

5 AALF patients sampled (Abstract P95 figure 3). Intra-hepatic levels of IL-10 (2 vs 0.6; $p=0.03$) and SLPI (442 vs 116; $p=0.004$) were higher in patients with AALF compared to controls, whereas no difference in TNF- α (24 vs 19; $p=0.3$) concentration was detected. The percentage of monocytes phagocytosing *E coli* was significantly reduced in ALF compared to HC (69 vs 92%; $p=0.008$).

Conclusion In AALF, circulating monocytes show modulations in intracellular signalling pathways compatible with ET and display reduced phagocytic capabilities. Our data also indicate that hepatic production of anti-inflammatory mediators, IL-10 and SLPI, may play a pivotal role in induction of ET monocytes and thus increase the risk of infection in AALF.

P96 SERUM PROTEIN N-GLYCOSYLATION AS A BIOMARKER OF PAEDIATRIC NAFLD

doi:10.1136/gutjnl-2011-300857a.96

¹E Fitzpatrick, ²B Blomme, ³A Quaglia, ⁴R D Bruyne, ²H V Vlierbergh, ¹A Dhawan. ¹Paediatric Liver, GI and Nutrition Centre, King's College Hospital; ²Department of Gastroenterology and Hepatology, Ghent University Hospital, Belgium; ³Institute of Liver Studies, King's College Hospital; ⁴Department of Paediatric Gastroenterology, Hepatology and Nutrition, Ghent University Hospital, Belgium

Introduction Serum protein N-glycosylation has previously been shown to distinguish adult patients with simple steatosis from those with non-alcoholic steatohepatitis (NASH). The pattern of the disease in paediatric patients is distinct from adults.

Aim The aim of this study was to characterise the glycomic profile of children with varying degrees of NAFLD to identify potential biomarker profiles of disease.

Method Children with biopsy proven non-alcoholic fatty liver disease (n=51) were recruited from a tertiary paediatric hepatology unit. Liver biopsy was scored according to NAFLD activity score. Blood was taken on day of biopsy for analysis. Serum protein N-glycosylation patterns were assessed with DNA-sequencer assisted fluorophore-assisted capillary electrophoresis (DSA-FACE) and compared with histology.

Results Median age at biopsy was 13.3 years (range 4.5–17.4). 31 were male. Median BMI z-score was 1.81. 23 children scored as simple steatosis/borderline NASH and 28 as true NASH. 18 children had no/minimal fibrosis (< F2) and 33 had significant fibrosis (\geq F2). Similar to previous work in adult patients with NAFLD, peak 1 (NGA2F) was the most significantly raised N-glycan in paediatric NASH patients with peak 5 (NA2) demonstrating the greatest decrease. The logarithmically transformed ratio of peak 1 to peak 5 (Glycomarker) was -0.85 (SD 0.22) in simple steatosis/borderline NASH and -0.73 (SD 0.12) in NASH ($p=0.02$). The biomarker correlated well with the amount of lobular inflammation with a consistent increase with ascending grade of inflammation. There was also a trend towards significance in differentiating patients with significant fibrosis \geq F2; -0.74 (SD 0.13) from patients with no/minimal fibrosis < F2; -0.86 (SD 0.24), ($p=0.06$). Glyco-analysis of immunoglobulin G (IgG) confirmed the undergalactosylation status with a significant increase in peak 1 (NGA2F; $p=0.024$) and a significant decrease of peak 6 (NA2F; $p=0.01$) on IgG. In multivariate analysis of the Glycomarker, GGT, AST and INR, only the Glycomarker displayed a significant result for distinguishing simple steatosis from NASH ($p=0.019$).

Conclusion In conclusion, the findings in this study are novel in that they represent the first Glycomic analysis of paediatric NAFLD. They validate findings in adults in that a Glycomarker can serve reliably as a biomarker of severity of disease in NAFLD. The same N-glycosylation alterations are observed in paediatric NASH patients when compared to an adult population and therefore the same biomarker can be used. B cells play a dominant role in the N-glycan alterations of NASH patients, both in an adult and paediatric population.

P97 LYMPHOCYTE-HEPATOCTE INTERACTIONS: HEPATITIS C VIRUS CHANGES THE RULES

doi:10.1136/gutjnl-2011-300857a.97

¹Z Stamataki, ¹O S Qureshi, ¹G M Reynolds, ²L Hibbert, ²J Waters, ²G R Foster, ³J Z Rappoport, ⁴S G Hubscher, ¹D H Adams, ¹J A McKeating. ¹MRC Centre for Immune Regulation, Institute of Biomedical Research, University of Birmingham, UK; ²The Liver Unit, Queen Mary's University of London, UK; ³School of Biosciences, University of Birmingham, UK; ⁴Department of Pathology, University of Birmingham, Edgbaston, UK

Introduction Hepatitis C Virus (HCV) is a major cause of liver disease worldwide. Innate and adaptive cellular immune responses play a critical role in resolving acute HCV infection. However, the majority of infections are not cleared, resulting in a progressive chronic liver disease consistent with inadequate immune control. Evidence from human and animal models suggest that T cells play a critical role in controlling acute HCV infection, yet the mechanism (s) behind their failure to control chronic HCV replication are unknown. HCV replicates predominantly in the liver and virus specific immune cells need to target infected hepatocytes to control virus replication. HCV specific effector cells have been reported to home to the liver, however, little is known on their subsequent trafficking and fate within the organ.

Aim Our aim is to investigate the role of HCV infection on lymphocyte-hepatocyte interactions, migration and immune cell effector function.

Method We used in vitro and ex vivo models to study the effect of HCV infection on lymphocyte-hepatocyte interactions. Primary lymphocytes and hepatocytes were used in combination with hepatoma cell lines and replication competent HCV clones. Ex vivo lymphocyte migration assays were performed using biopsy material and tissue from explanted liver. Results were confirmed by in vivo observations using tissue sections from patients with end stage liver disease of viral and non-viral origin. Experimental techniques included immunohistochemistry, flow cytometry, fixed and live cell time-lapse confocal microscopy.

Results We demonstrate: (1) A role for hepatocyte ICAM-1 in mediating T-lymphocyte adhesion and migration; (2) T-lymphocytes migrate spontaneously through hepatocyte monolayers via cell-cell junctions; (3). HCV enhances T-cell transmigration and pro-inflammatory cytokine expression. Our data demonstrate the existence of novel interactions between T cells and hepatocytes that are modulated in HCV infection. The nature of the T cell-hepatocyte interactions may have an impact on T-cell effector function and the outcome of anti-viral immune responses.

Conclusion Interaction with HCV-infected hepatocytes alters T-cell trafficking and cytokine expression, providing a novel mechanism for HCV to persist in the liver.

P98 THE EFFECTS OF TH17 CYTOKINES ON LIVER PARENCHYMAL CELLS SHAPE THE MICROENVIRONMENT FOR LOCAL GENERATION OF TH17/Tc17 IN INFLAMMATORY LIVER DISEASE

doi:10.1136/gutjnl-2011-300857a.98

¹E H Humphreys, ¹G M Muirhead, ¹R H Bhogal, ²B Eksteen, ¹S C Afford, Ye H Oo, ¹D H Adams. ¹Centre for Liver Research, and NIHR Biomedical Research Unit, Institute of Biomedical Research, University of Birmingham, UK; ²Snyder Institute of Infection, Immunity and Inflammation, Health Research and Innovation Centre, Calgary, Canada

Introduction IL-17 secreting T cells (Th17_{h17} and Tc17_{c17}) are subsets of T lymphocytes that have been implicated in autoimmunity, inflammatory disease and provide a link between the innate and adaptive immune responses. High numbers of IL-17-producing T cells are found in close proximity to bile ducts in several liver diseases

including primary biliary cirrhosis. IL-17 related cytokines have multiple effects on parenchymal cells in different tissues and may be involved in both effector responses and repair and regeneration. We recently reported increased numbers of liver infiltrating IL-17 secreting CD4 and CD8 T cells in chronic inflammatory liver disease. These cells also secrete IL-21 and IL-22 cytokines reported to promote epithelial repair. In the present study we report the pattern of IL-17, IL-21 and IL-22 receptor expression on hepatic parenchymal cells and demonstrate cell-specific effects of T_{H17} cytokines on these target cells.

Aim To assess the functional impact of Th17/Tc17 associated cytokines on hepatic parenchymal cells.

Method Primary human cholangiocytes, hepatocytes and sinusoidal endothelial cells were assessed for IL-17, IL-21 and IL-22 receptor expression. The effects of stimulation with recombinant IL-17, IL-21, IL-22, TNF-α or IFN-γ alone or in combination were compared on apoptosis, proliferation and cytokine secretion using flow cytometry with annexin or 7-AAD staining and in situ Ki67 staining and measurement of IL-1b, IL-6, IL-23 and TGF-β1 secretion by ELISA.

Results All the parenchymal cell types expressed IL-21R and IL-22R. Th17 and Tc17 cytokines did not cause apoptosis but alone and in combination led to parenchymal cell proliferation. Cholangiocytes and hepatocytes responded best to IL-17, whereas sinusoidal endothelial cells were responsive to IL-22. Cholangiocytes responded to Th17/Tc17 cytokines by secreting high levels of IL-1b, IL-6, IL-23 and TGF-β1 all cytokines that support the survival of Th17 and Tc17 cells.

Conclusion Liver parenchymal cells express IL-17, IL-21 and IL-22 receptors and proliferate in response to Th17/Tc17 cytokines. Cholangiocytes also respond to such cytokines by secreting Th17 polarising cytokines. Thus IL-17 related cytokines secreted by infiltrating lymphocytes may activate the epitheliome to generate a local environment characterised by cholangiocyte proliferation and Th17 cell survival. This response may contribute to the bile duct proliferation and persistent chronic inflammation that characterised many liver diseases.

P99 **FXR STIMULATION WITH INT-747 IN A RAT BILIARY DRAINAGE MODEL PROTECTS FROM HEPATOCELLULAR INJURY AFTER LOSS OF ENTEROHEPATIC CIRCULATION**

doi:10.1136/gutjnl-2011-300857a.99

¹ R G J Visschers, ¹ K V K Koelfat, ¹ C M Hodin, ² R Shiri-Sverdlov, ¹ W A Buurman, ⁴ R Mookerjee, ¹ K Lenaerts, ¹ S W M O Damink. ¹Department of Surgery, Maastricht University Medical Centre+, Maastricht University, Maastricht, the Netherlands; ²Nutrition and Toxicology Research Institute Maastricht, Maastricht University, the Netherlands; ³Department of Molecular Genetics, Maastricht University Medical Centre+, the Netherlands; ⁴UCL Hepatology, Royal Free Hospital, University College London, London, UK; ⁵Department of HPB Surgery and Liver Transplantation, Royal Free Hospital, University College London, London, UK

Introduction Enterocutaneous fistula are often associated with development of intestinal failure associated liver disease (IFALD), ultimately leading to liver damage. We hypothesise that this is caused by reduced farnosoid X receptor (FXR) stimulation, due to interruption of the enterohepatic circulation and consequent impact on bile acid synthesis.

Aim We aimed at investigating the effect of specific stimulation of the farnosoid X receptor (FXR) with INT-747, a synthetic agonist, in a rat biliary diversion model.

Methods Four groups of rats (n=6–8) were studied; two groups underwent bile duct cannulation and externalisation to achieve continuous biliary drainage, daily receiving either vehicle or INT-747 (Intercept Pharm Ltd); two groups underwent laparotomy without cannulation also receiving either vehicle or INT-747. Loss of bile was recorded daily. After 7 days, plasma, serum and liver and intestinal tissue were collected. Alanine aminotransferase (ALT), aspartate

aminotransferase (AST), alkaline phosphatase (AP), γ glutamyl transpeptidase (GGT) and total bilirubin were assessed, together with histological scoring of necrosis, inflammation, bile duct proliferation, fibrosis and steatosis. Expression of genes involved in the FXR pathway, and lipid and cholesterol metabolism were quantified by qPCR.

Results Loss of bile was significantly reduced in the INT-747 group compared to the vehicle group. Serum levels of ASAT, ALAT, AP, GGT, bilirubin (total and direct) were all significantly increased in the bile drainage group when compared to controls (p<0.05), suggesting hepatocellular damage. Interestingly, all parameters were significantly decreased in the bile drainage group receiving INT-747 (p<0.05). Histological analyses showed normal liver histology in both sham groups. In contrast, large necrotic areas were observed in the biliary diversion group receiving vehicle with a high number of infiltrating inflammatory cells, which decreased significantly in the biliary diversion group receiving INT-747 (p<0.05). Biliary diversion induced hepatic fibrosis and bile duct proliferation, which were both attenuated by INT-747 supplementation (p<0.05). Although genes involved in the FXR pathway (FXR, SHP, CYP7A1 and CYP8B1) were influenced by bile drainage, no significant change was observed when rats received INT-747.

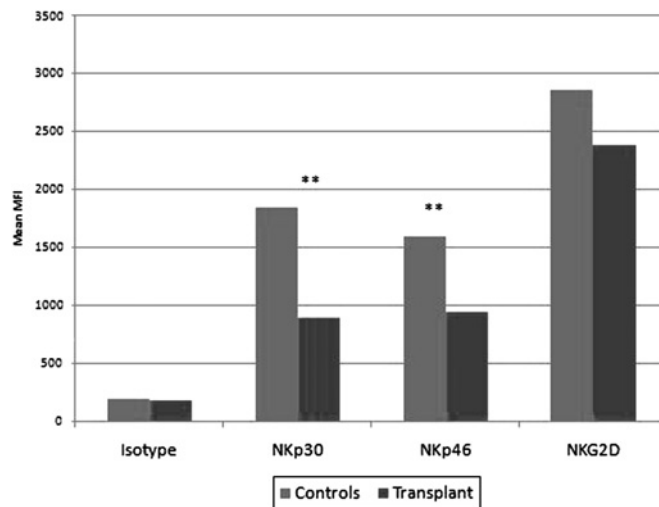
Conclusion The present data demonstrate that stimulation of FXR with INT-747 can attenuate hepatocellular damage in an experimental model of IFALD. The results suggest a role of FXR in the development of hepatocellular damage, hepatic fibrosis and necrosis during biliary diversion. FXR stimulation has the potential to be a novel therapy for patients with IFALD.

P100 **LIVER TRANSPLANTATION (LT) RESULTS IN REDUCED RECIPIENT NATURAL KILLER (NK) CELL ACTIVATION WITH ASSOCIATED DOWN REGULATION OF ACTIVATING RECEPTORS NKP30 AND NKP46 BUT NOT NKG2D**

doi:10.1136/gutjnl-2011-300857a.100

¹ K M Jamil, ¹ K S Cheent, ² K Agarwal, ³ G J Alexander, ² M A Heneghan, ¹ S I Khakoo. ¹Imperial College London; ²King's College Hospital, London; ³Addenbrookes Hospital, Cambridge

Introduction In solid organ transplantation, the effect of the allograft on recipient NK cell function is poorly understood. NK cells recognise self through inhibitory receptors for HLA class I, so that they



Abstract P100 Figure 1 Expression of activating receptors on NK cells.