Methods All patients diagnosed with pancreatic adenocarcinoma over a 2-year period who underwent trial dissection or resection after routine staging with CT and EUS were included in the study. CT and EUS images were retrospectively reviewed by two radiologists in a double blinded manner and the findings were compared with operative findings and final histology in those patients who underwent radical resection. Sensitivity, Specificity, Positive Predictive value (PPV), Negative predictive value and Accuracy were determined for assessing major vessel involvement which in most cases preclude radical resection.

Results 23 patients (M:F=13:10; mean age=68; range=56–78) underwent trial dissection or radical resection over a 2-year period. 13 were inoperable (nine inoperable due to locally advanced tumour, 1 inoperable due to liver mets, three both locally advanced and liver mets) and 10 underwent radical resection (three resected with cuff of portal vein (all R1), seven resected with six of them R1). Predictably EUS had superior sensitivity and accuracy over CT for both major vessel involvement (88% vs 53% & 87% vs 65%) and nodal involvement (43% vs 10% & 56% vs 30%). However CT was superior to EUS in excluding major vessel involvement (specificity = 100% vs 86%) and comparable to EUS in ruling out nodal disease (specificity = 100%). Importantly, three patients declared as having major vessel involvement by either of the modality underwent radical resection, two of them with PV resection. One patient who was staged as resectable with no vascular involvement was found to have major vessel involvement and underwent resection (R1).

Conclusion Though CT and EUS have important role in staging of patients with pancreatic cancer, a significant minority of patients will still be amenable to radical surgery and should be offered trial dissection with a view to radical surgery as surgery is the only realistic curative therapeutic option.

Competing interests None declared.

Basic science (liver)

PMO-112 IS PRIMARY BILIARY CIRRHOSIS A STEROID SENSITIVE AUTOIMMUNE DISEASE?

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Introduction Primary biliary cirrhosis (PBC) is a classic T cell mediated autoimmune disease: an autoantigen has been described and high levels of antigen specific liver infiltrating auto-reactive CD4⁺ T cells found. However, unlike in other autoimmune conditions steroid therapy is not considered effective in PBC although there is existing evidence that it can improve histological and biochemical parameters.¹ We sought further evidence that PBC is a steroid sensitive disease by using two in vitro measures of steroid sensitivity.

Methods We have applied an in vitro dexamethasone (Dex) inhibition of lymphocyte proliferation assay (DILPA), which correlates well with clinical steroid sensitivity and outcome in ulcerative colitis² and alcoholic hepatitis,³ to 20 patients with PBC diagnosed by liver biochemistry, antibodies and liver histology (when performed). The DILPA assesses peripheral blood mononuclear cell (PBMC) sensitivity to treatment with steroids in vitro. We also examined the role of CD14⁺ monocytes, which produce pro-inflammatory cytokines to recruit T cells to the tissue of inflammation. PBMCs were isolated from peripheral blood by density gradient centrifugation over Ficoll. CD14⁺ cells were obtained by positive microbead selection and cultured with 300 ng/ml lipopolysaccharide in the presence or absence of Dex 1×10⁻⁶M for 24 h. Supernatants were then collected and interleukin (IL)-1β, IL-6 and TNFα were measured by cytokine bead array (BD biosciences)

according to manufacturer's instructions. Suppression of cytokine production by Dex was calculated.

Results In 20 patients with PBC, just one individual demonstrated in vitro steroid resistance by DILPA, and peripheral lymphocytes were sensitive to steroids in all other study subjects. Suppression of lymphocyte proliferation by Dex was significantly greater in patients with PBC compared to 37 healthy volunteer controls (86% vs 76%, p=0.04). Furthermore, Dex induced a 40%–100% suppression of IL-1β, IL-6 and TNFα (mean 75%, 74% and 79%, respectively) in the supernatants of CD14⁺ monocyte cultures. This suggests that both peripheral blood lymphocytes and monocytes in patients with PBC are steroid sensitive.

Conclusion Using a validated measure of lymphocyte steroid sensitivity and a further assessment of monocyte steroid sensitivity we have demonstrated that PBC is a steroid sensitive disease. Together with existing clinical studies of glucocorticoids in PBC¹ our in vitro evidence suggests that steroid treatment should not be dismissed outright as it may provide a useful option in selected patients with PBC.

Competing interests None declared.

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PMO-113 FN14 IS EXPRESSED ON CHOLANGIOCYTES AND PROMOTES BILIARY DUCTULAR REMODELLING VIA APOPTOSIS AND REACTIVE OXYGEN SPECIES AFTER INTERACTION WITH TWEAK

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Introduction The mortality from chronic liver disease in the UK has increased by 50%.¹ The prevalence of cholangiopathies, diseases of the bile ducts, has increased fourfold.² These include primary biliary cirrhosis, primary sclerosing cholangitis and allograft rejection after transplantation.^{3–4} It is increasingly observed in livers donated for transplantation after cardiac death, a source of organs on which the NHS is becoming more reliant.^{5–6} It is characterised by inflammation and destruction of intrahepatic bile ducts.⁷ When sustained it may drive portal fibrosis to end-stage liver disease when the only therapeutic option for patients is liver transplantation.⁸ The novel TNF superfamily member TNF-like weak inducer of apoptosis (TWEAK) and its cognate receptor FGF-inducible protein 14 (Fn14) are implicated in hepatic inflammation and remodelling.^{9–10} TWEAK is mainly secreted as a soluble cytokine by myelomonocytic cells.¹¹ Fn14-TWEAK interaction in other systems promotes cell growth, apoptosis, autophagy and transdifferentiation via activation of TRAF and NF-κB pathways.¹²

Aim To demonstrate the expression of Fn14 and TWEAK on cholangiocytes and the functional significance of Fn14/TWEAK interaction on biliary ductular remodelling.

Methods Human liver samples were obtained with consent from the Queen Elizabeth Hospital liver transplant programme. Sections were stained for Fn14 and TWEAK using immunohistochemical techniques. Expression of Fn14 and TWEAK on cholangiocytes stimulated with TNF-α, IFN-γ and FGF was established quantitatively using flow cytometry. Cholangiocytes stimulated with FGF were exposed to TWEAK for 48 h. Apoptosis and reactive oxygen species production at this time point were determined by flow cytometry using annexin and dichlorofluorescein assays respectively.

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 IFN γ , IL17 and TGF β -producing CD39⁺Tregs was determined by intracellular cytokine staining.

Results The frequency of CD39⁺Tregs was markedly reduced in AISC (0.31 $\pm$ 0.11) compared to AIH (4.30 $\pm$ 0.89, p <0.01) and HS (7.02 $\pm$ 1.28, p <0.01). AISC patients also had fewer CD39⁺FOXP3⁺Tregs (0.03 $\pm$ 0.02) and CD39⁺CD127⁺Tregs (0.05 $\pm$ 0.02) than AIH patients (FOXP3⁺: 0.14 $\pm$ 0.03, p =0.05; CD127⁺: 0.42 $\pm$ 0.10, p <0.01) and HS (FOXP3⁺: 0.20 $\pm$ 0.04, p =0.03; CD127⁺: 0.49 $\pm$ 0.07, p =0.01). Analysis of cytokine profiles showed that in AISC there was a higher frequency of CD39⁺Tregs producing IFN γ (0.23 $\pm$ 0.15) and IL17 (0.22 $\pm$ 0.14) and a lower frequency of CD39⁺Tregs producing TGF β (0 $\pm$ 0) than in AIH (IFN γ : 0.04 $\pm$ 0.02, p =0.05; IL17: 0.03 $\pm$ 0.01, p =0.03; TGF β : 0.03 $\pm$ 0.01, p =0.09) and HS (IFN γ : 0.07 $\pm$ 0.03 p =0.13; IL17: 0.06 $\pm$ 0.03, p =0.11; TGF β : 0.02 $\pm$ 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 β 1-integrin-mediated survival signals. Anti- β 1-integrin antibodies have been shown to reduce apoptosis of rat hepatocytes¹ and improve their survival in allogeneic transplantation.² The purpose of this study was to determine the effect of β 1-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- β 1-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 $\times$ 10⁶ 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 β 1-integrin subunit on human hepatocytes was 86.8% (MFI 46.8). Hepatocytes treated with β 1-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). β 1-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