Supporting Information

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SI Methods

Mice. Six- to 8-week-old WT C57BL/6 (B6), BALB/c, DBA/2, CB17 SCID, and (B6 x BALB/c) F1 mice were from Charles River Japan Inc. C3H/HeJ and NOD mice were from CLEA Japan Inc. A/J mice were from Japan SLC Inc. B6 IFN-γ-deficient (IFN-$\gamma^{-/-}$) and Rag-2-deficient (RAG-$2^{-/-}$) mice were derived as previously described (8, 14). B6 TRAIL-deficient (TRAIL$^{-/-}$), DR5-deficient (DR5$^{-/-}$), or Bid-deficient (Bid$^{-/-}$) mice were kindly provided by Dr. Jacques Peschon, Dr. Astar Winoto, and Dr. Stanley J. Korsmeyer, respectively. All mice were maintained under specific pathogen-free conditions and used in accordance with the institutional guidelines of Juntendo University and the Peter MacCallum Cancer Centre.

Human Samples. Tissue specimens were obtained with consent from patients who were diagnosed with PSC (n = 10), PBC (n = 17), or CBD obstruction by biliary stones or drug-induced hepatic injury (n = 5) at Juntendo University Hospital and Kanazawa University Hospital. Normal samples (n = 10) were obtained from tumor-free portions of surgical resection of hepatic metastasis. This study was reviewed and approved by the ethical review committee of our universities and had the informed consent from all patients.

Histological Examination. Mouse liver and extrahepatic bile duct were removed 4 days after the last anti-DR5 mAb treatment or the indicated days after CBD ligation. All mice and human sections were prepared as the formalin-fixed paraffin embedded sections. Hematoxylin/eosin staining was performed as described (14). For immunohistochemistry, sections (3 μm) were autoclaved at 121°C for 10 min in target retrieval solution (DAKO). Endogenous biotin was blocked by avidin/biotin blocking kit (Vector Laboratories), and endogenous peroxidase was blocked by 0.3% H_2O_2 in methanol. Mouse DR5 was visualized with MD5–1 and biotin-conjugated goat anti-hamster IgG (Vector Laboratories) by using a catalyzed signal amplification (CSA) method with a CSA kit (DAKO) according to the manufacturer's instructions. Mouse TRAIL was visualized with biotin-conjugated anti-mouse TRAIL mAb (eBioscience) and CSA methods. Human DR5, human TRAIL, or mouse cytokeratin 19 were visualized with first anti-body (rabbit anti-human DR5 Ab [Ab-1]) (Calbiochem), mouse anti-human TRAIL mAb (R&D Systems), or rabbit anti-mouse cytokeratin 19 (Novus Biologicals), and biotin-conjugated goat anti-rabbit IgG (DAKO) or biotin-conjugated goat anti-mouse IgG (Vector Laboratories) by using avidin-biotin-peroxidase method. Apoptotic cells were stained by using *in situ* TUNEL method. Quantification of apoptotic cells was recorded as TUNEL positive cell number/cholangiocyte area (mm^2). All sections were counterstained with hematoxylin. To define myofibroblasts, α smooth muscle actin was visualized by avidin-biotin-peroxidase method using mouse anti-human smooth muscle actin mAb (1A4) (DAKO) and M. O. M. Kit (Vector Laboratories) according to the manufacturer's instructions. Fibrosis was examined by Azan staining.

Quantitative Analysis of Immnohistochemical Samples. Immunostained sections were quantified with a KS400 image analysis system (Carl Zeiss Imaging Solutions) for optical MEAND, and staining intensity was presented as the reciprocal. Mean intensity on cholangiocytes of bile ducts (three to five in each section) was compared with that of unstained cells in the same section. Sections were also analyzed using a TissueFAXS (TissueGnostics) image cytometer, and entire samples were recorded as digital samples. Cellular analysis was restricted to the regions of interest set on cholangiocytes. Identification of individual cholangiocytes and measurement of cell specific diaminobensidine density was performed by the analysis software HistoQuest (TissueGnostics). Results were displayed in scatter grams.

Quantitative Real-Time PCR. Total RNA was isolated from intrahepatic biliary tree prepared from mice using RNA STAT-60 (TEL-TEST Inc.) and first-stranded cDNA was prepared using oligo dT primers and TaqMan reverse transcription reagents (Applied Biosystems). Quantitative PCR was performed following manufacturer's instructions. Briefly, 7500 Real-Time PCR System (Applied Biosystems) was used with Assays-on-Demand gene expression products (Applied Biosystems) of mouse target genes, DR5 (Mm00457866_m1), FLIP (Mm01255576_m1), c-Myc (Mm00487803_m1), Caspase 8 (Mm00802247_m1), DcTRAILR1 (Mm00656375_m1), DcTRAILR2 (Mm00445826_m1), Cytokeratin 19 (Mm00492980_m1), or an endogenous control GAPDH (Ma99999915_g1) and TaqMan Universal PCR Master Mix (Applied Biosystems). The expression levels of respective molecules were shown as a ratio compared with GAPDH in the same sample by calculation of cycle threshold (Ct) value in amplification plots with 7500 SDS software (Applied Biosystems). Relative expression levels of respective molecules compared with BALB/c mice were calculated by relative quantification ($\Delta\Delta$Ct) using SDS v1.2 with RQ software (Applied Biosystems) according to manufacturer's instructions. Data are represented as the mean ± SD calculated from three independent experiments.

A

ALP (IU/L)
4000
3000
2000
1000
0
0 5 10 15 20
Days after 1st αDR5 mAb treatment

B

T-bil
B6
C3H
B6 x BALB/c F1
A/J
BALB/c
CB17 SCID
DBA/2
NOD

D-Bil
B6
C3H
B6 x BALB/c F1
A/J
BALB/c
CB17 SCID
DBA/2
NOD

0 10 20 30 40 50
(mg/dl)

C

DR5: B6, C3H, B6 x BALB/c F1
FLIP: B6, C3H, B6 x BALB/c F1
Myc: B6, C3H, B6 x BALB/c F1
Caspase 8: B6, C3H, B6 x BALB/c F1
DcTRAIL R1: B6, C3H, B6 x BALB/c F1
DcTRAIL R2: B6, C3H, B6 x BALB/c F1
Cytokeratin 19: B6, C3H, B6 x BALB/c F1

0 1 2
Relative expression of mRNA compared with BALB/c

Fig. S1. Contribution of apoptosis-relating molecules to anti-DR5 mAb-induced cholangitis. (*A*) Partial contribution of mitochondrial pathway to anti-DR5 mAb-induced cholestasis. Serum ALP level was examined in WT (squares), $Bid^{-/-}$ (*circle*), and $DR5^{-/-}$ (triangles) mice given a course of repeated intraperitonial injections of anti-DR5 mAb (n = 5 in each group). (*B*) Serum T-bil and D-bil levels of various mice strains after anti-DR5 mAb treatments (n = 5 in each group). (*C*) Relative expression of TRAIL-induced apoptosis-related molecules in intrahepatic biliary tree preparation from the indicated mouse strains compared with that of BALB/c mice.

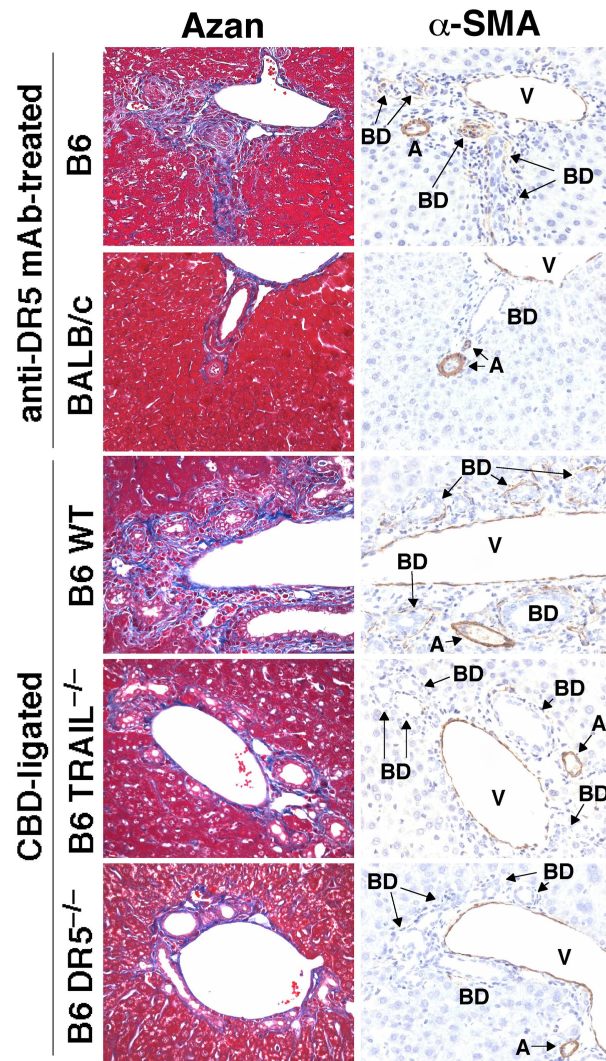

Fig. S2. Fibrosis and myofibroblasts around intrahepatic bile ducts in anti-DR5 mAb-treated B6 mice and CBD-ligated B6 WT mice. Fibrosis was examined by Azan staining on the liver sections from B6 and BALB/c mice treated with anti-DR5 mAb three times and the indicated mice 9 days after CBD ligation. To define myofibroblasts, immunohistochemical examination for α smooth muscle actin (α-SMA) was also performed. A, artery; BD, bile duct; V, vein. Original magnifications: ×40.

Fig. S3. TRAIL and DR5 expression and apoptosis in cholangiocytes of PSC and PBC patients. Immunohistochemical examination for DR5 and TRAIL was performed on a paraffin liver section of indicated subjects and normal liver controls. Arrows indicate bile ducts. TUNEL staining was also performed and the arrows indicate apoptotic cholangiocytes. Original magnifications: ×40.