Supplementary Materials and Methods

Cell Isolation and Culture

Liver cells were isolated from normal rats and mice as described previously. Briefly, after in situ perfusion of the liver with pronase (Boehringer Mannheim, Indianapolis, IN) followed by collagenase (Crescent Chemical, Hauppauge, NY), dispersed cell suspensions were layered on a discontinuous density gradient of 8.2% and 15.6% Accudenz (Accurate Chemical and Scientific, Westbury, NY). The resulting upper layer consisted of >95% stellate cells. Kupffer cells were further purified by selective plating as we have done previously.[1] The viability of cells was verified by phase-contrast microscopy as well as the ability to exclude propidium iodide. The viability of all cell cultures utilized for study was >95%.

Isolation of Human Peripheral Blood Monocytes

Monocytes were isolated from whole blood obtained from normal human volunteers as HLA-DR^{+}CD14$^{+/dim}$CD3^{-}CD20^{-}CD56^{-} cells by fluorescence-activated cell sorting using modifications of a published protocol.[2] Briefly, K3 EDTA blood was divided into 100-μL aliquots and the red blood cells were lysed for 15 minutes at room temperature with 2 mL fluorescence-activated cell sorting Lysing Solution (BD Bioscience, Franklin Lakes, NJ). After the incubation, 2 mL nominally calcium- and magnesium-free Corning cellgro Hanks' Balanced Salt Solution (CFH; Mediatech, Inc., Manassas, VA) containing 3% fetal bovine serum (FBS; Lonza, Walkersville, MD) was added to the lysed blood and the cell suspension was centrifuged at 300*g* for 5 minutes. Supernatant was removed by aspiration and the white blood cells were resuspended in 100 μL CFH + 3% FBS. Cells were stained with fluorochrome-conjugated antibodies (Supplementary Table 2) for 15 minutes at room temperature in the dark. Optimal antibody concentrations were determined by flow cytometry using a BD Bioscience LSR II analytical instrument equipped with lasers emitting at 407 nm, 488 nm, and 635 nm and filter sets appropriate for the fluorochromes used. Stained cells were washed with 3 mL CFH + 3% FBS and centrifuged at 300*g* for 5 minutes. The pellets were re-suspended in CFH + 5% FBS, combined, and monocytes were sorted on a BD Biosciences FACSAria III sorting cytometer configured to match the LSR II instrument according to the gating scheme illustrated in Supplementary Figure 2. Monocyte isolation was performed in 5 independent sessions yielding 410,000−580,000 cells each. Data files were analyzed by FlowJo software (Treestar, Ashland, OR).

Measurement of Hepatic Vascular Permeability In Vivo

Mice were injected intravenously with 1% Evan's Blue dye (40 mg/kg; Sigma-Aldrich) 30 minutes before sacrifice. Liver was perfused with 4°C phosphate-buffered saline then dye was quantified from liver tissue by spectrophotometry at 620 nm. Results were calculated from a standard curve of Evans Blue (0.05−25 μg/mL) and expressed as μg/g dry liver.

Real-Time Polymerase Chain Reaction

Messenger RNA levels were quantified by real-time reverse transcription polymerase chain reaction (PCR) per the manufacturer's specifications (Stratagene, Mx3000P real-time PCR). Primer sequences are listed in Supplementary Table 1. Total RNA was extracted from cells or whole livers using TRIzol (Invitrogen, Carlsbad, CA). One microgram RNA was reverse-transcribed by using random primers and Superscript RNase H-reverse transcriptase (Invitrogen). Samples were incubated at 65°C for 5 minutes, 50°C for 60 minutes; reverse transcriptase was inactivated by heating at 70°C for 20 minutes and cooling at 4°C for 15 minutes. Amplification reactions were performed with a SYBRgreen PCR master mix (Applied Biosystems, Carlsbad, CA). Five microliters diluted complementary DNA samples (1:5 dilution) was used for quantitative 2-step PCR (a 10-minute step at 95°C, followed by 50 cycles of 15 seconds at 95°C and 1 minute at 65°C) in the presence of 400 nM specific forward and reverse primers, 5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris buffer (pH 8.3), 200 μM deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and 400 μM deoxyuridine triphosphate and 1.25 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer Applied Biosystems). Each sample was analyzed in triplicate.

Migration Assay (Boyden Chamber)

Modified Boyden chambers (Becton Dickinson, Heidelberg, Germany) were used with filters (8-μm pores; Neuro Probe, Gaithersburg, MD) coated with collagen type-I (50 μg/mL). Human recombinant VEGF (10 ng/mL) or vehicle was added to the lower chamber and 3000 cells in 50 μL serum-free media were added to the upper chamber. After 3 hours incubation at 37°C, cells remaining on the upper surface of filters were scraped off with a cotton swab and cells on the lower surface were fixed with ethanol and stained with 4′,6-diamidino-2-phenylindole. Cells were counted using the ImagePro program in 4× low-power fields per filter.

Caspase 3/7 Assay

To evaluate apoptosis, human HSC and LX-2 (1×10^4/well) were used for caspase 3/7 assay using the manufacturer instructions (Apo-One Homogeneous Caspase 3/7 Assay; Promega, Madison, WI).

Supplementary References

1. Yang SQ, Lin HZ, Lane MD, et al. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. Proc Natl Acad Sci U S A 1997;94:2557–2562.
2. Autissier P, Soulas C, Burdo TH, et al. Evaluation of a 12-color flow cytometry panel to study lymphocyte, monocyte, and dendritic cell subsets in humans. Cytometry A 2010;77:410–419.