P64 HEPATITIS B VIRUS UPREGULATES HEPATOCYTE EXPRESSION OF PD-L1 TO EVADE HEPATOXIC ADAPTIVE IMMUNE RESPONSES

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Introduction Hepatitis B virus employs a variety of strategies aimed at overwhelming, evading or neutralising the host immune response to infection resulting in chronicity. We have previously shown that the Programmed Cell Death Pathway (PD-1/PD-L1) is an inhibitory T-cell pathway implicated in the homeostasis of immune responses and the balance between cytolytic and non-cytolytic CD8+ T-cell effector functions.
Aim The aim of this study was to investigate the impact of hepatitis B virus (HBV) infection on hepatocytic PD-L1 expression.
Method A human hepatoma cell line that constitutively expresses HBV-DNA (HepG2215), its parent cell line (HepG2) were cultured. A human hepatoma cell line (Huh7) was transfected with a plasmid containing an HBV head-to-tail dimer using Fugene 6 reagent. We also cultured a further HepG2 cell line (AD38) that produces full infectious virus under the control of a tetracycline (Tet)-responsive promoter. HBV-DNA and PD-L1 were quantitated longitudinally. Intracellular and secreted HBV-DNA was quantified with qRT-PCR. PD-1/PD-L1 expression was assessed by FACS and qRT-PCR. Co-cultures between virus-specific CD8+ T-cell lines and hepatocytes producing HBV were also established and analysis of T cell functions performed.
Results The hepatoma cell lines which constitutively produce HBV virions (HepG2215) had significantly higher basal levels of PD-L1 expression compared with their parent cell line (HepG2) ($p=0.01$). A significant increase in intracellular and secreted HBV-DNA levels confirmed successful transfection of Hepatitis B virus. Following transfection there was a significant increase in PD-L1 levels ($p=0.01$) on infected hepatocytes, which was not observed following transfection with an empty vector. A significant correlation was observed between PD-L1 expression and both intracellular HBV-DNA ($r=0.98$, $p=0.01$) and secreted HBV-DNA ($r=0.908$, $p=0.046$) following transfection. Following activation of HBV-DNA expression in the AD38 cell line (-Tet), PD-L1 expression increased. Moreover, subsequent fluctuations in HBV-DNA in the absence/presence of Tet was temporally associated with the expression of PD-L1 ($r=0.83$, $p<0.001$). Hyperexpression of PD-L1 on hepatocytes was associated with a predominance of non-cytolytic T cell functions.
Conclusion These results demonstrate that HBV-DNA drives PD-L1 expression on infected hepatocytes. As we have previously demonstrated, upregulation of PD-L1 impairs adaptive immune responses to HBV infection, and this novel function of HBV may reflect an important strategy by which hepatitis B virus extends the life-span of target hepatocytes and escapes an effective immune response contributing to the development of chronicity.

P65 FAILURE TO CONTROL HEPATITIS B VIRUS REPLICATION DESPITE CONTROL OF HUMAN IMMUNODEFICIENCY VIRUS REPLICATION IN CO-INFECTED PATIENTS ON TENOFOVIR-CONTAINING ART REGIMEN CAUSE FOR CONCERN IN HEPATITIS B VIRUS MONOINFECTION?

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Introduction Cross-resistance between human immunodeficiency virus (HIV) and hepatitis B virus (HBV) can play an important role in failure to control HBV replication in co-infected individuals resulting in suboptimal control of HBV. Tenofovir (TDF) based therapy should be effective for suppression of both HIV and HBV replication.
Aim To evaluate HBV factors in HBV/HIV co-infected subjects with undetectable HIV and persistently detectable HBV following 48 weeks of TDF based anti-retroviral therapy (ART).
Method Our prospectively maintained clinical database was interrogated. 113 HIV/HBV coinfected patients were identified; 14 (12.4%) patients had detectable HBV DNA (but undetectable HIV RNA) after 48 weeks of TDF based ART. 8/14 (57%) were eAg +ve, 12 patients were male, median age was 44.2 (39.9, 48.8) yrs. Direct sequencing of HBV polymerase was performed at baseline and 48 weeks. HBV DNA ($\log_{10}$ IU/ml) testing with Roche Cobas Ampliprep/Taqman v2 (LL<20 IU/ml) at baseline and appropriate time-points.
Results Baseline median HBV DNA was 7.74 (5.1, 8.0) $\log_{10}$ IU/ml. HBV genotype was A in 7/14 (50%), E in 4 (29%), G in 2 (14%) and D in 1 pt. 9 pts (64%) had lamivudine (3TC) monotherapy for a median of 19.9 (8.3, 60.5) months prior to switching to a TDF regime; 3/9 pts also had a period of TDF monotherapy. All 14 patients received combination therapy of TDF and 3TC/FTC. At baseline 5/14 (36%) had evidence of 3TC resistance as shown by the M204V mutation alone or with L180M and/or V173L. 9/14 had no known mutations; no pt displayed TDF resistance. After 48 weeks of TDF based therapy HBV DNA was 1.95 (1.6, 3.2) $\log_{10}$ IU/ml, in 8/14 pts HBV DNA was <2 $\log_{10}$ IU/ml. HBV DNA became undetectable in 9/14 pts (64%) after median 175 weeks of therapy but 5/14 pts (36%) still had detectable HBV DNA a median of 199 weeks after TDF was started. At resistance testing after 48 weeks TDF: 8/14 pts had HBV DNA below limit of amplification, in 6/14 pts 2 showed persistent 3TC mutations, 1 pt showed wild type despite previous 3TC resistance and 3 showed no known mutations. No patient developed the A194T mutation conferring TDF resistance.
Conclusion Despite optimal adherence to TDF treatment, as evidenced by control of HIV, 14 pts failed to achieve undetectable HBV DNA after 48 weeks of treatment. In 5/14 pts, HBV DNA remained detectable at a low level nearly 4 years into TDF treatment, but no patient developed TDF HBV resistance. The long-term clinical significance of low level HBV viraemia in this population is unclear. This may be an HIV specific issue or reflect cumulative HBV resistance allowing replication fitness. Further investigation by phenotypic analysis and/or ultra-deep pyrosequencing is warranted.

P66 SVR WITH TELAPREVIR, PEGINTERFERON ALFA-2A AND RIBAVIRIN IN HCV PATIENTS WITH WELL-CHARACTERISED PRIOR NULL RESPONSE, PARTIAL RESPONSE, VIRAL BREAKTHROUGH OR RELAPSE AFTER PEGINTERFERON+ RIBAVIRIN

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Introduction Study107 is an open-label rollover study of telaprevir (T) with peginterferon+ ribavirin (PR) in genotype-1 HCV patients who did not achieve SVR following PR treatment in telaprevir Phase 2 studies.

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Method Null responders (<1-log_{10} HCV RNA decrease at week-4 or <2-log_{10} at week-12), partial responders (=2-log_{10} decrease at week 12, detectable at week 24), patients with viral breakthrough and relapsers from PROVE1/2/3 PR arms were eligible for treatment. Initially all patients received T 750 mg q8h plus PR at standard doses for 12 weeks, followed by 12 weeks of PR (T12/PR24). Protocol was amended to allow partial responders, viral breakthroughs and relapsers with undetectable HCV RNA at weeks 4 and 12 (eRVR) to receive T12/PR24. Partial responders, viral breakthroughs and relapsers with detectable HCV RNA at week 4 and/or week 12 and null responders received an additional 24 weeks of PR (T12/PR48).
Results Of 117 patients included in an ITT analysis, 97 (83%) had baseline HCV RNA=800 000 IU/ml, (69) 59% had genotype subtype 1a, 44 (38%) had cirrhosis or bridging fibrosis, and 9 (8%) were black. Viral breakthrough and relapse rates occurred in 25%, 23% of prior null responders; 10%, 22% of prior partial responders; 13%, 0% of prior viral breakthroughs; and 0%, 4% of prior relapsers.
Conclusion Patients with prior relapse, breakthrough and partial response exhibited high SVR rates after 24 weeks of telaprevir-based regimen. High SVR rates were also observed in patients with previous null response after 48 weeks of therapy.

Abstract P66 Table 1 Results: Patients achieving SVR

	T12/PR24 n=80	T12/PR48 n=35	Unassigned n=2*
Overall: n, %	47 (59)	18 (52)	2 (100)
Prior null responders: n/N, %	3/23 (13)	**16/28 (57)**	–
Prior partial responders: n/N, %	**15/25 (60)**	0/3 (0)	1/1 (100)
Prior relapsers: n/N, %	**23/25 (92)**	2/3 (67)	1/1 (100)
Prior viral breakthrough: n/N,%	**6/7 (86)**	0/1 (0)	–

*One prior partial responder and one prior relapser who discontinued all treatment prior to reaching week 12 of dosing were designated "unassigned" to treatment group.
The most frequent AEs (=20%) were fatigue, flu-like-syndrome, nausea, diarrhoea, pruritus, rash, headache, insomnia and anaemia. Grade 3 rash and Grade 3 anaemia were observed in 6 (5%) and 6 (5%) patients, respectively. Ten (9%) patients discontinued due to AEs, 5 (4%) due to rash and 2 (2%) to anaemia.

P67 COMBINED INNATE AND ADAPTIVE IMMUNE RESPONSES ARE NEEDED TO CONTROL HEPATITIS B VIRUS REPLICATION IN CHILDREN WITH INFANCY-ACQUIRED INFECTION

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Introduction Innate/adaptive immunity interplay is crucial to control hepatitis B virus (HBV) replication. In infancy-acquired chronic hepatitis B, low viral load is associated with high numbers of natural killer cells (NKC), high expression of their activation markers/receptors (CD69, CD107a, CD161 and NKG2D) and possibly of their inhibitory receptor NKG2A, but there is no information on NKC functional subsets and their interaction with adaptive immunity.
Aim To investigate NKC functional subsets ex-vivo and after exposure to K562 cells in relation to HBV-specific Th1 immune response and viral load.
Method 30 infancy-acquired chronic hepatitis B children (median age 13 y, 14 boys) divided into: group A (HBeAg+/HBsAg+/normal ALT; n=8), group B (HBeAg+/HBsAg+/elevated ALT; n=8), group C (HBeAg-/HBsAg+; n=9) and group D (HBeAg-/HBsAg-; n=5). NKC were obtained by negative magnetic bead isolation from PBMC and after exposure to K562 cells (post-K562). CD107a degranulation, IFN-g intracellular staining and NKC receptor expression (NKG2A/2D) were assessed concomitantly by flow cytometry. HBV-specific immune response was tested by IFN-g intracellular staining after PBMC incubation with HBV core antigen (HBcAg) and HBV DNA viral load by real-time PCR.
Results Frequency of NKC producing IFNg only (CD107a-IFNg+), polyfunctional NKC (CD107a+IFNg+) and NKC expressing NKG2A or NKG2D only (CD107a-NKG2A/D+) was higher in group D than in groups A–C, both ex-vivo ((%CD107a-IFNg+: 24.5vs7.3, 11.3, 16.3, p=0.03) (%CD107a+IFNg+: 25.5vs14.6, 18.6, 20.2, p=0.04) (%CD107a-NKG2A+: 10.9vs3.1, 4.5, 4.6, p=0.02) and (% CD107a-NKG2D+: 27.3 vs 16.7, 20.2, 22.6, p=0.05)) and post-K562 ((%CD107a-IFNg+: 31.1 vs 13.2, 16.4, 19.6, p=0.04) (% CD107a+IFNg+: 39.5 vs 20.3, 24.1, 31.1, p=0.05) (%CD107a-NKG2A+: 12.3 vs 3.7, 4.1, 5.6, p=0.03) and (%CD107a-NKG2D+: 35.3 vs 20.1, 22.6, 23.4, p=0.05)). % NKG2D+CD107a+ NKC was higher in group D than in groups A-C ex-vivo (32.4 vs 17.1, 20.1, 22.4, p=0.03) and post-K562 (39.7 vs 17.9, 21.7, 26.2, p=0.04), while NKG2A+/CD107a+ NKC number was similar in all groups. % HBcAg-specific IFN-g producing cells was higher in group D than groups A–C (CD4+/IFN-g+: 7.2±1.2 vs 2.3±0.3, 2.7±0.5, 3.1±0.9, p=0.04). Polyfunctional NKC CD107a+/IFN-g+ number correlated with that of HBcAg-specific IFN-g producing cells (r=0.5, p=0.04) and negatively with HBV DNA viral load (r=−0.42, p=0.05).
Conclusion High numbers of NKC producing IFN-g, polyfunctional, and with high NKG2D expression are associated with low HBV DNA replication. The strong correlations between polyfunctional NKC and HBV-specific T-helper 1 cells and HBV DNA viral load indicate a joint action between innate and adaptive immunity in controlling HBV infection.

P68 PACIFIC: A PHASE III, RANDOMISED, MULTICENTRE, DOSE ESCALATION, EFFICACY AND SAFETY STUDY EXAMINING THE EFFECTS OF TREATMENT WITH PEGINTERFERON ALFA-2A IN PATIENTS WITH CHILD'S A OR B CIRRHOSIS IN CHRONIC HEPATITIS C VIRUS INFECTION

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Introduction Trials have found conflicting results about the efficacy of pegylated interferon α (PIFN), with or without pretreatment including ribavirin, as an antifibrotic agent in patients with established cirrhosis due to persistent HCV infection. We have investigated the use of an escalating dose of PIFN2a monotherapy for 48 weeks in the treatment of patients with established cirrhosis due to persistent HCV infection.
Method A multicentre, randomised prospective controlled trial of escalating dose PIFN2a treatment of patients with HCV infection and Child's A or B cirrhosis. 39 patients were enrolled at 5 UK centres and randomised to standard clinical care, or 48 weeks treatment with PIFN2a at 90 mcg p.w. escalating each month by 45 mcg to 180 mcg p.w. if tolerated and followed for 140 weeks. Primary outcomes were liver related death; "liver related morbidity" including variceal haemorrhage, ascites and SBP, hepatocellular cancer, transplantation and all cause mortality. Secondary outcomes were health related quality of life (HRLQ).
Results There was no significant difference in the baseline characteristics between treatment and control groups (male 71:77%; mean age 55.2:52.1; Child's score 5.35:5.32; MELD 8.23:7.95). Treatment was well tolerated. 15/17 (88%) completed 48 weeks treatment; 1 at 45 mcg; 1 at 90 mcg; 2 at 135 mcg; 11 at 180 mcg.

There were no differences between groups in HRQL except pain scores that were increased in the treatment group (Score=50.7:70.5, p=<0.01). Recruitment to the study was halted by the DSMC on publication of HALT-C and EPIC trial results.

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