Role of the *bsr* gene in the Intracellular Survival of *Bordetella bronchiseptica*

Hira Hasan [1], Monica Cartelle-Gestal [2], Eric Harvill [2]
[1] Louisiana State University, Baton Rouge, LA
[2] Center for Vaccines and Immunology, University of Georgia College of Veterinary Medicine, Athens, GA

Introduction

Bordetella pertussis and *B. parapertussis* are Gram-negative bacteria *that* cause whooping cough in humans, while *B. bronchiseptica* (BB) causes bronchitis in mice, dogs, and horses.

The wild type strain of BB, RB50, contains a gene (*bsr)* encoding a putative sigma factor that is up-regulated when BB is exposed to blood. To test the role of this gene in pathogen-host interactions, a knock-out mutant called RB50Δ*bsr* was made in our lab. Preliminary results showed that RB50Δ*bsr* survives longer within macrophages (immune cells which engulf and destroy pathogens) than RB50. The mutant also confers sterilizing immunity against further BB and *B. pertussis* infection in mice, which are excellent models for human infection.

The aim of this study is to determine if there is a difference between how RB50 and RB50Δ*bsr* are internalized by macrophages. We believe there is a delay in lysosome formation in RB50Δ*bsr*-infected macrophages and that RB50Δ*bsr* causes less damage to these immune cells, suggesting that the *bsr* gene can be targeted to produce a better vaccine against *Bordetella* infections.

Methods & Materials

Mouse macrophages in 96-well plates (RPMI media) were challenged with either RB50 or RB50Δ*bsr* bacteria at MOI 100. The cells were then incubated at various time points. [For time points greater than 15 minutes, gentamicin (300 ug/ ml) was added to the cell media after 1 hour of incubation.] The following assays were conducted:

- Confocal microscopy (Fig. 1 & 2) using fluorescent-labeled bacteria to determine co-localization with endosomes or lysosomes[1]
- pHrodo assay (Fig. 3) to detect acidification associated with lysosome formation[2]
- Phagocytosis assay (Fig. 4) to study the survival of engulfed bacteria overtime
- Intracellular assay (Fig. 5) to quantify live bacteria within macrophages over a 24-hour period
- Cytotoxicity assay (Fig. 6) to evaluate macrophage death caused by bacteria
- Electron microscopy (Fig. 7, 8 & 9) to quantify live bacteria within macrophages at specific time points

Results

a) b)

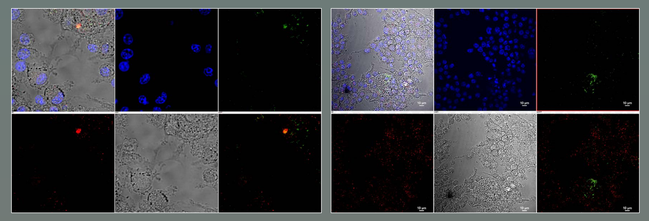


Figure 1: a) RB50 (green) colocalizes with lysosome marker LAMP (red) while b) RB50Δ*bsr* does not.

a) b)

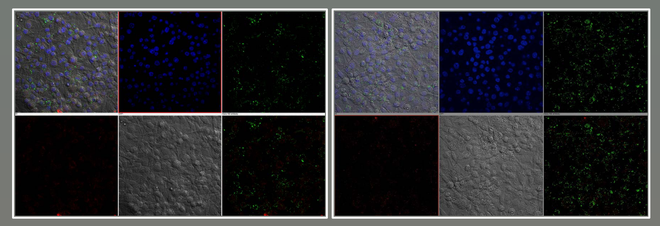
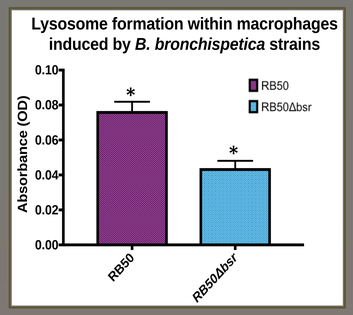


Figure 2: Less endosomal marker EEA1 (green) surrounds a) RB50 (red) than b) RB50Δ*bsr* (red).

Figure 3: Bacteria were stained with pHRodo Red, which produces an absorbance signal in acidic environments, such as lysosomes. The absorbance OD value is greater for RB50-challenged macrophages.



a) b) c)

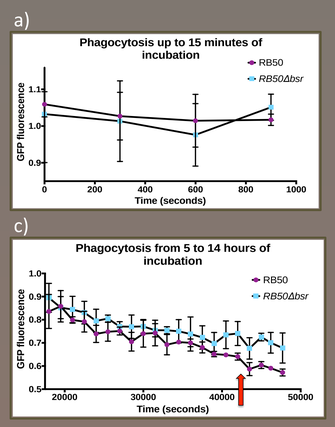
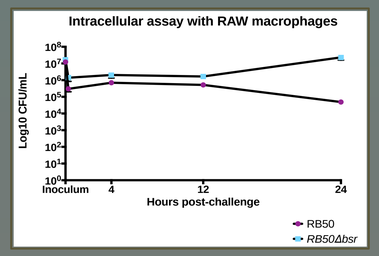


Figure 4: Levels of RB50 and RB50Δ*bsr* are a) stable at 15 minutes, followed by b) an increase in RB50Δ*bsr* at the 4 hour mark. After c) 12 hours, levels of intracellular RB50Δ*bsr* are higher than levels of RB50.

Figure 5: More RB50Δ*bsr* is recovered from macrophages at all time points. After 12 hours, RB50Δ*bsr* levels begin to rise while RB50 levels drop.



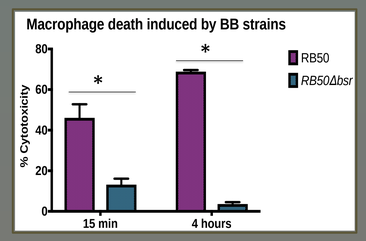


Figure 6: Less death is observed among RB50Δ*bsr* challenged macrophages than RB50-challenged cells at both time points.

a) b)

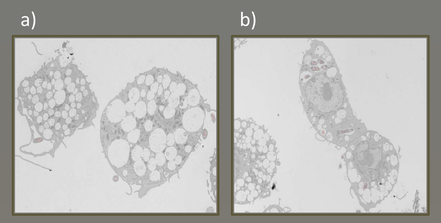
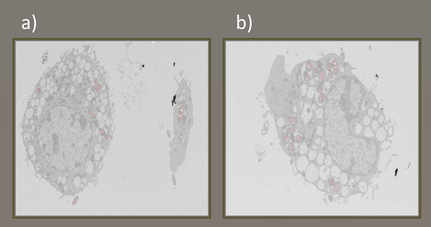


Figure 7: Individual macrophages (with bacteria in red) at 15 minutes. a) RB50 remains outside the cells, while b) RB50Δ*bsr* is internalized.

Figure 7: Individual macrophages (with bacteria in red) at 4 hours. The amount of intracellular b) RB50Δ*bsr* is nearly double the amount of a) RB50.

a) b)



a) b)

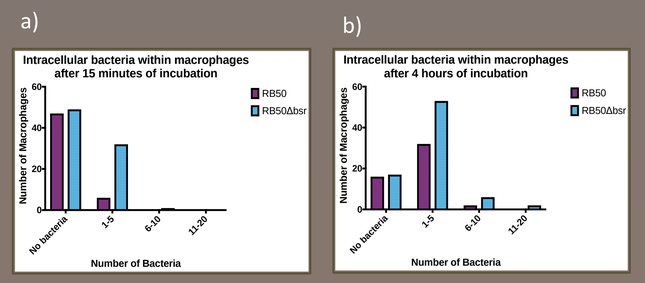


Figure 9: After a) 15 minutes, a greater number of macrophages engulf RB50Δ*bsr* than RB50. This trend continues after b) 4 hours, with some macrophages engulfing more than 10 RB50Δ*bsr.*

Conclusions

- More RB50Δ*bsr* than RB50 survives within macrophages overtime.
- RB50 enters into lysosomes readily while RB50Δ*bsr* does not show the same behavior after internalization.
- Infection by RB50Δ*bsr* results in less macrophage death overtime than infection by RB50.

Based on the results of this study, *bsr* plays a vital role in macrophage response to BB infection. Since macrophages are involved in activating several other immune system components, manipulating *bsr* leads to an overall change in the persistence of *Bordetella* infections.

References

1. Lamberti et al. "Proteome Analysis of Bordetella Pertussis Isolated from Human Macrophages." Journal of Proteomics 136 (2016): 55-67.

2. Guha, Sonia et al. "Approaches for Detecting Lysosomal Alkalinization and Impaired Degradation in Fresh and Cultured RPE Cells: Evidence for a Role in Retinal Degenerations." Experimental Eye Research 126 (2014): 68–76.

Acknowledgements

Support for this research was provided by the National Science Foundation (grant #1659683) through the Population Biology of Infectious Diseases Undergraduate Research program, based in the Odum School of Ecology at the University of Georgia.

Special thanks to the Harvill lab at the UGA Center for Vaccines and Immunology for welcoming and supporting me throughout this process.