(AIH-2), a severe disease often leading to end-stage liver damage despite immunosuppression. T-regs specific for cytochrome P450IID6 (CYP2D6), the target autoantigen in AIH-2, can be expanded in vitro and exert a stronger suppressor function over damaging effector T-cells than non antigen-specific T-regs.

Aim To explore the mechanisms controlling antigen-specific T-reg suppressor function in AIH-2.

Method 13 AIH-2 patients positive for the HLA-DR7 and DR3 predisposing MHC alleles, were studied. Peptide-pulsed semi-mature dendritic cells (smDCs) were obtained from CD14pos cells following treatment with IL-4, GM-CSF, interferon-λ and CYP2D6 peptides. CYP2D6-T-regs were obtained after CD4posCD25high cell purification from CD14neg cells cultured for 8 days in presence of CYP2D6 peptide, high dose interleukin-2 (IL-2) and T-cell expander. Frequency of interferon (IFN)-γ, IL-2, IL-17, IL-4, IL-10 and TGF-β producing cells within CYP2D6-T-regs was tested before and after 2-day co-culture with smDCs (smDC-CYP2D6-T-regs) by intracellular staining; suppressor function was determined by proliferation assay after T-reg addition to CD25neg target cells.

Results There was no difference in the frequency of IL-2, IL-17, IL-4, IL-10 and TGF-β producing CYP2D6-T-regs in the absence or presence of smDCs, while IFN-γ-producing cells were more frequent in the absence of smDCs (1.39±0.3 vs 0.4±0.1; p=0.046). Treatment with anti-IFN-γ neutralising antibody decreased the frequency of IFN-γ-producing cells within CYP2D6-T-regs to 0.46±0.2 (p=0.041) and enhanced their suppressor function over CD25neg cell proliferation from 23% to 45.6% (p=0.04), this value being similar to that obtained after adding smDC-CYP2D6-T-regs (50%). As engagement of the signalling molecule B7-H1 on smDCs inhibits IFN-γ secretion, we tested whether its blockage affects the number of IFN-γ-producing cells and/or smDC-CYP2D6-T-reg suppressor function. Interestingly, blockage of B7-H1 did not affect smDC-T-regs ability to suppress despite incrementing the frequency of IFN-γ-producing cells (1.63±0.08; p<0.001), suggesting that smDCs enhance antigen-specific T-reg function independently of IFN-γ.

Conclusion In AIH-2 T-reg suppression ability is enhanced by control of IFN-γ production and by co-culture with peptide-pulsed smDCs. These manoeuvres should be considered to obtain highly potent clinical-grade T-regs for immunotherapy. The mechanism through which smDCs augment suppression remains to be clarified.

P37 DEVELOPMENT OF A STABLE, CLINICALLY RELEVANT, FULLY MONITORED AND MANAGED REPRODUCIBLE MODEL OF PARACETAMOL INDUCED ACUTE LIVER FAILURE IN THE PIG

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Introduction Acute liver failure (ALF) is a rare but devastating clinical condition, a common cause of which is paracetamol overdose. Advances in treatment options for ALF have been hampered by the lack of a representative large animal model.

Aim This study was designed to develop a clinically relevant, fully monitored and managed model of ALF that would develop the clinical, biochemical, haemodynamic and inflammatory characteristics of the human equivalent.

Method Initial studies were performed in 36 landrace pigs to define a dose, which resulted in 100% mortality. We developed the model in 3 female pigs that were subjected to full intensive care but without any paracetamol administration and 8 pigs that were treated with paracetamol. After an overnight fast, 11 pigs [weighing 35-45 kg] were intubated and ventilated under general anaesthetic for the duration of the experiment. Catheters were placed for blood pressure monitoring, haemofiltration, urine measurement and triple lumen central intravenous lines for fluids, drugs and sampling. An ICP bolt was inserted and a separate catheter for cerebral microdialysis. Placebo (n=3) or a loading dose (0.25 g Kg-1) then hourly bolus' of paracetamol (n=8) were given via the jejunostomy to keep the serum paracetamol concentration 350 - 450 mg dl-1. Paracetamol was stopped when Quick index of 30% was reached. Animals were supported with fluids, glucose, fresh frozen plasma, inotropes, renal haemofiltration and mechanical ventilation until time of death.

Results The paracetamol overdosed animals developed typical changes of ALF manifested by attenuated mean arterial pressure requiring large amounts of fluid resuscitation (2.5 litres per hour) and inotropic support (noradrenaline; 15-150 μg kg-1 hr-1) and increased intracranial pressure (p<0.001 compared to sham operated pigs). There were increases in PT to >160 s, creatinine (0.58±0.17 vs 1.45±0.13 mg kg-1, p<0.01), ammonia (41.6±7.4 vs 120.6±48.1 μM L-1), lactate (2.1±0.4 vs 7.1±1.7 mM L-1, p<0.05) together with decreases in albumin (23±2 vs 2±0.4 mg L-1, p<0.01), urine (91±14 vs 6±5.5 ml hr-1 p<0.01) compared to baseline. The animals developed progressive albumin dysfunction (IMAR 0.014±0.002 vs 0.45±0.17, p<0.01) and endotoxaemia (0.57±0.17 vs 2.0±0.18 EU ml-1). They required increased ventilatory support and death was by respiratory failure following raised ICP. The mean time from paracetamol administration to ALF was 32±4.4 and from ALF to death 15.8±2.4 hrs.

Conclusion We have developed a stable, fully monitored and managed model of paracetamol induced ALF which exhibits the clinical, haemodynamic, biochemical and inflammatory characteristics of ALF that is suitable for interventional studies of novel therapies for this devastating rare disease.

P38 MATRIX STIFFNESS REGULATES PROLIFERATION, DIFFERENTIATION AND CHEMOTHERAPEUTIC RESPONSIVENESS IN HEPATOCELLULAR CARCINOMA

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Introduction The majority (80%) of hepatocellular carcinomas (HCC) develop within the context of advanced liver fibrosis and cirrhosis. Recent studies with ultrasound elastography have demonstrated that increased liver stiffness is a strong predictor of HCC.

Aim To establish whether alterations in matrix stiffness regulate the phenotype and chemotherapeutic response of HCC cells.

Method Experiments were conducted using a system of ligand-coated polyacrylamide gels of variable stiffness. Matrix stiffness (expressed as shear modulus) was modelled across a physiologically-relevant range (1−12 kPa), corresponding to values encountered in normal and fibrotic livers. Experiments were conducted in two HCC cells lines (Huh7/ HepG2).

Results In each cell type, there was a consistent morphological response to changes in matrix stiffness. There was increased cell spreading on stiff gels in association with both stress-fibre and mature focal adhesion formation. Increasing matrix stiffness promoted cellular proliferation. The proliferative index (assessed by Ki67 staining) of Huh7 and HepG2 cells was 2.7-fold (p<0.001) and 12.2-fold (p<0.001) higher, respectively, when the cells were cultured on stiff (12 kPa) vs soft (1 kPa) supports. Cells cultured on soft supports developed a quiescent (dormant) phenotype with marked reduction in cyclinD1/ D3 expression, without upregulation of p21/p27. We postulated that altered sensitivity to mitogenic growth factors mediates the stiffness-dependent regulation of proliferation. Matrix stiffness modulated both the magnitude and time-course of mitogenic signalling in response to HGF, with lower

baseline and HGF-induced FAK, ERK and STAT3 pathway activation. Increasing stiffness results in upregulation of mesenchymal markers (including N-cadherin and vimentin), consistent with mesenchymal shift, and down-regulation of differentiated hepatocyte markers (including albumin, α-1-antitrypsin and HNF4). Following treatment with cisplatin, cells cultured on soft supports were more susceptible to apoptosis (PARP/ Caspase-3 cleavage). However, in both Huh7 and HepG2 cells, surviving cells from soft supports had 2.2-fold ($p<0.05$) and 2.4-fold ($p<0.001$) higher clonogenic capacity respectively, than surviving cells from stiff supports. This was associated with upregulation of cancer stem cell markers (Oct4, NANOG, CD44, CD133, c-kit and CXCR4).

Conclusion HCC is a tumour that develops within an altered biomechanical niche. Increasing matrix stiffness regulates HCC mitogenic signalling, proliferation, differentiation and chemotherapeutic resistance. However, a soft microenvironment (as may be encountered by disseminated tumour cells) promotes stem cell characteristics following chemotherapy. This provides a possible explanation for the failure of systemic chemotherapy both in relation to treatment of primary HCCs and the eradication of disseminated tumour cells that give rise to metastases. The selective targeting of the cytoskeleton represents a potentially novel approach to the treatment of HCC.

P39 ABSTRACT WITHDRAWN

P40 EX VIVO TREATMENT OF NEUTROPHILS WITH A P38-MAPK AGONIST IN PATIENTS WITH LIVER FAILURE IMPROVES THEIR BACTERIOCIDAL CAPACITY

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Introduction Ammonia reduces neutrophil phagocytic and bacteriocidal capacity. Neutrophils swell in response to ammonia exposure and activation of the p38-MAPK pathway has been implicated in protecting the neutrophil against osmotic shock and in normalising neutrophil phagocytic dysfunction. (Shawcross *et al.*, *Hepatology* 2008) In patients with acute and chronic liver failure with elevated arterial ammonia, we hypothesised that treating neutrophils ex vivo with isoproterenol, a β adrenergic receptor and p38-MAPK agonist would improve neutrophil phagocytic capacity.

Aim Within the context of an ongoing longitudinal study of neutrophil function in 100 patients with acute (ALF) and chronic liver failure we investigated the role of ex vivo incubation of neutrophils with an exogenous ammonia load, a p38-MAPK agonist (isoproterenol) and the specific p38-MAPK antagonist SB203580 (Calbiochem) on neutrophil function in a cohort of 10 patients (5 ALF and 5 chronic) all with elevated arterial ammonia concentration.

Method Phagocytic capacity in heparinised whole blood was quantitatively determined by flow cytometry using FITC-labelled opsonised *E. coli* at baseline, and following incubation for 90 min with ammonium chloride (200 μmol/l), isoproterenol (2 μmol/l) or SB203580 (40 μmol/l) at 37°C.

Results The ALF patients (drug-induced n=3 and seronegative n=2) had a median SOFA score of 17 (IQR 16–18) and APACHE II score of 24 (22–28). The patients with cirrhosis (alcohol n=3; HCV; NASH) had a median MELD score of 10 (7–27). The median arterial ammonia level in the ALF group was 96 (49–158) and in the chronic group was 97 (63–122) μmol/l. Baseline bactericidal capacity was 49% (36–57) in the ALF group and 75% (67–84) in the chronic group compared to >85% in healthy controls and a median of 82% in a septic control group without liver disease. The exogenous ammonia load further reduced neutrophil phagocytic capacity by a median of 8% (3–16). The p38-MAPK agonist significantly abrogated the ammonia-induced phagocytic impairment and improved bacteriocidal capacity ($p=0.003$). The p38-MAPK antagonist however, exacerbated the ammonia-induced reduction in neutrophil phagocytic capacity by a median of 15% (4–24); $p=0.0015$.

Conclusion These data show that in patients with acute and chronic liver failure and elevated arterial ammonia that neutrophil bacteriocidal capacity is disabled. Activation of the p38-MAPK pathway serves as an important cellular protective mechanism against ammonia-induced impairment and further trials of p38-MAPK agonists in patients with liver failure are warranted.

P41 GENETIC VARIATION IN BILIARY TRANSPORTERS AS A SUSCEPTIBILITY FACTOR FOR CHOLANGIOCARCINOMA

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Introduction Cholangiocarcinoma (CC) is increasing in incidence globally but its pathogenesis remains poorly understood. Chronic inflammation of the bile duct and cholestasis are major risk factors but most cases in the West are sporadic. Genetic polymorphisms in biliary transporter proteins have been implicated in benign biliary disease and, in the case of progressive familial cholestasis, have been associated with childhood onset of CC. A recent case-control study of a single nucleotide polymorphism c.3972C>T (rs3740066) in ABCC2, reported an association with CC.

Aim To investigate five biologically plausible candidate genes as susceptibility factors for cholangiocarcinoma; ABCB11 (BSEP); ABCB4 (MDR3); ABCC2 (MRP2); ATP8B1 (FIC1) and NR1H4 (FXR).

Method Germline DNA was collected from 172 Caucasian individuals with confirmed CC. A control cohort of 256 healthy Caucasian patients was included in the analysis. 73 SNPs were selected using the HapMap database in Haploview 4.1 (build 22; MAF >0.05, pair-wise comparisons only) to capture the majority of common genetic variation around the five candidate loci. Genotyping was undertaken with a competitive allele-specific PCR based robotic genotyping system. Confirmation of Hardy-Weinberg equilibrium and Cochran-Armitage trend testing were performed using PLINK v1.07. Haplotype frequencies were compared using haplo.stats v1.4.4.

Results All 73 SNPs were in Hardy-Weinberg Equilibrium. Four SNPs in ABCB11 were associated with altered susceptibility to CC, including the V444A polymorphism (c.1331T>C, rs2287622, $p<0.007$) but these associations did not retain statistical significance after Bonferroni correction for multiple testing. Haplotype analysis of the genotyped SNPs in ATP8B1 identified significant differences in frequencies between cases and controls (global p value 0.005). None of the SNPs in ABCC2, including rs3 740 066, showed association with CC. Haplotype analysis in ABCC2 failed to detect significant association.

Conclusion This is the largest study to date of biliary transporter polymorphisms as susceptibility factors for CC. The previously reported association between SNP rs3 740 066 in ABCC2 and CC was not replicated. Haplotypes in ATP8B1 demonstrated a significant difference between CC and control groups. There was also a trend towards significant association of V444A with CC. V444A has been strongly implicated in other cholestatic diseases. Given the biological plausibility of polymorphisms in ABCB11 and ATP8B1 as risk modifiers for CC, further study in a validation cohort is required.