**Macrophages are Associated with Intrahepatic Bile Duct Regeneration**

**Kevin Song**1,2, Holly Poling1, Kari Huppert1, and Stacey Huppert1,3,4

1Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children’s, 2University of Tennessee College of Medicine, 3Divison of Developmental Biology, Cincinnati Children’s, 4Department of Pediatrics, University of Cincinnati

**Introductory Statement:**

Intrahepatic bile duct (IHBD) regeneration involves a complex cascade of cytokines and inflammatory cells. No group of molecules or cell population has proven to be key regulators in this process.

**Aims and Hypotheses:**

We hypothesize that the hepatoprotective cytokine levels are inversely related to hepatodestructive cytokine levels during IHBD regeneration. In order to test our hypothesis, we aim to determine the populations of cells, cytokines, and chemokines that are associated with IHBD regeneration.

**Methods:**

To investigate IHBD regeneration, a mouse model of bile duct insufficiency was generated. RBP-J, the Notch pathway DNA-binding partner co-factor, and HNF6, an Onecut transcription factor were specifically deleted from the liver using Albumin-Cre. Cytokines and chemokines from serum and liver were measured at different time points through Luminex technology (Millipore, Billerica, CA). Different immune cell populations were visualized through immunohistochemistry (IHC). Serum total bilirubin was assessed by colorimetric endpoint assay (TecoDiagnostics, Anaheim, CA). The biliary system was visualized through resin casting technique.

**Results:**

The chronic deletion of Notch and HNF6 results in no peripheral IHBD being formed during development and newly formed communicating IHBDs by postnatal day 120 (P120). Both cholestasis and infiltration decrease with time in DKO mice. In serum samples, P60 MIP-1β and IL-6 levels are elevated in DKO when compared to control. In liver samples, the following trends are observed: P30, P60, and P120 MCP-1, IL-6, and IL-33 levels are higher in DKO when compared to control; P120 IL-1α and IL-1β are higher in DKO when compared to control. IHC shows that F4/80, a marker for macrophages, expressing cells are increased in DKO compared to control and localized to the regions of CK19, a marker for cholangiocytes, expressing cells at P60. DKO F4/80+ cells decreases from P60 to P120.

**Conclusion:**

Current mechanistic models of IHBD regeneration propose that myofibroblasts expressing Jagged1, a Notch ligand, induce hepatic progenitor cells toward cholangiocyte differentiation\*. Our data show that IHBD regeneration can occur in the absence of Notch and HNF6 and that F4/80+ cells are in the vicinity of CK19+ cells. This suggests that macrophages may play in important role in inducing the cholangiocyte fate, and that the process of IHBD regeneration is more complicated than the current models theorize.

**Acknowledgments:**

This study was supported in part by NIH grants T32 DK58837, M01 RR08084, and T35 DK 60444, and R01 DK078640