

Complement C5a receptor-dependent macrophage polarizations regulate pathogenesis in human and experimental mouse models of biliary atresia and fibrosis

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Introduction: Biliary atresia (BA) is a rapidly progressing obliterative disease of the extra- and intra-hepatic bile ducts and represents an extreme spectrum of neonatal cholestasis. Children who develop BA are born jaundice-free; however, within the first weeks of life, the extrahepatic biliary tree develops inflammation leading to duct obstruction and loss of bile flow. Surgical intervention by Kasai portoenterostomy (KPE) is the *only treatment option*, which removes the entire fibrosed biliary tree and surgically recreates an intestinal anastomosis to establish bile flow. The biliary atresia murine-mouse model of rotavirus (RRV)-induced experimental atresia has identified temporal activation of the complement system. Dr. Shivakumar's laboratory has identified an "activation circuitry" involving activated complement proteins (*C1q_{a/b/c}, C1r, C1s, C3b, Cfb, Properdin, C6, Masp2, Mbl1, Mbl*).

Hypothesis: 1) Loss of C5a signals skews intrahepatic populations of M1 and M2 macrophages. **Aim:** Determine myeloid and macrophage-derived immune cells populations expressing M1 and M2 markers in livers of mice with experimental BA, **2)** Markers of M1 and M2 polarized macrophages are differentially expressed in a newly established mouse model of biliary fibrosis. **Aim:** Establish the profiles of M1 and M2 marker expressions potentially driving biliary fibrosis.

Methods: Neonatal Balb/c wild-type (WT) mice were challenged with saline or RRV after 3 days of birth. Intrahepatic gene expression was determined using real-time PCR. Histology was performed using H/E and Sirius Red stained sections of livers. Immunofluorescence consisted of dual staining of CD68+ and PanCK. Preliminary identification of markers of macrophage polarization in our fibrosis model was achieved via real-time PCR.

Results: Differential expressions of M1 and M2 macrophage subsets in WT-RRV and WT-RRV mice treated with pharmaceutical therapies (anti-complement antibodies). Identification of these unique sets of immune cells are of immense importance to determine how different macrophages potentially regulate hepatobiliary pathogenesis. Administration of pharmaceutical inhibitors exhibited key downregulation of genes which modulate hepatic and biliary fibrosis. LOX, Timp1 & Col1a1 were markedly downregulated in mice which received pharmaceutical therapies. Histological analysis revealed extensive portal inflammation and duct injury in WT-RRV mice and reduced injury with the use of pharmaceutical agents. In these mice, it is seen to have reduced fibrosis and overall improved phenotype.

Conclusions: A key modulator of biliary atresia is hepatic and biliary fibrosis. Key gene regulators which are shown to be increased in our newly established fibrosis model are integral to understanding the pathophysiology in BA. Our fibrosis model with administration of pharmaceutical therapies shows key downregulation of hallmark genes (LOX, Timp1, Col1a1) in our treated groups. We also have demonstrated data that shows a statistically significant difference in surface area of fibrosis between our fibrosis model and treated groups.

Acknowledgements: This project was supported in part by NIH grant T35DK060444.