Group B *Streptococcus* β–hemolysin/cytolysin modulates intestinal tight junction protein gene expression

Kristen Domínguez[1], April Lindon[1], Sophie Darch[1], Tara Randis[1,2]
1.Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL
2.Division of Neonatology, University of South Florida, Tampa, FL

USF Health UNIVERSITY of SOUTH FLORIDA

Background

Significance

Group B *Streptococcus* (GBS) is a leading cause of neonatal sepsis worldwide. GBS primarily colonizes the gastrointestinal tract in exposed newborns. Mechanisms of GBS translocation and invasion remain poorly understood.

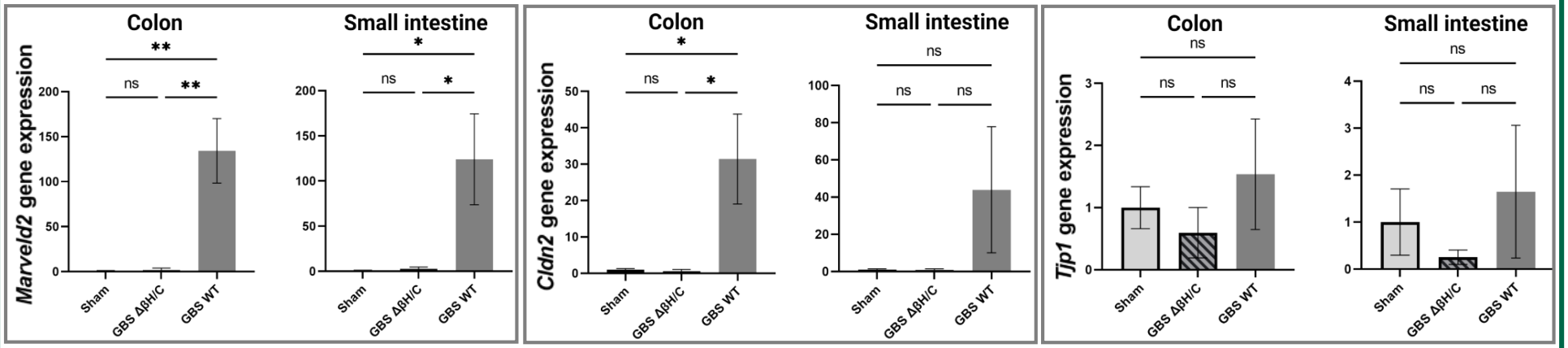
Rationale

- β-hemolysin/cytolysin (βH/C) is a pore-forming toxin produced by GBS capable of disrupting epithelial barriers; its role in GBS translocation across the intestinal epithelium remains unexplored.
- Tight junction gene expression indicates barrier function.
- Enteric pathogens alter the expression and function of tight junction proteins via toxin-mediated signaling pathways.

Hypothesis: ***GBS βH/C alters intestinal tight junction protein gene expression.***

Main Results

GBS induced changes in tight junction gene expression are dependent on the βH/C toxin



Relative gene expression of *Tjp-1*, *Cldn2*, and *Marveld2* normalized to *β-actin* in proximal colons and distal small intestines.
[ΔΔ-CT method, normalized to β-actin; One-way ANOVA with Šídák's multiple comparison test. * $p < 0.05$, ** $p < 0.005$]

- *Marveld2* gene expression was significantly increased in the intestinal tissues of pups colonized with WT GBS.
- *Cldn2* gene expression was significantly increased in colonic samples of pups colonized with WT GBS.
- There were no significant differences in *Tjp-1* expression among study groups.

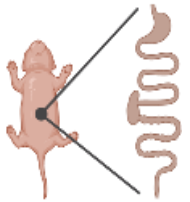
There was no significant difference in GBS burden in intestinal tissues between GBS WT and GBS ΔβH/C (data not shown).

Methods

Oral gavage day 10 of life

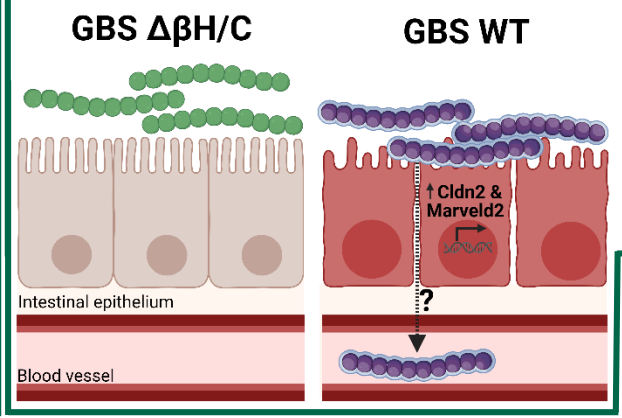
PBS (Sham)
GBS ΔβH/C
GBS WT

4 days post-gavage



Animals were gavaged with GBS wild type strain COH-1 (WT, n=7), its isogenic, βH/C-deficient mutant (ΔβH/C, n=4), or PBS (sham, n=4) on day 10 of life. Intestinal tissues were harvested 4 days post exposure and processed to determine GBS burden and for RNA isolation. We used selective media to determine GBS burden in tissues and RT-qPCR to compare candidate tight junction protein gene expression. Candidacy was based on human-mouse homology during this developmental period and their altered expression with other enteric pathogen exposure.

Conclusions



Overall, GBS induced changes in tight junction gene expression are dependent on the βH/C toxin.

- GBS βH/C altered intestinal *Cldn2* & *Marveld2* gene expression.
- βH/C may play a critical role in compromising intestinal barrier function in GBS colonized infants.

Work in progress: confocal microscopy (distribution of tight junction proteins in intestines) & unbiased approaches (RNA-seq, proteomics)

Acknowledgments

We would like to acknowledge the Les and Pamela Muma Endowment to the Division of Neonatology for funding this work, βH/C-deficient strain gifted by A.J. Ratner the Interdisciplinary Research Building vivarium staff, and BioRender.com for the graphics.