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Characterizing the role of TdfH extracellular loops in gonococcal zinc acquisition from calprotectin

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Background

Neisseria gonorrhoeae (Ngo), a Gram-negative diplococcus, is a human-specific pathogen that causes the sexually transmitted infection, gonorrhea. Ngo can undergo high-frequency antigenic and phase variation and has developed resistance to existing antimicrobial therapies. In addition, there is no effective gonococcal vaccine available. A promising approach for developing preventives and better therapeutics against Ngo is to investigate outer-membrane TonB-dependent transporter (TDTs) that enable direct binding and extraction of metals from host metal transporter/scavenger proteins in an energy-dependent mechanism. One of the 8 TDTs, TdfH, has minimal sequence diversity among strains, is not subject to phase variation, and therefore, is potentially an ideal vaccine target. Our lab has previously characterized the binding interactions between TdfH and human calprotectin (hCP) and showed the Zn piracy from hCP supports gonococcal growth and survival within neutrophil extracellular traps (NETs) in a zinc-dependent manner. Human calprotectin is part of the S100 family of proteins involved in nutritional immunity, whereby the host limits access to bacterial nutrients including iron, zinc, and manganese.

Aim/Methods

Our aim in this study is to understand and characterize the binding interface between hCP and TdfH to inform preventative and/or therapeutic development. To test our hypothesis, Ngo strains with deletions and point

mutations in the extracellular loop regions of TdfH were generated and evaluated for hCP binding and function.

Results
The mutated TdfH proteins were expressed ectopically behind an IPTG-inducible promoter and monoclonal antibodies against TdfH confirmed surface exposure similar to wild-type TdfH. TdfH-hCP binding was evaluated using whole-cell dot blot assays using HRP labeled hCP, which demonstrated that the TdfH-hCP binding is impaired in some TdfH mutant strains. Additionally, when hCP was provided as a sole zinc source, some mutants, when compared to the TdfH complement, were unable to grow or impaired in growth.

Conclusions
Altogether, these results indicate that mutation in specific residues from extracellular loops of TdfH inhibits hCP binding and, therefore, prevents Zn internalization. This study will inform future work, which will include characterizing the immune response and protection when TdfH is used as an antigen in hCP- transgenic mouse immunization studies.