Meningeal barrier breakdown and local immune response during neonatal bacterial meningitis

Sol (Sophia) Kim[1], Luke Joyce[2], Brady Spencer[2], Julia Derk[1], Kelly Doran[2], Julie Siegenthaler[1]

[1]Department of Pediatrics, Section of Developmental Biology, University of Colorado Anschutz Medical Campus, Aurora, CO

[2]Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, CO

OBJECTIVES

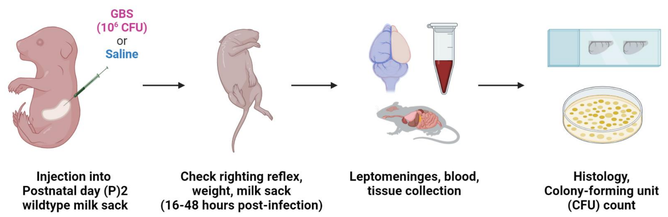
- Developing a neonatal bacterial meningitis model to study the functional role and response of the arachnoid barrier in CNS diseases.
- Investigating how leptomeningeal border-associated macrophages respond to infection and alter the arachnoid barrier integrity.

INTRODUCTION

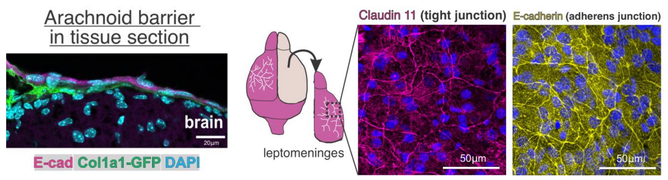
- The central nervous system (CNS) is protected by several types of barriers, one of which is blood-cerebrospinal fluid barrier (B-CSFB).
- One component of the B-CSFB is the arachnoid barrier (AB), the outer arachnoid layer that consists of epithelial-like cells connected by extensive junctional proteins (E-cadherin/Claudin-11) and regulates cellular and molecular movement between the CSF and the periphery.
- *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) is the leading cause of life-threatening meningitis in newborns and can lead to long-term neurological deficits in children who survive the initial infection during infancy.
- In the absence of AB prenatally, a significantly higher burden of GBS has been observed in the leptomeninges.
- Border-associated macrophages (BAMs) (CD206$^+$/LYVE-1$^+$) are among the most abundant resident immune cells in the meninges. Upon inflammation, BAMs become phagocytic and express inflammatory cytokines.
- Current gaps in the field include: 1) *Is the AB functionally perturbed in bacterial meningitis and what are the cellular and molecular mechanisms driving AB breakdown?*, and 2) *How do meningeal BAMs respond to GBS infection and do they contribute to the altered AB in diseased-state?*

METHODS

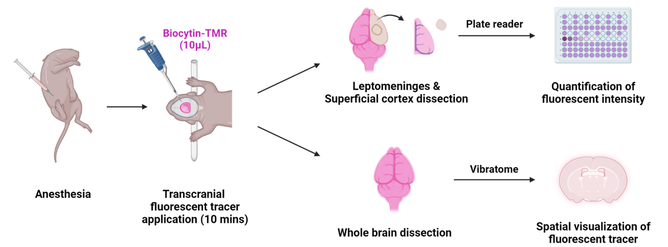
A. Acute Neonatal GBS Infection Model and Sample Collection



B. Histological Analysis of Arachnoid Barrier Post-Infection



C. Tracer Dye-Based Arachnoid Barrier Integrity Assay (ABIA)



ARACHNOID BARRIER JUNCTIONAL PROTEIN MISLOCALIZATION POST-INFECTION

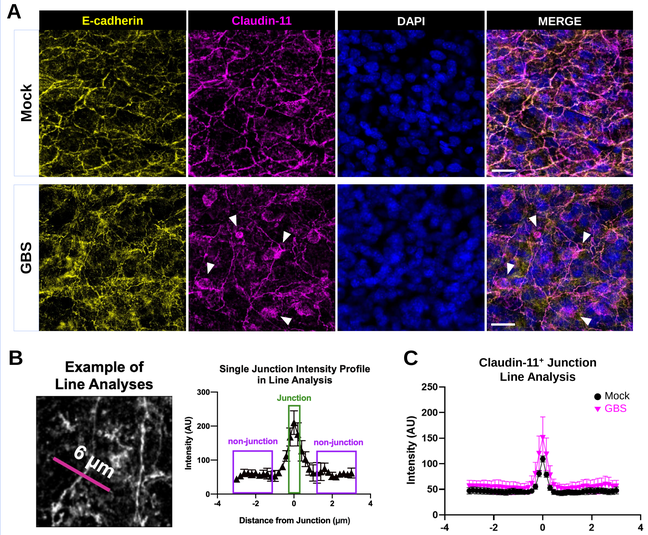


Fig 1. The AB junctional protein Claudin-11 is mislocalized after GBS infection, but its overall expression level remains unchanged. (A) Histological result showed no difference in E-cadherin$^+$ AB junctions between the mock (top) and the GBS-infected (bottom) group. However, the localization pattern of Claudin-11 proteins appeared aberrant in the infected group with non-junctional, nuclear localization (arrowheads). **(B)** Example of single junction intensity quantification. A 6μm-line was drawn across a single junction (left) and the central peak, the junctional intensity, was quantified (right). **(C)** Line intensity analysis of Claudin-11$^+$ junctions showed no significant change in expression level post-infection. (Scale = 20μm)

LOSS OF ARACHNOID BARRIER INTEGRITY FOLLOWING GBS INFECTION

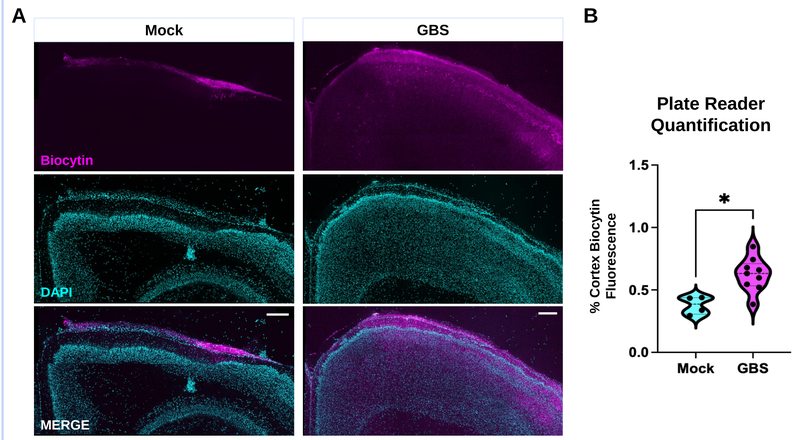
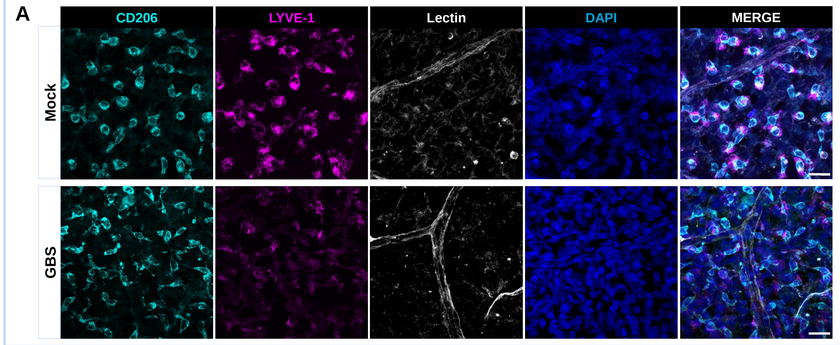


Fig 2. GBS infection drives AB breakdown. (A) AB integrity assay showed leakage of tracer dye (Biocytin-TMR, 893 Daltons) into GBS-infected superficial cortex (right), but not mock (left). **(B)** Quantification of tracer dye content in the superficial cortex confirmed dye leakage into cortices following infection. (Scale = 200μm)

ALTERED LEPTOMENINGEAL BAM MORPHOLOGY AND LYVE-1 EXPRESSION POST-INFECTION



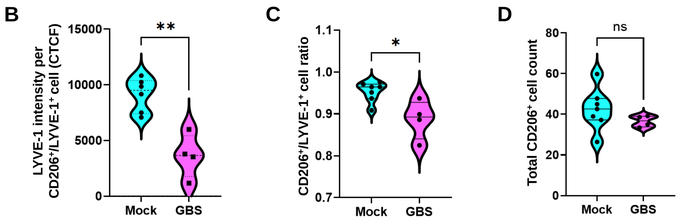


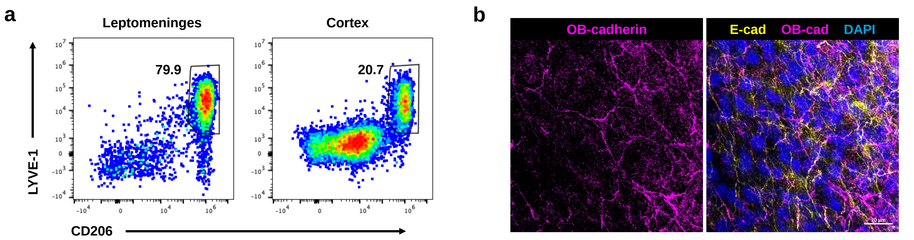
Fig 3. Leptomeningeal BAM morphology is altered and LYVE-1 expression is decreased following GBS infection **(A)** The morphology of CD206$^+$ BAMs was notably altered in GBS-infected leptomeninges (bottom), from a round to elongated form characteristic of activated macrophages. **(A)** Histological and **(B)** quantitative analysis showed a significant reduction in the expression level of LYVE-1 in BAMs in the GBS-infected group. **(C)** The number of CD206$^+$/LYVE-1$^+$ double-positive BAMs was also decreased post-infection, but the total density of BAMs was not different **(D)**. (Scale = 20μm)

CONCLUSIONS

- We developed a neonatal bacterial meningitis model using GBS to study the acute response at the B-CSFB, specifically the arachnoid barrier (AB).
- Infection with GBS did not alter the expression level of AB junctional proteins but resulted in aberrant localization pattern of Claudin-11$^+$ junctions.
- Tracer dye assay testing AB integrity showed leakage of tracer into superficial cortex post-infection, supporting a GBS infection-induced AB breakdown.
- Upon infection, the morphology of BAMs was notably altered, from a small, round form to cells with processes as seen in activated macrophages.
- Although the overall density of BAMs did not differ, the number of CD206$^+$/LYVE-1$^+$ double-positive BAMs and the expression level of LYVE-1 was significantly decreased after infection.

FUTURE DIRECTIONS

- Investigate acute and chronic local immune response during meningeal barrier breakdown following neonatal GBS infection (**a**: pilot flow data on mock-injected animals).
  - Acute: immune cell response (neutrophils, infiltrating monocytes) and local cytokine production
  - Chronic: structural and functional integrity of AB and BAM profile after meningitis resolution
- Investigate the relevance and mechanism of LYVE-1 downregulation in BAMs during infection.
- Investigate the cellular and molecular mechanism underlying the mislocalization of Claudin-11 and potentially other AB junctional proteins (**b**: OB-cadherin, Type II cadherin expressed at AB cell junctions).



ACKNOWLEDGMENTS

- **Siegenthaler Lab** (@SiegenthalerLab) | CU Anschutz
- Doran Lab (@KdoranLab) | CU Anschutz
- Cell Biology, Stem Cells, and Development (CSD) Program | CU Anschutz
- Funding support for this project from **NIH/NINDS**