Supplemental Data:

Interleukin 2 promotes hepatic regulatory T cell responses and protects from biliary fibrosis in murine sclerosing cholangitis

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Material and Methods

Mononuclear Cell Isolation: At the time of harvest, livers were perfused with 10mL of 1mg/mL collagenase D (Roche Diagnostics, Indianapolis, IN) in RPMI (Gibco, Grand Island, NY) via the portal vein. Tissue processing was performed with Miltenyi Biotec disintegrator (Miltenyi Biotec, Cambridge, MA) with incubation in collagenase/RPMI. Single-cell preparation of liver tissue was performed using MACS isolation buffer, 33% Percoll (Sigma-Aldrich, St. Louis, MO), and Red Cell Lysis steps, as described by our group before (1).

Flow Cytometric Studies: Isolated single cells were re-suspended in 2%FCS/PBS and stained with antibodies from: BD Pharmingen (CD3e clone 145-2C11); BioLegend (CD3e clone 145-2C11, CD39 clone Duha59, CD45 clone 30-F11, CD73 clone TY/11.8, GITR clone DTA-1, ICOS clone C398.4A, TCRβ clone H57-597); eBioscience (CD4 clone RM4-5, CD8a clone 53-6.7, CTLA4 clone UC104B9, PD1 clone J43, CD69 clone H1.2F3); Miltenyi Biotec (CD25 clone 7D4); Novus (A2AR clone 7F6-G5-A1); R&D Systems (NKG2D clone 191004). Flow cytometry of mononuclear

cells (MNC) were acquired on a BD Canto III, BD LSRFortessa, or BD Accuri (BD Biosciences, Franklin Lakes, NJ). Samples were analyzed utilizing FlowJo software (version 7.6.5 and 10.2; Tree Star, Inc., Ashland, OR).

Quantitative Real-Time Polymerase Chain Reaction: Total RNA from liver tissue was obtained and TaqMan-based gene amplification and analysis with normalization to HPRT or 18S was performed as previously described (2). Multiple gene analysis profiles were evaluated using the 88- multi-cytokine and chemokine Mouse Immune Panel TaqMan (ThermoFisher Scientific, Waltham, MA) and run on 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA), as described by us before (3). The ΔΔCT method was used for computation of relative mRNA concentrations.

Protein blot analysis for TNFα: Liver samples were processed for immunblot by using tissue lyser, followed by SDS-PAGE and transfer to nitrocellulose membrane, as described by our group before (4). For detection of the trimeric form of Tnfα (MW: 52kDa), membranes were incubated with a primary antibody against TNFa (clone: ab1793; Abcam; 1:1000 dilution) followed by incubation with the secondary HRP-conjugated antibody (clone W402B; Promega; 1:2000). Beta-actin, as loading control, was detected with an HRP-conjugated antibody (SC-47778; Santa Cruz; MW of 43 kDa).

Plasma Biochemistries and Cytokines: Alanine aminotransferase (ALT) was determined from plasma samples using Discretpak reagents (C164-0A; Catachem, Inc., Bridgeport, CT). Alkaline phosphatase (ALP) levels in serum samples was assessed using VETSPEC (ALP) Kit C (C174-0C;

Catachem, Inc., Bridgeport, CT). Plasma Tnfα levels were measured in a Milliplex™ assay (Millipore), as described by us before (3).

Histology, Immunohistochemistry, and Immunofluorescence: CK19 immunohistochemistry was performed on FFPE liver tissue as described previously (2). FFPE liver sections were also used for osteopontin (Opn) staining (clone AF808, R&D Systems, 1:200) following antigen retrieval with a pressure cooker and Sodium Citrate 10mM pH. Immunoreactivity was visualized using the R.T.U. Vectastain *Elite* ABC Reagent (Vector: PK-7100), and color development with DAB reagent (Vector Laboratories SK-4100). Immunofluorescent staining was performed with CK19 (clone M-17, Santa Cruz Biotechnology) and CD8α (clone 53-6.7, Acris).

References

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4. Carey AN, Zhang W, Setchell KDR, Simmons JR, Shi T, Lages CS, Mullen M, et al. Hepatic MDR3 expression impacts lipid homeostasis and susceptibility to inflammatory bile duct obstruction in neonates. Pediatr Res 2017;82:122-132.