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TonB-dependent iron transporters in Neisseria gonorrhoeae infection of primary human PMNs

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Background

Neutrophils are first responders to Neisseria gonorrhoeae (Gc) infection. Like other cell types, neutrophils require iron for biological function and release proteins like lactoferrin to sequester iron from pathogens. Gc evolved to express TonB-dependent transporters (TdTs) that hijack metals directly from host metal-sequestering proteins. Several TdTs–including TbpBA, TdfF, and TdfG–are iron regulated, suggesting they have roles in iron transport. tbpBA encodes two proteins that collaborate to sequester iron from human transferrin (hTf): the TbpA transporter and the TbpB lipoprotein that increases TbpA's affinity for hTf. TbpBA does not exhibit phase nor antigenic variation and is essential for virulence in a human infection model, making it a promising vaccine candidate. TdfF and TdfG are recently discovered TdTs with unknown ligands. We hypothesize that Gc hijacks iron from neutrophils using TbpBA, TdfF, and TdfG.

Aim/Methods

FA1090 Gc that do not express Opa proteins (Opaless) were used as the wild type (WT) for these studies. WT Gc were exposed to primary human neutrophils, and at indicated time points, RNA was collected from the infection mix, or neutrophils were lysed and Western blotted for proteins of interest. tbpBA, tdfF, and tdfG single knockout mutations were backcrossed into Opaless FA1090. Primary human neutrophils were isolated and infected with WT or tbpBA knockout Gc. At indicated times post infection, neutrophils were lysed to enumerate CFU.

Results

Using dual species transcriptomics, Gc exposed to neutrophils upregulated the expression of tbpBA, tdfF, tdfG, and other iron-regulated genes, although to lower levels than Gc grown in the absence of neutrophils. This suggests that the bacteria may be able to obtain iron from neutrophils during infection. The tbpBA knockout strain of Gc exhibits normal growth in RPMI+10% fetal bovine serum (FBS) but demonstrates a survival defect compared to WT Gc when incubated with primary human neutrophils in RPMI+10%FBS.

Conclusions

TbpBA is important in survival from human neutrophils, but Gc downregulates TbpBA in the presence of neutrophils. The roles of TdfF and TdfG remain unknown. Future work will determine if neutrophils are a source of iron for Gc during infection and elucidate the role of TdTs in Gc iron acquisition.