Methods We performed western blotting for ZEB1, E-cadherin, vimentin and α-tubulin to identify the epithelial-mesenchymal status of eight primary HCC cell lines. IHC was undertaken on paraffin sections from 40 patients who underwent resections for primary HCC between May 1997 and November 2010 and scored by two independent pathologists. Clinicopathological data were collated retrospectively and patient survival calculated using the Kaplan–Meier method. We transfected ZEB1 into Huh7 and HepG2 cell lines by electroporation and assessed EMT related changes in cell motility using Boyden chambers (pore size: 8 μm) and serum as chemo-attractant.

Results Western blotting of proteins from eight HCC cell lines demonstrated reciprocal expression of ZEB1 and E-cadherin, suggesting EMT promotes a migratory phenotype in HCC. ZEB1 also significantly increased cell motility as a threefold increase in cell migration was observed after ZEB1 transfection into Huh7 cells (23±4 vs 79±5). ZEB1 positivity was detected in 11/40 specimens analysed by IHC. Statistical analysis highlighted ZEB1 as an independent prognostic marker favouring a significant reduction in cancer specific (41 vs 16 months, p Conclusion Our results suggest that ZEB1 induced EMT promotes tumour progression and metastasis in HCC, and that over-expression of ZEB1 may represent an independent prognostic biomarker in patients with HCC.

Competing interests None declared.

Abstract PMO-135 Figure 1

PMO-136
DESTRUCTIVE INHIBITORY MOLECULES EXPRESSION MAY CONTRIBUTE TO BREAKDOWN OF TOLERANCE CHARACTERISTIC OF AUTOIMMUNE LIVER DISEASE

doi:10.1136/gutjnl-2012-302514b.136

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Introduction In autoimmune hepatitis (AIH) CD4posCD25high regulatory T-cells (T-regs), a subset central to immune-tolerance, are numerically defective and impaired in their ability to control effector cell function. At variance with CD4 effectors, T-regs, classically known as CD25high and FOXP3pos, express low levels of the activation marker CD127. The aim of the current study was to provide a phenotypic and functional profile of CD4posCD25highCD127low T-regs (CD127lowT-regs) in AIH and to explore to what extent absence or low levels of CD127 impact on T-reg ability to suppress.

Methods 20 ANA/SMA+ AIH patients and 12 healthy subjects (HS) were studied. T-reg phenotype was determined by flow cytometry using antibodies to CD4, CD25, CD127, CTLA-4 and Galectin-9, a molecule linked to T-reg ability to suppress. T-reg transcription factor and cytokine profile were assessed by intracellular staining. CD127low T-regs ability to suppress was evaluated in a proliferation assay following co-culture with CD25pos target cells.

Results In AIH CD4posCD25high cells contained fewer CD127low cells than in HS. Compared to conventional CD4posCD25high (c-T-regs), CD127low-T-regs from both AIH and HS had a) higher numbers of FOXP3pos, CD25pos, Galectin-9pos and IL-10pos cells; b) lower numbers of T-betpos, RORCpos, IFNγpos and IL-17pos cells; and c) similar numbers of TGF-βpos cells. In AIH, CD127low-T-regs contained fewer FOXP3pos, CD25pos, Galectin-9pos, IL-10pos and TGF-βpos cells and higher frequencies of T-betpos, RORCpos, IFNγpos and IL-17pos cells than in HS. CD127low T-regs inhibited CD25pos cell proliferation more effectively than c-T-regs, though less markedly in AIH than in HS. In AIH, treatment with anti-IFNγ and anti-IL-17 neutralising antibodies ameliorated the suppressive ability of c-T-regs, while leaving unchanged that of CD127low-T-regs, exposure to anti-IL-10 neutralising antibodies reduced c-T-reg suppression in HS, but not in AIH.

Conclusion CD127low T-regs bear the phenotypic and functional signature of “true T-regs”. Low numbers and reduced suppressive function of CD127low-T-regs in AIH may contribute to breakdown of
immune-tolerance by permitting effector cells to perpetuate hepatocyte damage.

**Competing interests** None declared.

**PMO-138** THE Molecular MECHANISMS OF B CELL AND B CELL LYMPHOMA RECRUITMENT TO THE HUMAN LIVER
doi:10.1136/gutjnl-2012-302514b.138

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**Introduction** There is gathering interest in the presence of B cells within liver tissue and their contribution to chronic inflammation and fibrosis but the recruitment signals for B cells into peripheral tissue is poorly understood. In addition a large proportion of lymphomas which infiltrate the liver are of B cell origin but again little is understood of the mechanism that underlies this process. Lymphocyte recruitment to the liver occurs within the hepatic sinusoidal channels. These low shear vascular beds are lined by specialised hepatic sinusoidal endothelial cells (HSEC). Our aim was to understand the molecular mechanisms of B cell and B cell lymphoma recruitment to the liver.

**Methods** We used isolated human HSEC in flow assays with purified peripheral blood B cells to elucidate the molecular mechanisms of B cell recruitment via HSEC. The contribution of conventional adhesion molecules, ICAM-1 and VCAM-1 and unconventional molecules VAP-1 and CLEVER-1/stabilin-1 was assessed by using function blocking antibodies. We repeated our experiments with two B cell lymphoma cell lines, CRL-2261 and Karpas B cell line. We assessed the contribution of chemokines by performing transwell assays and adding chemokines to our flow assays. We also tracked the motility of B cells and lymphoma cell lines on HSEC using tracking software.

**Results** B cells were captured from flow and firmly adhered to HSEC, the primary adhesion receptor on HSEC was VCAM-1. B cells also underwent transendothelial migration which was mediated by a combination of ICAM-1, VAP-1 and CLEVER-1/stabilin-1. Lymphoma cell line recruitment shared several features of primary lymphocyte homing, firm adhesion was mediated by ICAM-1 and VCAM-1 and they demonstrated shape-change and crawling behaviour which was ICAM-1 dependent. The lymphoma cell lines did not undergo transendothelial migration and this could not be initiated with the addition of SDF-1x.

**Conclusion** There is increasing evidence that B cells play an important role in chronic inflammatory liver diseases. The recruitment signals we have identified for B cells in this study may provide potential therapeutic targets for liver disease. Furthermore we have demonstrated preserved lymphocyte homing mechanisms in malignantly transformed B cells. These properties could be therapeutic targets to prevent lymphoma dissemination to the liver.

**Competing interests** None declared.

**PMO-139** HUMAN CYTOMEGALOVIRUS INFECTION OF HUMAN HEPATIC SINUSOIDAL ENDOTHELIAL CELLS PROMOTES CD4 T CELL ADHESION AND TRANSMIGRATION
doi:10.1136/gutjnl-2012-302514b.139

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**Introduction** Animal studies suggest that sinusoidal endothelial cells and not hepatocytes are the site of cytomegalovirus (CMV) latency and reactivation in the liver and the source of secondary viral spread. Furthermore, murine CMV infection of sinusoidal endothelium is able to break immunotolerance and induce a strong T cell effector response. The aim of this study was to investigate, whether CMV infection of human hepatic sinusoidal endothelial cells (HSEC) modulates the ability of the liver to recruit and activate lymphocyte. Methods Recombinant endotheliotropic eGFP-labelled CMV was propagated in RPE-1 cells and purified by ultracentrifugation in tartrate/glycerol gradients. Primary HSEC were isolated from explanted livers, grown to confluence and infected with CMV over 2 h. Infection was confirmed by fluorescence microscopy and plaque assay on fibroblasts. Chemokines and adhesion molecules were quantified by ELISA. Isolated primary lymphocytes and CMV-specific CD4 T cell clones were perfused over HSEC monolayers under constant flow simulating physiological shear stress and adhesion and transmigration recorded using phase contrast microscopy. Trans-well assays were used to study the phenotype of transmigrating cells using flow cytometry.

**Results** Human sinusoidal endothelial cells were permissive to CMV infection. CMV infection induced secretion of CXCL10 and CCL5 as well as an up-regulation of VCAM-1 and ICAM-1 surface expression. Early CMV infection resulted in a fourfold increase in the adhesion of allologenic lymphocytes to infected HSEC monolayers compared with mock-infected endothelium. Under flow, transendothelial migration of CMV-reactive CD4 T cell clones was increased through CMV-infected endothelium and could be significantly reduced by the use of anti-CXCL10 antibodies. Transmigrated allologenic CD4 CD45R0+ T cells and CMV-reactive T cell clones displayed increased expression of the early activation marker CD69 after transendothelial migration through CMV-infected HSEC.

**Conclusion** CMV infection of HSEC facilitates the up-regulation of cell-adhesion molecules and chemokines resulting in increased adhesion, transmigration and activation of CD4 T cells. This may explain how human CMV infection not only provokes significant hepatitis but also increases hepatic immune activation in graft rejection.

**Competing interests** None declared.

**PMO-140** ANALYSIS OF EUS-GUIDED CYST ASPIRATE HAS NO IMPACT ON SURGICAL MANAGEMENT OF SUSPECTED PANCREATIC CYSTIC TUMOUR
doi:10.1136/gutjnl-2012-302514b.140

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**Introduction** Preferred strategies for evaluation and management of patients with pancreatic cysts remain controversial. EUS-guided fine needle aspiration (FNA) of suspected pancreatic cyst tumours for CEA and cytology is often recommended to evaluate malignant potential in order to guide further management. Aim To evaluate the clinical impact of EUS guided cyst aspirate on surgical management of patients with suspected pancreatic cystic tumours.

**Methods** Outcome data of all patients having undergone EUS guided FNA of suspected pancreatic cystic tumours from March 2004 to November 2011 were retrospectively reviewed. Data were collected on demographics, EUS findings, radiological findings, biochemical and cytological findings, clinical outcomes and management. The mean follow-up was 24.5 months.

**Results** Of 123 patients (74F:49M; 64<192 g/l and 1 (7%) with EUS FNA. The majority of patients had low grade dysplasia (n=7), MCN (n=1), pancreatic neuroendocrine tumour (n=1) and serous cystadenoma (n=1). Only 3(12%) patients with CEA >192 μg/l and 1 (7%) with