Aim To determine the effect of increasing age upon telomere length in healthy liver within hepatocytes, Kupffer cells, stellate cells, CD4 or CD8 lymphocytes and cholangiocytes.
Method Q-FISH was performed on paraffin-embedded tissue from 73 "time zero" liver biopsies obtained at liver transplantation from liver donors selected carefully for the absence of reperfusion injury and to include a wide age range (5–79 years). Hepatocytes, Kupffer cells, hepatic stellate cells, CD4 or CD8 lymphocytes and cholangiocytes were identified with monoclonal antibodies to Hepar-1, CD68, SMA, CD4, CD8 and CK-19, respectively; nuclei were counterstained with DAPI and telomeres identified with a specific Cy5-labelled PNA probe. Image acquisition and data analysis were performed with Olympus ScanR microscope and software. Mean telomere fluorescent intensity was measured and analysed using linear regression on GraphPad PRISM 5.0.
Results The Q-FISH methodology enabled large volume analysis of telomeres in various cell lineages within paraffin-embedded liver sections. A mean 40 000 hepatocytes, 30 000 Kupffer cells, 15 000 cholangiocytes and 4000 hepatic stellate cells, intrahepatic CD4 and CD8 lymphocytes were analysed per liver. Hepatic stellate cells had the longest telomeres at all ages whilst the others cell types had similar telomere lengths. Telomeres within Kupffer cells and stellate cells in healthy liver shortened, as might be expected, with increasing age ($p=0.024$ and $p<0.0001$, respectively). In contrast, telomeres in hepatocytes, cholangiocytes and lymphocytes in healthy livers did not shorten with increasing age.
Conclusion Cells within the healthy liver do not age equally. The disparity between age-related telomere shortening in Kupffer cells and stellate cells and maintained telomere length with age in cholangiocytes, hepatocytes and intrahepatic lymphocytes was unexpected but may reflect low turn over in hepatocytes and cholangiocytes. The absence of an effect of age on these cell lineages suggests that the potential for regeneration is maintained with age and has important implications for the choice of control tissues in studies of hepatic ageing.

OP21 THE ROLE OF TOLL LIKE RECEPTOR-4 IN THE PATHOGENESIS OF HEPATORENAL SYNDROME IN A BILE DUCT LIGATED MODEL OF CIRRHOSIS IN THE RAT

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N Shah, D Dhar, F Mohamed, R Mookerjee, N A Davies, R Jalan. *Institute of Hepatology, University College London, London, UK*

Introduction Hepatorenal syndrome (HRS) is a frequent presenting complication in patients with cirrhosis and usually occurs with a superimposed infection/inflammation. It is associated with high morbidity and mortality. The pathogenesis of the HRS is not delineated. Toll like receptors (TLR's) play an important role in the recognition of molecules derived from microbes, which upon stimulation leads to activation of NFkB and pro inflammatory cascade. We hypothesised that cirrhosis associated endotoxaemia primes the kidney resulting in upregulation of TLR4 making the kidney susceptible to further endotoxaemia.
Aim The aims of this study were to determine whether (1) cirrhosis is associated with an upregulation of TLR4, NFkB and proinflammatory cytokines in the kidney and (2) whether selective decontamination would result in a reduction in TLR4 expression, attenuate NFkB and cytokines and make the kidneys less susceptible to further endotoxemic insult.
Method Six groups of Sprague–Dawley rats were studied; n=6 in each group (Sham operated, Sham-operated+LPS; BDL (4 weeks), BDL+LPS (1 mg/kg); BDL+Norfloxacin and BDL+LPS+Norfloxacin. The Norfloxacin groups were pretreated with Norfloxacin, 20 mg/kg administered orally daily for 10 days. The animals were studied 3 h after administration of placebo or LPS. Blood was for collected for biochemistry and cytokines. Kidney was collected for protein expression of TLR4 and NFkBp65 on Western blot and immunohistochemistry.
Results TLR4 and NFkBp65 protein expression was significantly upregulated in BDL rat kidney compared to sham ($p=0.03$ and 0.02), respectively, this increased further on LPS administration ($p=0.02$ and $p=0.01$). TLR4 was mainly localised in the proximal tubule and its brush border with ongoing apoptosis as evident by the presence of caspase-3 positivity on immunohistochemistry. Selective decontamination with Norfloxacin attenuated the inflammatory response by reducing the protein expression of TLR4 and NFkBp65 in BDL vs BDL group administered with LPS ($p=0.02$ and $p=0.01$). It also led to an improvement in the creatinine in BDL and BDL+LPS group administered Norfloxacin ($p=0.02$ and $p=0.03$), respectively. Norfloxacin ameliorated the kidney cytokine surge in BDL and BDL +LPS group (TNF $p=0.09$ and $p=0.04$, respectively).
Conclusion Our data provide strong evidence indicating an important pathogenic role of TLR4 in mediating susceptibility to the development of HRS in an experimental model of cirrhosis following inflammation/infection. Selective gut decontamination attenuates this pathological inflammatory process and prevents renal failure induced by LPS. TLR4 antagonist may be a therapeutic target for HRS.

OP22 A SIGNIFICANT REDUCTION IN "PRO-INFLAMMATORY" CD14LO/CD16HI MONOCYTES IN PARACETAMOL-INDUCED ACUTE LIVER FAILURE: ASSOCIATION WITH OUTCOME AND CCR2 EXPRESSION

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D Abeles, C G Antoniades, M S Longhi, N J Taylor, D L Shawcross, G Auzinger, W Bernal, N Heaton, J A Wendo, D Vergani. *King's College Hospital, London, UK*

Introduction Acute liver failure (ALF) shares many clinical and immunological features with severe sepsis. In severe sepsis the relative proportion of "pro-inflammatory" CD14lo/CD16hi monocytes (MØ) is reduced, possibly due to recruitment into inflamed tissue. No data exist on monocyte subsets in paracetamol-induced ALF (PALF). Animal models of ALF suggest the importance of CC chemokine receptor (CCR) 2 mediated MØ recruitment and in human PALF high circulating levels and hepatic expression of CC chemokine ligand 2 have been observed.
Aim Investigate the relative composition of "pro-inflammatory" CD14hi/CD16lo, "intermediate" CD14hi/CD16hi and "classical" CD14lo/CD16hi MØ subsets and the association of CCR2 expression, in patients with PALF.
Method Blood was taken from five healthy controls and 20 patients with PALF within 24–48 hours of admission. Monoclonal antibodies against CD14, CD16 and CCR2 were used to determine MØ subsets and CCR expression. Results are expressed as the percentage proportion of total MØ and mean fluorescence intensity of CCR2 expression.
Results Compared to healthy controls, a lower proportion of CD14lo/CD16hi MØ was observed in PALF (2% vs 10%, $p=0.01$), CD14hi/CD16lo MØ tended to be more highly represented (95% vs 82%, $p<0.08$) but there were no differences in the proportion of CD14hi/CD16hi MØ (3% vs 8%). In PALF, CCR2 expression was up-regulated on CD14hi/CD16lo and CD14hi/CD16hi MØ subsets compared to controls (CD16lo: 811 vs 454; CD16hi: 3376 vs 855, $p<0.05$) but remained low at control levels on CD14lo/CD16hi M&Oslash.

11 patients survived spontaneously (PALF-S) and nine died or required liver transplantation (PALF-NS). PALF-NS had higher peak INR (13.9 vs 7.4, p<0.001), SOFA score (13 vs 6, p<0.001), MELD (46 vs 35, p<0.05) and APACHE II score (20 vs 6.5, p<0.001). There were no differences in the total monocyte counts between PALF-NS and PALF-S (0.2 vs 0.43 X10 000 cells/dl). However, a significant reduction in the proportion of CD14lo/CD16hi MØ was observed in PALF-NS compared to PALF-S (0.5% vs 2.7%, p=0.01) and this was predictive of outcome (AUROC 0.838; values greater than 2% giving a sensitivity of 81%, specificity 89% for survival with medical management).

Conclusion These are the first reported data defining the relative proportions of MØ subsets in PALF. They show that the proportion of CD14lo/CD16hi MØ is significantly reduced in PALF, the degree of which is predictive for outcome. This reduction may be due to early sequestration of these MØ within the liver or other organs through CCR2 independent pathways.

OP23 BACTERIAL TRANSLOCATION AND REGULATORY T LYMPHOCYTES IN PATIENTS WITH LIVER CIRRHOSIS

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M Márquez, M Montes de Oca, C Rodríguez-Ramos, C Fernández-Gutiérrez, M J Blanco, S P Corchado, J A Girón. *Internal Medicine Digestive, Universtiario Puerta del Mar, Spain*

Introduction Increased prevalence of bacterial infectious diseases has been observed in cirrhotic patients, classically attributed to immunosuppression-associated liver cirrhosis. Conversely, advanced states of liver cirrhosis predispose to increased antigenic load. A possible role has been ascribed to the translocation of bacteria and endotoxins (lypopolysaccharide, LPS) from the gut. LPS increases the plasma levels of LPS-binding protein (LBP), the principal plasma protein responsible for transporting LPS to immune effector cells. High serum LBP has been proposed to identify a subset of cirrhotic patients with ascitis characterised by an activation of the immune cells to producer proinflammatory cytokines. Moreover, raised levels of circulating LBP or proinflammatory cytokines have been implicated in the endothelial activation and haemodynamic derangement observed in cirrhosis. A chronic antigenic stimulus will induce a monocyte and lymphocyte activation.

Aim Intestinal permeability and bacterial translocation and their influence on T lymphocytes activation and differentiation in T reg were analysed in patients with compensated and decompensated liver cirrhosis. In particular, the regulation of T cell activation, mediated by co-stimulatory molecules, the expression of activation markers and the proportion of T CD4+ regulatory cells, as a function of bacterial translocation, were studied.

Method 40 patients with liver cirrhosis, 20 of them without previous decompensation (CC) and 20 with ascetic decompensation (DC), and 20 healthy controls (HC) were studied. Bacterial translocation was analysed by serum concentrations of lypopolysacharide-binding protein (LBP). Membrane expression of co-stimulatory molecules (CD28), activation markers (CD25 and CD122) and proportion of T regulatory cells (defined as those CD4+CD25highintracellular FoxP3+) were studied by flow cytometry with specific antibodies. Values of the variables were expressed as median (interquartile range). Comparisons between variables were made by the Mann–Whitney U test. Associations between variables were analysed by the Pearson's correlation coefficient.

Results Serum concentrations of LBP were significantly elevated in patients with compensated (7.7 (5.7–9.1 microg/ml) and decompensated (28.2 (10.7–40.6)) cirrhosis when compared with healthy controls (3.4 (2.7–4.2)) (p<0.001). Significantly higher concentrations of LBP were detected in those patients with higher portal hypertension. Those patients with decompensated cirrhosis shows an activation state characterised by increased percentages of CD25+ and CD122+ expression on CD4+ T cells. A decrease of CD28 expression was detected in T CD4+ lymphocytes from patients with decompensated cirrhosis (DC, 94% (89–98%); CC, 97% (92–98%); HC, 98 (96–99), DC vs HC: p=0.010). Moreover, T reg lymphocytes, expressed as a proportion of global T CD4+ cells, were significantly increased in patients with compensated and decompensated cirrhosis (DC, 14.7% (13.3–16.1%); CC, 10.3 (10.1–11.2); HC, 8.4 (7.2–8.7), p<0.001 in each case). A significant and positive correlation was detected between serum LBP concentration and percentage of CD4+ T reg (r=0.787, p<0.001).

Conclusion Patients with liver cirrhosis, fundamentally those with previous decompensation, shows increased intestinal permeability and chronic systemic antigenic stimuli. As a response to those, T lymphocyte activation is detected. Probably as a mean to decrease the continuous antigenic stimuli, a diminution of co-stimulation and an expansion of suppressor populations are observed in them.

Viral hepatitis

OP24 VASCULAR ENDOTHELIAL GROWTH FACTOR ACTIVATION OF LIVER SINUSOIDAL ENDOTHELIAL CELLS VIA VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 REGULATES HEPATOCELLULAR HEPATITIS C VIRUS REPLICATION

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I Rowe, P Lalor, D Adams, P Balfe, J McKeating. *University of Birmingham, Birmingham, UK*

Introduction Hepatitis C virus (HCV) is a major concern for human health, with an estimated 180 million people infected worldwide. HCV primarily infects hepatocytes in the liver and the majority of infected subjects develop progressive liver disease. Treatment options remain limited and hence, there is an urgent need for new therapies that target viral and host cell pathways. Vascular endothelial growth factor (VEGF) is a multifunctional cytokine that is produced by a variety of cell types in response to low oxygen and viral infections. VEGF targets vascular endothelial cells that are present in many tissues, including liver sinusoidal endothelial cells (LSEC). LSEC are in close apposition to hepatocytes in the liver and VEGF is known to regulate LSEC proliferation and function.

Aim We recently demonstrated that HCV promotes VEGF expression in hepatocytes (Mee et al, 2010 *Gastroenterology*) and the aim of this study was to investigate the role of VEGF in LSEC-hepatocyte interactions in HCV infection.

Method Using primary human LSEC we established direct LSEC-hepatocyte co-culture models to recapitulate the hepatic microenvironment. The effects of VEGF on LSEC and hepatocytes were analysed in both monoculture and co-culture.

Results Initial studies demonstrated that LSEC do not express the full complement of HCV receptors or entry factors and fail to support HCV replication. However, in vitro co-culture of LSEC and hepatocytes to model the hepatic epithelial-endothelial cell environment demonstrated that LSEC significantly reduce the permissivity of hepatocytes to support HCV replication. Interestingly, this effect was abrogated by inclusion of a neutralising antibody or a drug antagonist targeting VEGF receptor-2 (VEGFR-2). Importantly, recombinant VEGF had no effect on HCV replication in hepatocyte monocultures, suggesting that VEGF stimulates endothelial cells to modulate expression of molecules