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.