Characterization of genes involved in the host specificity of *Salmonella enterica* serovars

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BACKGROUND

Typhoid fever is a disease specific to humans caused by infection with the bacteria *Salmonella enterica* serovar Typhi. Due to the strict host specificity of the bacteria, the mouse models available to study typhoid fever are limited to humanized mice, which are expensive and time consuming to develop, or the use of the related bacterium *Salmonella enterica* serovar Typhimurium that results primarily in gastroenteritis. This study aims to characterize the function of select genes present in *S. Typhimurium* and absent in *S. Typhi* to better understand their involvement in host specificity. We generated a *S. Typhimurium* deletion mutant for the itaconate response operon (S. Typhimurium ΔripABC*lgl*) and used a competitive index infection model to determine whether the deletion of the ripABC and lgl genes decrease the ability of the bacteria to replicate in vivo relative to the wildtype (WT) strain. The deletion mutant for ripABC*lgl* had an impaired ability to replicate relative to the WT strain when co-inoculated with equal amounts. This finding was consistent in the gastrointestinal tract (cecum contents and feces) as well as in the systemic organs (spleen and liver). An in vitro assessment of growth in the presence of the antimicrobial compound itaconate, produced by host macrophages showed that the *S. Typhimurium ΔripABC*lgl* was inhibited by itaconate. This suggests that in the murine host, these genes play an important role in itaconate resistance and confer greater overall fitness for the bacteria. By better understanding the function of genes implicated in *Salmonella* host specificity, we hope to contribute to the development of a typhoid fever model through addition of genes such as ripABC and lgl, combined with alterations in the host model to increase susceptibility to infection.

**Hypothesis:** The deletion of ripABC*lgl* genes from *S. Typhimurium* will decrease their ability to infect and replicate within the mouse.

**FIGURE 1. Mutant Construction**

**FIGURE 2. Decreased replication of *S. Typhimurium* ΔripABC*lgl* in the gastrointestinal tract and systemic organs of mice**

(A) Timeline for competitive index infection of C57Bl/6 mice. (B) Bacterial load in faces and (C) cecum following a competitive index infection. Mice were pre-treated with 20 mg of Streptomycin, then infected with a 1:1 ratio of *S. Typhimurium* WT (Cm*) and *S. Typhimurium* mutant (Kan*) via oral gavage for a total bacterial inoculation of 1×10⁹ CFU/mouse. Wildtype and mutant strains of *S. Typhimurium* were generated in a virulent and an avirulent background (*simA*Δ*upB*) as a negative control to demonstrate conditions of low inflammation and low itaconate. (D) Bacterial load in the systemic organs of liver and spleen following a competitive index infection, as described in (B).

**FIGURE 3. Itaconate inhibits the growth of *S. Typhimurium* ΔripABC*lgl* in vitro**

Growth of *S. Typhimurium* WT and ΔripABC*lgl* inoculated with or without itaconate in minimal media supplemented with acetate for 48 hours. *S. Typhimurium* WT growth is unaffected by itaconate while *S. Typhimurium* ΔripABC*lgl* growth is inhibited.

**CONCLUSIONS**

- *S. Typhimurium ΔripABC*lgl* had decreased growth in vivo within the gastrointestinal tract and systemic organs when co-infected with *S. Typhimurium* WT in mice.
- The growth of *S. Typhimurium ΔripABC*lgl* was inhibited in the presence of physiological concentrations of itaconate in vitro.

**FUTURE DIRECTIONS**

We aim to insert the ripABC*lgl* genes into *S. Typhi* and infect mice to determine if there is an increased ability of the bacterial to replicate in vivo.

**PRELIMINARY DATA**

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