

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

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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/stablin-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/stablin-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

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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

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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

CA 19–9 >1000 U/l had surgery. Similarly only 3 (30%) with abnormal cytology were considered for surgery. Although most patients (n=9) who underwent surgery were symptomatic, 18/113 (16%) patients in the conservatively managed group also had symptoms related to the cystic tumours. The size of the cystic tumour in the surgical group, however, was significantly larger than that in the conservative group (4.9 cm vs 2.9 cm, $p<0.01$).

Conclusion Surgical decision making process in patients with pancreatic cystic lesions is complex with multiple factors influencing the choice of surgery. Our data indicate the limited role of pancreatic fluid analysis compared with symptoms and cyst size. Factors that guide and influence the need for surgical resection of pancreatic cystic tumours should be further evaluated.

Competing interests None declared.

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PMO-141 FLAGELLIN-INDUCED IL-6 PRODUCTION IS SELECTIVELY IMPAIRED IN PATIENTS WITH CIRRHOSIS

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Introduction The innate immune response is an important determinant of progression in chronic inflammatory liver diseases such as alcoholic (ALD) and non-alcoholic fatty liver disease (NAFLD). Sepsis is a frequent cause of hepatic decompensation and some authors suggest a role for toll-like receptor (TLR) sensing of gut-derived pathogens (motile and gram-negative organisms) in disease progression. Paresis of the innate immune system has been described in patients with decompensated liver disease, but the function of TLRs in compensated disease has received scant attention. Our aim was to assess TLR responsiveness in patients with compensated ALD and NAFLD using a combinatorial experimental design, measuring LPS- and flagellin-induced TNF α and IL-6 production.

Methods Consenting adult outpatients with compensated ALD or NAFLD were recruited. Diagnoses were confirmed by casenote review. Exclusion criteria included alternative aetiologies, decompensated disease, other systemic immune-related illnesses, and immunosuppression (including steroids). Normal healthy volunteers without liver disease were also recruited. Monocytes isolated from peripheral blood mononuclear cells were stimulated with low dose LPS and flagellin. The production of TNF- α and IL-6 was assayed in supernatants and in patient sera by ELISA.

Results We included 28 patients and six normal controls. Patients with compensated cirrhosis have a selective defect in flagellin-induced IL-6 production (330 ± 117 pg/ml) compared to patients with non-cirrhotic ALD or NAFLD (896 ± 146 pg/ml; $p=0.01$) or healthy controls (764 ± 96 pg/ml). There were no differences in flagellin-induced TNF α nor in LPS-induced cytokine production. There were no differences between the three groups in serum concentrations of TNF, IL-6 and RANTES.

Conclusion Paresis of the innate immune response is not universal; there is selective impairment of TLR5-mediated IL-6 production in patients with compensated cirrhosis compared to non-cirrhotic patients. These data identify potential signalling pathways that may be involved in the progression of liver disease or in the susceptibility of patients with cirrhosis to bacterial infections.

Competing interests None declared.

PMO-142 EVIDENCE REFUTING THE TROJAN-HORSE HYPOTHESIS OF BRAIN SWELLING IN ACUTE LIVER FAILURE: L-TYPE GLUTAMINASE IS EXCLUSIVELY NEURONAL

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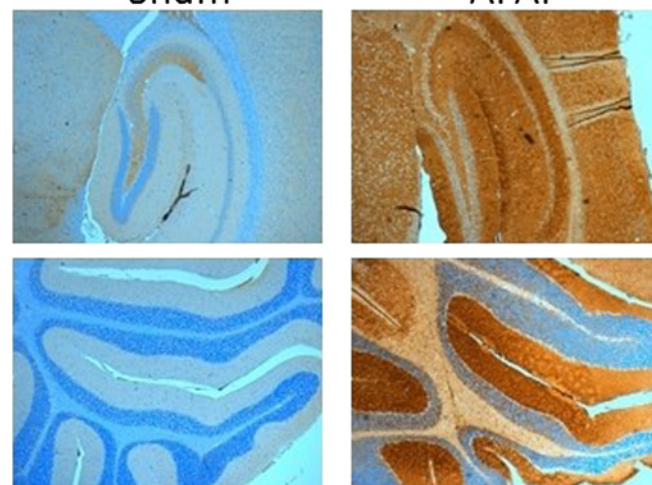
Introduction Astrocytic swelling is the characteristic feature of hyperammonemia and acute liver failure (ALF) which is thought to result from accumulation of glutamine due to the action of astrocytic glutamine synthetase (GS). It has been suggested that glutamine may not be a benign amino acid and may act as “Trojan horse” which leads to astrocytic apoptosis as it is metabolised by Glutaminase (GLN) yielding glutamate and ammonia. In vivo proof for this hypothesis is lacking. In health, GLN is mainly neuronal and generates glutamate and GABA. The aims of the study were to define the expression of the ammonia metabolising enzymes, GS and GLN in the brain of ALF animals.

Methods Two groups of CD1 mice were studied, sham: n=6; paracetamol (500 mg/kg IP): n=7. The animals were maintained normothermic and resuscitated with fluid and glucose and sacrificed at 8 h after injection of APAP or before development of coma. Arterial ammonia (COBAS) and frontal cortex brain water (dry weight technique) were measured. The brain sections were stained for GS and K and L-type GLN.

Results Arterial ammonia was significantly higher in the ALF group compared with controls (345 ± 32 vs 132 ± 11 $p=0.002$) and brain water did not reach significance (83.6 ± 2.3 vs 76.3 ± 2.6 $p=0.05$). GS protein expression was observed in the astrocytes in the dentate fascia in both groups and was not different but was also seen in the oligodendrocytes only in ALF group. L-type GLN was expressed only in the neurons and not in the astrocytes and was significantly higher in the ALF animals (++++) compared with controls (+). The most marked areas were the striatum and dentate fascia and interestingly the staining was mainly cytoplasmic. K-type GLN was not different between groups and limited to brain capillaries.

Conclusion Conclusion: The results of this study refute the Trojan-horse hypothesis and show for the first time increased protein expression of L-type GLN which is exclusively neuronal. From the pathophysiological perspective, this may function to generate excessive ammonia in the neuron thereby producing neuronal cell death.

L-glutaminase : sham vs APAP brain



Abstract PMO-142 Figure 1