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

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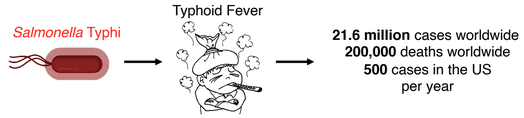
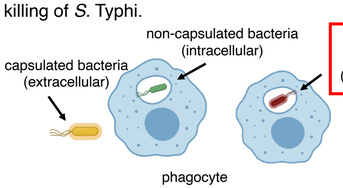
Background

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.



21.6 million cases worldwide
200,000 deaths worldwide
500 cases in the US
per year

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.

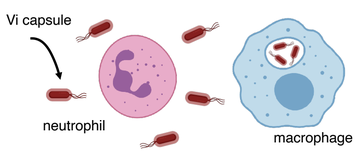


***S.* Typhi** capsulated (intracellular)

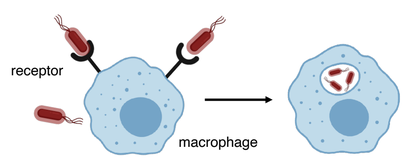
Why does an intracellular pathogen, such as *S.* Typhi, possess an anti-phagocytic capsule?

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.

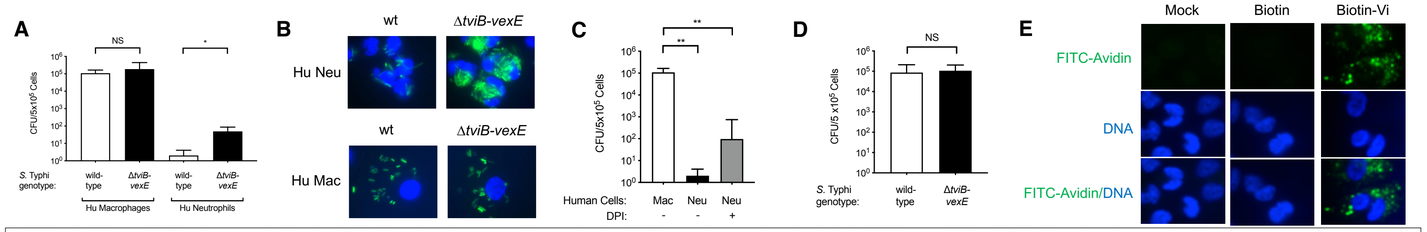


Is there a macrophage-specific receptor that directly binds to the Vi capsule to facilitate uptake?

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

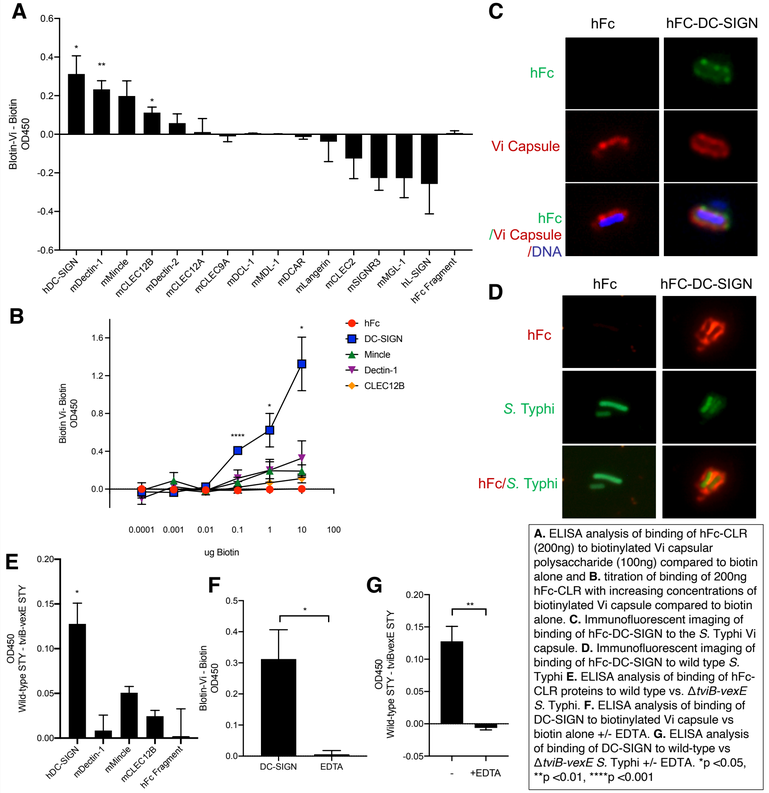
The Vi capsule selectively prevents phagocytosis of *S.* Typhi by neutrophils but not macrophages



A. Uptake of wild-type vs. Δ*tviB-vexE* (non-capsulated mutant) *S.* Typhi by primary human macrophages and neutrophils **B.** Immunofluorescent imaging of uptake of wild type vs. Δ*tviB-vexE S.* Typhi by primary human neutrophils and macrophages. **C**. Uptake of wild-type *S.* Typhi by primary human neutrophils when treated with DPI, a NADPH oxidase inhibitor. **D.** Uptake of wild-type and Δ*tviB-vexE S.* Typhi by the THP-1 cell-derived macrophages (human cell line). **E.** Immunofluorescent imaging of uptake of biotin alone or biotinylated Vi capsule by THP-1 cell derived macrophages. *p <0.05, **p <0.01

Figure 2

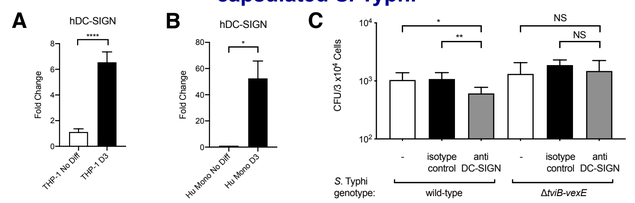
The C-type lectin receptor DC-SIGN binds to the *S.* Typhi Vi Capsule



A. ELISA analysis of binding of hFc-CLR (200ng) to biotinylated Vi capsular polysaccharide (100ng) compared to biotin alone and **B.** titration of binding of 200ng hFc-CLR with increasing concentrations of biotinylated Vi capsule compared to biotin alone. **C.** Immunofluorescent imaging of binding of hFc-DC-SIGN to the *S.* Typhi Vi capsule. **D.** Immunofluorescent imaging of binding of hFc-DC-SIGN to wild type *S.* Typhi **E.** ELISA analysis of binding of hFc-CLR proteins to wild type vs. Δ*tviB-vexE S.* Typhi. **F.** ELISA analysis of binding of DC-SIGN to biotinylated Vi capsule vs biotin alone +/- EDTA. **G.** ELISA analysis of binding of DC-SIGN to wild-type vs Δ*tviB-vexE S.* Typhi +/- EDTA. *p <0.05, **p <0.01, ****p <0.001

Figure 3

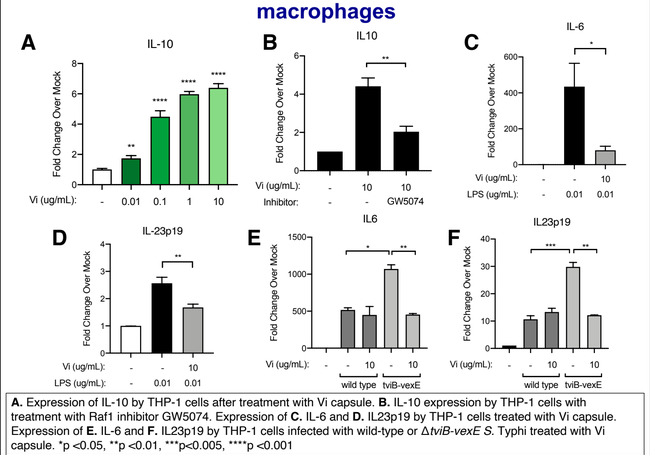
Blocking DC-SIGN results in decreased phagocytosis of capsulated *S.* Typhi



qPCR analysis of expression of DC-SIGN in **A.** undifferentiated vs differentiated (macrophage-like) THP-1 cells and **B.** undifferentiated vs macrophage differentiated primary human monocytes. **C.** Uptake of wild type and Δ*tviB-vexE S.* Typhi by THP-1 cells with and without DC-SIGN blocking Ab treatment. *p <0.05, **p <0.01, ****p <0.001

Figure 4

The Vi Capsule induces an anti-inflammatory response in macrophages



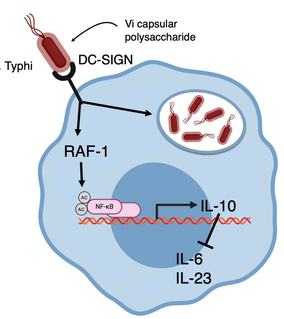
A. Expression of IL-10 by THP-1 cells after treatment with Vi capsule. **B.** IL-10 expression by THP-1 cells with treatment with Raf1 inhibitor GW5074. Expression of **C.** IL-6 and **D.** IL23p19 by THP-1 cells treated with Vi capsule. Expression of **E.** IL-6 and **F.** IL23p19 by THP-1 cells infected with wild-type or Δ*tviB-vexE S.* Typhi treated with Vi capsule. *p <0.05, **p <0.01, ***p<0.005, ****p <0.001

Conclusions

- The *S.* Typhi Vi capsular polysaccharide selectively prevents 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 is a receptor that is expressed on macrophages that binds directly to the Vi capsule to facilitate phagocytosis of *S.* Typhi.
- Blocking DC-SIGN results in decreased phagocytosis of *S.* Typhi, but not non-capsulated *S.* Typhi.
- Binding of the Vi capsule by macrophages induces an anti-inflammatory response.

S. Typhi binds to DC-SIGN expressed by macrophages and signals through the RAF_1 pathway to induce expression of anti-inflammatory cytokines, such as IL-10, which inhibits expression of pro-inflammatory cytokines such as IL-6 and IL-23. This contributes to the ability of *S.* Typhi to evade detection by the host immune system.

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.



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