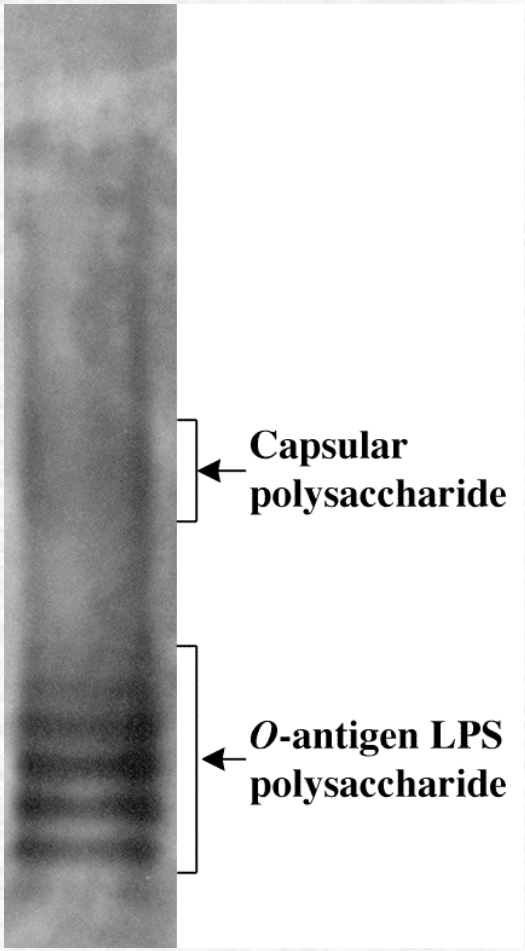
Fractionation Scheme for Polysaccharides

KS-804 HPLC of 576 Polysaccharides/Acetolysis/Sugar Phenol-H_2SO_4 100 μl of Fraction



- A, B, C, D: Pullulan Stds 400, 50, 10, 5 kD
- E: Mannose, MW 180
- Fractions Pooled: #12-14
- Dry weight: 8 mg
- Total neutral sugar content 40 μmole Glc equivalents, 7.2 mg

Western Blot of 576 Polysaccharide-CRM_{197} Conjugate



- Antigen 576PS - CRM_{197} polysaccharide conjugate
- Ab - 576 whole cells - Polyclonal
- Detection ECL HRP
- MW of the conjugate - > 60kD - 200kD

CONCLUSIONS

- We isolated polysaccharides (capsular and LPS) from *B. pseudomallei* in milligram quantities
- We successfully conjugated the above polysaccharides to a carrier protein CRM_{197}
- Western blot analysis of the conjugate indicated that the polysaccharides are derived from the capsule and LPS
- Experiments are in progress to test this polysaccharide-CRM_{197} conjugate for protection against glanders and melioidosis.

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