(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