innate and adaptive immune responses. High numbers of IL-17-producing T cells which also secrete IL-21 and IL-22 are found in close proximity to bile ducts in several liver diseases. $T_{h17}$ related cytokines have multiple effects and may be involved in both effector responses and repair and regeneration.

**Methods** Primary human parenchymal cells were assessed for cytokine receptor expression by western blotting. The effects of stimulation with recombinant IL-17, IL-21, IL-22, TNFa or IFN-g alone or in combination were compared for apoptosis using annexin staining, proliferation was measured by in situ Ki67 staining and adhesion molecule expression was assessed by flow cytometry. Secretion of IL-1b, IL-6, IL-23 and TGF-b1 was assessed by ELISA.

**Results** All parenchymal cells expressed IL-17R, IL-21R and IL-22R. $T_{h17}$ related cytokines did not cause apoptosis but led to parenchymal cell proliferation. Cholangiocytes and hepatocytes responded best to IL-17, whereas sinusoidal endothelial cells were responsive to IL-22. Endothelial cells upregulated adhesion molecules in response to $T_{h17}$ related cytokines. Cholangiocytes responded to $T_{h17}$ cytokines by secreting high levels of IL-1b, IL-6, IL-23 and TGF-b1 all cytokines that support the survival of $T_{h17}$ and $T_{c17}$ cells.

**Conclusion** Liver parenchymal cells express IL-17, IL-21 and IL-22 receptors and proliferate in response to $T_{h17}$ cytokines. Upregulation of adhesion molecules by sinusoidal endothelial cells promotes lymphocyte recruitment and retention. Cholangiocytes also respond by secreting $T_{h17}/T_{c17}$ polarising cytokines. Therefore $T_{h17}$ related cytokines secreted by infiltrating lymphocytes may activate the epitheliome to generate a local environment characterised by cholangiocyte proliferation and $T_{h17}/T_{c17}$ cell survival, thus contributing to bile duct proliferation and persistent chronic inflammation that characterises many liver diseases.

**Competing interests** None declared.

## PMO-119 PHENOTYPICALLY AND FUNCTIONALLY DISTINCT MONOCYTE SUBSETS AND THEIR ROLE IN HUMAN LIVER DISEASE

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**Introduction** Chronic liver inflammation is a leading cause of morbidity and mortality world wide, characterised by a dysregulated tissue repair driven by uncontrolled inflammation that leads to fibrosis, cirrhosis and hepatocellular carcinoma. We have investigated the role of different monocyte subsets: classical (CD14++CD16−/Mon1), intermediate (CD14++CD16+/Mon2) and non-classical (CD14+CD16++/Mon3) monocytes in human liver disease.

**Methods** Liver-infiltrating and peripheral blood mononuclear cells (MNC) were isolated from normal individuals or patients with liver disease (ALD/NASH/PSC/AIH) and sorted into phenotypic subsets that were studied for their differentiation in response to Th1/Th2 cytokines by flow cytometry, migration across TNFα/IFNγ stimulated hepatic sinusoidal endothelial cells (HSEC) under physiological flow and phagocytic activity of zymosan bioparticles. The ability of different monocytes to activate hepatic stellate cells (HSC) was assessed using QRT-PCR (aSMA and COL1α1 gene expression changes). In this study monocyte subsets are defined as Mon1 (CD14++CD16−), Mon2 (CD14++CD16++) and Mon3 (CD14+CD16++). In functional experiments Mon2 and Mon3 were studied together and are defined as total CD16+ monocytes.

**Results** Mon1 comprised 80% of MNC in the blood of healthy subjects and patients with liver disease but only 50% of MNC in both normal and diseased liver. Mon2 comprised 9% and 14% of MNC in normal and diseased blood respectively, but were significantly increased in normal and diseased livers (42 and 30% of MNC, respectively). Transmigration of total CD16+ monocytes across inflamed HSEC was 2.3-fold higher compared to CD14+ respectively. In vitro stimulation of Mon1 with TGFβ1 or IL-10 for 5 days induced 16- and 20-fold increases in CD16 expression. Liver infiltrating Mon2 expressed higher levels of CD163 and HLA-DR compared to Mon1, colocalized with CD68 and demonstrated high phagocytic activity, indicative of a macrophage phenotype. Diseased-liver-derived total CD16+ monocytes secreted higher levels of CCL2, IL-6, IL-8 and IL-13 and induced a twofold increase in aSMA and COL1a1 expression in co-cultured HSC.

**Conclusion** Compared with normal livers, diseased livers harbour fewer CD14+ but significantly more CD16+ monocytes that secrete profibrotic cytokines and are able to activate HSC. CD16+ monocyte accumulation in the liver is the result of enhanced recruitment from blood and also local differentiation from CD14+ in response to TGFβ1 and IL-10 present in the fibrotic microenvironment.

**Competing interests** None declared.

## PMO-120 KUPFFER CELL DERIVED INTERLEUKIN (IL)-18 INDUCES HEPATIC INFLAMMATION BY PROMOTING LYMPHOCYTE SUBSETS RECRUITMENT ON HEPATIC ENDOTHELIAL CELLS

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**Introduction** IL-18, known as interferon-γ inducing factor, is a potent pro-inflammatory cytokine implicated in liver allograft rejection, viral hepatitis and hepatocellular carcinoma progression, where it plays an important role in cell-mediated immune responses and inflammatory injury. We hypothesise that IL-18 promotes hepatic inflammation by supporting effector T cells migration across hepatic sinusoidal endothelium. In this study, we investigated the expression and cellular regulation of IL-18 secretion in the human liver and demonstrated a role in promoting T cell recruitment to the liver.

**Methods** IL-18 mRNA expression levels were measured in normal and diseased human livers using QRT-PCR and tissue localisation assessed by immunohistochemistry and confocal microscopy. Human hepatic sinusoidal endothelial cells were treated with Il-18 in vitro and flow cytometry used to assess induction of adhesion molecules. The functional significance of these responses to IL-18 was investigated in flow based adhesion assays using IL-18 treated HSEC and CD4 and CD8 T cell subsets under physiological flow.

**Results** IL-18 mRNA expression was significantly higher in liver tissue form patients with ALD (19-fold), PBC (7.6-fold) and seronegative hepatitis (30.6-fold) ($p<0.05$) compared with normal liver. IL-18 protein expression was restricted to hepatic sinusoids where it colocalized with $CD68^+$ Kupffer cells, whereas $CD31^+$ endothelial cells were $IL\text{-}18^{neg}$. HSEC stimulated with IL-18 lead upregulation of cell adhesion molecules ICAM-1 and VCAM-1 which translated into a 2.5-fold increase in their functional ability to recruit CD4 and CD8