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T regulatory cells promote bile duct regeneration through modulating ductular reaction in a model of cholestatic liver injury

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Background and Aims: Reduced regulatory T cells (Tregs) and increased bile duct senescence are observed in primary sclerosing cholangitis (PSC) patients, with the degree of cholangiocyte senescence linking to disease severity and prognosis. Cholangiocytes can act as facultative liver progenitor cells through ductular reaction during extensive liver damage, whether this process is impaired during PSC remains to be investigated. The role of Tregs in modulating tissue resident progenitor cells have been shown in multiple organs, but this remains unclear in the context of liver regeneration. We aim to use transgenic murine models to investigate the cause of reduced Tregs in the liver and whether the lack of Tregs in the liver affect bile duct regeneration and senescence.

Method: Foxp3^{GFP}DTR transgenic mice were used to reduce Tregs number in a dose dependant manner. 50% of Tregs were depleted to avoid triggering systematic autoimmunity whilst cholestatic liver injury was induced by the feeding of 3,5-diethoxycarbonyl-1,4-dyhydrocollidine (DDC) diet and compared to the control group with intact Tregs population. We generated the Foxp3^{GFPCreERT}tdTom^{loxSTOPlox} mice to investigate Tregs stability. Tamoxifen was injected intraperitoneally to induce tdTom expression in Foxp3 Tregs and cell fate was investigated after DDC diet to determine Tregs stability. CD4 T-cells were isolated and co-cultured with intrahepatic cholangiocytes organoids to confirm the effect of CD4 T-cells on cholangiocytes.

Results: Mice with reduced Tregs have a lower tolerance to the feeding of DDC diet, with rapid weight loss and two times higher periportal fibrosis than the control group. Histological findings showed that the reduction in Tregs decrease the magnitude of Ck19^{+} ductular reaction by 30%. A two-fold increase in Ck19+p21+ senescing cholangiocytes was observed in the group with reduced Tregs after DDC induced liver injury. Transcriptional analysis of liver tissue revealed downregulation of *Yap1*, *Sox9* and *Ctgf*, suggesting the Yap pathway is affected following Tregs reduction. This is further confirmed with immunohistochemistry showing a two-fold reduction in the number of Yap and Sox9 expressing Ck19+ cholangiocytes. The Foxp3 fate mapping

experiments showed that the labelled Tregs population reduces Foxp3 expression after DDC diet indicating that the stability of Tregs decreases during liver injury.

Conclusion: Our results demonstrated that the role of Tregs in promoting bile duct regeneration by modulating ductular reaction through the Hippo-Yap pathway. Furthermore, the observation that Foxp3 Tregs become unstable in an injured microenvironment in mice may explain the lack of Tregs seen in PSC patients. These show the potential of using Tregs to promote liver regeneration but also highlights the stability of Tregs should be taken into consideration when designing cell based Tregs therapy.