Progress Report

Grant: PSC Partners Seeking a Cure Foundation

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Regulation of hepatic lymphocyte responses in pediatric sclerosing cholangitis

1) PROGRESS DURING THE THE RESEARCH AWARD

I made significant progress in the proposed aims of my research plan.

Hypothesis 1: **Tregs inhibit effector lymphocyte responses and constrain biliary injury in murine SC.**
Aim 1.1) To determine whether antibody-mediated Treg depletion enhances CD8 and NK lymphocyte activation and aggravates hepatobiliary injury in mdr2-/- mice.
Aim 1.2) To examine whether administration of IL-2/anti-IL-2 complexes boosts hepatic Treg responses resulting in inhibition of effector lymphocyte activation and attenuation of the SC phenoytype in these mice.
Progress: Treatment with IL-2/anti-IL2 complexes (IL2C) significantly increased the number of hepatic Tregs compared with untreated mdr2-/- control mice. Expansion of hepatic Tregs was associated with decreased serum alkaline phosphatase levels, diminished ductal proliferation, inflammation and fibrosis on histological analysis of liver sections, and decreased number of hepatic CD8 cells. Furthermore, depletion studies using anti-CD8 antibodies linked CD8 cells to bile duct epithelial injury and fibrosis. The results were presented at the AASLD and NASPGHAN annual meetings and the manuscript is currently under preparation.
During the second year of funding we were investigating which metabolic factors controlled hepatic Treg expansion in murine sclerosing cholangitis. In the context of these studies we treated mdr2-/- mice with SC435, a minimally absorbed inhibitor of ASBT, which dramatically reduced serum bile acid, ALT and bilirubin levels, and reduced hepatic inflammation and fibrosis. RNAseq studies on hepatic RNA revealed down-regulation of proinflammatory and fibrogenic genes in SC435 treated compared with control mdr2-/- mice. Importantly, SC435 treatment resulted in almost 3-fold expansion of hepatic Tregs. Whereas the results on the impact of SC435 on the sclerosing cholangitis phenotype were recently published in HEPATOLOGY, its effects on Treg homeostasis under cholestatic conditions prompted another line of investigations.
Hypothesis 2: Hepatic DCs promote lymphocyte activation in murine SC through B7 dependent costimulation.
Aim 2.1) To elucidate whether ablation of hepatic DCs abrogates effector lymphocyte activation in murine SC.
Aim 2.2) To find out whether costimulatory blockade with CTLA4-Ig reduces hepatic lymphocyte activation and blocks progression of SC in mdr2-/- mice.

Our studies showed that costimulatory blockade with Orencia® early after birth resulted in reduction in percentage of hepatic effector memory CD4+ cells from 60 to 20% by 14 days of life when compared with age-matched IgG treated control mice. This reduction was associated with lower ALT levels and reduced ductal proliferation. The results were presented at the 2015 AASLD Annual Meeting in San Francisco.

Hypothesis 3): Frequency of hepatic Tregs in relation to effector lymphocytes decreases with disease progression and CTLA4-Ig blocks DC-dependent activation of lymphocytes in vitro in pediatric SC.
Aim 3.1) To determine the immunohistochemical phenotype of the inflammatory infiltrate in children with PSC.
Aim 3.2) To test whether CTLA4-Ig blocks in vitro activation of naïve CD8 cells by hepatic DCs
We performed a detailed analysis of clinical course, liver histology and findings of MRI based imaging of intra- and extrahepatic bile ducts in a retrospective cohort of 50 children and young adults with PSC and IBD who received medical care at our institution between the years 2009 and 2014. Surprisingly, we found that the clinical presentation in regards to initial laboratory measurements (GGT and Alkaline Phosphatase), distribution and severity of ductal injury on MRCP imaging, and liver histology (periductal T cell inflammation vs fibrosis) significantly differed between the patients dependent on the type of associated bowel disease (Crohn disease vs ulcerative colitis vs no IBD). The results were presented at the 2014 DDW Annual meeting and at the 2015 Monothematic Conference on Autoimmune Liver Disease in London. Immunohistochemical studies to characterize the lymphocyte infiltrate are ongoing. Of note, we started to collaborate with Dr. Bufler at Haunersche Children's Hospital in Munich, Germany, to validate our findings in an independent cohort.

2) Lay summary

The effects of regulatory T cells and dendritic cells on progression of bile duct injury in mdr2 knockout mice, a mouse model of PSC, are unknown. In our experiments using antibodies and cytokines to change the number and function of these cells in the liver of these mice we found that regulatory T cells dampen immune-mediated liver injury: when these cells were removed from the liver, the disease worsened, when these cells were increased in number, the liver was protected. Similarly, when we blocked the function of dendritic cells we reduced the activation of T lymphocytes and protected liver cells from injury. In order to understand how these inflammatory processes in the liver were affected by bile acids, which are retained in the liver in PSC, we blocked reuptake of bile acids in the intestine using a small molecule compound. This treatment greatly reduced serum bile acid-, ALT- and alkaline phosphatase levels, and diminished liver fibrosis (scarring). Importantly, these changes were associated with downregulation of genes driving liver inflammation and expansion of regulatory T cells in the liver. These studies will help to interpret results of ongoing clinical trials on the safety and efficacy of bile acid reuptake inhibitors in PSC and may facilitate design of future trials targeting regulatory T and dendritic cells in the liver.

In a translational study we reviewed clinical course, liver biopsies and MRI studies from 50 children and young adults who received care for PSC and PSC/AIH overlap at our center. We found that their type of liver disease was dependent on the type of concomitant bowel disease (Crohn disease, Ulcerative colitis, or no IBD).

3) Information about published results.

Our findings were presented at several national meetings:

1. Menchise A, Simmons J, Lages C, Almanan M, Chougnet C, Zhang W, Setchell K, Shivakumar P, **Miethke AG.** Oral Presentation: Initiation of murine sclerosing cholangitis involves effector lymphocytes and regulatory T cells. Annual Meeting: North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) October 10, 2013 in Chicago

2. Jones K, Bufler P, **Miethke AG**. Oral Presentation: The types of Inflammatory Bowel Disease (IBD) predispose to distinct clinical phenotypes of Primary Sclerosing Cholangitis (PSC) in children. Annual Meeting: Digestive Disease Week (DDW) May 6, 2014 in Chicago

3. Simmons J, Taylor A, Shanmukhappa SK, Keller B, **Miethke AG**. Oral presentation: Pharmacological inhibition of intestinal bile acid reuptake blocks inflammatory liver injury and fibrosis in a murine model of sclerosing cholangitis. Annual Meeting: American Association for the Study of Liver Diseases (AASLD) November 10, 2014 in Boston.

4. Keaton J, Pirringer L, Wallihan D, Makeschin MC, Mayr D, Bufler P, **Miethke AG**; Disease course of autoimmune sclerosing cholangitis (AISC) in two cohorts in Europe and North America (NA). EASL Monothematic Conference on Autoimmune Liver Disease. September 5, 2015 in London, UK.

5. S. Lages C, Shi T, Bolcas P, Simmons J, Maddox A, Shanmukhappa K, **Miethke AG**; Poster Presentation: Hepatic CD4+ lymphocyte responses and initiation of biliary injury in mdr2 knockout mice depend on dendritic cell costimulation. Annual Meeting: AASLD; November 15, 2015 in San Francisco.

6. Taylor AE, Menchise A, S. Lages C, Simmons J, Shanmukhappa K, Zhang W, Oehrle M, Setchell KD, **Miethke AG**. Poster Presentation: Expansion of regulatory T cells reduces hepatic lymphocyte responses and hepatobiliary injury in murine sclerosing cholangitis. Annual Meeting: AASLD; November 15, 2015 in San Francisco.

Our findings were published:

Miethke AG, Zhang W, Simmons J, Taylor AE, Shi T, Shanmukhappa SK, Karns R, et al. Pharmacological inhibition of apical sodium-dependent bile acid transporter

changes bile composition and blocks progression of sclerosing cholangitis in multidrug resistance 2 knockout mice. *Hepatology* 2015.

Alexander G. Miethke, MD
Assistant Professor of Pediatrics
Division of Gastroenterology, Hepatology and Nutrition
Cincinnati Children's Hospital Medical Center