Modification of LPS by EptB Inhibits Intelectin Binding and Increases Systemic Inflammation During *Salmonella* Infection

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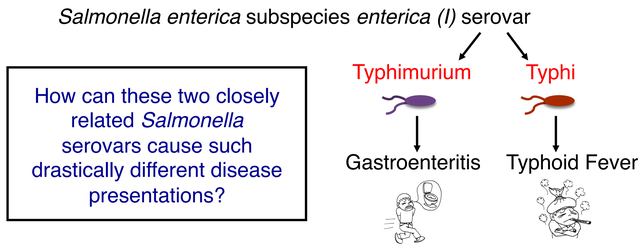
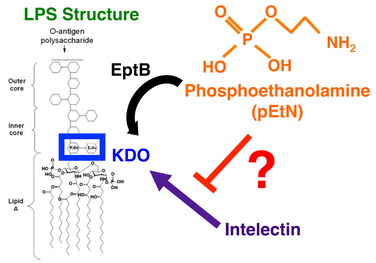
Background

Salmonella enterica is a highly diverse species of Gram-negative bacteria that can be grouped into typhoidal and non-typhoidal serovars. Non-typhoidal serovars, such as *S.* Typhimurium, cause gastroenteritis and inflammatory diarrhea, whereas typhoidal serovars, such as *S.* Typhi, cause systemic disease with a comparatively decreased inflammatory response. However, the properties that distinguish these two closely related groups remain poorly understood.



Previously, comparative analysis of *Salmonella* genomes revealed that typhoidal serovars contain a higher number of pseudogenes than non-typhoidal serovars. One such pseudogene is *eptB,* which codes for a phosphoethanolamine transferase that can specifically modify the outer keto-deoxyoctulosonate (KDO) residue of lipopolysaccharide (LPS).

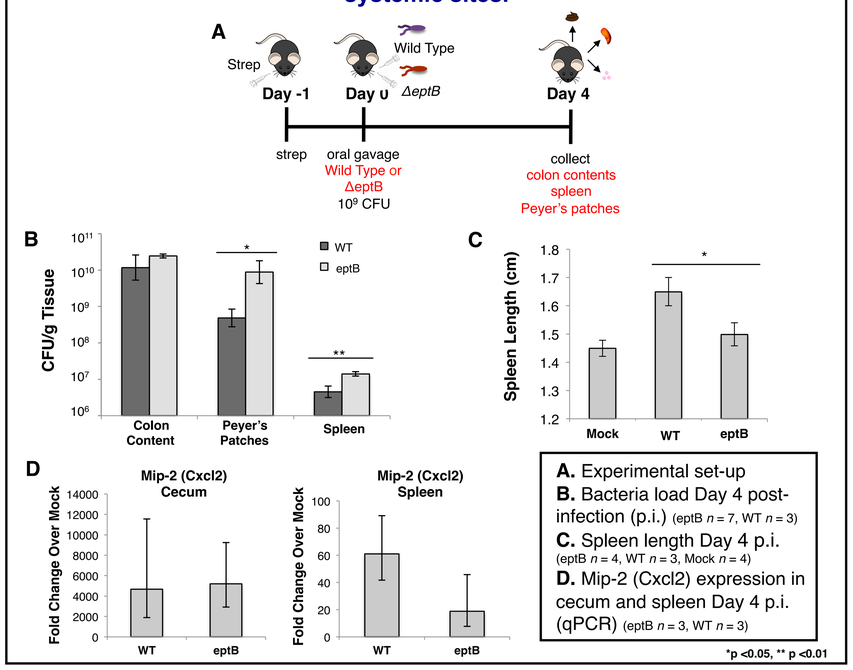


Does EptB prevent intelectin binding to *Salmonella* LPS? Does intelectin binding "detoxify" LPS and decrease systemic inflammation?

Human intelectin-1 is known to bind to and recognize multiple microbial glycan epitopes, including KDO, and has been proposed to function in innate immunity. Interestingly, previous studies have shown that *S.* Typhimurium LPS is not bound by intelectin, despite possessing KDO residues.

Results

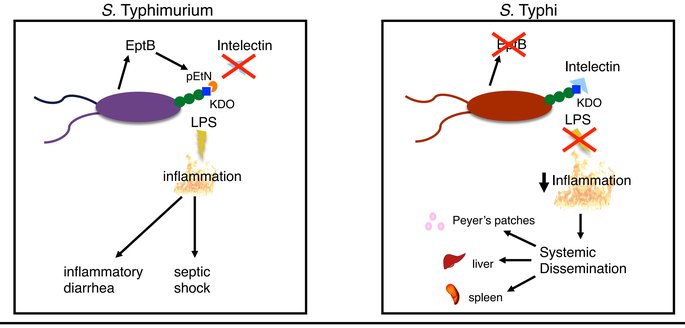
Loss of *eptB* function in *S.* Typhimurium results in decreased expression of pro-inflammatory cytokines and increased bacterial dissemination to systemic sites.



A. Experimental set-up
B. Bacteria load Day 4 post-infection (p.i.) (eptB $n = 7$, WT $n = 3$)
C. Spleen length Day 4 p.i. (eptB $n = 4$, WT $n = 3$, Mock $n = 4$)
D. Mip-2 (Cxcl2) expression in cecum and spleen Day 4 p.i. (qPCR) (eptB $n = 3$, WT $n = 3$)

*p <0.05, ** p <0.01

Working Model



Conclusions

- Infection of mice with a *S.* Typhimurium *eptB* mutant results in increased bacterial burden in the spleen and Peyer's patches, compared to infection with wild-type *S.* Typhimurium.
- The size of the spleen is significantly decreased in mice after infection with an *eptB* mutant compared to mice infected with the wild-type strain.
- There is a significant decrease in expression of inflammatory cytokines, such as Mip-2 (Cxcl2) in the spleen in mice infected with an *eptB* mutant compared to mice infected with the wild-type strain.

Together, these results suggest that in the absence of EptB, intelectin is able to bind to and detoxify *S.* Typhimurium LPS, leading to decreased systemic inflammation during infection.

These results have broad implications for how pathogens such as *S.* Typhimurium induce systemic shock during infection and may also help to explain a mechanism for how *S.* Typhi is able to evade immune detection and enhance dissemination to systemic sites.

Future Directions

- Infection of intelectin-1 KO mice with WT vs. eptB mutant *S.* Typhimurium
- Generation of an even stealthier *S.* Typhimurium mutant: combine the *eptB* mutation with other mutations that trigger a decreased inflammatory response (Vi capsule)
- Generation of *eptB* mutations in other bacteria (*E. coli*)

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