The World's Smallest Escape Artists

Manipulation of the host innate immune response by *Bordetella bronchiseptica*

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Background

- *Bordetella* spp. are gram negative bacteria that cause respiratory disease. It is well documented that *Bordetella* spp. are able to sense and adapt to the host immune response.
- *B. pertussis* causes whooping cough in humans and is limited to that host. Whooping cough is a serious childhood illness, and cases are on the rise due to decreased vaccination rates and shortcomings of the vaccine.
- *B. bronchiseptica* causes illness in many mammalian species. It is a natural pathogen of mice and is very closely related to *B. pertussis*, which makes it an excellent model to study host/pathogen interactions.
- When studying blood and serum responsive genes, we identified a putative sigma factor up-regulated in both conditions, and we hypothesized that this is a regulator that dictates adaptation to pressure from the immune system. This gene has been named the Bordetella Sigma Factor, or *bsr.*

Objective and Hypothesis

Studying bacterial mechanism to hijack the innate immune response.

RB50Δ*bsr* is unable to respond to the presence of macrophages in the same manner as RB50, resulting in its inability to manipulate the host immune response.

Methods

Intracellular Assays: RAW macrophages were challenged with RB50 or RB50Δ*bsr* at an MOI of 100. Samples were collected at different time points, and dilutions were plated to count CFUs or samples were fixed and further imaged by Georgia Electron Microscopy. The number of macrophages and intracellular bacteria were counted. Confocal microscopy was used to image samples.

Cytotoxicity Assay: Performed following standard methods to measure LDH release.

MicroRNA Analysis of Infected Macrophages: RAW macrophages were challenged with RB50 or RB50Δ*bsr* at an MOI of 100. After 15 minutes RNA was extracted. Gentamycin was added 1 hour post infection. RNA was extracted again at 4 hours post infection. Data was sequenced by Mr. DNA.

Results

1. *bsr* increases macrophage and bacterial death

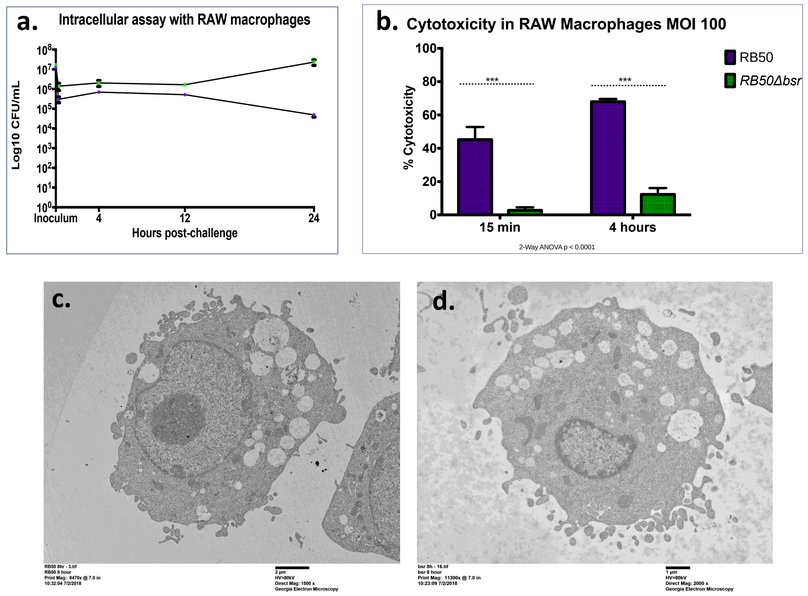

Figure 1. Macrophages challenged with RB50Δ*bsr* have more intracellular bacteria and experience increased cytotoxicity. a) Intracellular assay performed by M.C. Gestal, showing more bacteria recovered from macrophages challenged with RB50Δ*bsr* . **b)** Cytotoxicity assay performed by H. Hassan and M.C. Gestal, showing that macrophages challenged with RB50 experience more cytotoxicity. **c)** Macrophage 8 hours post gentamycin treatment challenged with RB50 and imaged using transmission electron microscopy. **d)** Macrophage challenged with RB50Δ*bsr*.

2. *bsr* impedes the accumulation of bacteria within RAW macrophages

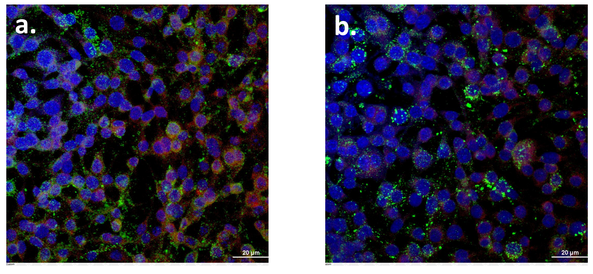

Figure 2. Macrophages 4 hours post challenge, imaged using confocal microscopy. a) Macrophages challenged with RB50. **b)** Macrophage challenged with RB50Δ*bsr*. Blue is DAPI, staining for the nucleus of the macrophages; green is GFP, staining for the bacteria; and red is LAMP, staining for endosomes. Macrophages were fixed 4 hours post challenged and stained by H. Hassan.

Conclusion

bsr is an important gene in regulating virulence factors in *B. bronchiseptica,* and when it is absent, the bacteria is unable to sense and respond to the host innate immune system efficiently.

3. *bsr* decreases persistence within macrophages

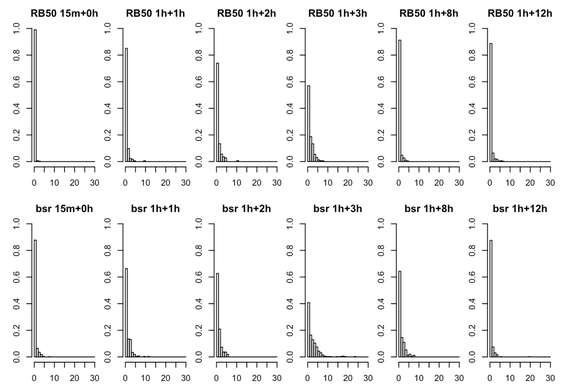

Figure 3. RB50Δ*bsr* is present in higher numbers along a time course. Intracellular assays were performed, and samples were collected at different time points. Data was compiled and analyzed by O. Restif using R

4. *bsr* manipulates macrophage signaling

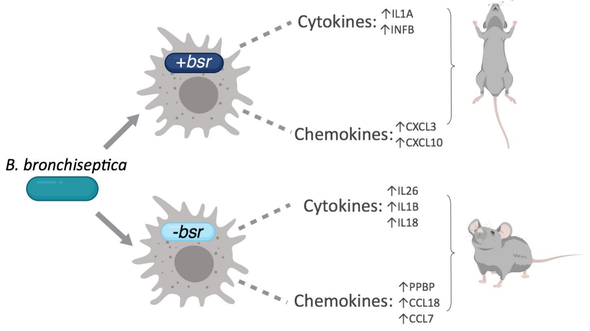

Figure 4. The presence of *bsr* alters cytokine and chemokine expression of macrophages. When *bsr* is present macrophages produce cytokines that increase fever and monocyte adhesion. When it is absent, proinflammatory cytokines and chemokines that create a more robust immune response are released.

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