directive information appearing on virology result slips; the vast majority of patients found to be hepatitis B carriers in the community are not referred for appropriate follow-up. In-hospital referral rates are significantly better. This is consistent with a concerning survey of London GPs regarding knowledge of hepatitis and indications for referral (Taylor *et al Gut* 2009;**59**(Suppl1):PTU-072) and indicates the need for improving education in this area.

P74 CD161 EXPRESSING CD8+ T-CELLS; ELUSIVE PLAYERS IN VIRAL HEPATITIS

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Hepatitis B (HBV) and C (HCV) -specific CD8+ T-cells are characterised by expression of the NK receptor CD161. CD8+ T cells with high levels of CD161 (CD161++) make up a mean of 12% of CD8+ T-cells in healthy controls and have distinct properties; they express the gut and liver homing chemokine receptors CCR6 and CXCR6, cytokines IL-17, IFN-y and IL-22 and have narrow TCR Vb usage (predominantly Vb 7.2 and 13), linking them to the mucosal-associated invariant T-cells of the gut. In healthy controls ~40% of this CD161++ subset does not express the co-receptor CD8 alpha-beta (CD8ab), but the co-repressor CD8 alpha-alpha (CD8aa), yet share key functional and phenotypic features of the subset.

Aim We aimed to the study the distribution and phenotype of CD8ab and CD8aa subsets in chronic hepatitis C (cHCV) and hepatitis B (cHBV).

Method Flurochrome-labelled antibodies were used for multi-colour FACS analysis of lymphocytes in whole blood from 24 cHCV, 6 e-antigen (Ag) +ve HBV and 14 eAg−ve HBV patients and 19 healthy controls (HC). Liver infiltrating lymphocytes (LILs) (obtained from explant material; 4 HCV patients with paired PBMCs, eight alcoholic liver disease and 1 PBC) were included in the study. FACS data were analysed using FloJo software (Tree Star, Inc) and statistics were performed using PRISM (Graftpad software, Inc).

Results CD8aa cells are exclusive to the CD161++ subset in HCs, cHCV and cHBV. In cHCV and eAg−ve cHBV there is a significant reduction in the proportion of cells in the CD161++CD8+ subset compared to HCs ($p \leq 0.05$). Within the CD161++CD8+ subset there is a further reduction in the fraction of CD8aa cells in cHCV patients (18.5% vs 34.13%, p=0.0086) compared to HCs. No difference is observed in cHBV. CD8ab and CD8aa CD161+ populations are found within human LILs in HCV, ALD and PBC. The CD161+CD8aa cell subset constitute a mean of 9.9% of the total CD8+ LILs. Relative enrichment of CD161+CD8aa cells is seen in the liver of patients with cHCV compared to peripheral blood (p=0.0079). In eAg−ve cHBV a distinct CD8a+blow population can be identified within the CD161+ and CD161− subsets. These populations are not seen in HCs, eAg+ve HBV and HCV ($p<0.05$).

Conclusion CD161++ CD8+ T-cells are lost from the peripheral blood in cHCV and eAg−ve HBV. Maintenance of this subset in eAg +ve HBV may reflect immuno-tolerance to virus at this stage of infection. In chronic HCV there is a relative enrichment of the CD161+CD8+ subset in LILs, indicating recruitment to and retention in the liver. The role of these cells in health, immunity and disease outcome in viral hepatitis requires further study. The emergence of a CD161+/CD161-CD8a+blow subset in eAg−ve HBV may reflect activation or exhaustion of these cells; their phenotype and function requires investigation.

P75 INFLUENCE OF VITAMIN D SUPPLEMENTATION ON OUTCOME IN THE TREATMENT OF CHRONIC HEPATITIS C

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Introduction Vitamin D, acting as an immune modulator, has recently been shown to increase the sustained virological response (SVR) in genotype 1 patients.

Aim To retrospectively examine the outcome of patients treated in our institution using pegylated interferon and ribavirin, and compare the effects of treatment with a Vitamin D preparation.

Method All patients in our treatment database who have received treatment for Hepatitis C using Pegylated Interferon were identified. Only those patients who were greater than 6 months post treatment were included. Data collected included genotype, fibrosis score (Ishak) and if they were prescribed Vitamin D preparations, The primary outcome was to attain a SVR, defined as persistently negative HCV PCR status 6 months after cessation of anti viral therapy.

Results Data were available for 206 patients treated over a 3 year period. Total SVR by genotype was as follows, Genotype 1−39 % (n=44), Genotype 2−71 % (n=8), Genotype 3−72% (n=151) and Genotype 4−100% (n=3).

27.5 % (n=57) of our patients received Vitamin D supplementation with Calcichew D3 Forte (Shire Pharmaceuticals, Hampshire, UK) during the course of treatment, an observed SVR rate of 72 % was seen in those receiving supplementation compared to 64 % in those not supplemented (p=0.281).

When examining patients by genotype, no patients with genotype 1 received Vitamin D therapy. Of Genotype two patients 25 % (n=2) were treated with Vitamin D, achieving a 50 % SVR compared to 83.3% for those not treated with Vitamin D. 34 % of Genotype 3 patients received Vitamin D (n=52) achieving an SVR in 77% of cases, compared to those who did not receive supplementation (n=99) with an SVR of 71% (p=0.414).

From the subset of genotype three patients, the SVR for fibrosis scores <4 and 5/6 were 78% and 53 % respectively. When these groups were analysed considering Vitamin D supplementation those with fibrosis scores of <4 receiving supplementation achieved an SVR of 87% compared to 74% in those not (p=0.183). Patients with fibrosis scores of 5/6 achieved an SVR of 53 % in both supplemented and non-supplemented groups.

Outcomes were also analysed using fibrosis scores, as expected those with less significant fibrosis achieved SVR more frequently, no significant differences were detected when the data were analysed using treatment with Vitamin D as a variable.

Conclusion Our data show that Vitamin D supplementation could improve the SVR in Genotype three patients with mild/moderate fibrosis, this has not been reported so far. We suggest routine testing of vitamin D levels prior to combination therapy and replacement during treatment for chronic hepatitis C.

P76 LENTIVIRAL VECTORS CO-EXPRESSING HEPATITIS B CORE AND VFLIP INDUCE POTENT CD8 T-CELL AND ANTIBODY RESPONSES IN HLA-A2 TRANSGENIC MICE

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Introduction The failure to clear persistent Hepatitis B viral (HBV) infection is characterised by an insufficient CD8 T-cell response to

several antigens including HBV core (HBc). Generating a strong T-cell response to this antigen to counter immunotolerant mechanisms in chronic HBV is an attractive therapeutic strategy. We have previously shown that lentiviral vaccines (LV) encoding antigen can generate potent CD8 and CD4 responses and these can be dramatically enhanced by co-expression of viral FLICE-like inhibitory protein (vFLIP) from Kaposi sarcoma-associated herpes virus. vFLIP is a potent stimulator of the NFKB pathway which matures and activates dendritic cells, enhancing expression of costimulatory molecules (including CD80, CD86 and ICAM1) and increasing IL-12 secretion.

Aim In this study we aimed to assess CD8 T-cell and antibody responses in HLA-A2 transgenic mice vaccinated with LV co-expressing vFLIP and HBc (A), expressing HBc alone (B) or HBc with an inactive vFLIP mutant (vFLIPa57l) (C).

Method HLA-A2 transgenic mice were vaccinated with LV 12 days before sacrifice and harvest of splenocytes. These were restimulated overnight with an HLA-A2 restricted HBc peptide (18—27) and/or overlapping HBc peptides. IFN? responses were measured by intracellular cytokine staining and by ELIspot. Antibody responses were assessed by ELISA on serum obtained at sacrifice.

Results Vaccination with LV co-expressing vFLIP and HBc (A) results in enhanced CD8-T-cell responses compared with mice vaccinated with LV expressing HBc alone (B) or HBc with an inactive vFLIP mutant (vFLIPa57l) (C). This was demonstrated on intracellular cytokine staining for IFN? of CD8+ve splenocytes re-stimulated overnight with HLA-A2 restricted peptide HBc 18—27 (A:B:C=5.35% :1.44% :0.84%). IFN? Elispot demonstrated twofold greater CD8 T-cell responses after restimulation with overlapping HBc peptides in splenocytes from mice vaccinated 12 days previously with LV co-expressing vFLIP and HBc compared with mice vaccinated with LV encoding HBc alone (p=0.006). LV expressing HBc also generated a strong antibody response comparable to vaccination with recombinant protein HBc virus-like particles (VLP). This was despite the presumed endogenous HBc antigen expression in transduced cells and no known mechanism of HBc secretion. LV encoding a mutant HBc p138g which does not multimerise into VLPs failed to raise an antibody response.

Conclusion LV encoding HBc are a potential means of generating both therapeutic CD8 T-cell and antibody responses in chronic HBV. Our data suggest that an intact VLP structure is essential for the generation of an antibody responses to HBc delivered with a lentiviral platform. We are now using this system to explore potential synergy between T-cell and B-cell responses to HBc in HBV infection.

P77 AN ANTI-VIRAL ROLE FOR CD4+ T CELLS IS OBSERVED IN ONLY A MINORITY OF PATIENTS SUCCESSFULLY TREATED FOR HEPATITIS C VIRUS

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Introduction CD4+ T cells are thought to play an important role in the control and elimination of non-cytopathic viral infections such as HCV. However their role in patients being treated with type I IFNs is not clear.

Aim To assess the role of HCV-specific CD4+ T cells in patients being treated for HCV infection.

Method 33 consecutive viraemic patients undergoing treatment had ex vivo (IFNg-producing) and cultured anti-viral CD4+ T cells intensively measured at multiple time points along with viral loads, alanine transaminase and serum cytokines (IL-2, -4, -5, -6, IL-10, TNFa and IFNg).

Results The patients could be divided almost equally into four groups depending on long term virus eradication or treatment failure and the magnitude of CD4+ cell responses: group 1: treatment failure, group 2—4 treatment success with group 2: no T cell responses, group 3: extremely weak transient T cell responses and group 4: strong robust responses. Early proliferation but not an ex vivo response was associated with rapid (k1 > 2 day-1) initial viral clearance and patients with robust early proliferation demonstrated reduced serum IL-10 compared to group 1 (p<0.0002). However the majority of successfully treated patients (groups 2 and 3) demonstrated variable rate of viral clearance, increased levels of IL-10 and a paucity of CD4+ T cell responses.

Conclusion Anti-viral CD4+ T cells may only have a role in a selected small group of patients in controlling viraemia, and in most patients the mechanisms of viral elimination awaits further studies.

P78 PREVALENCE OF VIRAL HEPATITIS IN PATIENTS UNDERGOING ANTI-TUBERCULOSIS THERAPY IN WEST LONDON

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Introduction Tuberculosis (TB) is prevalent in over a third of the world's population, with Asia (31%) and Africa (55%) accounting for most cases. The rising UK incidence of TB is partly due to increasing immigration from these regions. The UK prevalence of Hepatitis B virus (HBV) is estimated at 0.1% and the prevalence of Hepatitis C Virus (HCV) at 0.4%. HBV & HCV are treatable, but are largely asymptomatic until advanced liver disease has occurred. Therefore, HBV and HCV screening are recommended in high risk groups. Patients with TB do not currently undergo routine screening for these viruses, but are offered screening for HIV. HBV & HCV share similar epidemiological hotspots with TB and studies from Asia suggest HBV/HCV are significantly associated with TB infection and Drug-Induced Liver Injury (DILI) from anti-TB therapy. Currently, no studies have investigated the prevalence of viral hepatitis in TB patients in Western Europe, and the risk this poses to DILI.

Aim To assess (1) the prevalence of viral hepatitis in patients undergoing anti-TB therapy in West London; (2) if patients with serological evidence of viral hepatitis are at increased risk of DILI.

Method This was a prospective study of 245 newly diagnosed active (n=167) and latent (n=78) TB patients embarking on anti-TB therapy. Liver Function Tests (LFTs) were performed prior to & 2 weeks after initiation of anti-TB therapy. Patients were offered both HIV and viral hepatitis screening. All patients were tested for viral markers, including HBsAg, HBeAg, HBcAg, anti-HBc, seropositivity to HCV, and to HIV. DILI was defined as ALT elevated twice above the upper limit of normal (2x >ULN (40 IU/L)) any time following normal pre-treatment LFTs.

Results 149 (61%) TB patients were from the Asian Subcontinent and Sub-Saharan Africa, while only 15 (6%) were from the UK, mimicking global TB prevalence. 49 (20%) patients had serological markers for HBV or HCV, of whom 7 (3%) were HBsAg positive (one patient was Asian, two South East Asian and four Sub-Saharan African). 37 patients (15%) had isolated antibody to HBcAg. Five (2.0%) patients were HCV positive (one Asian, two Sub-Saharan African and two UK Caucasian). 17% of those with viral markers had raised pre-treatment ALT. 2.6% of patients tested for HIV were positive for viral hepatitis. 18% of active TB patients and 23% of latent TB patients had markers for viral hepatitis. Ten (5.4%) patients were diagnosed with DILI, of whom only 1 (0.5%) had