Conclusion In conclusion, we demonstrate for the first time global alterations in cellular expression of glucose and lipid transporter proteins in human NAFLD. We confirm that VAP-1 is elevated in disease and that SSAO activity of VAP-1 results in enhanced hepatic lipid and glucose uptake and changes in transporter expression. Thus we propose that bioactive metabolites of SSAO activity contribute to the metabolic derangement evident in fatty liver disease.

P89 OSTEOPONTIN PROMOTES LYMPHOCYTE RECRUITMENT IN STEATOHEPATITIS

doi:10.1136/gutjnl-2011-300857a.89

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Introduction Steatohepatitis is the critical step in the progression to fibrosis, and is characterised by increased inflammatory cell recruitment from the circulation. The cytokine Osteopontin (OPN) is intricately involved in cell-recruitment and tissue-repair, and we reported that OPN is significantly upregulated during non-alcoholic steatohepatitis (NASH).

Aim Thus, we hypothesised that OPN promotes steatohepatitis by supporting leucocyte migration across hepatic sinusoidal endothelium.

Method Wild-type mice were fed chow or methionine-choline deficient (MCD) diet to induce NASH. After 4 weeks, mice were sacrificed; severity of disease assessed by serum aminotransferase (AST), hepatic OPN quantified by QRTPCR and immunohistochemistry, serum OPN measured by ELISA. In separate experiments, MCD-fed mice were treated with anti-OPN or IgG, and flow cytometry used to quantify numbers of liver infiltrating lymphocytes (LIL). Primary human hepatic sinusoidal endothelial cells (HSEC) were stimulated with recombinant (r) OPN (0–1000 ng/ml), and expression of adhesion molecules (ICAM-1, VCAM-1, CD31) quantified by western blot. To assess lymphocyte transendothelial migration, lymphocytes were perfused over rOPN- or vehicle-treated-HSEC, with or without TNFa (20 ng/ml) + IFNa (100 ng/ml). In separate experiments, TNFa+IFNa stimulated-HSEC were treated with sham or OPN-aptamers and total lymphocyte adhesion recorded. Human livers with NASH were immunostained for OPN, and FACS used to quantify LIL isolated from control or NASH-cirrhotic patients.

Results In mice, diet-induced NASH upregulated expression of hepatic OPN by threefold ($p<0.05$), serum OPN by twofold ($p<0.05$), and increased intrahepatic CD4 by 2.2-fold, CD8 by 4.5-fold, and NKT cells by 3.2-fold ($p<0.05$). MCD-fed mice treated with anti-OPN accumulated fewer CD3, CD4, CD8 and NKT cells ($p<0.05$), and exhibited attenuated injury (ALT: threefold reduction; $p<0.02$). rOPN induced expression of ICAM-1, VCAM-1 and CD31 on human HSEC, enhanced lymphocyte recruitment under conditions of flow (41%), and amplified recruitment capacity of TNFα +IFNγ stimulated HSEC (23%), while OPN neutralisation with RNA-aptamers reduced lymphocyte recruitment by 50% (all $p<0.05$). In humans, expression of OPN was significantly upregulated in NASH; livers from NASH-cirrhosis harboured twofold more CD4 and threefold more CD8 and NKT cells ($p<0.05$) than normal.

Conclusion OPN is upregulated during steatohepatitis in mice and humans, and promotes lymphocyte recruitment across HSEC. Neutralisation of OPN significantly reduces lymphocyte recruitment and liver injury. Our results suggest that OPN is a promising anti-inflammatory target in steatohepatitis.

P90 α-1 ANTITRYPSIN (A1AT) POLYMERS CAUSE EXTREME HEPATOCYTE AGEING

doi:10.1136/gutjnl-2011-300857a.90

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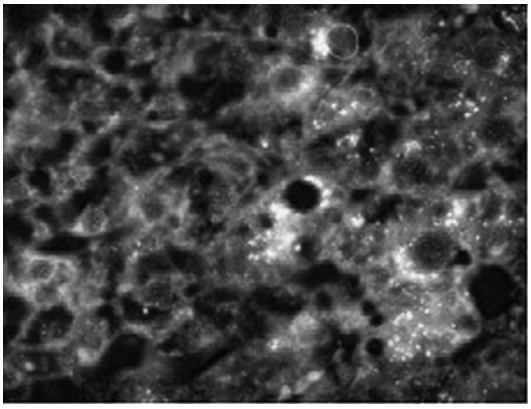
Introduction a1AT, synthesised predominantly in the liver, is the archetypal inhibitor of the serpin protein family. a1AT deficiency is a common inherited disorder; Glu342Lys substitution causes abnormal folding of mutant protein, which may polymerise and aggregate in the endoplasmic reticulum. a1AT aggregates are the histological hallmark of a1AT-related liver disease but it is unclear how aggregates induce liver injury.

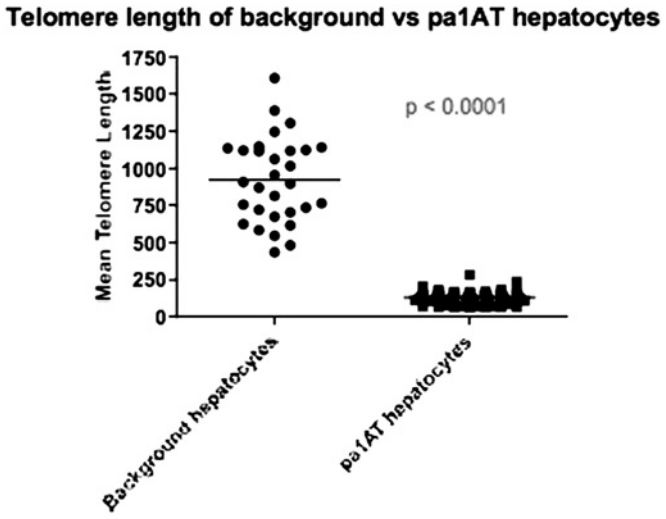
Aim To determine whether hepatocytes containing polymerised-a1AT (pa1AT) had accelerated ageing manifest as shortened telomeres.

Method Liver biopsy sections were studied from 60 patients with a1AT- related liver disease with a broad spectrum of fibrosis, recruited from the Cambridge metabolic liver clinic (30 were homozygous and 30 heterozygous). Comparison was made with sections from 20 age and sex matched time zero biopsies obtained at liver transplant. Mean hepatocyte telomere length, a reflection of age, was measured by quantitative fluorescent in situ hybridisation (QFISH) with a PNA Cy5 probe. Nuclei were identified with DAPI, hepatocytes with antibody against hepar-1 and pa1AT with specific mouse monoclonal antibody (2C1). Images were obtained and analysed using the Olympus ScanR software system (Abstract P90 figure 1). Statistical analysis used Graph Pad Prism.

Results Hepatocyte nuclei were larger in patients with both homozygous and heterozygous a1AT deficiency ($p=0.002$) and had shorter telomeres ($p<0.0001$) than age and sex matched controls. Homozygous patients had shorter hepatocyte telomeres than heterozygous patients ($p=0.003$). Hepatocyte nuclei in both homozygous and heterozygous a1AT deficiency were larger in cells with pa1AT compared to neighbouring cells without pa1AT ($p=0.002$). Hepatocyte telomeres were far shorter in cells that contained pa1AT than neighbouring hepatocytes without pa1AT ($p<0.0001$, Abstract P90 figure 2). Hepatocytes with pa1AT showed additional telomere shortening with increased age ($p=0.0002$). Fibrosis stage was related to telomere shortening- telomeres shortened as the stage of fibrosis increased.



Abstract P90 Figure 1 QFISH image which highlights the a1AT polymers (shown as bright white speckles), surrounding some of the affected hepatocytes.



Abstract P90 Figure 2 Hepatocyte telomere length in patients with a1AT deficiency with or without polymerised a1AT.

Conclusion Senescence, characterised by increased nuclear size and shortened telomeres, was present in hepatocytes from patients with both homozygous and heterozygous a1AT related liver disease. The novel 2C1 antibody showed that these markers of senescence were even more marked in cells expressing pa1AT. The steps between pa1AT expression and accelerated senescence are a clear target for therapeutic intervention.

P91 DUAL MECHANISM OF NK CELL INHIBITION BY THE HCV CORE 35–44 PEPTIDE

doi:10.1136/gutjnl-2011-300857a.91

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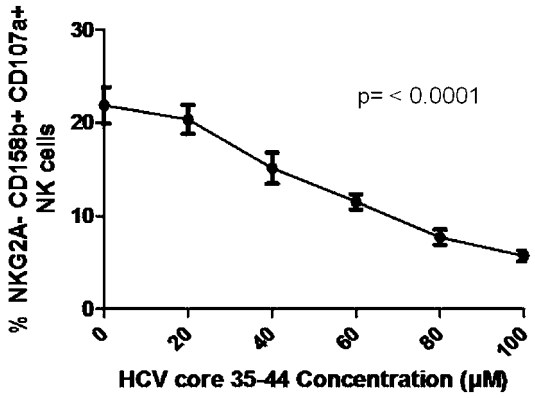
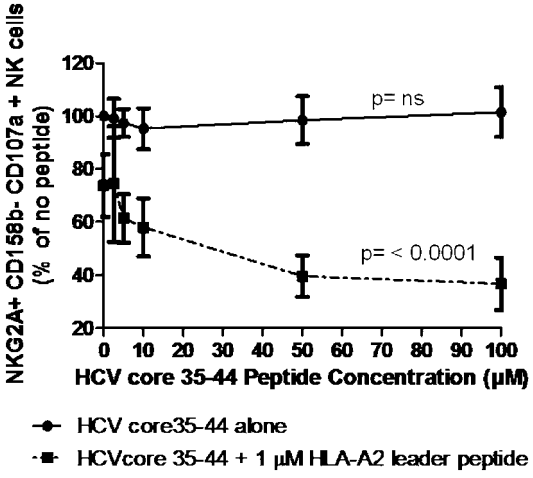
Introduction Disease association studies have shown that the inhibitory killer cell immunoglobulin receptor KIR2DL3 and its HLA-C1 allotype ligands are protective against chronic HCV infection. A number of studies have shown increased expression of another inhibitory natural killer (NK) cell receptor, CD94:NKG2A, in chronic HCV infection. The ligand for this receptor, HLA-E, usually binds to leader peptides derived from other HLA class I molecules. Up-regulation of HLA-E with concomitant inhibition of NK cells has been thought to be induced by peptides derived from several viruses including one derived from HCV (HCV $core_{35–44}$) which thus represents a mechanism of immune escape.

Method To determine the effects of viral peptides on NK cell inhibition, we pulsed the TAP-deficient 721.174 cell line with host and virus derived peptides and used these as target cells in flow cytometry assays of NK cell degranulation (measured by CD107a expression).

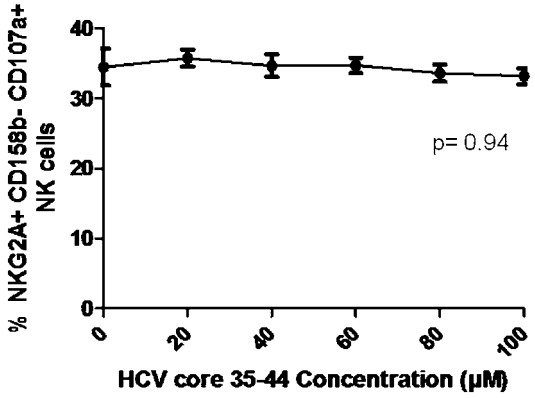
Results In functional experiments HCV $core_{35–44}$ inhibited degranulation of the total NK cell population. Unexpectedly, this was due to inhibition of KIR2DL2/3-positive NK cells (one way ANOVA $p<0.0001$), but not NKG2A-positive NK cells ($p=0.94$). However, HCV $core_{35–44}$ peptide increased cell surface expression of both HLA-C and HLA-E. To rationalise this with the previous findings we performed peptide mix experiments using the natural peptide ligands for HLA-E. Such peptides, derived from the leader sequence of host HLA class I molecules, efficiently inhibited NKG2A-positive NK cells. In the presence of a low concentration of a host HLA class I leader sequence peptide, HCV $core_{35–44}$ had a synergistic effect in suppressing NK cell activity ($p<0.0001$). A synergistic effect at CD94:NKG2A was also demonstrable for peptides derived from EBV and HIV viruses, which in isolation did not inhibit NK cells, implying that this is a general mechanism for NK cell inhibition.



Abstract P91 Figure 2 HCV core 35–44 alone does not inhibit via CD94:NKG2A.



Abstract P91 Figure 1 HCVcore 35–44 induced inhibition of KIR2DL2/3 (CD158b) + NK cells.



Abstract P91 Figure 3 However, in the presence of a host HLA (HLA-A2) derived leader peptide, HCV 35–44 induces synergistic inhibition at CD94:NKG2A.