increased the number of cells per field of view in the livers of mice following transplantation (1.6 vs 0.7, $p=0.017$).

Conclusion β 1-integrin blocking antibodies increase survival of isolated hepatocytes and improve their ability to remain adherent to HSEC under flow resulting in increased engraftment of transplanted human hepatocytes in mouse liver. The use of β 1-integrin blocking antibodies may provide a means to increase the efficacy of human hepatocyte transplantation.

Competing interests None declared.

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PMO-116 EUS GUIDED SAMPLING OF PANCREATIC MALIGNANCY

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Introduction Endoscopic ultrasound (EUS) guided sampling of malignant pancreatic lesions is increasingly performed to confirm malignancy prior to chemotherapy and/or radiotherapy. Historically lesions have been sampled by fine needle aspiration (FNA) yielding cells for cytological analysis. Cook Medical has recently developed the ProCore needle for EUS guided fine needle biopsy (FNB). This study compared the diagnostic yield of Procore FNB and FNA in patients undergoing EUS guided sampling of suspected pancreatic malignancy in a tertiary EUS centre.

Methods All patients with suspected pancreatic malignancy undergoing EUS guided tissue sampling over a 1-year period from 1st January 2011 to 31st December 2011 were retrospectively identified from endoscopy records. Note was made of whether FNA or FNB were performed. Electronic records were reviewed to determine the results of FNA/FNB. Standard statistical tests were used to compare the diagnostic yield of FNA and FNB.

Results EUS guided sampling was performed on 51 suspected malignant pancreatic mass lesions. FNA was performed on 27 occasions; FNB was performed on 40 occasions. Fifteen lesions were sampled by both FNA and FNB. FNA yielded a sample sufficient for cytological analysis in 19 (70%) cases. Of these samples, cytology confirmed malignancy in 17 (89%) of cases. FNB yielded a sample sufficient for histological analysis in 27 (68%) cases. Of these, histology confirmed malignancy in 26 (96%) cases. There was no statistically significant difference in either the yield of analysable tissue or the yield of positive cytology/histology between FNA and FNB. In cases where both FNA and FNB were performed, both modalities confirmed malignancy in eight cases (53%) and both modalities failed to yield diagnostic tissue in three cases (20%). In two cases FNA was positive with insufficient tissue from FNB, and in two cases FNB was positive with FNA yielding insufficient tissue. The overall yield of FNB was only one in five of patients undergoing repeat sampling where the initial sample had been non-diagnostic, compared to two in three for FNA.

Conclusion An advantage of Procore FNB is that cytology specimen preparation in endoscopy is not required and the larger sample allows more extensive histological assessment. The overall positive yield of FNB in patients who underwent EUS guided sampling of a suspected pancreatic malignancy was 65% compared to 63% for FNA. The limitation to higher yield appears to be acquisition of sufficient tissue for histological analysis. The yield of FNB in repeat sampling is low suggesting that combined FNA & FNB should be

performed in such situations, ideally with on site microscopy assessment to ensure adequate tissue acquisition.

Competing interests None declared.

PMO-117 PERTURBATION OF THE MITOCHONDRIAL NETWORK ARCHITECTURE IN AN IN VITRO MODEL OF ALCOHOL-INDUCED LIVER TOXICITY

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Introduction Mitochondria are central to many cellular processes and are dynamic organelles that exist as a network in the form of elongated filaments and respond to the demands of the cell through cycles of fusion (binding of mitochondria) and fission (mitochondrial fragmentation) which are driven primarily through multiple mitochondrial shaping proteins. Mitochondrial function is intimately associated with their morphology and while mitochondrial dysfunction has been previously correlated with alcohol consumption, there is a paucity of understanding regarding the impact of alcohol on the dynamic balance between fusion/fission and on mitochondrial morphology. The aim of this study was to investigate the impact of alcohol-induced liver damage on mitochondrial morphology, dynamics and to identify the precise mechanisms driving these changes.

Methods Ethanol metabolising-human hepatoma cell lines VL-17A (positive for alcohol-dehydrogenase and CYP2E1) were cultured in the presence of increasing doses of ethanol (EtOH), reflecting real-life alcohol consumption. Cells were cultured with EtOH at 10 mM (safe levels), 50 mM, 250 mM (high levels) and 500 mM (highly toxic levels). Cultures were incubated in presence/absence of EtOH for 24, 48, 72 and 96 h. Post-treatment the levels of mitochondrial shaping proteins including Mitofusin-1 (Mfn-1), Mitofusin-2 (Mfn-2) and Dynamin-related protein-1 (Drp-1) were analysed by detecting protein and mRNA levels. Dynamic changes in the morphology of mitochondria were assessed by confocal microscopy.

Results In the absence of alcohol, we observed no changes in the mitochondrial shaping proteins and no changes in the mitochondrial network over time. At 24 h, cells treated with 50 mM ethanol induced profound modifications in the mitochondrial network with a spot-like presentation which correlated with increased levels of Drp-1. At higher toxic levels of 500 mM, the cells display features of mitochondrial toxicity characterised by fragmentation reflecting the high level of cell death observed with this concentration. This toxicity was associated with reduced expression of Mfn-1 and Mfn-2.

Conclusion For the first time we show that alcohol can profoundly perturb the equilibrium between fusion and fission which directly affects the mitochondrial morphology. This study reveals a novel finding in the pathogenesis of alcohol-induced liver toxicity.

Competing interests None declared.

PMO-118 THE EFFECTS OF TH17 CYTOKINES ON LIVER PARENCHYMAL CELLS SHAPE THE MICROENVIRONMENT FOR LOCAL GENERATION OF TH17/TC17 IN INFLAMMATORY LIVER DISEASE

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Introduction IL-17 secreting T cells have been implicated in auto-immunity, inflammatory disease and provide a link between the

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, TNF α or IFN- γ 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- β 1 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- β 1 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 TNF α /IFN γ 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 COL1 α 1 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 TGF β 1 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 TGF β 1 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 from patients with ALD (19-fold), PBC (7.6-fold) and sero-negative 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-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