Progress Report 2021

The proposed experiments in this project are based on preliminary findings that CXCL1 is upregulated in both animal models of ileus and in trauma patients who develop ileus. The objective of this project is to explore the effects of CXCL1 on intestinal smooth muscle contractile activity and the interactions between PAK1 and CXCL1 using both a primary intestinal smooth muscle cell model and an animal model of ileus. The original hypothesized mechanism states that CXCL1 was upstream of PAK1 activation. However, evidence in PAK1 knockout mice show that CXCL1 is downstream of PAK1. In addition, this project focused on the effects of CXCL1 in intestinal smooth muscle cells. In light of the data collected during this grant period, we have expanded our studies in a new direction to include the effects of mechanotransduction on CXCL1 expression in intestinal macrophages.

Results:

PAK1 knockout mice grow normally and have no gross anatomic abnormalities of the gastrointestinal system; no significant changes in intestinal length were detected. The overall inflammatory response to gut manipulation is not altered by the absence of PAK1, as exhibited by similar increases in intestinal smooth muscle myeloperoxidase activity, mast cell protease, and gut wet to dry weight ratios (indicating edema development) in wildtype and PAK1 knockout mice. Of note, litter sizes in the PAK1 knockout mice tend to be smaller. Thus, generating the data fin PAK1 knockout mice has taken longer than expected. When individual cytokines were screened using an antibody array assay, Il-1b, TNF-a, and other cytokines, which have been shown by others to be upregulated in response to gut manipulation, were unaltered by PAK1 knockdown compared to wildtype mice. However, CXCL1 was up-regulated in wildtype mice but not PAK1 knockout mice.

As we recently published in Docsa et al. (Neurogastroenterol Motil 32(3), 2020), contraction amplitude decrease significantly in the ileus model in a tetrodotoxin-indepedent manner (indicating smooth muscle dysfuction) 12 hours after gut manipulation. CXCL1 was increased in mechanically activated smooth muscle cells and macrophages. CXCL1 inhibited intestinal contractile amplitude and decreased agonist-induced (carbachol) contractile. CXCL inhibited agonist-induced contractile activity in a CXCR1 receptor-dependent manner.

In PAK1 knockout mice, the decreased contractile activity induced by gut manipulation is attenuated, i.e. intestinal contractile activity does not decrease in response to gut manipulation in PAK1 knock mice as it does in wildtype mice. Interestingly, inhibition of PAK1 with IPA-3 did not prevent CXCL1-induced inhibition of agonist-induced contractile activity. Thus, we concluded that CXCL1 changes are downstream of PAK1.

Primary human intestinal smooth muscle cells (hISMC) were subjected to control cyclical stretch (CCS) or increased cyclical stretch (ICS) mimicking physiological conditions and intestinal wall distension during the development of ileus, respectively. Treatment of the hISMC with CXCL1 resulted in decreased myosin light chain phosphorylation after ICS but not CCS. Pretreatment of cells with IPA.3 did not prevent the decreased myosin light chain phosphorylation-induced by

CXCL1. Thus, we are examining the effects of PAK1 on CXCL1 mRNA and protein expression in macrophages.

Conclusion:

Our evidence supports the involvement of CXCL1 downstream of PAK1. PAK1 knockdown prevents the gut manipulation-induced decrease in intestinal contractile activity. While PAK1 knockdown does not interfere with the inflammatory response to gut manipulation in the intestinal smooth muscle, PAK1 knockdown does prevent the increased CXCL1 levels induced by gut manipulation. CXL1 inhibits intestinal contractile activity and pretreatment with a PAK1 inhibitor does not prevent CXCL1 this inhibition.

Next steps:

The results of the proposed research are highly clinically relevant and translatable. The results of this project can be applied in two areas: the development of new drugs to treat ileus and the identification of biomarkers for the early detection of ileus in trauma patients. The following steps will be taken to exploit the results of this project.

1. CXCR2 antagonists already exist and have already undergone clinical safety testing. If our upcoming experiments confirm the importance of CXCL1 in the development of ileus, preclinical testing of CXCR2 antagonists can be conducted in our animal models of ileus. The effects of the reversible CXCR2 antagonist Danirixin on chronic obstructive pulmonary disease (COPD) have been investigated in clinical studies [2] with few adverse events, even in elderly patients [3]. This drug could be repurposed for the treatment of ileus. Thus, one possible next step is to examine the effects of Danirixin on the development of ileus.

2. Understanding the molecular mechanisms by which CXCL1 affects intestinal motility will facilitate the identification of other drug targets for treating or preventing ileus. Thus, we will explore the role of CXCR2 signaling in ileus to identify other possible drug targets that can be exploited to develop new drugs for the treatment of ileus.

3. The results of our studies will facilitate the identification of biomarkers for the development of ileus. The early detection of ileus may prevent many of the downstream effects of ileus. We will examine the association of CXCL1 and any other biomarkers discovered in this study with the development of ileus. In collaboration with Dr. Charles Wade from the University of Texas Health Science Center, we will test blood samples collected within the first 24 hours of hospital admission (before the development of ileus) (samples collected at Memorial Hermann Hospital (MHH) in Houston under an existing approved protocol. MHH is a level one trauma center with 1000s of trauma patients admitted per year.)

Students:

Baffin Kola, a stipendium student, worked un the effects of PAK1 knockdown in cells on CXCL1 signaling. Baffin obtained is Masters degree from my laboratory in 2019. Baffin is currently a PhD student in my laboratory and is working on the effects of mechanical stretching on CXCL1 expression in intestinal macrophages. The student, Bence Gergely, who was working on this project, has graduated with his MS in Spring of 2021. Work in the PAK1 knockout mice was the

basis for his Master's thesis. Bence presented this work at the Debrecen University TDK in 2021. In a separate project, Luca Varga earned her Master's degree studying the effects of bacterial metabolites on intestinal motility, Ephraim Acquah and Dauren Abilkassym, who are biochemical engineering students, both did their undergraduate thesis work in my laboratory.