Understanding the Role of the *Salmonella* Typhi Vi Capsular Polysaccharide in Neutrophil and Macrophage Phagocytosis

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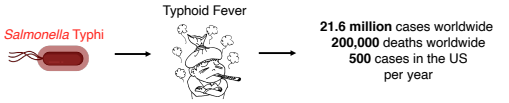
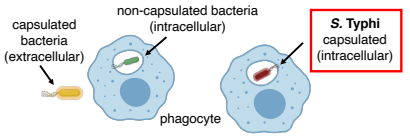
Background

Salmonella Typhi is the causative agent of typhoid fever, which is a life-threatening, systemic disease, with an estimated global disease burden of 21.6 million cases annually, resulting in about 220,000 deaths. Due to the absence of convenient animals models to study *S.* Typhi and other typhoidal *Salmonella* serovars, our understanding of typhoid fever pathogenesis is still incomplete.



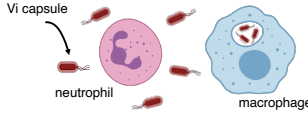
Like other Salmonella serovars, *S.* Typhi is phagocytosed by host macrophages and survives and replicates intracellularly within these macrophages. Interestingly, one important virulence factor of *S.* Typhi is the polysaccharide capsular antigen Vi, which, like many of the bacterial capsules produced by extracellular bacteria, has long been thought to play a role in preventing phagocytosis and complement killing of *S.* Typhi.



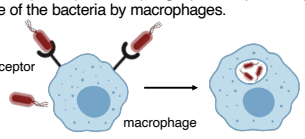
Why does an intracellular pathogen, such as *S.* Typhi, possess an anti-phagocytic capsule?

Thus, we encounter a paradox in which a bacteria that survives and replicates within macrophages as part of its life cycle, also possesses an anti-phagocytic capsule, which is more characteristic of an extracellular pathogen.

Is there a difference in phagocytosis of *S.* Typhi depending on cell type?
Macrophages
vs.
Neutrophils



Here, we demonstrate that the *S.* Typhi Vi capsule selectively prevents or allows phagocytosis and uptake of the bacteria depending on the host cell type. The Vi capsule prevents phagocytosis by neutrophils, but does not prevent uptake of the bacteria by macrophages.

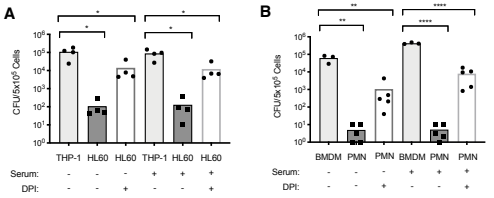


Is there a macrophage-specific receptor that directly binds to the Vi capsule to facilitate uptake?

Instead, we propose that macrophages possess cell surface receptors that specifically bind to and recognize polysaccharides present in the Vi capsule, thereby facilitating engulfment.

Figure 1

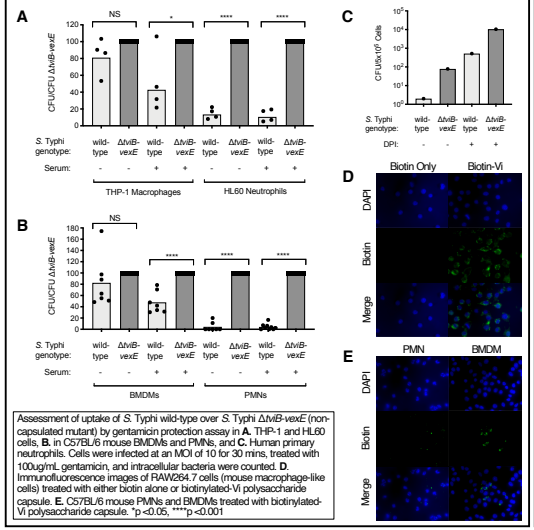
There is a decrease in phagocytosis of capsulated *S.* Typhi in neutrophils compared to macrophages



Assessment of *S.* Typhi uptake by gentamicin protection assay in **A.** human macrophage-like cells (THP-1) and human neutrophil-like cells (HL60) and **B.** in C57BL/6 mouse bone marrow-derived macrophages (BMDM) and mouse neutrophils (PMN). Cells were infected at an MOI of 10 for 30 mins, treated with 100ug/mL gentamicin, and intracellular bacteria were counted. *p <0.05, **p <0.01, ****p <0.001

Figure 2

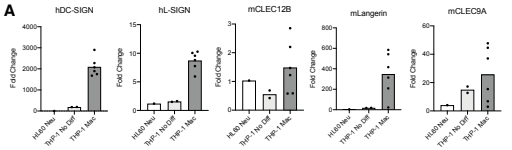
The Vi capsule selectively prevents phagocytosis of *S.* Typhi by neutrophils but not macrophages



Assessment of uptake of *S.* Typhi wild-type over *S.* Typhi *ΔtviB-vexE* (non-capsulated mutant) by gentamicin protection assay in **A.** THP-1 and HL60 cells, **B.** in C57BL/6 mouse BMDMs and PMNs, and **C.** Human primary neutrophils. Cells were infected at an MOI of 10 for 30 mins, treated with 100ug/mL gentamicin, and intracellular bacteria were counted. **D.** Immunofluorescence images of RAW264.7 cells (mouse macrophage-like cells) treated with either biotin alone or biotinylated-Vi polysaccharide capsule. **E.** C57BL/6 mouse PMNs and BMDMs treated with biotinylated-Vi polysaccharide capsule. *p <0.05, ***p <0.001

Figure 3

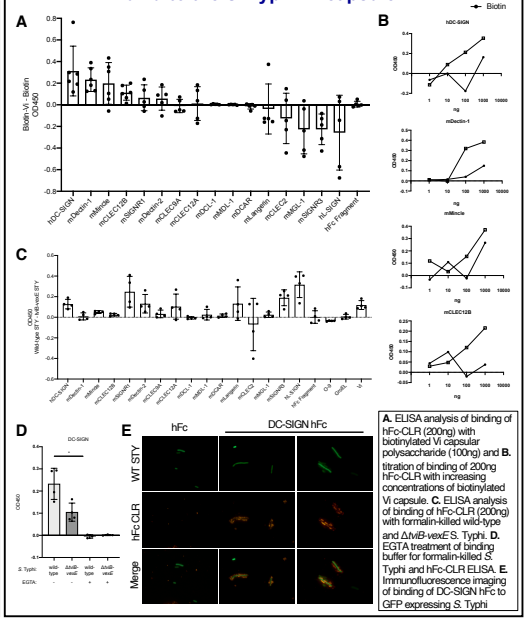
C-type lectin receptors are differentially expressed between neutrophils and macrophages



A. qPCR analysis of expression of C-type lectin receptors in HL60 human neutrophil-like cells, undifferentiated THP-1 cells (human monocyte-like), and differentiated THP-1 cells (human macrophage-like).

Figure 4

C-type lectin receptors expressed on macrophages bind to the *S.* Typhi Vi capsule



A. ELISA analysis of binding of hFc-CLR (200ng) with biotinylated Vi capsular polysaccharide (100ng) and **B.** titration of binding of 200ng hFc-CLR with increasing concentrations of biotinylated Vi capsule. **C.** ELISA analysis of binding of hFc-CLR (200ng) with formalin-killed wild-type and *ΔtviB-vexE S.* Typhi. **D.** EGTA treatment of binding buffer for formalin-killed *S.* Typhi and hFc-CLR ELISA. **E.** Immunofluorescence imaging of binding of DC-SIGN hFc to GFP expressing *S.* Typhi

Conclusions

- The *S.* Typhi Vi capsular polysaccharide excludes phagocytosis by neutrophils, but does not prevent phagocytosis by macrophages
- Differences in expression of C-type lectin receptors and other cell surface receptors between macrophages and neutrophils may contribute to the selective phagocytosis of *S.* Typhi by macrophages and not neutrophils
- DC-SIGN (mSIGNR1), Mincle, Dectin-1, and CLEC12B are potential receptors expressed on macrophages that bind directly to the Vi capsule and facilitate phagocytosis of *S.* Typhi.

These findings that the Vi capsule of *S.* Typhi interacts differently with different host phagocytes represents a step forward in our understanding of how typhoidal *Salmonella* serovars interface with host immunity and will provide important new insights into the pathogenesis of typhoid fever.

Future Directions

- Verify the macrophage receptor(s) that recognize and bind directly to the Vi capsule of *S.* Typhi and evaluate the contribution of these receptors to uptake
  - Receptor knockdown/knockout *in vitro*
  - Gain of function in non-phagocytic cells
  - Receptor knockout mice
- Determine the downstream effects of receptor binding on inflammatory pathways
- Confirm findings using primary human neutrophils and monocytes/macrophages

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