controls (n=8). All patients were treatment naïve and had stable disease. FACS analysis was performed on both cell surface antibody staining using a panel of activation/exhaustion markers (CD25, CD38, CD69, HLA-DR, PD-1, CD8b) and intracellular cytokine staining following PMA/ionomycin stimulation. CD8b^{high} T cells were freshly isolated from health controls and sorted using magnetic beads, prior to culture in a variety of conditions and subsequent FACS analysis.

Results In chronic HBV infection, regardless of eAg status, CD161-CD8b^{low} T cells express significantly lower CD28 (eAg+ve p=0.005, eAg−ve p=0.0001) and significantly higher HLA-DR (eAg+ve p=0.002, eAg−ve p=0.009) than their CD161-CD8b^{high} counterparts. The CD161-CD8b^{low}CD28^{low}HLA-DR^{high} population represents a mean of 38% of total CD8 T cells in chronic HBV. The CD161-CD8b^{low}CD28^{low}HLA-DR^{high} population produce significantly more IFN-g on stimulation with PMA/ionomycin than their CD8b^{high}CD28^{high}HLA-DR^{high} counterparts (p<0.0001). No difference was observed in IL-2 production. In addition we are able to demonstrate ready transition of sorted CD161-CD8b^{high} to CD161-CD8b^{low} T cells from healthy controls cultured overnight in culture media alone, which provides an insight into the potential mechanisms involved in the generation of this population and an in-vitro model for use in further study.

Conclusion CD161-CD8b^{low}CD28^{low}HLA-DR^{high} T cells are a functionally distinct, prominent T cell population in chronic HBV infection that are likely to profoundly influence the immune environment with important implications for the development of immunotherapy and treatment. The absence of this T cell subset in chronic HCV indicates a disease-specific rather than liver-related effect.

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Basic science

OP14   IDENTIFICATION AND CHARACTERISATION OF A NOVEL LY-6C^{INTERMEDIATE} INTRAHEPATIC MACROPHAGE POPULATION WHICH MEDIATES THE RESOLUTION OF LIVER FIBROSIS, IS INDUCED BY PHAGOCYTOSIS AND CAN BE MANIPULATED THERAPEUTICALLY IN VIVO

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Introduction Macrophages are critical for the progression and resolution of hepatic fibrosis. Studies have identified Ly-6C^{hi} macrophages as the pro-fibrogenic subset in mice. However, the identity of the pro-resolution hepatic macrophage population is unknown.

Aim We aimed to identify and characterise the macrophage population mediating the resolution of hepatic fibrosis.

Method We established a model of reversible murine hepatic fibrosis by administering 4 weeks of CCl$_4$, followed by tissue harvests at serial timepoints after the final dose.

Results Histological analysis identified maximal fibrosis resolution between 72 and 96 h after the final CCl$_4$ dose. Flow cytometry of hepatic macrophages showed that during maximal fibrosis resolution there was a loss of pro-fibrotic Ly-6C^{hi} macrophages and large increase in a Ly-6C^{intermediate} macrophage population, which were the most numerous macrophage subset identified at any timepoint during fibrogenesis and recovery. Using CD11B-DTR mice, macrophages were depleted during the rapid resolution phase, resulting in a failure to remodel the hepatic scar. Critically, this depletion strategy selectively ablated the Ly-6C^{int} subset, the degree of depletion correlating significantly with the amount of persistent fibrosis.

A series of bone marrow transplantation, adoptive transfer and in situ labelling experiments identified that the pro-resolution Ly-6C^{int} macrophage population derives from recruitment of Ly-6C^{hi} monocytes, a common origin to the pro-fibrotic Ly-6C^{hi} macrophages, indicative of a phenotypic switch in situ.

Microarray profiling of FACS sorted Ly-6C^{int} macrophages in comparison to pro-fibrotic Ly-6C^{hi} macrophages, demonstrated a novel phenotype outside the M1/M2 macrophage paradigm, with down regulation of pro-inflammatory and pro-fibrotic genes, upregulation of matrix degrading enzymes and enrichment for phagocytosis related pathways. Confocal microscopy indicated that the Ly-6C^{int} population contained more intracellular apoptotic debris, confirming the post-phagocytic nature of these cells.

Feeding primary murine macrophages with hepatocyte debris in vitro induced a similar phenotypic switch to that seen in vivo. Furthermore, this phagocytosis-induced switch could be modelled by feeding macrophages with liposomes in vitro. Critically, systemic administration of liposomes to mice during maximal fibrosis resolution increased the number of hepatic Ly-6C^{int} macrophages and accelerated the resolution of fibrosis.

Conclusion In summary, we have identified the specific Ly-6C^{int} macrophage subset which mediates the resolution of hepatic fibrosis. Extensive characterisation demonstrated this macrophage phenotype is produced by the phagocytosis of dead cells and thus can be manipulated in vivo by the induction of phagocytic behaviour with a beneficial effect on fibrosis resolution.

OP15   DISCOVERY OF NEW LIVER FIBROSIS MARKERS IN HEPATITIS C PATIENTS USING PROTEOMICS

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Introduction Liver biopsy is the reference standard for assessing liver fibrosis, and no reliable non-invasive diagnostic approach is available to discriminate between the intermediate stages of fibrosis. Therefore suitable serological biomarkers of liver fibrosis are urgently needed.

Aim The objective of this study was to use proteomics to identify novel fibrosis biomarkers in hepatitis C patients with different degrees of liver fibrosis.

Method Proteins in plasma samples from healthy control individuals and patients with hepatitis C virus (HCV) induced cirrhosis were analysed using a proteomics technique—two dimensional gel electrophoresis (2-DE). This technique separated the proteins in plasma samples of control and cirrhotic patients and by visualising the separated proteins we were able to identify proteins which were increasing or decreasing in hepatic cirrhosis. Identified markers were validated across all Ishak stages and compared to the markers used in FibroTest, Enhanced Liver Fibrosis (ELF) test, Hepascore and FIBROSpect by western blotting.

Results 44 candidate biomarkers for hepatic fibrosis were identified of which 20 were novel. western blot validation of all candidate markers using plasma samples from patients across all Ishak fibrosis scores showed that the markers which changed with increasing fibrosis most consistently included lipid transfer inhibitor protein, complement C3d, corticosteroid-binding globulin, apolipoprotein J and apolipoprotein L1. These five novel markers which are secreted in blood showed a promising consistent change with increasing fibrosis stage when compared to the markers used for the FibroTest, ELF test, Hepascore and FIBROSpect.

Conclusion This study identifies 20 novel fibrosis biomarker candidates. The proteins identified by these improved approaches may help to assess hepatic fibrosis and eliminate the need for invasive liver biopsies.