Spatial heterogeneity of macrophage activation in the biliary epithelium

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Introduction: Biliary atresia (BA) is a fibro-obliterative cholangiopathy that leads to the obliteration and obstruction of the extrahepatic bile duct in newborns and is the most common indication for pediatric liver transplant. Although the etiology of BA remains unknown, studies have shown dysregulation of innate and adaptive immunity in the developing liver. The role of biliary epithelial cells, their immediate interacting environments, and the spatial heterogeneity of their interaction with immune cells remain unexplored. Here, we hypothesize that cholangiocyte organoids from mouse extrahepatic bile ducts (mEHBO) will stimulate a greater pro-inflammatory response when co-cultured with macrophages than intrahepatic biliary organoids (mIHBO).

Methods: Livers and common bile ducts were harvested from adult Balb/c mice, dissociated, and embedded in Matrigel for organoid culture. mEHBO and mIHBO were co-cultured with lipopolysaccharide (LPS) activated Raw264.7 Balb/c macrophages. All treatment and control groups were plated under the same conditions. Co-culture systems were harvested at 24 and 48 hours and macrophages were analyzed via flow cytometry to assess activation (intracellular TNFa, IL6, and iNOS). Previous studies have identified aberrant tight junctions and loss of apical-basal polarity in organoids derived from BA patients. To determine the effect of activated macrophages on cholangiocyte morphology, tight junction (ZO-1), apical (F-actin), and basolateral (B-catenin) proteins were assessed by whole mount immunofluorescent staining of mouse IHBO and EHBO after 48 hours of co-culture with LPS-activated macrophages.

Results: LPS-activated macrophages co-cultured with mEHBO (Mco-EO) showed higher expressions of the pro-inflammatory markers of TNFa, IL6, and iNOS than co-cultures with mIHBO (Mco-IO) at 24 and 48 hours. Flow cytometry revealed higher expression of TNFa in Mco-EO (61.8%) compared to TNFa-expressing cells in Mco-IO (19.2%) at 24 hours. Mco-EO TNFa expression decreased at 48 hours, with sustained TNFa levels in Mco-IO. Expression levels of IL6 were elevated in Mco-EO (7.93%), when compared to Mco-IO (1.49%) at 24 hours of co-culture. At 48 hours, we identified a 2-fold decrease in IL6 expression in Mco-EO. Expression of IL6 in Mco-IO was unchanged at 48 hours. iNOS expression increased in Mco-EO (64.7%) relative to Mco-IO (24.2%) and declined in Mco-EO at 48 hours (53.7%). iNOS levels continued to increase in Mco-IO (37.2%) at 48 hours identifying a biological regulation of immune responses distinct from mEHBO. Assessing the effect of macrophages on cholangiocyte function, preliminary whole-mount staining identified damaged tight junctions and nuclear apposition specific to Mco-EO with no morphological changes in Mco-IO. Data collection is ongoing.

Conclusion: Our results identify a tissue-specific heterogeneity in macrophage activation by extra- and intra-hepatic cholangiocytes. mEHBO induced a robust and durable pro-inflammatory response in macrophages by increasing TNFa and IL6 expressions, while appreciating damaged junctions and aberrant polarity. These findings further expand our understandings of cellular damage in BA pathogenesis.

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