

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

doi:10.1136/gut.2010.223362.73

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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 lymphocyte 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 transmigration 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 sinusoids 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

doi:10.1136/gut.2010.223362.74

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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 immunofluorescence (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 overwhelmingly 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

doi:10.1136/gut.2010.223362.75

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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 (MHCII^{low}CD86^{low}) 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