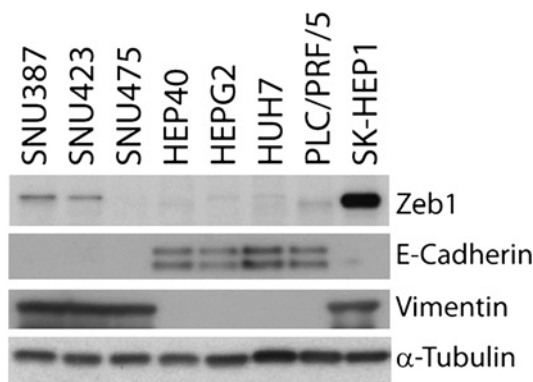


Methods We performed western blotting for ZEB1, E-cadherin, vimentin and α -tubulin to identify the epithelial-mesenchymal status of eight primary HCC cell lines. IHC was undertaken on paraffin sections from 40 patients who underwent resections for primary HCC between May 1997 and November 2010 and scored 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 μ m) 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 DEFECTIVE 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 Autoimmune hepatitis (AIH) is a severe hepatopathy often progressing to end-stage liver disease. Evidence implicates the involvement of both CD4 and CD8 T cell responses in its pathogenesis. There are a number of different inhibitory molecules expressed by T cells that can attenuate T cell receptor signalling. These include cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed death-1 (PD-1), and the recently described T cell immunoglobulin and mucin domain-3 (Tim-3). Whether a disturbed expression of these inhibitory molecules can result in an increased susceptibility to autoimmune liver disease is unknown.

Aims to evaluate the expression of CTLA-4, PD-1, and Tim-3 by CD4 and CD8 T cells in patients with autoimmune hepatitis.

Methods 12 ANA/SMA+ AIH patients (6 females, median age: 14 years) and 6 healthy subjects (HS, four females, median age:

26.4 years) were studied. Phenotype of CD4 and CD8 T cells was determined by flow cytometry using monoclonal antibodies against CD4, CD8, PD-1 and Tim-3. Expression of CTLA-4 was determined by intracellular staining.

Results The frequency of Tim-3^{pos} and PD-1^{pos} cells within CD4 and CD8 T cells was lower in AIH (CD4^{pos}Tim-3^{pos}: 1.6 ± 0.3 ; CD4^{pos}PD-1^{pos}: 4.8 ± 0.5 ; CD8^{pos}Tim-3^{pos}: 9.6 ± 1.6 ; CD8^{pos}PD-1^{pos}: 6.7 ± 0.7) than in HS (CD4^{pos}Tim-3^{pos}: 6.2 ± 0.8 , PposPD-1^{pos}: 8.1 ± 1.9 , P=0.04; CD8^{pos}Tim-3^{pos}: 15.8 ± 1.8 , P=0.04; CD8^{pos}PD-1^{pos}: 12.6 ± 1.4 6.7 ± 0.7 , Ppos cells between the two groups of subjects. While in health dually Tim-3 and PD-1 positive populations are recognisable (CD4^{pos}Tim-3^{pos}PD-1^{pos}: 0.7 ± 0.1 ; CD8^{pos}Tim-3^{pos}PD-1^{pos}: 1.7 ± 0.4), they are reduced in AIH (CD4^{pos}Tim-3^{pos}PD-1^{pos}: 0.4 ± 0.1 , p=0.007; CD8^{pos}Tim-3^{pos}PD-1^{pos}: 0.9 ± 0.3 , p=0.03).

Conclusion AIH patients have fewer PD-1 and Tim3 positive cells within both CD4 and CD8 T cells. Defective expression of these negative immune-regulatory molecules may contribute to breakdown of tolerance, possibly accounting for the initiation and/or perpetuation of the autoimmune liver attack.

Competing interests None declared.

PMO-137 PHENOTYPIC AND FUNCTIONAL SIGNATURE OF CD4^{POS}CD25^{HIGH}CD127^{LOW} REGULATORY T-CELLS IN AUTOIMMUNE HEPATITIS

doi:10.1136/gutjnl-2012-302514b.137

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Introduction In autoimmune hepatitis (AIH) CD4^{pos}CD25^{high} 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 CD25^{high} and FOXP3^{pos}, express low levels of the activation marker CD127. The aim of the current study was to provide a phenotypic and functional profile of CD4^{pos}CD25^{high}CD127^{low}T-regs (CD127^{low}T-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. CD127^{low}T-reg ability to suppress was evaluated in a proliferation assay following co-culture with CD25^{neg} target cells.

Results In AIH CD4^{pos}CD25^{high} cells contained fewer CD127^{low} cells than in HS. Compared to conventional CD4^{pos}CD25^{high} (cT-regs), CD127^{low}T-regs from both AIH and HS had a) higher numbers of FOXP3^{pos}, CTLA-4^{pos}, Galectin-9^{pos} and IL-10^{pos} cells; b) lower numbers of T-bet^{pos}, RORC^{pos}, IFN γ ^{pos} and IL-17^{pos} cells; and c) similar numbers of TGF- β ^{pos} cells. In AIH, CD127^{low}T-regs contained fewer FOXP3^{pos}, CTLA-4^{pos}, Galectin-9^{pos}, IL-10^{pos} and TGF- β ^{pos} cells and higher frequencies of T-bet^{pos}, RORC^{pos}, IFN γ ^{pos} and IL-17^{pos} cells than in HS. CD127^{low}T-regs inhibited CD25^{neg} cell proliferation more effectively than cT-regs, though less markedly in AIH than in HS. In AIH, treatment with anti-IFN γ and anti-IL-17 neutralising antibodies ameliorated the suppressive ability of cT-regs, while leaving unchanged that of CD127^{low}T-regs; exposure to anti-IL-10 neutralising antibodies reduced cT-reg suppression in HS, but not in AIH.

Conclusion CD127^{low} T-regs bear the phenotypic and functional signature of "true T-regs". Low numbers and reduced suppressive function of CD127^{low}T-regs in AIH may contribute to breakdown of

immune-tolerance by permitting effector cells to perpetrate 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-1 α .

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 transmigrated 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 allogeneic 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 allogeneic CD4 CD45RO+ 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 \pm 2.7 years) with suspected pancreatic cystic tumours, only 10 (8%) patients had surgical resection for IPMN with 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 μ g/l and 1 (7%) with