Results Immunohistochemistry reveals Fn14 on the intra-hepatic small bile ducts of inflamed livers, especially around the Canals of Hering. Fn14 expression is increased on cholangiocytes in vitro by 26% after stimulation with FGF. Exposure of cholangiocytes to TWEAK for 48 h induces apoptosis and upregulation of reactive oxygen species in FGF-activated cholangiocytes.

Conclusion Fn14 is expressed on cholangiocytes in inflamed human livers. Activation of the Fn14/TWEAK receptor-ligand system induces apoptosis using a novel mechanism partly dependent on the generation of reactive oxygen species.

Competing interests None declared.

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PMO-114 LOW CD39 EXPRESSION MARKS SEVERE REGULATORY T CELL IMPAIRMENT IN PATIENTS WITH AUTOIMMUNE SCLEROSING CHOLANGITIS

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Introduction Autoimmune hepatitis (AIH)/sclerosing cholangitis overlap syndrome (autoimmune sclerosing cholangitis, AISC) is a severe hepatopathy that, in addition to the serological and histological features typical of AIH (hyper γ globulinaemia, autoantibody seropositivity and interface hepatitis), presents with bile duct abnormalities. Both conditions are associated with numerical and functional impairment of $CD4^+CD25^{high}$ regulatory T cells (Tregs), a lymphocyte subset central to immune-tolerance. It remains unclear whether the two conditions can be distinguished on the basis of specific immune-regulatory T cell defects. To this end, we have explored a subset of Tregs expressing CD39, an ectoenzyme that contributes to Treg suppression by hydrolysing pro-inflammatory nucleotides and whose polymorphisms are associated with autoimmune disease in humans.

Methods We studied 10 patients with AISC (2 females, median age: 14.5 years), 24 with AIH type 1 (12 females, median age: 16 years) and 25 healthy subjects (HS; 15 females, median age 36 years). The frequency and phenotype of circulating $CD4^+CD39^+CD25^{high}$ cells ($CD39^+$Tregs) was assessed by flow cytometry using monoclonal antibodies to CD4, CD25, CD39, CD127 and FOXP3. The frequency of IFNγ, IL17 and TGFß-producing $CD39^+$Tregs was determined by intracellular cytokine staining.

Results The frequency of $CD39^+$Tregs was markedly reduced in AISC (0.31±0.11) compared to AIH (4.30±0.89, p<0.01) and HS (7.02±1.28, p<0.01). AISC patients also had fewer $CD39^+FOXP3^+$Tregs (0.03±0.02) and $CD39^+CD127^-$Tregs (0.05±0.02) than AIH patients ($FOXP3^+$: 0.14±0.03, p=0.05; $CD127^-$: 0.42±0.10, p<0.01) and HS ($FOXP3^+$: 0.20±0.04, p=0.03; $CD127^-$: 0.49±0.07, p=0.01). Analysis of cytokine profiles showed that in AISC there was a higher frequency of $CD39^+$Tregs producing IFNγ (0.23±0.15) and IL17 (0.22±0.14) and a lower frequency of $CD39^+$Tregs producing TGFß (0±0) than in AIH (IFNγ: 0.04±0.02, p=0.05; IL17: 0.03±0.01, p=0.03; TGFß: 0.03±0.01, p=0.09) and HS (IFNγ: 0.07±0.03 p=0.13; IL17: 0.06±0.03, p=0.11; TGFß: 0.02±0.01, p=NS).

Conclusion Compared to AIH and health, $CD39^+$Tregs in AISC are reduced in frequency and display a more proinflammatory cytokine profile. These findings suggest that immune-regulation impairment is more severe in AISC than AIH and implicate CD39 as a marker to differentiate immune-regulatory T-cell defects in the two conditions.

Competing interests None declared.

PMO-115 ANTI-B1-INTEGRIN ANTIBODIES IMPROVE SURVIVAL OF ISOLATED HUMAN HEPATOCYTES SIGNIFICANTLY INCREASING BOTH ADHESION TO HEPATIC SINUSOIDAL ENDOTHELIUM UNDER FLOW AND ENGRAFTMENT IN MURINE LIVER FOLLOWING TRANSPLANTATION

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Introduction Hepatocyte transplantation is a potential alternative to orthotopic liver transplantation but is limited by poor survival of transplanted cells. This may be partly due to apoptosis of isolated hepatocytes following detachment from extracellular matrix with loss of β1-integrin-mediated survival signals. Anti-β1-integrin antibodies have been shown to reduce apoptosis of rat hepatocytes[1] and improve their survival in allogeneic transplantation.[2] The purpose of this study was to determine the effect of β1-integrin blocking antibodies on the survival and initial engraftment of transplanted human hepatocytes.

Methods Hepatocytes were isolated from tissue obtained with ethical approval from Queen Elizabeth Hospital Birmingham. Integrin expression was confirmed using flow cytometry. Cells were incubated in suspension with anti-β1-integrin blocking antibodies or isotype matched control for 1 h. Viability and caspase three activity were assessed using flow cytometry and cleaved caspase 3 ELISA respectively. A modified flow adhesion assay was used to investigate the resistance to flow of cells adherent to sinusoidal endothelium (HSEC). An FC blocking agent was used to exclude the possibility of antibody-treated cells binding via antibody-FC receptor interactions. 1×10^6 fluorescently labelled cells were injected into C57BL/6 mice via the portal vein under general anaesthesia and the mice culled after 15 min. The livers were immediately frozen and sectioned and the number of fluorescent cells per field of view counted.

Results Mean surface expression of the β1-integrin subunit on human hepatocytes was 86.8% (MFI 46.8). Hepatocytes treated with β1-integrin antibodies showed increased viability (85.4% vs 79.0% p=0.02) and reduced caspase 3 activity as demonstrated by a decrease in cleaved caspase 3 (mean 450 nm absorbance 1.37 vs 1.90, p=0.02). β1-integrin blockade significantly increased the mean percentage of cells remaining adherent to HSEC under flow compared to IgG control (30.6% vs 12.7%, p=0.03) and significantly

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 β1-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 β1-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 autoimmunity, inflammatory disease and provide a link between the