Oral Presentation 4 - Title: Gut-Brain Links in Necrotizing Enterocolitis: Impact of G-CSF on Neuronal Synaptic Connections

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Background: Necrotizing enterocolitis (NEC) is an inflammatory and infectious gastrointestinal process that significantly increases the risk of neurodevelopment delay in patients, whereby surgical NEC patients have the greatest risk for developing cognitive impairments. In a graded mouse model of NEC, we find that concentrations of the cytokine G-CSF in the liver, plasma and brain correlate with the severity of NEC induced by the protocol. From this result, we hypothesize that G-CSF may be a key factor linking NEC with neurodevelopment. The purpose of this study is to determine whether G-CSF affects neuronal function and understand the cellular mechanisms involved.

Methods/Research Design: Ex vivo organotypic hippocampal slice cultures were made from postnatal day 4 (P4) C57BL/6 mice. On day in vitro 3 (DIV3), slices were incubated in G-CSF (1ng/mL, 10ng/mL, 100ng/mL) or vehicle (BSA) for 72 hours. Using whole-cell patch clamp, miniature excitatory postsynaptic currents (mEPSCs) for AMPA receptors (AMPARs) were recorded from CA1 pyramidal neurons. These cells were filled with biocytin, which allowed for imaging of neuronal morphology. Neurons were imaged using confocal microscopy and spine density was calculated. Baseline physiological properties of neurons were also measured. Immunohistochemistry was performed against Iba1 to determine if G-CSF affects microglia. Statistical tests used include one-way ANOVA and student's t-test, performed in Microsoft Excel and GraphPad Prism 10.

Results (or Preliminary Results, as applicable for a project in progress): G-CSF treatment of organotypic hippocampal slice cultures significantly decreased AMPAR mEPSC frequency in the presence of 10ng/mL and 100ng/mL G-CSF without affecting mEPSC amplitude or baseline resting membrane potential. Both concentrations of G-CSF caused the same degree of decrease in mEPSC frequency, indicating that the effect is not concentration-dependent. G-CSF application did not affect spine density. However, G-CSF treatment significantly increased microglia number, suggesting that the decrease in functional synapses may result from increased microglia activity.

Conclusion (or Preliminary Conclusion, as applicable for a project in progress): In conclusion, we show that G-CSF treatment of organotypic hippocampal slice cultures decreases functional excitatory neuronal synapses without affecting neuronal cell health. Furthermore, G-CSF increases microglia, a major neuroimmune cell involved in synaptic pruning and cortical refinement during neurodevelopment. These findings suggest that G-CSF may serve as a mediator between intestinal dysfunction and brain function and development in NEC patients.