days with clodronate for the following 3 weeks, mice after 20 weeks of CDD received clodronate every 4-5 days for the following 5 weeks while continuously fed CDD. B. Liver sections stained with F4/80 antibody from wt mice administered vehicle or clodronate as described in A. Representative photomicrographs at 400x magnification presented. C. Hepatic T_h1 and T_h2 cytokine expression as assessed by real-time PCR in vehicle or clodronate treated animals. Values are mean ± SEM, *p<0.05. n=4-6 animals per group.

Figure 7. Kupffer cells contribute to steatotosis-induced hepatic NKT cell depletion. Hepatic mononuclear cells were isolated from vehicle or clodronate treated wild type mice fed choline deficient diet for indicated time periods and subpopulations of lymphocytes assessed by flow cytometry. Results are representative of 4-6 different experiments, p<0.01.

Figure 8. Hepatosteatosis is associated with decreased numbers of NKT and NK cells in humans. Human liver biopsies were divided into three groups as described in methods. Slides were stained for CD3 and CD57, a marker for human NK cells. CD3 positive cells stained blue, NK positive cells present red. Double positive cells are termed NKT cells. A. Representative microphotographs of stained tissue are presented, NKT cells are pointed out by arrows. B. Quantitation of NKT cells were counted from at least 10 400x pictures of the tissue. Representative results for n = 3-5 per group. Values are means ± SEM.

Supplemental Materials

Total RNA Isolation and Real Time Reverse Transcriptase-PCR

Total RNA was isolated from whole liver, subjected to reverse transcription and real time PCR and gene expression quantitated as previously described [23]. Primer sequences for IL-12, TNFα, IL-4, IFNγ, IL-10, and β actin have been previously reported [12]. Primer sequences used for CD14 are as follows: Forward: 5' - AGG GTA CAG CTG CAA GGA CT – 3', Reverse: 5' – CTT CAG CCC AGT GAA AGA CA – 3'.

Mononuclear cell isolation and cell surface labelling

Hepatic mononuclear cells or splenocytes were isolated and labelled as described previously [12]. Anti-mouse TCRβ-PECy5 and NK1.1-PE were obtained from eBioscience (San Diego, CA). Additionally, some cells were labelled with TCRβ-PECy5 and PE labelled CD1d-tetramer/PBS57 (generously provided by the National Institute of Allergy and Infectious Disease MHC Tetramer Core Facility, Atlanta, GA) to specifically identify CD1d restricted NKT cells. Labelled cells were subject to flow cytometry (Becton Dickinson, Palo Alto, CA) and data analyzed by Summit V4.2 (DakoCytomation, Fort Collins, CO). Gates were set using_single stained samples, either TCRβ or NK1.1 or CD1d-tetramer.

Enzyme-Linked Immunosorbent Assay (ELISA)

IL4 concentration was determined in liver tissue as previously described [23] using a quantitative sandwich enzyme immunoassay (ELISA) technique (Quantikine®; R&D Systems, Minneapolis, MN). Concentrations were expressed as pg/mg protein.

Tissue Pathology Evaluation

Formalin-fixed paraffin embedded liver sections were stained with hematoxylin and eosin to assess steatosis, inflammation, and necrosis. Slides were viewed by light microscopy and images captured with an Olympus DP-70 digital camera.

Triglyceride Assay

Liver tissue (150mg) was incubated overnight at 55°C in 350μl ethanolic KOH. After centrifugation, the supernatant was combined with 50% ethanol to 1.2ml and vortexed. A 200μl aliquot was transferred to a new tube and 215μl of 1M $MgCl_2$ was added. Samples were incubated on ice (10min) and centrifuged at 15000xg. A 30μl aliquot of supernatant was incubated with 1ml Triglyceride (GPO Trinder) Reagent A (#337-40A, Sigma) for 15min and the absorbance measured at 540nm (Shimadzu UV-160, MD). Liver triglycerides were calculated using a standard curve of glycerol (Sigma).