Development of a Multivalent Subunit Vaccine That Provides Sterilizing Immunity Against Melioidosis

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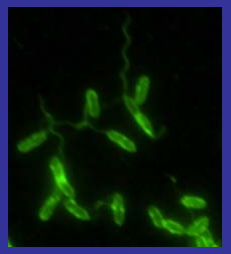
Burkholderia Genus

- ~50 *Burkholderia* species have been identified
- Most are soil commensals and phytopathogens
- A select few cause disease in humans and animals
  - *B. gladioli* (food poisoning)
  - *B. cepacia* complex (opportunistic infections)
  - *B. pseudomallei* (melioidosis)
  - *B. mallei* (glanders)

Burkholderia pseudomallei

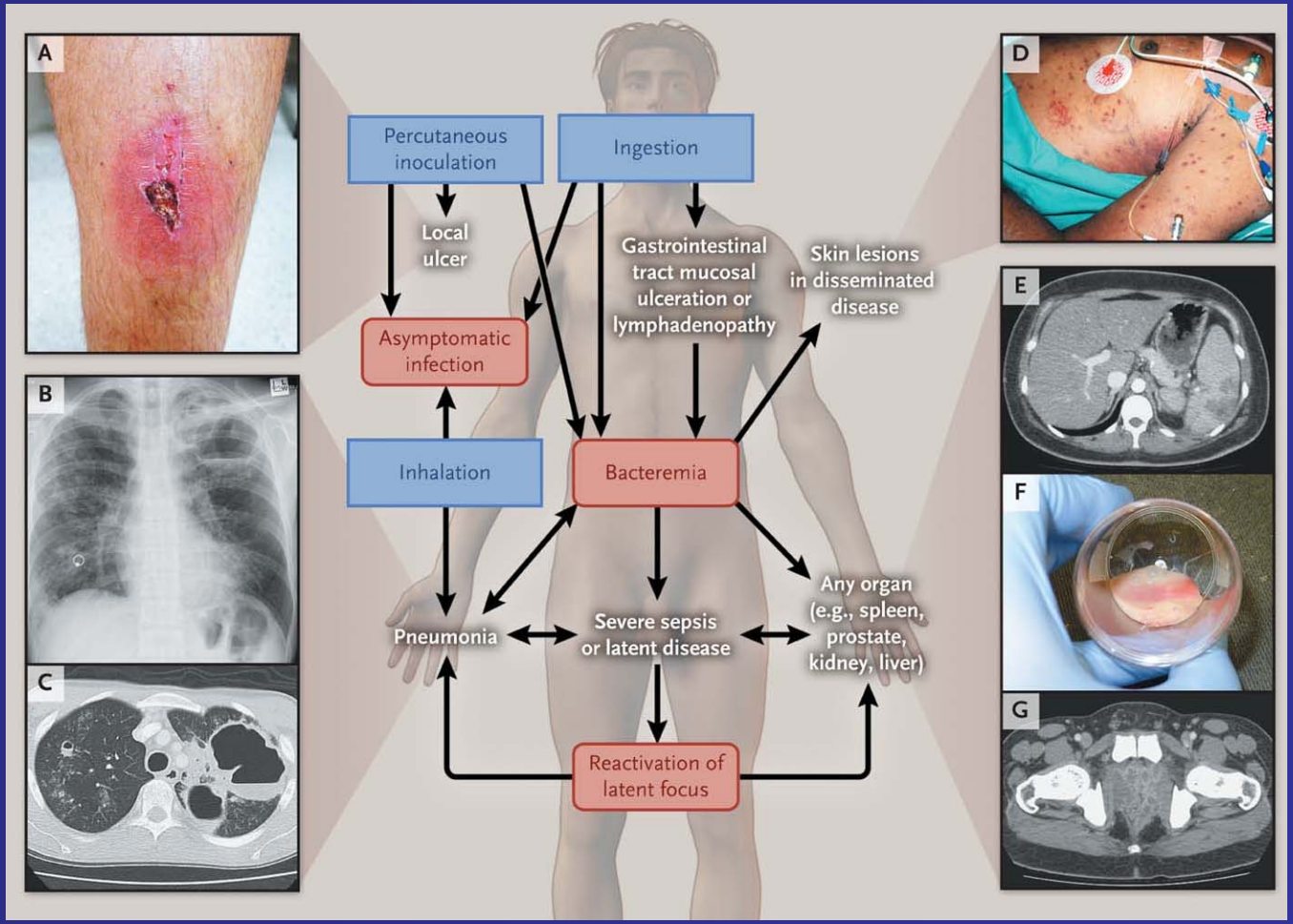


- Motile, aerobic, facultative-intracellular, Gram negative bacillus
- Environmental saprophyte
- Etiologic agent of melioidosis
  - causes severe disease in both humans and animals
- CDC Tier 1 select agent
  - resistant to many classes of antibiotics
  - high mortality associated with acute disease
  - aerosol risk of infection
  - potential biothreat agent

Global Distribution

- Endemic to equatorial regions
- High incidence of disease in northern Australia and South Asia
- Accounts for ~20% of community acquired septicemias in northeastern Thailand
- High incidence of disease during the rainy season

Summary of Clinical Events



Wiersinga et al, 2012

Global Morbidity and Mortality

nature microbiology

LETTERS

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Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis

Direk Limmathurotsakul[1,2,3*], Nick Golding[1], David A. B. Dance[4,5], Jane P. Messina[6], David M. Pigott[1], Catherine L. Moyes[1], Dionne B. Rolim[7], Eric Bertherat[8], Nicholas P. J. Day[2,5], Sharon J. Peacock[2,9,10] and Simon I. Hay[1,11,12]

Estimated number of cases per year: ~165,000

Estimated number of deaths per year: ~89,000

Melioidosis Vaccines

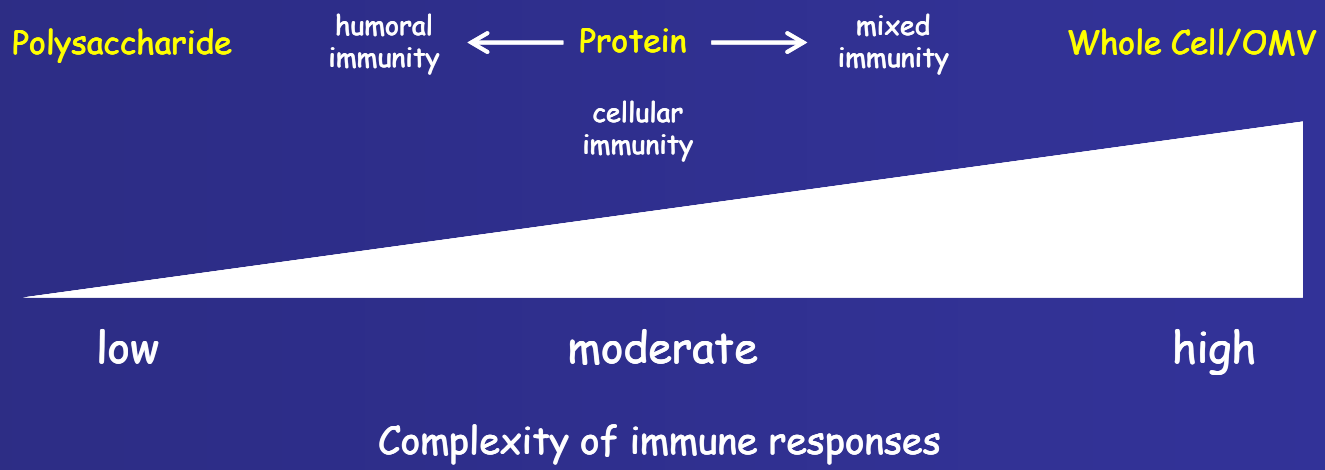
- Adaptive immunity to *B. pseudomallei* infections is complex
- Humoral responses are important for controlling early stages of an infection → extracellular phase
- Cellular responses are important for controlling later stages of an infection → intracellular phase
- A vaccine that elicits both types of responses will likely be required to provide full protection against disease

Subunit Vaccines

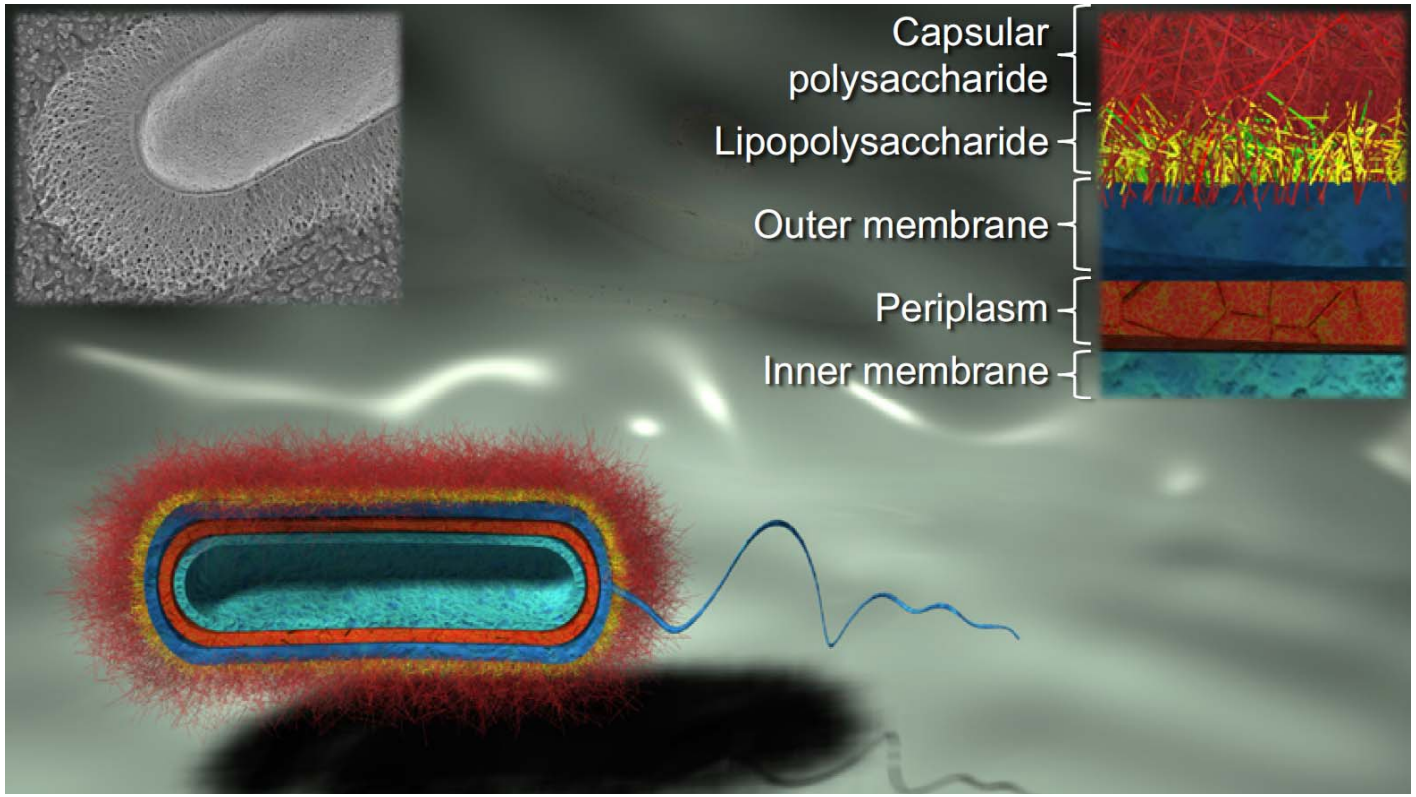
- Rationally designed
  - modular
  - broad protective capacity
  - promote specific immune responses
- Antigenically defined
  - minimize QC issues
  - attractive from a licensing standpoint
- Safe
  - endotoxin free
  - minimize the risk of undesirable side effects

Immune Responses Against Vaccines

- The less complicated a vaccine formulation, the greater the likelihood that specific correlates of vaccine-induced immunity can be defined

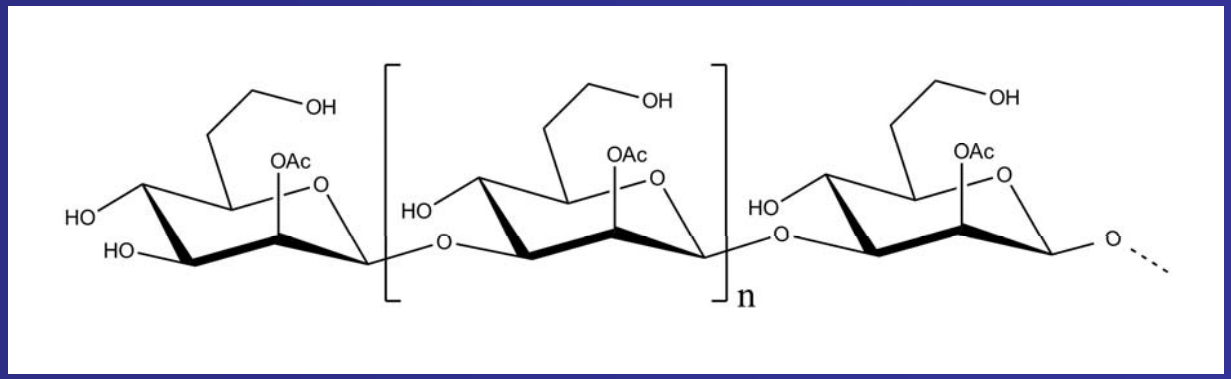


Cell-Surface Polysaccharides



Capsular Polysaccharides (CPS)

- Five distinct CPS antigens have been identified
- All virulent isolates of *B. pseudomallei* express a common protective antigen



2-*O*-Ac-6-deoxy-*manno*-heptan

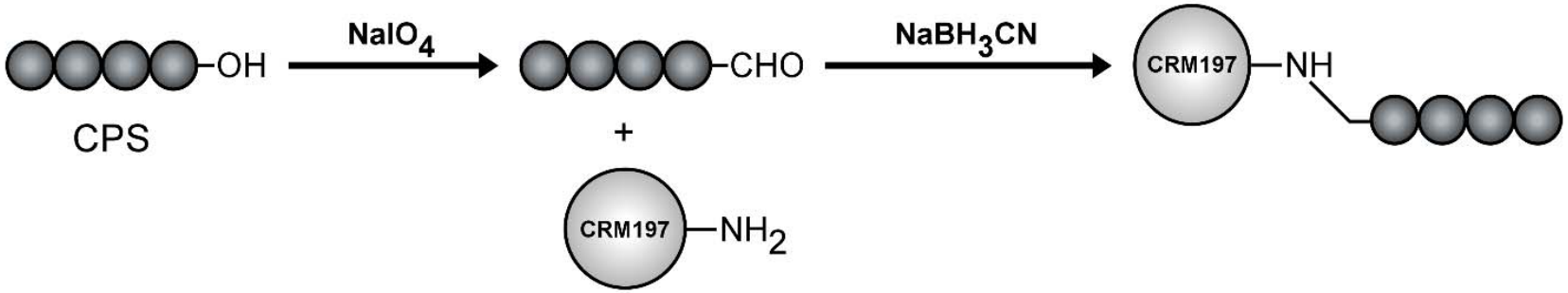
Thymus Independent Type 2 (TI-2) Antigens

- Polysaccharides such as CPS are TI-2 antigens
- Unlike proteins, TI-2 antigens do not enable APCs to engage/activate T-cells
- Poorly immunogenic
- Disadvantages of immunizing with TI-2 antigens
  - memory responses are not generated (boosting ineffective)
  - isotype switching and affinity maturation may not occur
  - failure to induce protective immune responses in infants

Glycoconjugate Vaccines

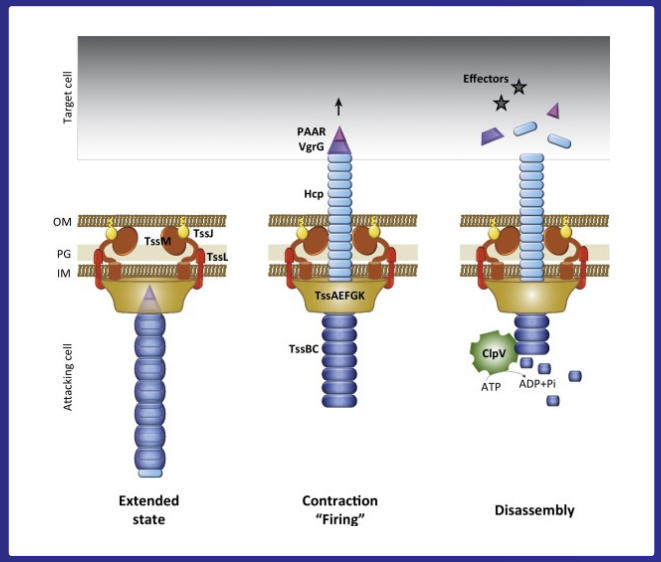
- Polysaccharides can be covalently linked to carrier proteins to form glycoconjugates
- Stimulate the production of T-cell dependent-like responses against the polysaccharide component
- Highly immunogenic
- Advantages of immunizing with glycoconjugates
  - boosting produces a secondary response (memory)
  - isotype switching and affinity maturation occur
  - protective immune responses are raised in infants

Conjugation of CPS to CRM197



Burtnick et al, 2017

Hemolysin Co-Regulated Protein 1 (Hcp1)

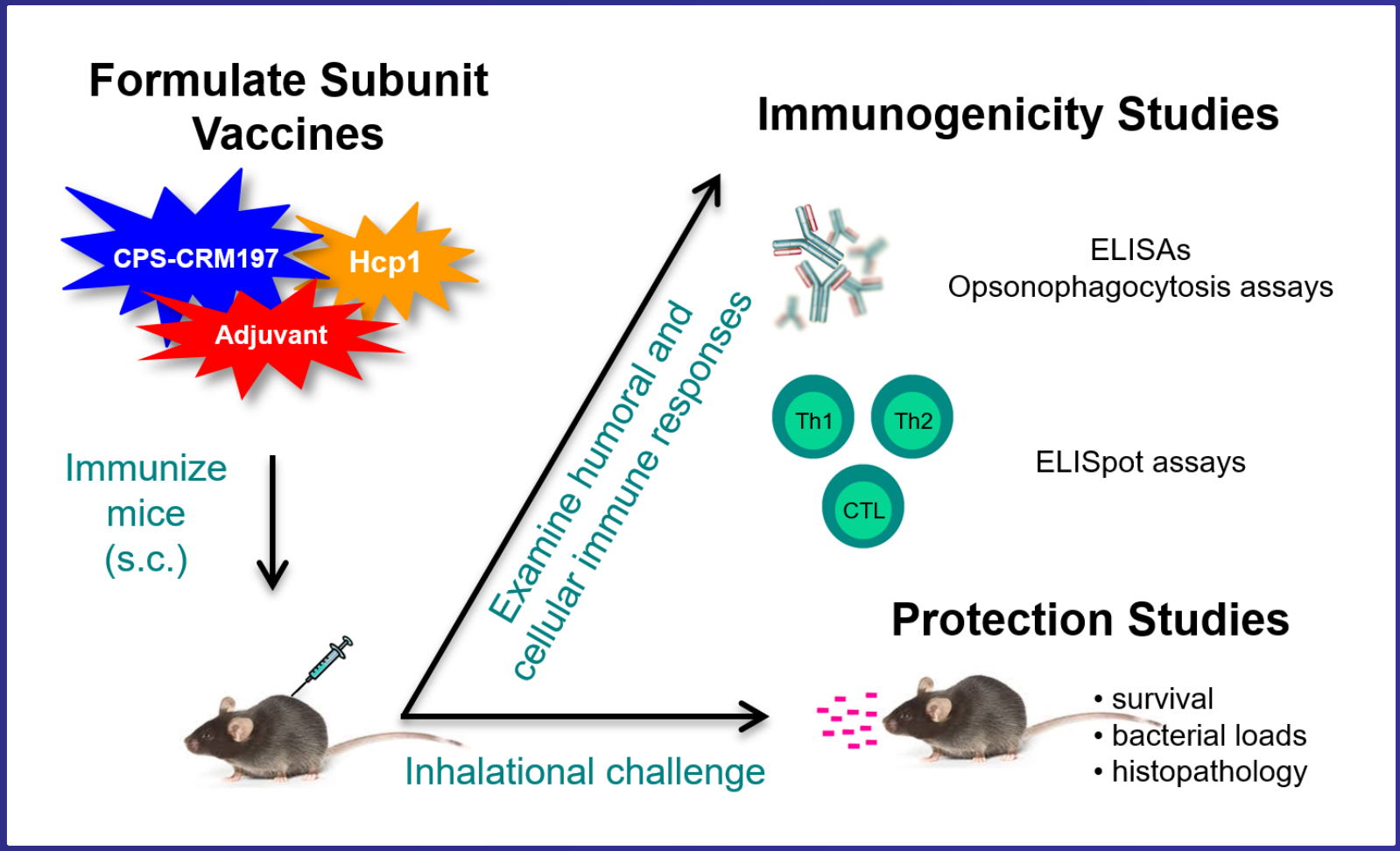


Type VI Secretion System 1 (T6SS-1)

Hcp1

- Highly conserved protein
- Expressed during active infections
- Known protective antigen
- Recombinant protein is very stable

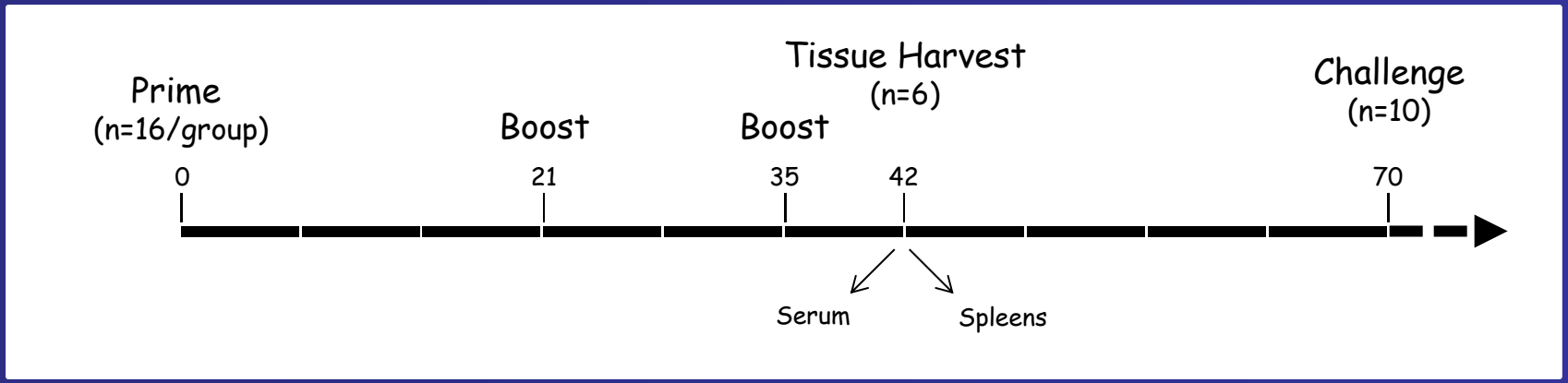
Development and Testing of Subunit Vaccines



Immunization Schedule

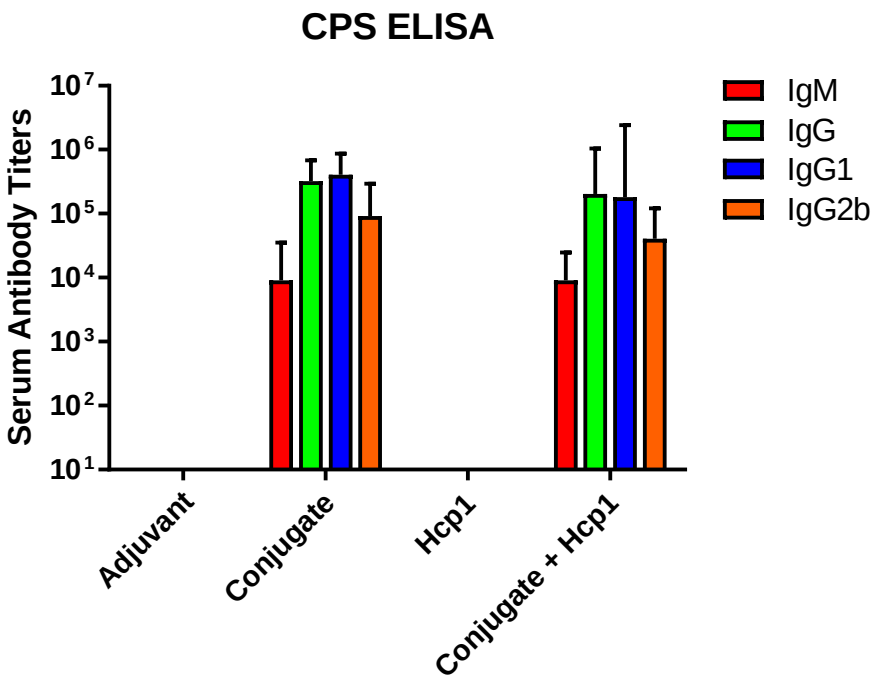
Formulations

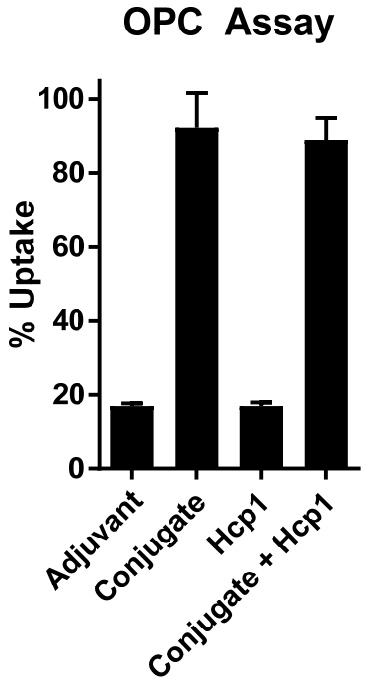
1. Adjuvant
2. Adjuvant + Conjugate
3. Adjuvant + Hcp1
4. Adjuvant + Conjugate + Hcp1



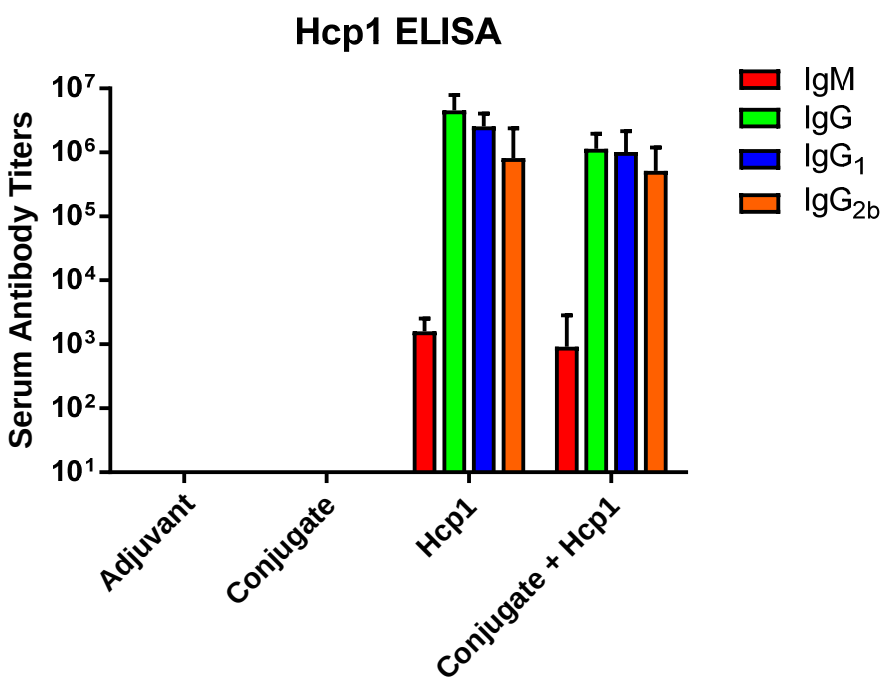
C57BL/6 mice are immunized with 2.5 μg of CPS as a conjugate and/or 5 μg of recombinant protein

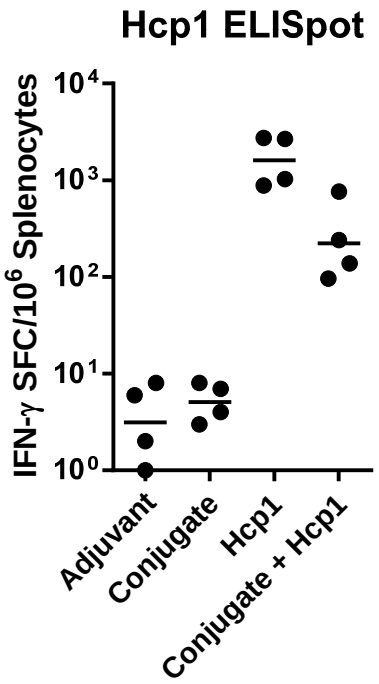
Immune Responses Against CPS-CRM197



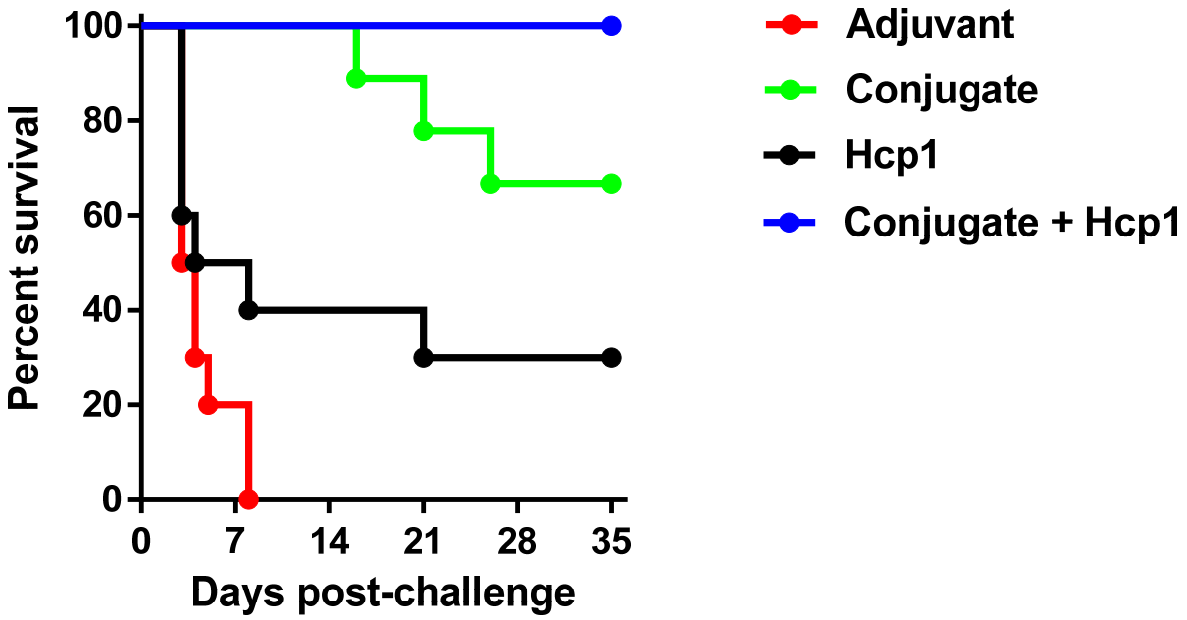


Immune Responses Against Hcp1



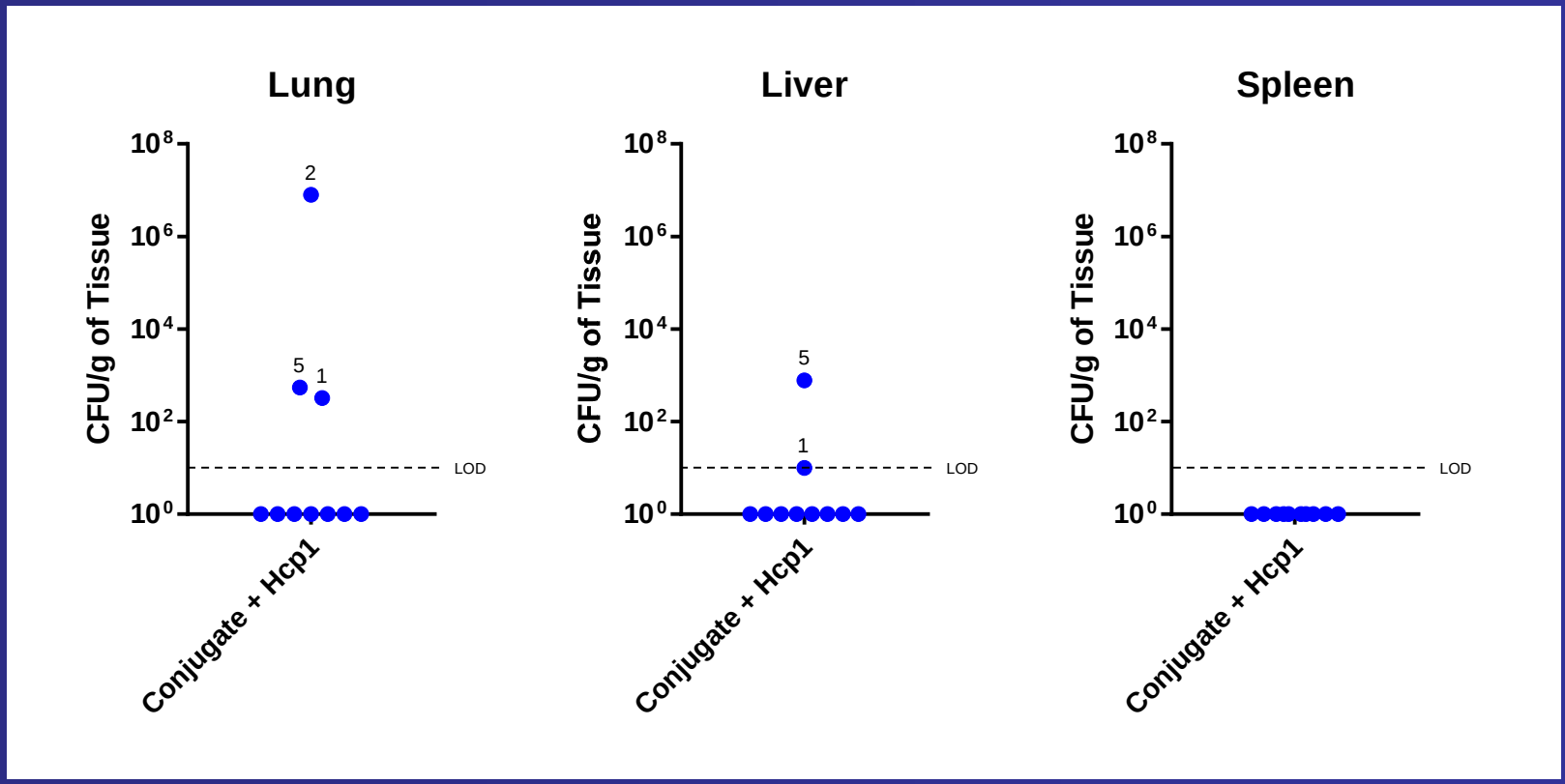


Challenge Study



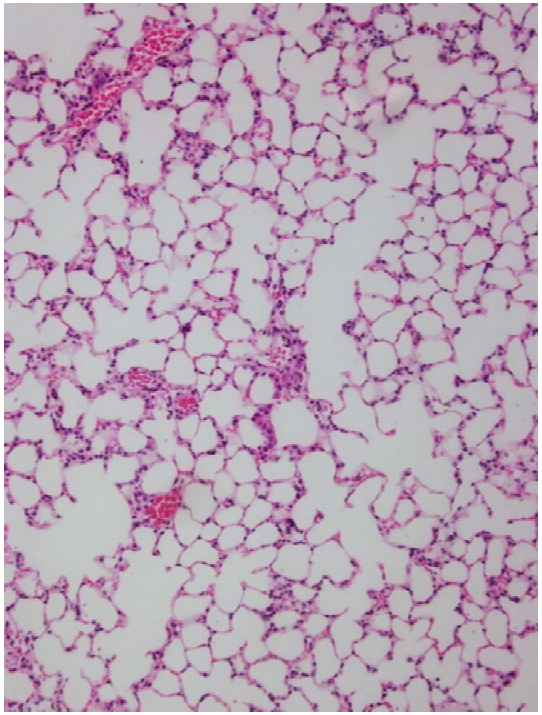
Inhalational challenge: ~10 LD_{50} *B. pseudomallei* K96243

Bacterial Tissue Loads

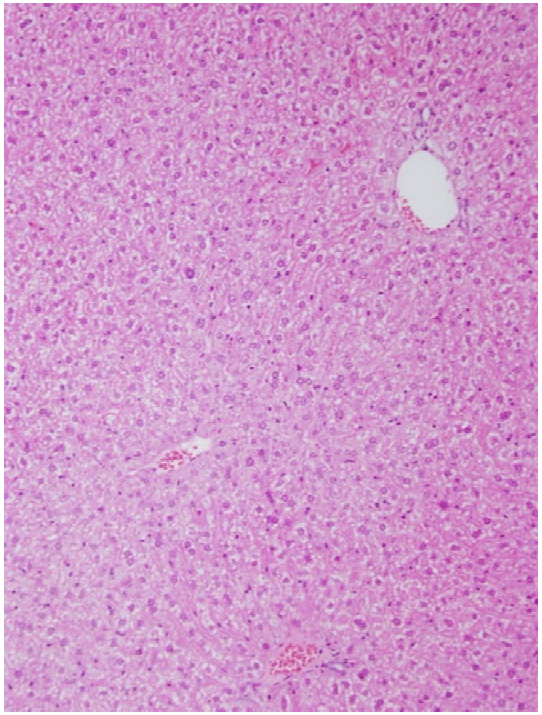


Histopathology

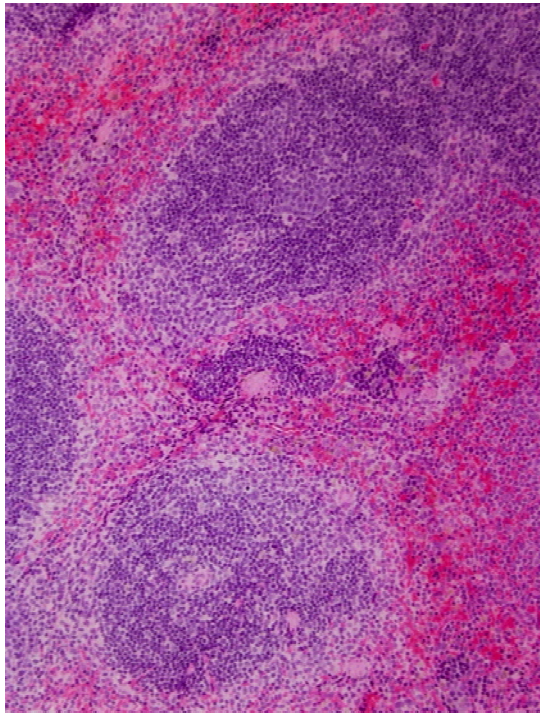
Lung



Liver



Spleen



Conclusions

- Robust protection can be achieved with a few as two antigens
- Sterilizing immunity can be achieved using a subunit-based vaccine approach
- A model has been developed to help identify specific correlates of antigen-induced immunity

Future Directions

- Optimize lead vaccine formulation
- Assess protective capacity of lead formulation in NHPs
- Advance our lead candidate into Phase I clinical trials
- Assess the protective capacity of additional CPS/protein formulations

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