and NK cells. Flow cytometric analysis was performed on a FACS Canto II. Biochemistry was measured spectrophotometrically.
**Results** Compared with sham animals, ALF was associated with widespread and profound abnormalities in cellular immune responses which were significantly improved in the group treated with TLR4 antagonist after APAP administration; significant improvement was observed in (STM28 vs APAP): total myeloid cells (39% vs 57%, p=0.0002), neutrophils (21% vs 37%, p=0.005), total granulocytes (28% vs 48%, p=0.0016), monocytes (6% vs 11%, p=0.0009) and resident monocytes, which are able to differentiate into macrophage (7% vs 15%, p=0.01). No significant differences were observed in subtypes of myeloid and plasmocytoid dendritic cells (54% vs 56% and 15% vs 10%, respectively), T-lymphocytes (16% vs 14%), B cells (7% vs 6%) and NK cells (1% vs 1%). These decrease in cellular inflammatory response was associated with a significant reduction in markers of liver injury (ALT: p<0.001; Ammonia: p<0.01).
**Conclusion** The results of this study suggest profound cellular immune dysfunction in APAP induced ALF mice which have a predominant pro-inflammatory phenotype. This dysfunction can be significantly improved by treatment with a TLR4 antagonist. This restoration of immune dysfunction is associated with significantly less liver injury indicating that TLR4 antagonism may have important therapeutic potential in APAP induced ALF.

## P34 THE MECHANISM BEHIND SYNERGISTIC ACTION OF L-ORNITHINE AND PHENYLACETATE TO REDUCE AMMONIA IN BILE-DUCT LIGATION RATS

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**Aim** This study was designed to test the hypothesis that OP has additional actions on the key ammonia regulating enzymes glutamine synthetase (GS) and glutaminase (GA), which results in the observed ammonia lowering effect of OP in cirrhotic rats.
**Method** 11.53 g: 4 sham operated, and 11 BDL. 5 BDL's received OP (5 days, IP 0.6 g/kg), 5 BDL's received ornithine (5 days, IP 0.6 g/kg), 5 BDL's received phenylacetate (5 days, IP 0.6 g/kg) and six received saline (IP). We measured plasma levels for: ammonia and standard biochemical markers. Expressions of GS, GA and ornithine amino transferase (OAT) were determined by Western blot (expressed as a % of sham values) and activity by end-point methods in liver, kidney, gut, muscle and lung.
**Results** Plasma ammonia was decreased in BDL-OP rats vs BDL-saline (58.97±6.02 vs 106.2±20.56 μmol/l). BDL-OP rats showed increased GS expression in liver (66% BDL-OP vs 55% BDL-saline; p<0.01) and showed further increased levels in the muscle (153% BDL-OP vs 142% BDL-saline). OP prevents the BDL related increases in glutaminase expression (124% vs 163%; p<0.05) and activity (0.45±0.16 mIU/mg protein BDL-OP vs 1.14±0.046 mIU/mg protein BDL-saline; p<0.01) in gut. We demonstrated that this prevention is due to effect of ornithine in glutaminase activity (0.46±0.17 mIU/mg protein BDL-O vs BDL-saline; p<0.05) and not to phenylacetate. OP treatment increased OAT expression in muscle (142 %BDL-OPvs.114% BDL-saline; p<0.01) and lung (103%BDL-OP vs 127%BDL-saline; p<0.01).
**Conclusion** OP treatment in BDL rats increased the conversion of glutamate to glutamine by stimulation of OAT and GS in the muscle and also resulted in normalisation of glutaminase expression and activity in the gut, indicating that OP effectively restricts the production of in vivo ammonia in a cirrhotic model explaining the lack of stoichiometry between ammonia reduction and excretion of phenylacetylglutamine. In summary, the mechanism by which OP reduces ammonia in cirrhosis is by increasing glutamine synthesis (action of "O") and its excretion as phenylacetylglutamine (action of "P") and concomitantly normalising gut glutaminase activity (action of "O"), demonstrating synergistic effect of "O" and "P".

## P35 TREATMENT WITH AN ALPHA 2A ADRENORECEPTOR ANTAGONIST MODULATES HEPATIC INFLAMMATION, MARKEDLY REDUCES PORTAL PRESSURE, AND IMPROVES ARTERIAL PRESSURE AND HEPATIC BLOOD FLOW IN CIRRHOTIC RATS

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**Introduction** Inflammation plays a pivotal role in modulating the severity of intrahepatic resistance in cirrhosis. Our studies have shown a close relationship between the activation of the sympathetic nervous system, inflammatory response and severity of portal hypertension. Stimulation of alpha 2a adrenergic (ADRA2a) receptors results in inflammation and vasodilation in resistance vasculature, and its antagonism has shown benefit in models of sepsis.
**Aim** The aim of the study was to test the hypothesis that treating bile duct ligated rats (BDL) with an ADRA2a antagonist reduces hepatic inflammation and improves the haemodynamic abnormalities associated with cirrhosis.
**Method** Male Sprague-Dawley rats (N=46) were studied 4-weeks after BDL surgery (N=29) or sham operation (N=17) and randomised to two doses of placebo or ADRA2a antagonist (BRL 44408, Sigma, UK, 10mg/kg s.c 24 hours prior to study). Portal vein and hepatic arterial blood flow, mean arterial (MAP) and portal pressure were measured directly. Plasma biochemistry was measured by colorimetry. ADRA2a and NFkB protein expression were determined by western blotting and immunohistochemistry (ADRA2a).
**Results** BDL rats had significantly increased hepatic protein expression of ADRA2a compared with sham operated rats and this was mostly shown to be located on hepatocytes by immunohistochemistry. Following treatment with ADRA2a antagonist there was a significant increase in the MAP (p<0.05) and a significant reduction in portal pressure as compared to the placebo treated group (11.4±3.4 vs. 18.0±3.7 mmHg, p<0.001). The hepatic arterial blood flow was markedly increased in the treated group without significant change in the portal venous blood flow resulting in a significant reduction in intrahepatic resistance post treatment (1.1±0.2 vs. 0.5±0.1 mmHg/ml/min, p<0.05). Biochemical analysis showed a significant reduction in plasma lactate (p<0.05), AST (p<0.05) and a trend towards reduction in creatinine in treated animals. Hepatic phosphorylated NFκB expression was increased in BDL animals and this reduced significantly with ADRA2a antagonist treatment (p<0.05).
**Conclusion** The results of this study show for the first time that modulating ADRA2a-mediated sympathetic tone and hepatic inflammation with an ADRA2a antagonist significantly improves systemic haemodynamics and reduces portal pressure, whilst also increasing hepatic blood flow. Our data provide the rationale for evaluating an ADRA2a antagonist in the treatment of portal hypertension.

## P36 BLOCK OF INTERFERON γ AND CO-CULTURE WITH SMDCS ENHANCE ANTIGEN-SPECIFIC T-REG SUPPRESSION ABILITY IN AUTOIMMUNE HEPATITIS TYPE 2

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**Introduction** CD4posCD25high regulatory T-cells (T-regs), central to immune homeostasis, are impaired in autoimmune hepatitis type 2

(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