P47 CLEVER-1 MEDIATES T REGULATORY CELL RECRUITMENT VIA HEPATIC SINUSOIDAL ENDOTHELIUM BY TRANSCELLULAR MIGRATION

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S Shetty, C Weston, Y Oo, N Westerlund, Z Stamataki, J Youster, S Hubscher, M Salmi, S Jalkanen, P Lalor, D H Adams. *Liver Research Group, University of Birmingham, UK*

Introduction Lymphocytes are recruited via the unique hepatic sinusoidal channels during chronic inflammatory liver diseases. This low shear vascular bed is lined by hepatic sinusoidal endothelium (HSEC) which lacks certain conventional adhesion molecules leading us to look for novel receptors involved in lymphocytes recruitment. HSEC express several receptors found on lymphatic endothelium including the scavenger receptor CLEVER-1 which has been implicated in lymphocyte migration to lymph nodes.
Aim We now show that CLEVER-1 is upregulated on human hepatic sinusoidal endothelium where it is involved in lymphocyte trans-endothelial migration.
Method We studied the expression of CLEVER-1 in normal and diseased human liver tissue and on isolated human sinusoidal endothelial cells. We used isolated HSEC in flow adhesion assays to study the functional role of CLEVER-1 in lymphocyte subset recruitment. Immunofluorescent staining and confocal microscopy were used to characterise the transmigration of lymphocytes across HSEC under conditions of flow.
Results CLEVER-1 was expressed at high levels within the sinusoids of chronically inflamed livers and hepatocellular carcinomas as well as at other sites of lymphocyte recruitment including neo-vessels and portal associated lymphoid tissue. Flow-based adhesion assays using human HSEC demonstrated that CLEVER-1 mediates trans-migration of CD4 but not CD8T cells with strong preferential activity for FoxP3+regulatory T cells. Confocal microscopy demonstrated that a large proportion of CD4 Treg transmigrated via the transcellular route through CLEVER-1 lined channels within the endothelial cell.
Conclusion This is the first report to implicate a specific adhesion molecule in the recruitment of T regulatory cells to tissue. CLEVER-1 appears to mediate the transcellular migration of Tregs through hepatic sinsuoids and is an organ specific target for therapy aimed at modulating Treg recruitment to the liver.

P48 HAEMATOPOIETIC BUT NOT MESENCHYMAL STEM CELLS CONTRIBUTE TO THE STROMAL MICROENVIRONMENT IN CHOLANGIOCARCINOMA AND DO NOT TRANSDIFFERENTIATE INTO MALIGNANT BILE DUCTS

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A Robson, K Samuel, A Pellicoro, J Iredale, S Forbes. *Centre for Inflammation Research, University of Edinburgh, UK*

Aim Cholangiocarcinoma (CC) is characterised by a pronounced inflammatory stroma consisting of tumour associated myofibroblasts, macrophages, immune cells and a modified extracellular matrix. Furthermore, in animal models of gastric cancer, reports have suggested that mesenchymal stem cells may contribute to the epithelial compartment of malignant tumours. The treatment options for CC are very limited. Therefore, an understanding of the source of tumour-associated cells in CC will inform therapy in the future.
Method The matrix and cellular composition of the tumour niche was studied in humans and the thioacetamide (TAA) rat model of CC over 10 months. To investigate the cellular origin of the tumour and associated stroma, reconstituted wild type recipients of GFP+ bone marrow were administered TAA. Extra-hepatic derivation of cells was studied using dual immunoflourescence (GFP and CK19 (biliary epithelium), SMA (myofibroblasts), ED1, ED2 (macrophages) and MPO (neutrophils).) Persistent expression of GFP+ BM in control transplanted animals was confirmed by qPCR for Y-chromosome genomic DNA. Flow cytometry of blood, spleen and BM was performed to investigate GFP+ donor reconstitution of the haematopoietic compartment in recipient rats. BM of transplanted animals was cultured in mesenchymal stem cell (MSC) selective media and flow cytometry analysis for co-expression of stro-1 (a marker of MSC) and GFP was performed.
Results In human and rat tissue a laminin rich extracellular matrix ensheathed neoplastic cholangiocytes. The tumour cellular micro-environment comprised of myofibroblasts, migratory macrophages (CD68+) and immune cells. In transplanted rats, GFP+ expression was persistent throughout the study period and chimerism was confirmed in BM, spleen and blood. GFP+ reconstitution of the haematopoietic and mesenchymal stem cell compartments was identified. Expression of stro1+GFP+ cultured cells was similar in transplanted animals and control GFP+ animals. In tumours, macrophages (ED1, ED2) and neutrophils (MPO) were over-whelmingly GFP+, whereas myofibroblasts (SMA) did not express GFP. Additionally, benign and malignant bile ducts were GFP negative.
Conclusion A stereotypical niche forms around cholangiocarcinoma in developing and malignant lesions. The TAA rat model provides close correlation to human intrahepatic lesions with formation of a pronounced tumour microenvironment. We found no evidence of a BM-derived stem cell contribution to the epithelial component of cholangiocarcinoma. The haematopoietic but not the mesenchymal components of the tumour stroma were of BM origin.

P49 THE STROMAL COMPARTMENT OF HEPATOCELLULAR CARCINOMA PROMOTES THE LOCAL DIFFERENTIATION OF TOLEROGENIC DENDRITIC CELLS

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A Bhatt, S M Curbishley, E L Haughton, C J Weston, M Blahova, D H Adams. *Centre for Liver Research, University of Birmingham, UK*

Introduction Hepatocellular carcinoma (HCC) stimulates an immune response but this fails to destroy the tumour which contains "stunned" effector T-cells and regulatory T cells (Tregs) that suppress antigen-specific responses. The number of Tregs in the tumour predicts survival in patients undergoing resection. The underlying mechanisms that suppress anti-tumour immunity are unknown. Because dendritic cells (DC) are critical for the induction and maintenance of immune activation we studied the effect of the tumour microenvironment on DC function in patients with HCC. We hypothesise that the tumour microenvironment (TM) modifies DC differentiation resulting in the suppression of anti-tumour immunity.
Aim In order to test this hypothesis we modelled the TM in vitro and studied its effect on the differentiation and function of DCs.
Method DCs isolated from HCC tumour cores and uninvolved human liver tissue were compared with human monocyte-derived DCs (MoDC) matured in tissue conditioned media (CM). DC function was studied in T cell activation assays and the effect of tumour on DC function modelled by co-culturing with either tumour tissue or tumour-derived fibroblasts.
Results Tumour-derived DCs had a tolerogenic phenotype (MHCIIlowCD86low) compared with DC isolated from matched uninvolved liver. This phenotype was recapitulated in vitro by culturing MoDCs in HCC CM after which levels of MHC II and CD86 were significantly lower than on MoDCs matured in matched non-tumour liver CM (p=0.001). Tumour-conditioned MoDCs

induced significantly less naive T cell proliferation compared with MoDC matured in CM from uninvolved or inflamed liver tissue. Furthermore tumour-conditioned DCs generated significantly more CD4+CD25+FOXP3+ Tregs (p=0.01) and IL10producing T-cells (p=0.01). To determine the cell type responsible for this effect naive MoDCs were co-cultured with fibroblasts (-smooth muscle actin +vimentin+CD90+) isolated from either tumour cores or uninvolved liver. DCs conditioned by tumour fibroblasts developed a tolerogenic phenotype (MHCIIlowCD86low) and the ability to induce Tregs. Culturing MoDCs in tumour stroma CM had a similar effect implicating soluble factors. Part of this effect was IL-6-dependent because depletion of IL-6 from tumour-fibroblast CM abolished the ability to generate tolerogenic DC.

Conclusion Tumour associated fibroblasts in HCC contribute to an IL-6-rich TM that drives differentiation of tolerogenic DCs. These DCs generate immunosuppressive Tregs and IL-10 secreting T-cells which inhibit anti-tumour immunity. Inhibition of IL-6 or downstream STAT-3 signalling could prevent tumour-associated immunosuppression and hence be an important immunotherapeutic strategy in HCC.

P50 SYNTHETIC LETHALITY IN LIVER CANCER CELL LINES TREATED WITH INHIBITORS OF DNA DOUBLE-STRAND BREAK REPAIR

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[1]H Reeves, [1]L Cornell, [1]J Munck, [1]F Budhisetiawan, [1]D Newell, [2]J Bardos, [3]D Manas, [1]C Nicola, [1]H Reeves. *[1]School of Clinical Medical Sciences, Newcastle University, UK; [2]KuDOS Pharmaceuticals Ltd, UK; [3]Hepatopancreatobiliary Unit, Freeman Hospital, UK*

Introduction DNA double-strand breaks (DSBs) are the most cytotoxic lesions induced by ionising radiation (IR) and anticancer drugs, such as topoisomerase II poisons (eg, doxorubicin). The major DSB repair pathways are non-homologous end joining (NHEJ) and homologous recombination (HR), in which DNA-Dependent Protein Kinase (DNA-PK) and ataxia telangiectasia mutated (ATM) are key components. DNA-PK in particular is up-regulated in hepatocellular carcinoma, (GEO profiles) possibly contributing to resistance to cytotoxic therapies.

Aim To assess DNA-PK and ATM as therapeutic targets for chemo- and radio-sensitisation in hepatoma.

Method Basal protein levels and activities were determined by Western blot analysis in hepatoma cell lines. DNA-PK and ATM activity following doxorubicin stimulation was measured using antibodies specific to phosphorylated Ser-2056 DNA-PKcs and phosphorylated Ser-1981 ATM. DSB repair was measured by immunofluorescence detection of γ-H2AX foci. Cell survival was determined by clonogenic assay.

Results We demonstrated high basal levels of DNA-PK in three hepatoma cell lines (Huh7, Hep3B and HepG2), with DNA-PK activation induced by 0.25 μM doxorubicin. Despite similar DNA-PK activation, we observed differential sensitivity to doxorubicin (7%, 49% and 75% survival at 10 nM doxorubicin in Huh7, Hep3B and HepG2, respectively). HepG2 cells with the greatest resistance to doxorubicin displayed a 10-fold activation of ATM relative to the other cell lines. The DNA-PK inhibitor NU7441, increased doxorubicin and ionising radiation (IR) induced cytotoxicity in all cell lines (1.3 up to fourfold), correlating with a reduction in DSB repair measured by γ-H2AX foci. Importantly, in doxorubicin resistant HepG2 cells, while incubation with NU7441 or the ATM inhibitor (KU55933) alone, had minimal effects on cell survival (91% and 86%, respectively), their combination in the absence of a cytotoxic agent markedly inhibited cell survival (21%; p<0.001, ANOVA). The addition of 10 nM doxorubicin reduced survival to less than 5% of colonies.

Conclusion These findings support the clinical application of DNA-PK and ATM inhibitors as chemo- and radio-sensitisors in hepatoma patients. Furthermore, these data suggest that hepatoma cell survival is dependent on up-regulation of DSB repair, effected by either DNA-PK or ATM, and that inhibition of both induces synthetic lethality—preventing DSB repair by both NHEJ and HR. The therapeutic implication is that in combination, these agents could be used to specifically induce cancer cell death, with minimal toxicity to surrounding liver tissues.

P51 INADEQUATE COMPENSATION BY GLUTAMINE SYNTHETASE AND INCREASED GLUTAMINASE ACTIVITY CONTRIBUTES TO HYPERAMMONAEMIA IN CIRRHOSIS

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[1]M Jover, [1]L Noiret, [1]A Habtesion, [1]V Balasubramaniyan, [1]Y Sharifi, [2]M Romero-Gomez, [1]N Davies, [1]R Jalan. *[1]Institute of Hepatology, University College London, UK; [2]University Hospital Valme, Seville, Spain*

Introduction In cirrhosis, the function of the urea cycle is compromised which leads to accumulation of ammonia. In this situation, ammonia metabolism is regulated by glutamine synthetase (GS) and glutaminase (GA) making them important therapeutic targets. The relative contributions of these enzymes in the different organs in regulating ammonia metabolism in cirrhosis are unclear.

Aim To study the protein expression and activity of glutamine synthetase (GS) and glutaminase (GA) enzymes in the different organs in a model of chronic liver disease (bile duct ligation: BDL).

Method Ten male Sprague—Dawley rats were studied (260.7±10.57) g: 4 sham operated, and 6 following bile duct ligation (BDL). We measured plasma levels for: ammonia and standard biochemical markers. Expression of GS and GA were determined by Western-blotting (described as % of sham expression) and activity by end point methods in liver, kidney, gut, muscle, lung and frontal cortex (brain).

Results Plasma ammonia was increased in BDL rats vs. Sham (45.97±14.72 vs 106.2±59.10) μmol/l). The most important organs for GS activity were the liver > lung = frontal cortex > muscle > kidney = gut. In cirrhosis, liver GS activity is reduced by 7 fold (62.61±8.29 SHAM vs 8.98±2.67* BDL). The most important organs for GA function in disease were: lung (0.70±1.4 SHAM vs 4.19±2.24* BDL) > kidney (1.24±0.09 SHAM vs 1.68±0.58* BDL) > gut (0.43±0.14 SHAM vs 1.14±0.51* BDL) (activities expressed as mIU/mg protein; *P<0.05).

Gut Liver Kidney Muscle Lung Frontal cortex Brain SHAM GS (0.78+0.67) (62.61+8.29) (0.87+1.24) (1.75+0.48) (2.98+4.26) (2.74+1.14) GA (0.43+0.14) (1.84+0.58) (1.24+0.09) (0.37+0.14) (0.70+1.4) (0.61+0.30) BDL GS (0.84+0.84) (8.98+2.67)* (0.86+0.78) (1.92+0.63) (2.15+3.14) (3.22+0.35) GA (1.14+0.51)* (0.52+0.16)* (1.68+0.58)* (0.38+0.11) (4.19+2.24)* (0.63+0.20).

Conclusion Inadequate compensation by GS and increased GA activity account for hyperammonemia observed in cirrhosis. For the first time, these data indicate the importance of the lung in regulating ammonia metabolism through GS and also GA, activities of both of which are increased in cirrhosis. In order to reduce ammonia levels in cirrhosis, it would be advantageous for novel drugs to target GS stimulation and Glutaminase inhibition simultaneously.

P52 NEUTROPHIL DYSFUNCTION: A POTENTIAL BIOMARKER OF POOR PROGNOSIS IN ACUTE LIVER FAILURE?

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N Taylor, A Nishtala, F Lin, R D Abeles, W Bernal, J Wendon, Y Ma, D Shawcross. *Institute of Liver Studies, King's College Hospital, UK*

Introduction In acute liver failure (ALF) an exaggerated systemic inflammatory response can result in neutrophil activation with