Supporting Data

Materials and Methods (continual)

Liver transplantation. Orthotopic liver transplantation was performed as described previously (10) with some modifications using a combination of suture and cuff technique under isoflurane anesthesia. The hepatic artery was not reconstructed. Immunosuppressive therapy was not administered to any of the liver recipients. Graft survival was determined by recipient survival and confirmed by histology. For depletion of sessile Kupffer cells, mice received one intravenous injection of 200 µl clodronate liposomes (Clophosome®-A, FormuMax, Palo Alto, CA) as previously described (27,28). The peak depletion of Kupffer cells was identified on post-injection day 3 as shown by immunochemical staining, and the animals were then used as liver donors.

Isolation of liver NPC. Mouse liver NPCs were isolated by collagenase digestion and centrifugal elutriation, as described (25) with some modifications. Briefly, the liver was perfused in situ via the portal vein with buffered collagenase solution (type IV, 1 mg/ml in HBSS; Sigma Chemical Co., St. Louis, MO). The liver was then removed, diced, and digested with collagenase at 37^{o}C for 30 min. The studies involving human HpSC have been reviewed by IRB of Cleveland Clinic, and qualified as exempt from further review under the federal exemption category 4, because the information is recorded by the investigators in such a manner that subjects cannot be identified. Human liver tissue was obtained from patients (age 25-45) undergoing liver resection. The liver specimens used demonstrated normal lobular hepatic architecture and were without evidence of fibrosis. Similarly, liver tissue was meshed and

agitated in collagenase IV. Cells were filtered through a nylon mesh. Parenchymal cells (hepatocytes) were removed using a low speed centrifugation (50g). NPC were further separated from parenchymal cells via Percoll (Sigma) gradient centrifugation. The isolated NPC demonstrated greater than 95% cell viability, estimated by trypan blue exclusion, and less than 5% hepatocyte contamination, determined by morphological examination. $CD45^-$ NPC were selected using anti-CD45 mAb beads (Miltenyi Biotec, San Diego, CA).

Isolation and preparation of LSEC and HpSC. LSEC and HpSC were separated by centrifugal elutriation as previously described (25,32). Briefly, the mouse liver $CD45^-$ NPC were placed onto two-step (25 and 50%) Percoll (Sigma) gradients and centrifuged at 900g for 20 min. LSEC, HpSC and Kupffer cells were then separated. The isolated LSEC were then plated onto 0.1% gelatin (Invitrogen, Carlsbad, CA) coated coverslips and incubated overnight in MCDB-131 (Invitrogen, Carlsbad, CA) containing 0.5% fetal bovine serum (Hyclone, Logan, Utah), 20 ng/ml murine VEGF (PeproTech, Rocky Hill, NJ), 50 μM ascorbic acid (Sigma, St, Louis, MO) 10 ng/mL EGF (PeproTech), 25μg/ml gentamycin (Invitrogen, Carlsbad, CA), and 1μg/ml hydrocortisone (Sigma, St. Louis MO.) The purity of LSEC (greater than 95%) was determined by uptake of fluorescently labeled acetylated low-density lipoprotein. The isolated HpSC were cultured in an uncoated plastic flask (Nunclon, Roskilde, Denmark) with RPMI-1640, supplemented with 10% FSC and 10% horse serum for 7-10 days as described. The purity of HpSC ranged from 90%-95% as determined by desmin immunostaining (25).

Bone marrow chimeras. BM was isolated from WT B6 mice and cell clumps were removed. Cells were adjusted to 6.5×10^7 cells/ml in PBS. B7-$H1^{-/-}$ mice were lethally γ-irradiated (900

rads) and immediately injected with 6.5 x 10^6 WT BM cells (i.v.). Chimeric animals were allowed to recover for five weeks before transplantation. Chimerism was confirmed by analysing B7-H1 expression on peripheral blood by flow cytometry, as shown in Fig. 4B.

Islet transplantation. 300 islets (BALB/c) alone or mixed with 3 x 10^5 LSEC or HpSC isolated from liver grafts were transplanted under the renal capsule of streptozotocin (180 mg/kg, Sigma-Aldrich, St. Louis, MO)-induced diabetic recipients (B6), as previously described (34,35). Transplantation was considered successful when blood glucose normalized ($\leq$150 mg/dL) for at least 4 days. The first day of two consecutive readings of blood glucose $\geq$350 mg/dL was defined as the date of graft failure. No immunosuppressive drugs were administered. In all animals with euglycemia for >100 days, the kidney bearing the islet allograft was removed, resulting in a prompt return to hyperglycemia, which confirmed the function of the transplanted islets.

Mixed leukocyte reaction (MLR): One-way MLR culture was performed in triplicate in 96-well round-bottom microculture plates (Corning, NY). Nylon wool-eluted spleen T cells (2 x 10^5/ well) from C3H mice were used as responders. T cell proliferation was elicited by irradiated (20Gy; X-ray source) DC (B6) at a T:DC ratio of 10:1 or indicated. Cultures were maintained in RPMI-1640 complete medium, for 3 days in 5% CO_2 in air. T cell proliferative response was determined by either thymidine uptake or CFSE dilution assay analyzed by flow cytometry. To examine suppressive effect, the regulator cells were added the MLR culture at the indicated ratio of regulator to effectors

Culture of DC. As previously described (35) 2 x 10^6/well BM cells from tibias and femurs of B6 mice were cultured in RPMI-1640 medium containing 10% FCS in the presence of mouse recombinant (r) GM-CSF (8 ng/ml) or GM-CSF (8 ng/ml) plus IL-4 (1000 U/ml) (both from Schering Plough, Kenilworth, NJ) for 5 days. The floating cells were harvested, washed, and re-suspended in RPMI-1640 medium. For further stimulation, cells were exposed to LPS at 1μg/ml for the last 18 hours of culture.

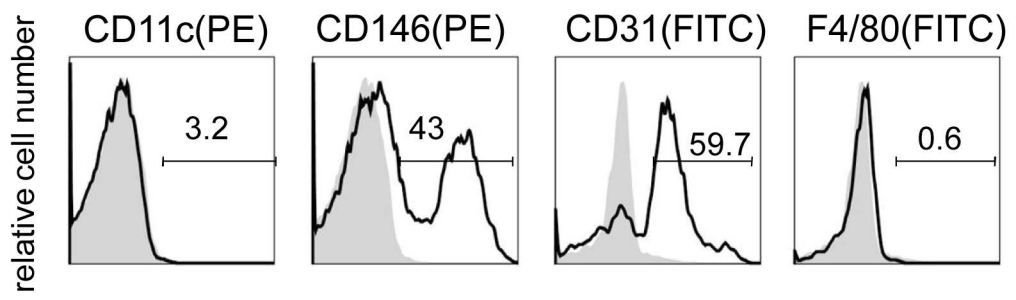
Flow cytometric analysis. Antibodies against CD4, CD8, CD11b, CD11c, CD25, CD31, CD40, CD45 CD86, CD146, F4/80, Gr-1, H-2K^b, H-2K^k, I-A^b, IFN-γR or annexin V were purchased from BD PharMingen (San Diego, CA), and against B7-H1/$Foxp^3$ from eBioscience (San Diego, CA). Intracellular staining protocols were followed for staining of Foxp3. For CFSE labeling, T cells (10^7/mL) were incubated with 0.5 mM CFSE (Molecular Probes, Eugene, OR) for 10 min at room temperature. The isotype and species matched irrelevant mAbs were used as controls. Flow analyses were performed with a BD FACSCalibur flow cytometer (BD Biosciences).

Immunohistochemistry. Immunofluorescence staining protocols were used for CD4, CD8, B7-H1, CD105, desmin and F4/80 staining in cryostat sections using anti-mouse specific mAb (BD PharMingen). The isotype and species matched irrelevant mAbs were used as controls. The color was developed by an enzyme reaction using avidin-biotin-alkaline phosphatase complex (ABC) as the substrate. To detect apoptosis, sections were stained for TUNEL using an in situ apoptosis detection kit (EMD Millipore, Billerica, MA) according to the manufacture instructions. The slides were counterstained with Harris's hematoxylin and mounted with Crystal mount (Biomeda

Corp, Foster City, CA). For quantification, the positive cells were counted on a microscope and a total of 30 high-power fields were randomly selected in each group.

Quantitative-polymerase chain reaction. Total RNA was extracted with TRIzol Reagent. cDNA was synthesized with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). The primers for IFN-gR1 were forward 5'-TCCTACATACGAAACATACGG-3', reverse 5'-TCTAACTTGCCAGAAAGATGA-3'. The mRNAs were measured using Applied Biosystems 7500 Fast PCR System in duplicate and were normalized to GAPDH mRNA.

Statistical Analysis. Graft survival between groups of transplanted animals was compared using the log-rank test. The parametric data were analyzed by Student *t* test (2-tailed). Values of $p<0.05$ were considered statistically significant.


relative cell number
CD11c(PE)
3.2
CD146(PE)
43
CD31(FITC)
59.7
F4/80(FITC)
0.6