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