Whole genome sequencing of colony variants of *Burkholderia pseudomallei* for use in a chronic infection model

Veterinary Research Scholars Program
University of Missouri

Leah Fray, Alexis Dadelahi, Jeffrey Adamovicz

Veterinary Pathobiology, Laboratory for Infectious Disease Research, College of Veterinary Medicine, University of Missouri

Laboratory for Infectious Disease Research
University of Missouri

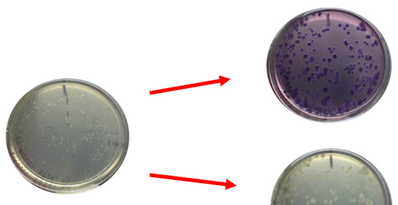
Veterinary Pathobiology
University of Missouri

Abstract

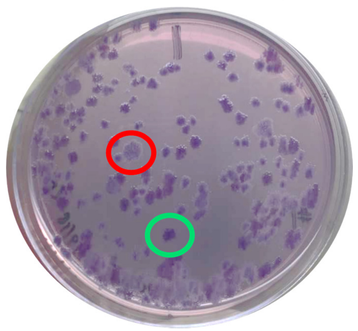
Burkholderia pseudomallei is a recognized biothreat agent and the causative agent of meliodosis, an infectious disease endemic in tropical areas such as northern Australia and Thailand. Meliodosis can present as either an acute or chronic infection, and there is no preventative vaccine. Our lab is interested in studying the changes in immune response during a chronic infection in a murine model. However, during pilot acute and chronic infection studies with *Burkholderia pseudomallei* strain 1026b, we observed less robust disease than others have reported. Upon plating spleen homogenates from acutely infected mice, only two of the twelve animals harbored viable bacteria. Further, we observed a threefold reduction in bacterial titer on Ashdown's media, a selective media for *Burkholderia*, as compared to growth on Luria-Bertani media. In addition, colonies growing on the LB media displayed two distinct colony morphologies. From these observations, it was decided to use replica plating to isolate colonies lacking the ability to grow on Ashdown's media and assess differences in virulence observed between the two morphotypes. It has been previously documented that *Burkholderia pseudomallei* has a particular affinity for mutation and horizontal gene transfer. We hypothesize that these genetic changes could relate to the lack of virulence in our original in vivo model. Thus, any differences identified between the two populations could potentially result in identification of novel in vivo determinants of virulence.

Replica Plating

- Purpose: To isolate colonies that grew on LB but not Ashdown's agar with intention to perform whole genome sequencing and compare variants



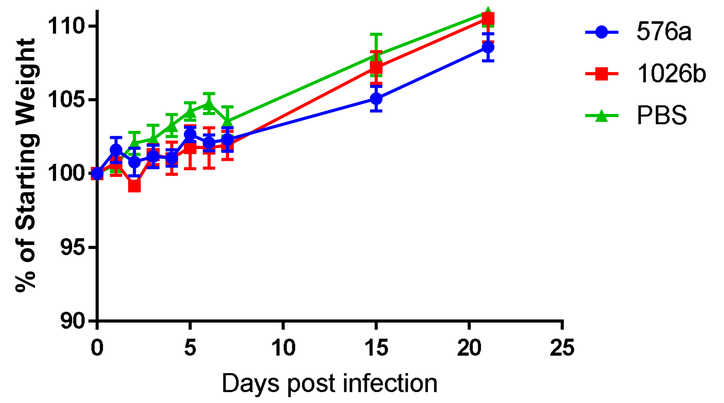
- Result: While varied colony morphology was observed on LB, isolation of Ashdown's negative colonies has not yet been achieved.



- Replica plating experiments are ongoing
- Chose to re-derive 1026b Ashdown's positive colonies for new working stock

Chronic Infection Trial

Results To Date:



Chronically infected mice gained weight more slowly than the controls within the first 5 days and then grew evened out with the controls in following days

Flow Cytometry Markers:

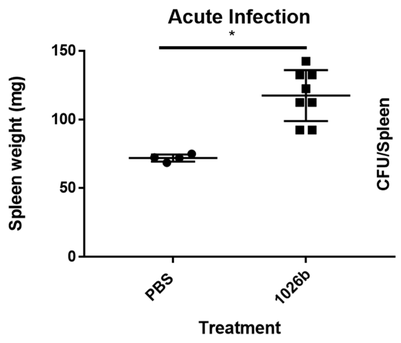
T Cell Exhaustion:

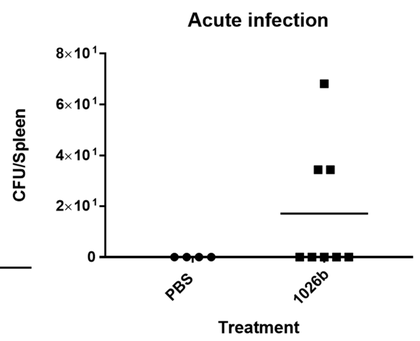
- Observed decline in robust T cell response in many chronic infections and in cancer progression
- Due to lack of sterilizing immunity observed in many vaccine and infection models of melioidosis, despite initial effective T cell responses, exhaustion may play a role:

CD4 T Cells	CD8 T cells
CD40 & CD44 - Activation	CD40 & CD44- Activation
IFN-γ – Function	IFN-γ – Function
PD-1, BTLA – Exhaustion	PD-1, Lag-3, Tim-3 – Exhaustion
Ly6C (al-21 clone), CD44 – long term memory	CD27, CD127, KLRG1l – long term memory

Acute Infection Trial

- Intranasal infection of 8 mice with 3.5 X 10^5 CFU *Burkholderia pseudomallei* strain 1026b
  - Delivery volume of 10 ul per nostril
  - 4 mice used for controls – administered same volume of PBS intranasally
- No mortality observed at day 7 as expected
- Significant increase in splenic weight observed compared to PBS controls
- Plated spleen homogenates & only 2 of the 12 mice harbored viable bacteria





Morphology Observations

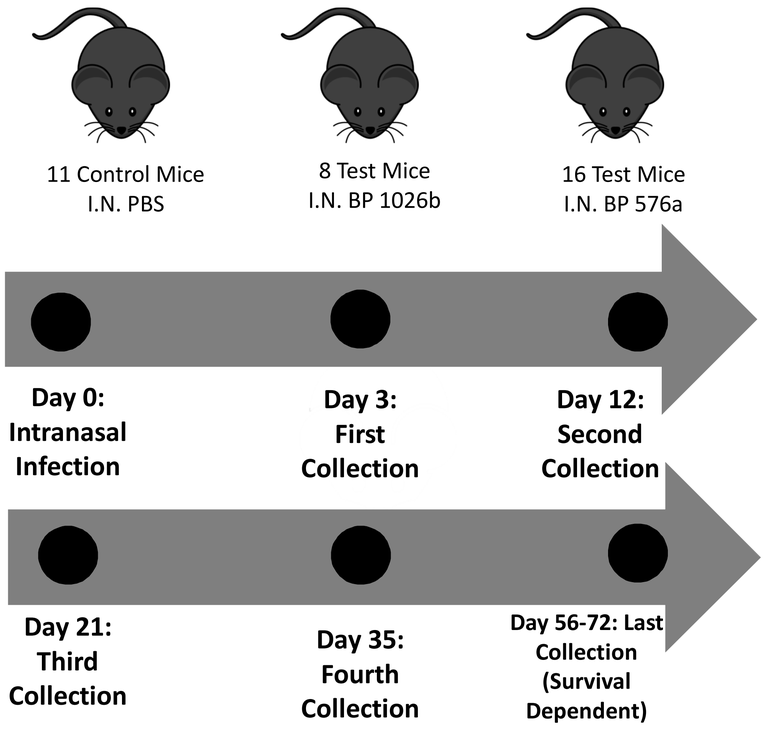
- Plated working stock of 1026b strain on both LB agar and selective Ashdown's media to rule out contamination
- Observed a three fold reduction in bacterial titer on Ashdown's vs LB
- Seed stock also displayed a three fold reduction in titer on Ashdowns's compared to LB
- Variants may be tied to reduced virulence of strain in murine model

Dilution plating on Ashdown's Agar

Chronic Infection Model

Experimental Approach

The objective of this study is to develop a chronic melioidosis infection model in order to observe changes in the antigen specific T-cell responses following intranasal challenge with *Burkholderia pseudomallei* strains 1026b or 576a. Challenge groups received 100CFU of either 1026b, or 576a *Burkholderia pseudomallei,* via intranasal administration of 10 ul of inoculum per nostril. Control groups were administered 10 ul of PBS per nostril and observed concurrently to compare morbidity and weight changes.



From animals sacrificed: Lungs, Liver and Spleens will be harvested and blood collected for flow cytometric analysis

Conclusions & Future Directions

- Due to titer and morphology setbacks, timeline was not started on time to achieve results at this time
- Expect to see activation and indication of a chronic infection followed by robust decrease in T cell activation, function and number at later timepoints
- Continue replica plating trials to try and isolate an Ashdown's negative colony for whole genome sequencing