The prevalence of 25(OH)D3 in the patients of South China with chronic HBV infection and high 25(OH)D3 related with low HBV DNA viral load

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Associations of HLA-DPB1 with CHB infection and HBV related HCC in Asia

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BACKGROUND/AIM: In the previous report, HLA-DPA1*01:03-DPB1*04:02 and HLA-DPA1*02:02-DPB1*05:01 showed significant associations with chronic hepatitis B (CHB) infection in Japanese as a protective and a risk haplotype, respectively [Kamatan et al., 2009]. However, there have been no reports of HLA-DP genes to be associated with disease progression from CHB to liver cirrhosis (LC) or HBV-related hepatocellular carcinoma (HCC). PATIENTS/METHODS: We conducted HLA-DP genotyping using a total of 4,830 samples (including Japanese [n=2,954], Korean [n=586], Hong Kong [n=661] and Thai [n=629]) for HBV patients (including CHB, LC and HCC), healthy controls and resolved individuals (HBsAg-negative and anti-HBc-positive), based on PCR-RFLP system according to manufacturer’s protocol. The Fisher’s exact test in a two-by-two cross table was applied to acquire exact P values. We used the DerSimonian-Laird method for a meta-analysis in four populations. The phase of each individual (i.e., a combination of two DPA1-DPB1 haplotypes) was estimated using PHASE software, assuming samples are selected randomly from a general population. RESULTS: A total of successfully genotyped 4,558 samples revealed one high-risk haplotype (HLA-DPA1*02:01-DPB1*09:01) and one protective haplotype (HLA-DPA1*01:03-DPB1*04:01) to be associated with CHB infection over the previously reported HLA-DP
haplotypes in Asian populations (P= 3.38x10^-6; OR=1.95; 95% CI, 1.46–2.64 for HLA-DPA1*02:01-DPB1*09:01; P= 1.17x10^-5; OR=0.32; 95% CI, 0.18–0.56 for HLA-DPA1*01:03-DPB1*04:01). Moreover, a significant association of DPB1*02:01 with protection against HBV infection but against disease progression from CHB to HCC, was identified in Asian populations (P= 1.55x10^-7; OR=0.50; 95% CI, 0.39–0.65). CONCLUSIONS: Trans-ethnic association study of HLA-DP in Asian populations revealed that specific HLA-DPB1 alleles (i.e. DPB1*02:01, *04:01, and *04:02) worked to be protective against HBV infection, and different alleles (i.e. DPB1*05:01, *09:01) worked to be susceptible to HBV infection. To determine all the associated DPB1 alleles for HBV infection would enable HBV infected individuals to divide into two groups who need treatment or not. Moreover, DPB1*02:01 allele was associated with disease progression as well as CHB infection in Asian populations. DPB1 alleles would be key host factors to recognize HBV derived antigen peptides, which will lead the following collaborative studies of HLA-DP molecules in the future.

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Background and Aim: Aldo-keto reductase family 1 member B10 (AKR1B10) is an enzyme that converts retinals to retinols; up-regulation of AKR1B10 reduces intracellular retinoid acid levels, resulting in inhibited cell differentiation. Because AKR1B10 is one of the genes with increased expression in human hepatocellular carcinoma (HCC), its involvement in hepatocarcinogenesis is intriguing. Recently, several studies demonstrated up-regulation of AKR1B10 in some chronic liver diseases such as chronic hepatitis C and steatohepatitis. In addition, the association between AKR1B10 expression and the risk of HCC development was reported. However, there have been few reports that have demonstrated AKR1B10 expression in hepatitis B virus (HBV)-infected patients. Therefore, the aim of the present study was to analyze AKR1B10 expression in HBV-infected patients and to elucidate its association with the risk of HCC development. Methods: The study included 109 consecutive chronic HBV-infected patients who underwent percutaneous liver biopsy. The expression of AKR1B10 in the liver was examined by performing immunohistochemical analyses, and it was quantified as a percentage of positive staining area by using image analysis software. Univariate and multivariate Cox proportional hazard analyses were used to estimate the hazard ratios of AKR1B10 expression for HCC development. The cumulative incidences of HCC development were evaluated by using Kaplan-Meier plot analysis and the log-rank test. Results: Of the 109 patients, 45 patients (41.3%) showed scarce AKR1B10 expression at 0%, similar to that observed in the normal liver tissues. However, the remaining 64 patients (58.7%) showed different degrees of AKR1B10 expression in the liver, and the maximum AKR1B10 expression observed was 81%. During the median follow-up time of 4.8 years, 10 of the 109 patients developed HCC. Multivariate Cox proportional hazard analysis demonstrated that age and AKR1B10 expression were independent risk factors for HCC development. The receiver operator characteristics curve analysis determined that AKR1B10 expression of ≥15% was a cutoff value for HCC development. The age-adjusted hazard ratio for AKR1B10 expression of ≥15% was 12.8 with a 95% confidence interval of 2.8–57.5 (P = 0.002). The 5-year cumulative incidences of HCC were 23.4% and 3.1% in patients with AKR1B10 expression ≥15% and AKR1B10 expression <15%, respectively (P < 0.001). Conclusion: AKR1B10 immunoreactivity in the liver could be a novel predictor of HCC development in HBV-infected patients. These results suggest the involvement of AKR1B10 in the early stage of HBV-related hepatocarcinogenesis.

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Association between the expression of aldo-keto reductase family 1 member B10 and the risk of hepatitis B virus-related hepatocellular carcinoma

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1698
Induction of hepatitis B virus-specific immune responses in immunologically humanized mice

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Background & Aims: Reconstitution of human immune cells is warranted in liver chimeric mice to address hepatitis B virus (HBV)-specific immune responses. Recently, we generated NOG-laj/p2m double KO mice which were NOG mice deficient in both MHC class I and II (DKO-NOG mice). In this study, we evaluated HBV-specific immune responses against HBV in human peripheral blood mononuclear cells (PBMC)-engrafted DKO-NOG mice. Methods: We used NOG mice and DKO-NOG mice which are deficient in both MHC class I and II. Human HLA-A2+ PBMC were injected into NOG and DKO-NOG mice via tail vein. We evaluated liver mononuclear cells (MNCs) isolated from these mice by flow cytometry to detect the engrafted human immune cells in mice. Next, we evaluated the production of anti-HBs antibody (anti-HBs) in sera of human PBMC-engrafted DKO-NOG mice after inoculation of hepatitis B vaccine. We also evaluated the induction of HBe-derived peptide-specific cytotoxic T lymphocytes (CTLs) by using specific HLA-A2+ binding tetramer after vaccination of HBe-derived peptide-pulsed dendritic cells (DCs) or hydrodynamic injection of HBV expressing vector. Results: After inoculation of human PBMC, both infiltration of human immune cells into liver and the damage of hepatocyte were observed in NOG mice, but not in DKO-NOG mice. Serum ALT levels were severely elevated in NOG mice, but not in DKO-NOG mice (510 ± 299 IU/l vs. 15 ± 4 IU/L at day 15, 939 ± 444 IU/l vs. 36 ± 20 IU/L at day 29, respectively). Seven of 8 NOG mice died within 2 months after injection of human PBMC whereas all DKO-NOG mice survived more than 70 days. On day 28 after injection of human PBMC, replacement rates of human immune cells in the liver increased up to 85% in DKO-NOG mice. Both CD4+ and CD8+ human T cells gradually increased in DKO-NOG mice. On day 15, the expressions of PD-1 and Tim-3 on human T cells from DKO-NOG mice were significantly lower than those from NOG mice and the frequencies of human B cells and DCs in DKO-NOG mice were significantly higher than those in NOG mice. Recombinant hepatitis B vaccine resulted in the production of anti-HBs in 50% of vaccinated mice. Vaccination of HBe-derived peptide-pulsed DCs induced generation of HBe-derived peptide-specific CTLs in vaccinated mice. Moreover, hydrodynamic injection of HBV vector resulted in significant increase of HBs-derived peptide-specific CTLs. Conclusion: The present study demonstrates that induction of hepatitis B virus-specific immune responses could be induced in the immunologically humanized mice.

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1700
A novel humanized cDNA-uPA/SCID mouse for the study of HBV and HCV infections

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Aim: Urokinase-type plasminogen activator-severe combined immunodeficiency (uPA/SCID) mice transplanted with human hepatocytes are available for the study of human hepatitis viruses. However, the percentage of human hepatocytes grad-
usually decreases in human hepatocyte chimeric mice because expression of uPA transgene disappears following homologous recombination and deletion of uPA transgene in a fraction of mouse cells, and such mouse cells lacking the uPA gene grow rapidly and replace the human hepatocytes. Recently, we developed a novel uPA transgenic (cDNA-uPA/SCID) mouse where the deletion of uPA cDNA rarely occurs. In this study, we infected hepatitis B virus (HBV) and hepatitis C virus (HCV) into humanized uPA/SCID and cDNA-uPA/SCID mice. Methods: uPA/SCID and cDNA-uPA/SCID mice were transplanted with frozen human hepatocytes obtained from the same donor. Twelve weeks after hepatocyte transplantation, mice were injected intravenously with 50 μL of either HBV- or HCV-positive human serum samples. Mouse serum samples were obtained every two weeks after virus infection, and HBV DNA or HCV RNA levels were measured by real-time PCR. The concentration of human serum albumin (HSA), which is correlated with the human hepatocyte repopulation index, was measured by ELISA. Results: Twelve weeks after transplantation of human hepatocytes, mouse serum HSA levels were significantly higher in cDNA-uPA/SCID mice (n=190) compared to uPA/SCID mice (n=340) (10.4 ± 3.8 vs 9.1 ± 1.8 g/dL, p<0.001). All 102 uPA/SCID and 40 cDNA-uPA/SCID HBV-inoculated mice became positive for serum HBV DNA 2 weeks after inoculation. Serum HBV DNA titers 8 weeks after HBV infection in cDNA-uPA/SCID (n=40) mice were significantly higher than in uPA/SCID mice (n=102) (9.2 ± 0.4 vs 7.9 ± 0.8 log copy/mL, p<0.001). 158 of 168 (92.3%) uPA/SCID and 53 of 54 (98.1%) cDNA-uPA/SCID mice became positive for HCV RNA 8 weeks after HCV inoculation. Despite similar frequencies of HCV viremia, serum HCV RNA titers 2 weeks after infection in cDNA-uPA/SCID were significantly higher than in uPA/SCID mice (6.6 ± 0.4 vs 8.1 ± 0.6 log copy/mL, p<0.001). Conclusion: Humanized cDNA-uPA/SCID mice thus provide a more robust animal model useful for the study of hepatitis virus virology and development of antiviral drugs.

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1701 A novel TK-NOG based humanized mouse model for the study of HBV and HCV infection

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Aim: Immunodeficient mice transplanted with human hepatocytes are available for the study of human hepatitis viruses. Recently, human hepatocytes were also successfully transplanted in herpes simplex virus type-1 thymidine kinase (TK)-NOG mice. In this study, we attempted to infect hepatitis viruses in humanized TK-NOG mice and uracilinoate-type plasmid activator-severe combined immunodeficiency (uPA-SCID) methods. Results: Eight-week-old TK-NOG mice were injected intraperitoneally with 6 mg/kg of ganciclovir (GCV) twice a day. Two days after the first injection, mice were re-infected with the same amount of GCV. Seven days after the first GCV injection, mice were transplanted with 1 x 10^6 of human hepatocytes. Eight weeks after hepatocyte transplantation, TK-NOG and uPA/SCID mice with HSA levels over 1.0 mg/mL were injected intravenously with 50 μL of either hepatitis B virus (HBV); or hepatitis C virus (HCV)-positive human serum samples. Mice serum samples were obtained every two weeks after virus infection, and HBV DNA or HCV RNA levels were measured by real-time PCR. The concentration of human serum albumin (HSA), which is correlated with the human hepatocyte repopulation index (RI), was measured by ELISA. Results: In TK-NOG mice (n=194), serum alanine aminotransferase (ALT) levels one week after GCV administration and HSA levels 8 weeks after hepatocyte transplantation showed a positive correlation, indicating that the higher the serum ALT level, the higher the RI. All humanized TK-NOG (n=43) and uPA/SCID mice (n=36) injected with HBV infected serum developed viremia irrespective of lower replacement index. Incidence of HCV viremia was also high in TK-NOG mice regardless of the RI. In contrast, the frequency of HCV viremia was much lower in uPA-SCID mice having low RI. Only 20% (1 of 5) of uPA-SCID mice with low RI (<70%) became positive for HCV, whereas 94.3% (50 of 53) of mice with high RI (>70%) became positive (p=1.07x10^-6). Eight weeks after infection, HBV DNA and HCV RNA titers increased to approximately 8 and 6 log copies/mL, respectively in both TK-NOG and uPA-SCID mice. The effects of drug treatment with entecavir or interferon-α were similar in both mouse models. Incidence of unexpected death in the early stage of viral infection (8 weeks after injection) was similar in both TK-NOG mice and uPA-SCID mice. Conclusion: TK-NOG mice transplanted with human hepatocytes is a useful model for the study of hepatitis virus virology and evaluation of antiviral agents.

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1702 A detailed systems biology study identifies unappreciated roles for B-cells in the differentiation between HBV clinical phases

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Background The natural history of chronic HBV infection (HBV) is characterized by 4 distinct phases: the immune tolerant (IT), immune active (IA), inactive carrier (IC) and HBeAg negative (NEEG) hepatitis phases. More profound understanding of the factors underlying the immunopathogenesis during each phase is needed to develop future treatment strategies aimed at eradicating the virus. Methods Untreated chronic HBV patients (n=71) attending the outpatient hepatology clinic of the Erasmus MC were requested to donate blood. Standardized
clinical criteria according to EASL guidelines were applied to categorize patients in each clinical phase. Patients were excluded if they had concomitant diseases, higher than F2 liver fibrosis, significant liver steatosis or other liver pathology on ultrasound or liver biopsy. MIPlex cytokine (n=29) measurement, quantitative HBsAg and HBeAg levels and HBV genotype and precore mutant analysis were performed on serum. Circulating leukocytes were phenotyped using multicolor flow cytometry. Whole blood transcriptomics profiles were generated with whole genome expression arrays. Genes with a two-fold difference in expression and a q-value<0.04 were considered as differentially expressed. Results: HBV viral load, HBeAg and HBSAg levels but not genotype differed significantly between the different phases (P<0.001). A set of 6 cytokines (MCP1, IL-12p40, IP10, MIP1b and the IL18/IL1a ratio) was able to distinguish IA, IC and IC patients from each of the other phases (P<0.05). The lowest number of HBV precore mutations were found in IT patients (P=0.0193). Despite extensive phenotypic characterization no significant differences were found in the composition of circulating leukocyte subpopulations. Nevertheless a set of 77 differentially expressed genes between clinical phases were identified using supervised analysis of the whole blood transcriptome. The gene signature distinguishing IA from IC patients was predominantly composed of highly up-regulated immunoglobulin encoding genes (47% and 36% respectively). Furthermore, gene set enrichment analysis corroborated abundant expression of B cell function-related genes in IA patients, while opposing activities of interferon stimulated genes and NK-cell related genes were observed for ENEG and IC patients, respectively. Conclusions: HBV clinical phases are characterised by distinct gene signatures in whole blood, but not by numerical differences in circulating leukocyte populations. The blood transcriptome of IA patients is dominated by B cell function-related transcripts, pointing towards a crucial differential role for B-cells in the immunopathogenesis of HBV.

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1704
Mutational profile of HLA-A2-restricted cytotoxic T lymphocyte (CTL) epitope env183-191 in chronic HBV-infected patients and the epitopic mutation’s influence on CTL activities
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Background and aims: The interplay between HBV and host immunity plays a key role for clinical outcome of HBV infection. The study aimed to investigate clinical relevance of HBV mutations on epitopes of cytotoxic T lymphocytes (CTL) and the impact of the mutations on CTL response. Methods: Sequence analysis of complete HBV genomes was performed for 516 HBV-infected patients with different clinical presentations. Among them, 188 HLA-A2-positive patients with genotype C HBV infection were further studied, including 51 with acute hepatitis B (AHB), 86 with chronic hepatitis B (CHB), and 51 with acute-on-chronic liver failure (ACLF). The mutations at 31 known HLA-A2-restricted epitopes were analyzed. Binding affinity of epitopic peptides were estimated by BIMAS and measured by T2 cell binding assay. S-gene vaccines containing wild-type or mutant env183-191 epitope-encoding sequence
were constructed and inoculated into HLA-A2/HBV transgenic mice. The epitope-specific CD8 T cells were detected by para-
tamers, IFN-γ ELISPOT, and cytotoxicity assay. Results: The incidences of 12 HLA-A2-restricted epitopic mutations were significantly different among ACLF, CHB and AHB patients. Biomarkers significantly reduced for 10 mutant epitopes and increased for 2 mutant epitopes compared to the wild-type. T2 cell binding assay verified the affinity change of the mutant epitopes. env183-191 (FLRTLRTI) had three mutational patterns, i.e., FLTTLRTI (K), FTSLTLRTI (S), and FSLTLRTI (SK). The K, S, and SK mutation incidences were 11.8%, 0%, and 0% in AHB patients; 1.2%, 16.3%, and 0% in CHB patients; and 15.7%, 11.8%, and 9.8% in ACLF patients. T2 cell binding assay showed that the K, S, and SK mutants had 52.2%, 3.5%, and 1.8% of the binding affinity of the wild-type, respectively. Both K- and S-mutant-immunized mice had moderate cross CTL response to wild-type epitope, but not to each other. SK-mutant-immunized mice had weak CTL response to S mutant and the wild-type epitopes, but not to K mutant epitope. The wild-type-immunized mice had much weaker CTL response to K and S mutant epitope compared to the wild-type epitope. ELISPOT assay showed that wild-type-immunized mouse had strong response to wild-type epitopic peptide stimulation, but spot number reduced 89%, 90%, and 93% to K, S, and SK mutant epitopic peptide stimulation respectively. The killing effect was significantly lower to the mutant target cells than the wild-type ones. Conclusion: HBV CTL-epitopic mutation might be a factor influencing disease progression of HBV infection. env183-191 mutations may decrease the binding affinity of the epitope to CTL and weaken the specific CTL response.

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1705
HBx interact with and modulates the expression of the DLEU2 IncRNA locus in HBV replicating cells
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Background: HBx regulatory protein is required for HBV cccDNA transcription/viral replication and contributes to HBV oncogenicity. HBx affects the epigenetic control of both HBV viral chromatin and cellular genes. ChIPSeq experiments in HBV replicating cells have shown that HBx specifically binds to a large number of genomic sites and potentially regulates the expression of several genes and noncoding RNAs. LncRNAs are endogenous cellular RNAs molecules longer than 200 nt capable to regulate gene expression at various levels, including chromatin modification, transcription and post-transcriptional processing. Objectives: Aim of this study was to identify and characterize IncRNAs targeted by HBx. Methods: High-throughput sequencing of anti-HBx ChIPenriched DNA (ChIPseq) was performed in HBV replicating HepG2 cells. Hits were validated in independent ChIP experiments by TaqMan real-time PCR using lncRNA specific primers. HBx-targeted IncRNAs expression was assessed both by PCR (isoforms evaluation) and real-time RT-PCR (quantification). Results: ChIPseq analysis identified 39 IncRNAs targeted by HBx. We focus here on HBx regulation of DLEU2 and the intragenic/overlapping TRIM13 gene, hsa-mir-15 and hsa-mir-16. Up-regulation of specific DLEU2 splicing variants correlates with HCC development whereas hsa-mir-15 and hsa-mir-16 are down-regulated in HCCs. We show that HBx binds to and induces increased histone acetylation at the DLEU2 promoter. HBx binding results in: a) a different DLEU2 splicing profile leading to over-expression of a shorter isoform; b) down-regulation of the hsa-mir-15 and hsa-mir-16; c) up-regulation of the antisense autophagic gene TRIM13. DLEU2 selective degradation by specific Groungers resulted in a reduced H4 acetylation on DLEU2 and TRIM13 promoters and a significant reduction of both DLEU2 and TRIM13 expression in HBV replicating HepG2 cells. These results directly link DLEU2 with TRIM13 transcriptional regulation in the presence of HBx. In silico analysis indicates that DLEU2 RNA species potentially binds the HBx protein and we confirmed the HBx-DLEU2 interaction using an anti-HBx RNA Immune Precipitation (RIP). Finally, we found that DLEU2 inactivation has a profound impact on HBV pgRNA transcription, thus unveiling a functional relevance of the DLEU2-HBx interaction in regulating HBV replication. Conclusion: Genome wide occupancy study revealed that HBx targets 39 IncRNA promoters. HBx binding to the DLEU2 promoter modifies its splicing profile and affects the expression of the intragenic/overlapping TRIM13 gene, hsa-mir-15 and hsa-mir-16. Moreover, a direct interaction of DLEU2 with the HBx regulates HBV replication.

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1706
Differential expression of Fc gamma receptors contributing to the diverse host immunity during HBV infection
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Backgrounds and Aims: Fc gamma receptors (FCGRs) are important in regulating immune responses. Most of the FCGRs are activating receptors including FCGR1, FCGR2A, FCGR3, whereas FCGR2B is the only inhibiting receptor. So far, there is no FCGRs study regarding hepatitis B virus (HBV) infection. Methods: whole-genome expression profiling of purified RNA of peripheral blood mononuclear cells (PBMC) from patients with acute self-limiting hepatitis B (AHB) and treatment-naive chronic HBV infection including immune tolerant(ITT) and HBeAg positive(HBeAg+) chronic hepatitis B(CHB) and healthy control (HC) was performed using microarrays. Gene signatures were developed through bioinformatics approaches and further evaluated and validated by real time RT-PCR and western blot. Results: Microarray data showed activating FCGRs namely FCGR1A and FCGR2A that were differentially expressed and validated by real time RT-PCR in the same patient cohort, which revealed higher expression level of FCGR1A and FCGR2A in AHB compared with chronic HBV infection, in HBeAg+ CHB compared with IT and in AHB compared with HC(all, P<0.001). Subsequent studies with an independent patient cohort of 25 AHB, 40 IT, 50 HBeAg+ CHB showed that FCGR1A and FCGR2A had the similar expression pattern which was most significant for FCGR1A mRNA expression( P<0.001) and protein expression( P<0.01), whereas FCGR3A expression was statistically insignificant. Also, we assessed mRNA expression of FCGR2B which revealed higher expression in IT compared with HBeAg+ CHB without statistical significance. The ratio of FCGR2A/ FCGR2B mRNA expression was calculated in 10 HBeAg+ CHB[10/50] before and after PEG-IFN therapy who achieved virological response. The result showed that the ratio FCGR2A/ FCGR2B was higher in those after treatment compared with those before treatment(5.88±5.12 vs 2.05±1.86, p<0.01).
Simultaneously, we examined mRNA and protein expression in liver tissue samples from 5 IT(5/40) and 6 treatment-naive HBsAg+ CHB(6/50). The results revealed that both FCGR1A mRNA and protein expression was significantly higher in treatment-naive HBsAg+ CHB compared with IT(p<0.01), however, FCGR2A, FCGR2B and FCGR3A expression did not acquire significant differences in two groups. Conclusions: Our study provides a unique representation of FCGRs expression during HBV infection. Especially, FCGR1A mRNA and protein levels on PBMC and in liver tissue are differentially expressed between IT and HBsAg+ CHB patients. Changes of the ratio of FCGR2A/FCGR2B mRNA expression before and after PEG-IFN treatment suggested that PEG-IFN treatment could shift monocyte balance toward the activating FCGRs.

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1707
No Detectable Resistance to Tenofovir Disoproxil Fumarate (TDF) in HBeAg+ and HBeAg- Patients with Chronic Hepatitis B (CHB) After Eight Years of Treatment
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Aim: To perform a cumulative evaluation of CHB patients who qualified for resistance surveillance over 8 years of treatment with tenofovir disoproxil fumarate (TDF). Methods: Patients in Studies GS-US-174-0102 (HBeAg) and GS-US-174-0103 (HBeAg+) were randomized 2:1 to receive TDF or adefovir dipivoxil (ADV) for 48 weeks followed by open-label TDF (OL-TDF) through year 8. Patients with HBV DNA >400 copies/mL (viremic) could add emtricitabine (FTC) at/after Week 72. Virologic breakthrough was defined as confirmed HBV DNA either >1 log10 from nadir or viremia after <400 copies/mL. Population sequencing of HBV pol/RT was attempted for all patients at baseline and, if viremic, annually, at time of study discontinuation, or FTC addition. TDF-treated patients were evaluated for the entire study; ADV-treated patients were evaluated after switching to TDF (week 48). Results: Of 641 enrolled patients, population sequencing was performed on 165 samples from (n=90) TDF-treated patients across 8 years of treatment. Over years 1-2, 9-11% of patients qualified for genotypic analysis mostly for viremia (≥73%) without virologic breakthrough. In contrast, over years 3-8, < 4% of patients qualified for testing and the majority had transient increases in HBV DNA. Protocol-defined virologic breakthrough occurred throughout the study; of the 41 episodes observed, the majority (n=29; 70%) were associated with confirmed nonadherence to study medication. Of the patients that experienced breakthrough and had an opportunity to resuppress, 56% of patients (22/39) achieved HBV DNA resuppression to <400 copies/mL. Across all patients who qualified for genotypic analysis, 36% had no sequence changes compared to baseline, 29% had polymorphic site changes, 7% had preserved site changes in pol/RT, and 28% were unable to be genotyped (mostly due to low viral load). There was no accumulation of conserved site changes with long-term TDF treatment and no sequence changes were associated with TDF resistance. Conclusions: In these long term studies of TDF for CHB treatment, the percentage of subjects qualifying for genotypic analysis declined over time. Virologic breakthrough was often transient and usually associated with nonadherence to study medication with subsequent resuppression of HBV DNA <400 copies/mL. There was no accumulation of conserved site changes and no evidence of TDF resistance. These results support the long-term use of TDF for CHB treatment.

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1708
Functional innate immune responses are restored with sequential NUC therapy following Pegylated Interferon–Alpha exposure and not with NUC monotherapy in Chronic Hepatitis B
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INTRODUCTION: Treatment strategies in Chronic Hepatitis B (CHB) are now focused on achieving HBsAg loss; therefore greater consideration is being given to combined/sequential therapeutic approaches comprising Pegylated-Interferon (PEG-IFN-α) and nucleo(s)ide analogue (NUC) therapy, to achieve this goal. We previously demonstrated boosting of NK cell responses in eAg+ patients treated with PEG-IFN-α (Micco et al, J. Hepatol, 2013), and postulated that this effect could be maintained with sequential NUC therapy, representing a superior strategy to NUC monotherapy. Differential NK cell responses in patients receiving a sequential NUC were compared to patients on NUC monotherapy to determine if there was a treatment advantage with PEG-IFN-α exposure. PATIENTS & METHODS: PBMC from 18 eAg+ patients during PEG-IFN-α therapy were utilised. 10/18 patients considered PEG-IFN-α non-responders after 48 weeks therapy progressed to sequential NUC therapy and were followed until virally suppressed. NUC monotherapy patients, without prior PEG-IFN-α exposure, were analysed for comparison. Phenotypic and functional analysis of NK cell subsets was performed by multicolour flow-cytometry. RESULTS: PEG-IFN-α expanded CD56bright NK cells by 3-fold (p=0.0001); this was maintained on sequential therapy but not seen with NUCs alone (p=0.03). NK cell expression of C-Type lectin and natural cytotoxicity receptors was analysed. All receptors, except NKG2D, were expressed at significantly higher levels on sequential NUCs vs. NUC monotherapy (p=0.05), with marked augmentation in the expression of NKP30 and NKP46 on CD56bright NK cells (p=0.0001 & 0.002 respectively). The proportion of CD56bright NK cells expressing TRAIL was 3-fold higher on sequential NUC therapy compared with NUC monotherapy (p=0.007). These NK cells during sequential therapy demonstrated ability to degranulate and produce IFN-γ; functional restorations not achieved on NUCs alone (p=0.0001 & 0.002 respectively). These changes were more dramatic in patients demonstrating eAg seroconversion +/- sAg decline on sequential NUCs CONCLUSIONS: The
potent expansion of activated CD56 bright NK cells induced by PEG-IFN-α is sustained on sequential NUC therapy, with high expression of NKP30, Nkp46 and TRAIL when compared to NUCs alone. Restoration of NK cell cytotoxic/effecter functions on sequential therapy is seen compared to NUC monotherapy. PEG-IFN-α non-responders exhibit innate boosting which is maintained with functional innate restoration on sequential NUC therapy. Further work is being undertaken to determine if this priming effect is present with shorter courses of PEG-IFN-α.

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1709 Mice with syngeneic human liver and immune system to study cellular immunity to hepatitis B virus
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Human liver chimeric mouse models have proven useful to study human liver disease, including hepatitis B (HBV) and C (HCV) virus infections. Independently, immunodeficient mice reconstituted with hematopoietic stem cells (HSCs) derived from fetal liver reliably develop human T and B lymphocytes. Combining these systems has long been hampered by the inability of human fetal hepatoblasts to reconstitute liver chimeric mice. Here we set out to engraft immunodeficient fah/-/- mice with human hepatoblasts with the goal of developing mice with a syngeneic human liver and immune system. Substitution of human oncostatin-M, which does not cross-react between mouse and human, enhanced liver engraftment with human hepatoblasts by 5-10 fold. Fetal hepatoblast engrafted mice had similar liver morphology as adult hepatocyte engrafted animals, and could support both HBV and HCV viremia. We next created immunodeficient fah/-/- mice with syngeneic human HSCs and fetal hepatoblasts. In contrast to mice singly engrafted with HSCs that predominantly develop lymphocytes, doubly engrafted mice contained physiological levels of intrahepatic human monocytes and NK cells in addition to human lymphocytes. Upon infection with HBV these animals displayed rising levels of pro-inflammatory human cytokines previously observed in patients. These doubly engrafted 'HIS-Hep' mice are a new small animal model to study human leukocyte functions in early HBV infection.

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1710 Effect of Immunosuppression and Antiviral Therapy in Persistent Intracellular Replication among Hepatitis B Virus and HIV Co-Infected Patients
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Covalently closed circular DNA (ccc-DNA) of hepatitis B virus (HBV) acts as a reservoir for reactivation of viral replication and whose quantification can be used as a marker of persistent intracellular replication. The determinants of intracellular levels of replication have rarely been evaluated in HBV-human immunodeficiency virus (HIV) co-infected patients. Sixty HIV-HBV co-infected patients with at least one liver biopsy during follow-up in the French HIV-HBV cohort were included. HBV ccc-DNA and total intracellular HBV-DNA were extracted from biopsies and quantified by real-time PCR. Risk factors of intracellular replication were determined using mixed-effect linear regression models. At the time of biopsy, 35 (61.4%) patients were HBeAg-positive and 23 (46.9%) had detectable serum HBV-DNA [median: 3.10 log10 IU/ml (IQR:2.75-5.38)]. Among the 22 patients undergoing tenofovir (TDF)-containing antiretroviral therapy, cumulative TDF-duration was at a median 17.8 months (IQR:5.7-31.0). Overall, median HBV ccc-DNA was -1.10 log10 copies/cell [IQR:-1.70, -0.29] and total intracellular HBV-DNA was 0.27 log10 copies/cell [IQR:-0.39, 2.00]. In multivariable analysis, patients with HBeAg-positive serology had significantly higher levels of HBV ccc-DNA (+0.76 log10 copies/ml; 95%CI:0.39, 1.13; p<0.001), whereas those with a nadir CD4+ cell count above 250/mm3 had significantly lower HBV ccc-DNA levels (-0.57 log10 copies/ml; 95%CI:-0.95, -0.19; p=0.004). Furthermore, patients with longer than 3 years of cumulative TDF-duration had significantly lower HBV ccc-DNA levels after adjustment (0.88 log10 copies/cell; 95%CI:-1.40, -0.35; p=0.001). Accordingly, when focusing on patients undergoing TDF with a biopsy at TDF-initiation and sometime during therapy (median duration: 35.3 months, range: 20.2-56.6), most exhibited strong declines in HBV ccc-DNA (median change in log10 copies/cell/year:-0.46, range:-0.67, 0; n=7). HBV ccc-DNA levels did remain detectable at the end of follow-up for all patients, yet at very low levels (median: 0.04 copies/cell, range:0.01, 0.31).

The results above were similar when using total intracellular HBV-DNA levels as an end-point. In conclusion, severe immunosuppression is associated with intracellular HBV replication in co-infected patients. Treatment with TDF is linked to large declines in ccc-DNA, yet replication within the hepatocyte still persists after long periods of treatment.

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1711
Bile acids receptor FXR agonists repress HBV replication in HepaRG cell
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Hepatitis B virus (HBV) is a “metabolivirus” intimately linked to bile acids (BA) metabolism. 1) BA nuclear receptor FXR binds to two response elements in the HBV core promoter region and its activation by ligands regulates the HBV core promoter activity. 2) HBx binds to Sirt-1, a deacetylase that regulates FXR activity and to PRMT1 transmethylase that is recruited by FXR upon its activation. 3) the Na1-taurocholate cotransporting polypeptide (NTCP) responsible of BA uptake was identified as a functional receptor for HBV and 4) reciprocally competition between virus and BA for NTCP induces a compensatory BA synthesis. We aimed at investigating the effect of FXR on HBV replication. First we screen HBV proteins interaction with FXR and found that among the HBV proteins, HBx was co-immunoprecipitated with FXR. Second we tested the effect of FXR modulators on HBV replication. Differentiated HepaRG cells that support a complete replication cycle were infected with HBV and treated from day 2 to 10 post infection with FXR modulators. Treatment with BA derived 6-ethyl-chenodeoxycholic acid (6-ECDCA) or synthetic non-steroidal agonists, but not with antagonists or ursodeoxycholic acid, strongly inhibited the secretion of HBV DNA, HBsAg, HBeAg and of HBeAg synthesis in a dose dependent manner (70 to 80 % inhibition at 1 or 10 micro-Mol) as well as the viral pregenomic RNA synthesis, cccDNA copies number and cellular total HBV DNA. Cyclosporine A, an NTCP ligand and HBV entry inhibitor, did not modify the effect of agonists suggesting that the effect did not depend on entry inhibition. Treatment consistently increased FXR activity as indicated by the increase of the small heterodimer partner (SHP) and decrease of the apolipoprotein-A1 mRNA expression, two FXR dependent genes, despite reduced FXR mRNA levels. In conclusion, BA-derived or synthetic agonists lead to a sustained repression of HBV replication in the HepaRG cell culture system. This effect is likely mediated by a modulation of FXR activation that could perturb the complex FXR network of transcription factors, which is highly targeted and controlled by HBx rather than by a competition between the virus and FXR agonist for NTCP and inhibition of virus entry. These data stress out the importance to exploit drug regulation of metabolism pathways in controlling HBV replication.
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1712
Toll-like receptor 3-activated non-parenchymal liver cells control hepadnaviral replication in HBV-transgenic mice lacking the surface antigen (HBsAg)

1713
Frequency and role of NKp46 and NKG2A expressing NK cells in patients with chronic hepatitis B
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Background and Aim: Natural Killer (NK) cells play important roles in innate immune response in viral infection. The activa-
tion of NK cells are controlled by various activating and inhibitory NK cell receptors and many NK cell receptors have been identified. In this study, we focused on NKp46, an activating receptor, and NKG2A, an inhibitory receptor, and investigated the frequencies and function of NKp46 and NKG2A expressing NK cells in chronic hepatitis B (CHB) patients. Methods: Sixty six CHB patients and 32 healthy subjects (HS) were enrolled in this study. Peripheral blood lymphocytes (PBL) were obtained from these subjects and the frequencies of NKp46 and NKG2A were assessed by flow cytometry. CD107a, which is a marker of degranulation and indicates cytotoxicity, and IFN-γ were measured for functional assay of NK cells by flow cytometry with co-culturing the NK-target cell line K562. Results: When CHB patients were divided into CHB high titer group (CHBH) and low titer group (CHBL) by the cut off level of serum HBVDNA 4 logcopies /ml, 18 patients were classified into CHBH and 48 were CHBL. CHBH were younger and showed higher ALT level than CHBL and HS. There were no statistical difference in the frequencies of NKG2A-positive cells and NKp46-positive cells among 3 groups. However, we identified unique subset which were strongly positive for both NKp46 and NKG2A [NKp46<sup>high</sup>NKG2A<sup>high</sup> subset]. The frequencies of this subset in NK cells were 4.9 ± 3.0%, 4.9 ± 3.0%, and 8.4 ± 4.1% in HS, CHBH and CHBL, respectively. The frequencies of this subset positively correlated with serum HBVDNA level. In functional assay upon co-culture with K562, the expressions of CD107a and IFN-γ were higher in this subset than in other subset [CD107a: 65.9/39.8%, IFN-γ: 39.0/25.1% in NKp46<sup>high</sup>NKG2A<sup>high</sup> subset/other subset, respectively]. In addition, we analyzed 5 CHB patients who received IFN-α therapy over 24 weeks and achieved improvement of serum ALT levels and HBV DNA levels. The frequencies of NKp46<sup>high</sup>NKG2A<sup>high</sup> subset in these patients were found to be significantly higher than those patients without therapy (37.3 ± 16.4% in CHB patients received IFN therapy, 5.9 ± 3.8% without therapy). Conclusion: The frequencies of NKp46<sup>high</sup>NKG2A<sup>high</sup> subset which possess the ability of high cytotoxicity and cytokine production were higher in CHBH than in CHBL. This subset was significantly increased upon IFN-α therapy with improvement of serum ALT and HBVDNA level. So our data suggests that IFN-α therapy boosts the insufficient innate immune response through increasing NKp46<sup>high</sup>NKG2A<sup>high</sup> subset.

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1715 HBV infection in humanized chimeric mice has multiphasic viral kinetics from inoculation to steady state and an HBV half-life of 1 hr

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Background: uPA-SCID chimeric mice with humanized livers (SCID-MhL) are a useful tool for studying HBV infection in the absence of an adaptive immune response. Aims: To estimate HBV clearance rate from circulation post-inoculation (p.i.) and characterize subsequent HBV kinetics from inoculation to steady state in the uPA-SCID model. Methods: Twenty-nine mice (25 SCID-MhL, 4 without humanized livers, SCID-M) were inoculated with HBV serum (Fig.1). Viral loads were frequently measured from blood up to 60 days p.i. HBV half-life (1/2) was estimated during the 1st phase (Fig.1) using a linear mixed-effects model. HBV DNA was measured using Real-Time PCR.

Results: When CHB patients were divided into CHB high titer group (CHBH) and low titer group (CHBL) by the cut off level of serum HBVDNA 4 logcopies /ml, 18 patients were classified into CHBH and 48 were CHBL. CHBH were younger and showed higher ALT level than CHBL and HS. There were no statistical difference in the frequencies of NKG2A-positive cells and NKp46-positive cells among 3 groups. However, we identified unique subset which were strongly positive for both NKp46 and NKG2A [NKp46<sup>high</sup>NKG2A<sup>high</sup> subset]. The frequencies of this subset in NK cells were 4.9 ± 3.0%, 4.9 ± 3.0%, and 8.4 ± 4.1% in HS, CHBH and CHBL, respectively. The frequencies of this subset positively correlated with serum HBVDNA level. In functional assay upon co-culture with K562, the expressions of CD107a and IFN-γ were higher in this subset than in other subset [CD107a: 65.9/39.8%, IFN-γ: 39.0/25.1% in NKp46<sup>high</sup>NKG2A<sup>high</sup> subset/other subset, respectively]. In addition, we analyzed 5 CHB patients who received IFN-α therapy over 24 weeks and achieved improvement of serum ALT levels and HBV DNA levels. The frequencies of NKp46<sup>high</sup>NKG2A<sup>high</sup> subset in these patients were found to be significantly higher than those patients without therapy (37.3 ± 16.4% in CHB patients received IFN therapy, 5.9 ± 3.8% without therapy). Conclusion: The frequencies of NKp46<sup>high</sup>NKG2A<sup>high</sup> subset which possess the ability of high cytotoxicity and cytokine production were higher in CHBH than in CHBL. This subset was significantly increased upon IFN-α therapy with improvement of serum ALT and HBVDNA level. So our data suggests that IFN-α therapy boosts the insufficient innate immune response through increasing NKp46<sup>high</sup>NKG2A<sup>high</sup> subset.

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1714 Hepatitis B virus enhances the endo-lysosomal pathway through the activation of the small GTPase Rab7 via an interaction between HBe and the Rab7 GAP, TBC1D15

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Background/aim: It has been shown that the late events of the hepatitis B virus (HBV) life cycle associate with late endosomes/multivesicular bodies (MVBs) in infected hepatocytes. Currently, however the trafficking pathways of viral particles/components through these organelle are poorly defined. Previously we have shown that HBV activates the small GTPase Rab7 to promote the transport of virus in MVBs to lysosomes resulting in the attenuation of viral secretion. It was revealed that among five individual HBV proteins, only HBe activates Rab7. The GOAL of this study was to analyze further the alteration of the endo-lysosomal pathway by HBV and to define how HBe activates Rab7. Results: Electron microscopy (EM) and/or fluorescence imaging showed many virus-like electron-dense particles in MVBs of HBV-expressing HepG2.2.15 cells, and interestingly, numerous tubules extended outward from the MVBs with a 10-fold greater frequency in the HepG2.2.15 cells than the parental HepG2. These tubules also formed in Huh7 cells transfected with the HBV genome while siRNA-mediated knockdown of Rab7 decreased tubule formation significantly. From these findings, we conclude that MVB dynamics are induced by HBV and are Rab7-dependent. Importantly, Rab7 knockdown decreased the colocalization of viral proteins and lysosomes, and increased the viral secretion. Although it was found that HBe activated Rab7, there was no evidence of direct interaction between HBe and Rab7. As Rab7 is regulated by the GTPase activating protein (GAP) TBC1D15, we tested for interactions between HBe and Rab7. GAP. TBC1D15. This interaction induces tubules extending from MVBs/APs and promotes the fusion with lysosomes resulting in the degradation of HBV particles in MVBs/APs. HBV is known as a ‘stealth’ virus, and the Rab7 activation by HBe, which attenuates the HBV secretion, may lead to a weakened immune responses for persistent infection.

Disclosures:  The following people have nothing to disclose: Jun Inoue, Eugene W. Krueger, Jing Chen, Hong Cao, Tooru Shimosegawa, Mark A. McNiven
1716

**Effect of bile acid on the entry of hepatitis B virus**

**via sodium taurocholate cotransporting polypeptide**

Jung Wha Chung, Eun Sun Jang, In Young Moon, Gi Hyun Kim, Kyeong Sam Ok, Jong Ho Lee, Sook-Hyang Jeong, Jin Wook Kim; Department of Internal Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnamsi, Republic of Korea

Purpose: Recently, sodium taurocholate cotransporting polypeptide (NTCP) has been reported as an entry receptor for hepatitis B virus (HBV) in susceptible hepatocytes. In the light of bile acid metabolism, NTCP has an important role to uptake conjugated bile acid at basolateral membrane of human hepatocytes. Moreover, bile acid can suppress NTCP expression in human hepatocellular carcinoma cell lines. This study was conducted to document whether bile acid could affect the HBV entry into hepatocytes, and whether NTCP mediates the change of viral entry amount after bile acid treatment. Methods: PH5CH8 cells, an immortalized non-neoplastic human liver cell line, and Huh-BAT cells, Huh7-derived cell line expressing human NTCP were used in our experiments. Supernatant of HepAD38 cells cultured in tetracycline-free medium was used for the generation of infectious HBV particles. After adding HBV concentrates on cells overnight, cells were grown in HBV-free medium and collected at Day 8. NTCP expression and HBV levels in the supernatant or cytosol of treated cells were measured by real-time PCR. Confocal immunofluorescence microscopy was performed to confirm the colocalization of HBV particle and NTCP. NTCP promoter activity was assessed by luciferase assay. Results: NTCP expression was confirmed in Huh-BAT and PH5CH8 cells but not in Huh-7 cells. After overnight HBV treatment, Huh-BAT and PH5CH8 cells showed more intracellular HBV particles compared to Huh-7 cells at Day 8. NTCP promoter activity was significantly enhanced by HBV S (1.72 folds), C (1.49 folds) and X (1.68 folds) proteins, and NTCP mRNA expression level tended to be correlated with intracellular HBV pgRNA levels whereas bile acid treatment without HBV infection suppressed NTCP mRNA expression compared to control in PH5CH8 cells. Conjugated bile acid treatment using glycocholic acid, glycochenodeoxycholic acid, taurocholic acid (TCA) and taurochenodeoxycholic acid (TCDCA) suppressed intracellular HBV rcDNA and pregenomic RNA (pgRNA) levels. Conclusions: Intracellular uptake of HBV was dependent on NTCP expression levels of susceptible cells. Expression of NTCP is upregulated by HBV, and HBV-induced NTCP overexpression might be an evolutionary adaptation strategy of HBV. In contrast, various doses of conjugated bile acid treatment suppress NTCP expression and HBV entry in human hepatocytes, and this phenomenon may be an innate defense mechanism of human liver in the course of acute icteric HBV infection.

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1717

**Distinct helper roles of dendritic cell subsets in NK cell-dependent HBV suppression in bystander infected cells**

Sacchiyo Yoshio, Tatsuya Kanto, Masaya Sugiyama, Hirotaka Shoji, Yohei Mano, Yoshihiko Aoki, Nao Nishida, Masaaki Korenaga, Kazumoto Murata, Masashi Mizokami; The Research Center for hepatitis and immunology, National Center for global health and medicine, Chiba, Japan

**BACKGROUND&AIMS:** Natural killer (NK) cells play crucial roles in HBV eradication by leading hepatocyte injury (cytolytic mechanism) or by suppressing HBV without causing collateral damage (non-cytolytic mechanism). In order to gain insights in immunological control of HBV, an exploration of NK-mediated HBV eradication has been anticipated. Dendritic cells (DCs) are an essential regulator of NK cells. However, coordinated roles of DCs and NK cells against HBV infection remain largely unknown. We thus aimed to clarify the potency of DC/NK interaction in HBV suppression, using a culture system simu-
lating later phase of HBV replication in hepatocytes. METH-
ODS: We utilized human hepatoblastoma cell line (Huh7) tran-
sfected with 1.24-length of HBV genome (genotype C) (HBV-Huh7). After the transfection, HBV-Huh7 produces HBs/ 
HBc/HBe antigens (AGs) and virions in the supernatant. NK 
cells, BDCA3+DCs and plasmacytoid DCs (pDCs) were sorted 
from PBMC of uninfected healthy donors. After NK cells and/ 
or DCs were co-culture with HBV-Huh7, HBV Ags and intra-
cellular HBV DNA were quantified. We assayed IFN-α/β/γ, 
cytokines and examined the profile of interferon-stimulated 
genes (ISGs) in HBV-Huh7. Simultaneously, LDH levels in 
the supernatants were monitored as a marker of cytolytic effect 
on Huh7 cells. RESULTS: In the co-culture of HBV-Huh7 and 
NK cells, the quantity of HBV Ags and HBV-DNA were sig-
nificantly reduced in an NK number-dependent manner. The 
levels of HBV markers were inversely correlated with IFN-γ 
and LDH, suggesting that activated NK cells inhibit HBV repa-
clication mainly by cytolytic mechanisms. The presence of pDCs 
with NK cells and HBV-Huh7 increased the levels of IFN-γ 
and LDH, resulting in an additional 28% reduction of HBV 
quantity. These results imply that the coexistence of pDCs with 
NK cells suppress HBV replication more significantly than NK 
alone by enhancing NK-dependent cytolyis. In the presence of 
BDCA3+DCs, pDCs and NK cells, the quantity of HBV was 
26% more reduced without elevation of LDH. In this setting, 
anti-viral ISGs, such as indolamine-2, 3-dioxygenase (IDO) or 
APOBEC3G, were strongly induced in HBV-Huh7, the degree of 
which was inversely correlated with HBV quantity. These 
results indicate that BDCA3+DCs are capable of enhancing 
NK-mediated, but non-cytolytic HBV suppression via induction 
of ISGs. CONCLUSIONS: Bystander DCs utilize NK cells to 
suppress HBV replication in infected hepatocytes. Plasmacytoid 
DCs enhance cytolytic NK activity, while BDCA3+ cells stimu-
late their non-cytolytic capacity of inducing ISGs, showing a 
proof of concept on immunological HBV control.

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1718 Establishment of a mouse model of acute hepatitis B by 
activation of human cytotoxic T lymphocytes

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Aims: Hepatitis B virus (HBV) infection occasionally causes 
massive liver damage. Although cytotoxic T lymphocytes (CTLs) 
play a critical role in hepatitis, the mechanism of massive 
liver cell damage by HBV infection has not been elucidated 
sufficiently due to lack of an animal model. In this study, we 
attempted to establish a hepatitis B animal model using human 
hepatocyte transplanted NOG mice and human peripheral 
blood mononuclear cells (PBMCs). Methods: Eight-week-old 
SCID-NOG mice were injected intraperitoneally with 6 mg/kg of ganciclovir 
(GCV) twice a day. Two days after the initial injection, mice were re-injected with the same amount of GCV. 
Seven days after the first GCV injection, mice were trans-
planted with human hepatocytes obtained from an HLA-A24 
donor. Eight weeks after hepatocyte transplantation, mice were 
injected intravenously with 50 μl of HBV-positive human serum samples. Eight weeks after HBV injection, mice were inoculated 
with 5 x 106 human PBMCs isolated from an HLA-A24 patient who recovered from acute severe hepatitis B. Two weeks after 
PBMC injection, liver pathology, microarray human albumin, which is correlated with the human hepatitis reopulation 
index (ELISA), HBV DNA levels (real-time PCR) and the pheno-
type of human PBMC (FACS) were analyzed. Results: Trans-
plantation of human PBMCs resulted in up to 82% human 
mononuclear cell chimerism in the liver. Massive hepatocyte 
damage and decrease in serum human albumin with a decline 
in HBV DNA levels were seen in HBV-infected mice, but not 
uninfected and PBMC-transplanted mice. The population of 
regulatory T cells was significantly lower in HBV-infected mice 
compared to that of uninfected mice. In HBV-infected mice, 
HBV-specific CTLs were detected by tetramer. Serum ALT, gran-
zyme A and interferon-gamma levels were elevated only in 
HBV infected and PBMC-transplanted mice. Two weeks after 
injection of human PBMCs, the value of hepatitis B surface 
(HBs) antigen decreased below the detectable limit, and anti-
HBs antibody became positive in all 6 mice. Such a decrease 
in HBs antigen was not observed in mice with only HBV infec-
tion.

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The following people have nothing to disclose: Takuro Uchida, Nobuhiko 
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shi Aikata, Yui Ishida, Chise Tateno, Katsutoshi Yoshizato

1719 Increased fucosyltransferase 2 gene expression in HBV 
infecion enhances HBV replication

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Background Glycans, located on the cell membrane, mediate 
various in vivo phenomena such as embryonic development 
and viral infection. Carcinogenesis often alters glycogene 
expression, which affects glycan structure. Hepatitis B virus 
(HBV) infection is a well-known cause of hepatocellular car-
cinoma; however, the interaction between HBV and glycans 
remains unclear. We therefore aimed to search for glycogenes 
that are specifically upregulated in HBV infection and define 
their function in the HBV lifecycle. Methods We made new 
cDNA microarray slides consisting of 118 human glycogene 
clones. Surgical specimens were obtained from 26 patients who 
underwent surgical treatment for hepatocellular carcinoma; 13 
HBV-related and 13 HCV-related. Surgical specimens of nor-
mal liver were obtained from 11 patients who underwent surgi-
cal treatment for other cancers such as colon or gastric cancer. 
Glycogene expression was analyzed using a cDNA chip. For 
in vitro analysis, we used HepG2 cells, HepG2.2.15 cells that 
contantly support HBV replication derived from HepG2 cells, 
HepAD38 cells that support HBV replication by removing tetra-
cycline, and stably Na+-taurocholate cotransporting polypep-
tide (NTCP)-overexpressing HepG2 cells. For gain-of-function 
and loss-of-function analyses, we generated or purchased the 
relevant plasmids and siRNA for transfecting the cells. We
then determined intra- and extracellular HBV DNA by RDT-PCR and gene expression levels by RDT-PCR and western blotting. Results We specified the glycoengenes specifically upregulated in HBV-infected patients with a focus on the fucosyltransferase 2 (Fut2) gene. Fut2 gene expression in HepG2.2.15 cells was significantly higher than in HepG2 cells. The tetracycline-off system revealed a significant increase in Fut2 gene expression in HepAD38 cells when HBV replication was propagated, and this expression was attenuated by entecavir or lamivudine treatment. We then investigated whether Fut2 gene expression has a positive effect on HBV replication. Fut2 overexpression in HepAD38 cells significantly increased HBV replication and silenced Fut2 gene expression reduced HCV replication. Moreover Fut2 overexpression increased HBV infection in hepatocytes, regardless of NTCP overexpression status. Conclusion HBV infection upregulates Fut2 gene expression, and the Fut2 gene induces HBV replication. Detailed functional analysis of the effect of Fut2 on HBV infection may be the key for defining the HBV life-cycle and may lead to the discovery of a new therapeutic target for HBV infection.

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The following people have nothing to disclose: Takayuki Shimoto, Masao Honda, Takayoshi Shirasaki, Kazuhisa Murai, Tetsuro Shimakami, Seishi Murakami

1720
Extensive Hepatitis B virus (HBV) Core Antigen Mutation Associated with Massive Intrahepatic Production of Germline Anti-core IgM and IgG Suggest a Major Role of Humoral Immunity in the Pathogenesis of HBV-Associated Acute Liver Failure (ALF)
Zhaochun Chen1, Ronald E. Engle1, Ashley B. Tice1, Zhileng Long2, Fausto Zamboni3, Giacomo Diaz4, Patrizia Facci1,2
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The pathogenesis of HBV-associated ALF is poorly understood. Access to multiple liver specimens and serum from 4 well-characterized Italian patients with HBV-associated ALF, who underwent liver transplant within 1 week of admission, provided a unique opportunity to investigate the role of viral and host factors in the molecular pathogenesis of ALF. Following our initial observation of an overwhelming B cell gene signature in ALF, with massive intrahepatic accumulation of plasma cells secreting IgG and IgM, here: i) we analyzed the biological and genetic characteristics of the HBV strains recovered from serum and liver of 4 patients with ALF; ii) we cloned and expressed HbsAg and HbcAg from the patients, which were used to screen the corresponding phage-display Fab libraries (IgG1 and IgM) generated from the liver of each patient to identify the molecular targets of the antibodies produced in the liver; and iii) we performed extensive sequence analysis of these antibodies to investigate their variable region usage and somatic mutation rates. The complete HBV sequence from each patient showed a 2-3% nucleotide mutation rate compared to a reference sequence. All patients harbored the pre-core stop mutation, and data from next-generation sequencing confirmed the presence of this mutation in almost 100% of the viral populations both in liver and in serum. HbcAg was the most variable region of the entire genome, with a mean number of amino acid changes of 12.75 (range 9 to 17) compared to a reference sequence, scattered throughout the protein, with clusters within B- and T-cell epitopes, particularly within the immunodominant B-cell epitope (amino acid 74-84), indicating that HbcAg is under strong immune pressure. By contrast, no AA changes within HbcAg were seen in reported sequences of patients with classic acute hepatitis B. Sequence analysis demonstrated that these anti-Hbc antibodies, albeit produced from unrelated individuals with ALF, display a restricted variable heavy chain (VH) repertoire for both IgG and IgM and lack somatic mutations, providing evidence that HbcAg is the target of antibodies in germline configuration likely produced by naïve B cells. Conclusions: In contrast to acute hepatitis B where liver damage is believed to be predominantly T-cell mediated, our data strongly suggest a major role of humoral immunity against HbcAg in the pathogenesis of HBV-associated ALF.

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The following people have nothing to disclose: Zhaochun Chen, Ronald E. Engle, Ashley B. Tice, Zhileng Long, Fausto Zamboni, Giacomo Diaz, Patrizia Facci

1721
Plasma Fibroblast Growth Factor 23 Concentration is Increased and Related with Liver Injury in Patients with HBV Related Acute-on-Chronic Liver Failure
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Background and aim: The liver expresses several fibroblast growth factors including FGF1, FGF2, FGF19, FGF21, FGF23. Fibroblast growth factor 23 (FGF23) is a circulating peptide whose role is to control phosphate homeostasis and calcitriol levels. FGF23 inhibits renal phosphate reabsorption and renal phosphate transporter expression. High plasma fibroblast growth factor 23 (FGF23) concentration predicts the risk of death and poor outcomes in patients with chronic kidney disease or chronic heart failure. We checked if FGF23 concentration could be modified in patients with HBV related acute-on-chronic liver failure (HBV-ACLF) and predict the relationship with liver injury. Methods: Fifty-two patients with HBV-ACLF, fifty-two patients undergoing chronic HBV hepatitis (CHB), and forty-four healthy controls were enrolled. Plasma FGF23 concentration was measured by enzyme-linked immunosorbent assays (ELISA). Correlations of variables with FGF23 were assessed by the Spearman rank correlation coefficients. Survival time was defined as the time from the date of FGF23 measurement to death or last follow-up. Survival rates were estimated by the Kaplan-Meier method. Biochemical parameters were measured using routine biochemistry laboratory methods. The Glomerular filtration rate (GFR) and Model for End-Stage Liver Disease (MELD) score was calculated with the use of the standard formula. Results: In comparison with healthy controls and CHB patients, a significant increase in plasma FGF23 concentration, which ranged from 4.95 to 240.73RU/ml (median 4.95RU/ml; P < 0.01), were observed in HBV-ACLF patients. No significant difference was observed between CHB patients and healthy controls. Plasma FGF23 concentration was negatively correlated with the levels of calcium, phosphate and sodium (P < 0.05), and was positive correlation with age, MELD score, MELD-Na score and refractory ascites (P < 0.05). We
analyzed the prognostic value of FGF23 levels. On Kaplan-Meier analyses, mortality was significantly associated with high FGF23 concentration (P<0.05). Conclusion: FGF23 concentration can be measured quite easily by enzyme-linked immunosorbent assay (ELISA) and should be enter in routine in many diagnosis laboratories in the next few years. FGF23 levels were increased in patients with HBV-ACLF, even in the absence of renal insufficiency and was the best predictor of the level of liver injury.

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The following people have nothing to disclose: Fangfang Liu, ZhiHong Wan, Hong Zang, Shaoli You, HongLing Liu, Shaojie Xin

1722
Virologic and clinical characters of hepatitis B virus mutations in basal core promoter and precore region in children with chronic hepatitis B and hepatitis B related liver cirrhosis
Yanwei Zhong, Hongfei Zhang, Shishu Zhu, Yi Dong, Zhiqiang Xu, Dawei Chen, Hui Dong, Fenglin Di, Limin Wang, Yu Gan, Fuchuan Wang; pediatric liver disease therapy and research center, 302 hospital, Beijing, China

Background/Aims: Chronic HBV infection may lead to chronic hepatitis B (CHB), which is linked to persistence of viral replication and evolution to liver cirrhosis (LC) and hepatocellular carcinoma (HCC). Chronic HBV infection develops in 90% of newborns, 20–40% of children and 5–10% of adults who were infected. Several studies have reported that the BCP/PC mutants may be associated with progression of fulminant hepatic failure. However, virologic and clinical features of children patients with CHB and LC have not been well documented. This study is to investigate virologic and clinical characters of basal core promoter (BCP) and precore (PC) region mutations in children with chronic hepatitis B and hepatitis B related liver cirrhosis. Methods: A total of 307 patients with a CHB infection, including 88 with hepatitis B related liver cirrhosis and 219 with chronic hepatitis B were enrolled. The HBV genotypes and the presence of mutations in the BCP/PC regions were determined by direct sequencing. Biochemical and serological parameters as well as HBV DNA level were routinely performed. Viral DNA was extracted and subjected to a nested PCR. Genotypes/subgenotypes were determined by direct DNA sequencing following by molecular evolutionary analysis of the viral sequences. Mutations at 11 interested sites of the BCP/PC region were compared among the two groups of patients. Results: 46/307 (14.98%) were infected with genotype B and 261/307 (85.02%) with genotype C. LC and CHB patients both had a significantly higher ratio of genotype C to B (81.9% vs. 70.1%–29.9%). The prevalence of BCP/PC wild-type virus was 54.3% in CHB patients in contrast to 4.8% in LC patients. In genotype C patients, the C1653T T1753C, A1762T, G1764A, G1896A mutations were significantly higher prevalent in LC patients. Genotype B virus had higher 1752 mutation frequency. Genotype C virus had higher prevalence of T1753C, T1758C, A1762T, G1764A, G1896A mutation frequency compared to genotype B virus. CHB patients with BCP/PC mutant virus had higher viral load, whereas LC patients with BCP/PC mutant virus had higher viral load and elevated alanine aminotransferase in comparison with those with the wild-type virus. Conclusions: Children patients with genotype C virus, BCP/PC C1653T, A1762T, G1764A, G1896A mutant virus were more susceptible to develop LC, whereas higher prevalence of the BCP/PC mutations was associated with CHB development. Disclosures: The following people have nothing to disclose: Yanwei Zhong, Hongfei Zhang, Shishu Zhu, Yi Dong, Zhiqiang Xu, Dawei Chen, Hui Dong, Fenglin Di, Limin Wang, Yu Gan, Fuchuan Wang.

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- Hiromi Abe, Tetsushi Sakuma, Masataka Tsuge, Nobuhiko Hiraga, Michio Imamura, C. Nelson Hayes, Hiroshi Aikata, Takashi Yamamoto

Table: GENOTYPE P-S vs GENOTYPE X-PC

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ID: Patient. S-P region (nucleotides [nt] 615-969). X-PC region (nt 1596-1912), In this region genotypes D and E are too similar to be distinguished therefore are classified as D/E. BA: Basal sample; UT: Sample after 1-2 years without treatment, LAM: Sample after 1-4 years treatment with Lamivudine. *Patient 9-UT: viral load level did not allow ultra-deep pyrosequencing analysis.

1724
Analysis of the effect on HBV life cycle by HBV genome editing using TALEN and CRISPR/Cas9 systems
Hiromi Abe1,2, Tetsushi Sakuma, Masataka Tsuge, Nobuhiko Hiraga, Michio Imamura, C. Nelson Hayes, Hiroshi Aikata1,2, Takashi Yamamoto2,1
1Hiroshima University, Hiroshima, Japan; 2Liver Research Project Center, Hiroshima University, Hiroshima, Japan; 3Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Hiroshima, Japan

Background and aim: In HBV infection, interferon and other antiviral drugs can control HBV replication. However it is still difficult to eradicate HBV completely because covalently closed circular DNA (cccDNA) stably remains in the nucleus of hepatocytes as mini-chromosomes. cccDNA works as a template for transcription for viral mRNAs after removal of nucleoside analogues and viral replication and worsening of hepatitis often occurs. Recently, new genome editing systems, TALEN and CRISPR/Cas9 systems have been developed to target specific regions of double stranded DNA sequences. The aim of this study is to investigate the efficacy of genome editing using TALEN and CRISPR/Cas9 systems to destroy HBV genomes.

Method: HepG2 cells were maintained with DMEM containing 10% FBS. Cells were seeded in 6 well-plates and co-transfected with 1.4×HBV genome and TALEN or CRISPR encoding plasmid in a 1:2 ratio. Three days after co-transfection, we harvested cells and culture medium to evaluate the efficacy of the genome editing by TALEN and CRISPR/Cas9 systems. The HBV DNA in culture medium was measured by qPCR. To examine viral replicative intermediates, we performed immunoprecipitation using anti-HBc antibody. After DNA purification, the core-associated HBV DNA was quantified by qPCR. TALEN plasmids were designed to target HNF4 binding sites in the core region. CRISPR plasmids were designed to target the S gene, polymerase and core region of HBV genome.

Results: We designed three sets of TALEN-encoding plasmids targeting the core region and confirmed the nuclease activity by reporter-based assay. When we co-transfected 1.4×HBV genome plasmids and TALEN encoding plasmid, we did not observe any suppressive effect of TALEN. As we observed loss of protein production by TALEN expression, we thought that poor effect of TALEN was due to loss of viability in TALEN transfected cells. In contrast, co-transfection of plasmid of 1.4×HBV genome with CRISPR/Cas9 plasmid showed apparent reduction of HBsAg and HBeAg production compared with control plasmids. Furthermore, core associated HBV DNA declined significantly by co-transfection with CRISPR/Cas9 plasmid.

Conclusion: Our results show that the CRISPR/Cas9 system is a possible candidate to target HBV DNA in infected cells. Further study is necessary to determine whether this system can reduce cccDNA in infected cells.

Disclosures:
- Kazuaki Chayama - Consulting: Abbvie; Grant/Research Support: Dainippon Sumitomo, Mitsubishi Tanabe, DAIICHI SANKYO, Toray, BMS, MSD; Speaking and Teaching: Chugai, Mitsubishi Tanabe, DAIICHI SANKYO, KYORIN, Nikken Medi-Physics, BMS, Dainippon Sumitomo, MSD, ASKA, Astellas, AstreaZeneca, Eisai, Olympus, GlaxoSmithKline, ZERIA, Bayer, Minophagen, JANSSEN, JIMRO, TSUMURA, Otsuka, Taiho, Nippon Kayaku, Nippon Shin'yaku, Takeda, AINOMOTO, Meiji Seika, Toray
- The following people have nothing to disclose: Hiromi Abe, Tetsushi Sakuma, Masataka Tsuge, Nobuhiko Hiraga, Michio Imamura, C. Nelson Hayes, Hiroshi Aikata, Takashi Yamamoto
1725 Serum Arginase-1 Levels Are Lower Than Expected in Hepatitis B-related Acute Liver Failure

Perry H. Dubin, Jody A. Balko, Michelle Gottfried, William M. Lee; 1Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX; 2Data Coordination Unit, Medical University of South Carolina, Charleston, SC

Background: Hepatitis B virus (HBV)-related liver injury is immune-mediated. The 1% of acute HBV infection evolving to acute liver failure (ALF) is presumed to result from over-exuberant immune responses. Arginase (ARG) is a non-antigen dependent immune-modulator that inhibits HBV specific CD8 T-cells, by depleting arginine necessary for T-cell-hepatocyte attachment. ARG released from within damaged hepatocytes may provide increasedmodulatory effect, thus limiting further hepatocyte injury. We hypothesized that ARG might be driving the evolution to ALF, if values of ARG were lower than expected in ALF patients, suggesting a muted immune response in this setting. Methods: Serum levels of ARG-1 isotype were measured using a sandwich type ELISA employing HRP-labeled antibody in 107 HBV patients with different phenotypes: HBV-ALF (non-immunosuppressed), acute HBV with recovery, chronic HBV (with and without flares of activity), and, as controls, 20 acetaminophen-related ALF, 10 chronic hepatitis C and 10 healthy subjects. Results: Healthy controls had median ARG of 5 ng/mL, chronic HBV and HCV ~25-30 ng/mL (Table), while acute HBV, HBV flares and HBV-ALF median levels were 89.2, 78.4 and 69.5 ng/mL, respectively, markedly lower than APAP median level of 968 ng/mL. HBV-ALF ARG levels were actually lower than acute or flare HBV despite comparableaminotransferase levels. Particularly low values for ARG were found in those who died of HBV-ALF (median 30.4 ng/mL; n=5). For chronic HBV phenotypes with relatively low AST values there was poor correlation of AST with ARG (Spearman rho); only flare or APAP patients showed strong correlations (rho >0.75). Summary/Conclusions: Despite massive hepatic necrosis, low ARG levels characterize HBV-ALF and, to a lesser extent, acute HBV patients, supporting the postulate of ARG-driven immunomodulation in hepatitis B. A genetically mediated alteration in ARG protein might account for the low levels observed. Further understanding of the significance of ARG levels in these settings will require mechanistic or genomic studies.

Median Arginase, AST Results and Correlationby Categories and Etiologies

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Disclosures: William M. Lee - Consulting: Eli Lilly, Novartis; Grant/Research Support: Gilead, Roche, Vertex, BI, Anadys, BMS, merck; Speaking and Teaching: Merck

The following people have nothing to disclose: Perry H. Dubin, Jody A. Balko, Michelle Gottfried

1726 Hepatitis B virus recurrence after liver transplantation and evolution of viral quasispecies

Maria Buti, David Tabernero, Rosario Casillas, Antoni Mas, Maria Homs, Martin Prieto, Fernando Casalot, Antonio Gonzalez, Manuel Miras, Jose Ignacio Herrera, Luis Castells, Rafael Esteban, Francisco Rodriguez-Frias; 1Liver Unit, Hospital Universitari Vall d’Hebron, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; 2Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain; 3Biochemistry, Hospital Universitari Vall d’Hebron, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; 4Liver Unit, Hospital Clinic, IDIBAPS, Barcelona, Spain; 5Hepatology Unit, Digestive Medicine Service, Hospital Universitari i Politècnic La Fe, Valencia, Spain; 6Gastroenterology and Hepatology Unit, Hospital Universitario Marqués de Valdecilla, Universidad de Cantabria, Santander, Spain; 7Digestive Medicine Service, Hospital Universitario Virgen de la Arrixaca, Murcia, Spain; 8Liver Unit, Clinica Universidad de Navarra, Pamplona, Spain

Background and aims: Some orthotopic liver transplantation (OLT) patients experience HBV recurrence with detectable HBV-DNA despite hepatitis B immune globulin + lamivudine (HBIG + LAM) prophylaxis. We analyzed changes in the HBV quasispecies in patients with recurrent HBV post-OLT. Methods: Twenty-nine OLT patients included in a previous study (1) to compare LAM vs. LAM+HBIG in preventing HBV recurrence were followed for >10 years (mean, 154.9 months [50-188]). In patients with recurrent HBV after OLT, defined as detectable HBV-DNA by real-time PCR, a region of HBV surface gene (S, codons s92-s200), including the “a” determinant, was studied by ultra-deep pyrosequencing (UDPS, GS-Junior, Roche). Results: Twelve (41%) of 29 patients had detectable HBV-DNA at some point after OLT. In addition, 4 of them were HBSAg positive. Among patients with recurrent HBV, one with, and three without HBSAg had available pre- and post-OLT samples with HBV-DNA above 10E3 IU/mL and were selected for UDPS analysis (Table). Conclusions: After OLT, the HBV quasispecies showed major changes in dominant genotype and variant composition: in the S gene, variants associated with low HBSAg detectability and prophylactic treatment escape were preferentially selected outside the “a” determinant, whereas the wild-type was preferentially selected inside. These changes may be enhanced by a bottleneck effect over HBV-quasispecies variant populations due to OLT, and prophylactic treatment pressure.


Disclosures: Maria Buti - Advisory Committees or Review Panels: Gilead, Janssen, Vertex, MSD; Grant/Research Support: Gilead, Janssen; Speaking and Teaching: Gilead, Janssen, Vertex, Novartis
Hepatitis B Virus Core Protein Amino Acids 77-78 Play Vital Roles in Capsid Formation and Function

Kai Deng1,2, Dong Jiang1,2, Lai Wei1,2; 1Peking University Hepatology Institute, Beijing, China; 2Beijing Key Laboratory of Hepatitis C and Immunotherapy for Liver Diseases, Beijing, China

Background: In HBV life cycle, viral core proteins, pregenomic RNA, reverse-transcriptase and host factors form icosaedral nucleocapsid, which plays an important role in HBV DNA replication and progeny virus production. Previous reports revealed that core protein-core protein hydrophobic interaction was required for capsid assembly initiation. In this study, we identified two acidic amino acids E77, D78 of HBV core proteins, located at the tip of the capsid spike, played vital roles in capsid formation and function. E77K/R, D78K/R mutations, which changed the charge of the region, completely blocked the capsid formation, while E77K/A, D78K/A mutations form irregular core protein aggregates with a larger molecular weight than wild type capsid. The corresponding core protein mutants (E77K/R, D78K/R) can also effectively interfere wild type capsid formation, HBV DNA replication and progeny virus production. Methodology: Based on Hbc capsid spatial structure (PDB Accession Number: 1QGT.), HBc E77, D78 spatial location was analyzed using SwissPdbViewer v4.0. Plasmid pHBV1.2 (AY518556) contained a 1.2-length HBV adw genome inserted into the vector PUC18. Plasmid pHBV1.2-core– was derived from pHBV1.2 by introducing a stop codon (TAT TAG) into the C gene at Y38 position, thereby preventing the production of core proteins. Plasmid 1-183flag directed the expression of the HBV core gene with a flag tag at the C terminal. Core protein mutations were generated by site-directed mutagenesis based on plasmid 1-183flag. HepG2 cells were cultivated in DMEM:F12 medium and all transient transfections were performed using FuGENE HD transfection agent. HBV capsid was detected by anti-core serum (DAKO B0586). Core particle related HBV DNA and pregenomic RNA were detected by Southern Blot and Northern Blot, respectively. Culture medium virion DNA was quantified by Real-time PCR assay. Results: E77K/R, D78K/R mutations fully abolished the capsid formation, viral pregenomic RNA encapsidation and DNA replication, while E77K/A, D78K/A mutations formed large aggregates with disruption of function still supporting replication. In addition, E77K/R, D78K/R core protein mutants were able to interact with wild type HBV core protein monomers, induced irregular core protein aggregates formation and block the correct capsid assembly, HBV replication and progeny virus production were also inhibited. Conclusions: HBV core protein acidic amino acids E77K, D78 were critical for HBV core protein interplay and capsid formation. Changing the charge round the region disrupts core protein assembly and function. Our work provides a new angle and framework for further exploring the novel antiviral strategy.

Disclosures:

Lai Wei - Advisory Committees or Review Panels: Gilead, AbbVie; Consulting: Gilead; Grant/Research Support: BMS, Roche, Novartis

The following people have nothing to disclose: Kai Deng, Dong Jiang

Cytokeratins 8 and 18 are responsible for intracellular distribution of the large hepatitis B virus surface protein

Martin Roderfeld1, Dirk Schröder1, Yury Chunin1, Dieter Glebe2, Elke Roeb1; 1Department of Gastroenterology, Justus Liebig University, Giessen, Germany; 2Institute of Medical Virology, National Reference Centre for Hepatitis B and D Viruses, Justus Liebig University, Giessen, Germany

Background: Chronic hepatitis B virus (HBV) infection causes liver cirrhosis and hepatocellular carcinoma. Host autoimmune reactions against the virus and infected cells as well as direct cytotoxic effects of viral components contribute to liver injury. The accumulation of the large HBV surface protein (LHBs) in the endoplasmic reticulum (ER) of hepatocytes leads to ER stress. Severely affected cells finally undergo apoptosis or transform into tumor cells. In this work we show that cytokeratins (CK) are responsible for the intracellular distribution of LHBs. Methods: DNA sequences of LHBs and the small surface protein of HBV (SHBs) were cloned separately into lentiviral vectors. The human hepatoma cell line HuH7 and the untransformed mouse fibroblast cell line NIH3T3 were stably transduced using these vectors. HBs expressing cells were treated with the phosphatase inhibitor okadaic acid (Oka) and the microtubule (MT) and microfilament (MF) disrupting substances nocodazole and cytochalasin D, respectively. Furthermore immunofluorescence staining, confocal microscopy, proximity ligation assay (PLA) and surface-plasmon resonance (SPR) were performed. We also analysed the effects of Oka on HBsAg secretion in a separate HBV infection and secretion experiment on primary hepatocytes from Tuapaia belangeri (PThs). Results: Whereas the accumulation of SHBs in both cell lines was finely distributed within the cells, the expression of LHBs in NIH3T3 led to formation of large intracellular aggregates of LHBs protein. In contrast, LHBs was finely distributed within HuH7. Treatment with Oka caused a breakdown of the LHBs together with the CK filament network followed by formation of perinuclear aggregates of CK18. An interaction of LHBs with CK18/18 together with LHBs. Interrupting MT and MF led to slight changes in LHBs distribution pattern only. Confocal microscopy and PLA of co-stained LHBs and CK8/18 in HuH7 expressing LHBs confirmed their colocalization. An interaction of LHBs with CK8/18 was seen by SPR technique. CK18 transfection in NIH3T3 expressing LHBs prevented the formation of large LHBs aggregates and led to a finely distributed LHBs pattern and strong colocalization with CK18. Contrarily, CK18 knockdown by RNAi caused perinuclear aggregates of CK and LHBs in HuH7 expressing LHBs. Treatment of PThs with Oka led to an increase in the infection rate by about two-fold, whereas no effect of Oka on HBsAg secretion could be seen in already HBV-infected PThs. Conclusion: CK 8 and 18 are responsible for intracellular distribution of LHBs and might be relevant for HBV infectivity. These new findings might be relevant for new therapeutic options in HBV therapy.

Disclosures:

The following people have nothing to disclose: Martin Roderfeld, Dirk Schröder, Yury Chunin, Dieter Glebe, Elke Roeb

Reference Centre for Hepatitis B and D Viruses, Justus Liebig University, Giessen, Germany
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**Hepatitis B virus infection efficiency and immune response decreases with cell density in primary cultured hepatocytes**

C. Nelson Hayes, Hiromi Abe, Sakura Akamatsu, Nobuhiko Hiraga, Michio Imamura, Masataka Tsuge, Daiki Miki, Hiroshi Aikata, Hidenori Ochi, Yuji Ishida, Chise Tateno, Kazuaki Chayama; Hiroshima University, Hiroshima, Japan

**Purpose**

The protective role of invariant Natural Killer T cells (iNKT cells) against hepatitis B virus (HBV) remains controversial. We sought to clarify the role of peripheral iNKT cells during chronic HBV infection. Methods 60 patients with chronic HBV infection were categorized into immune tolerance phase group (n=16), immune tolerance phase group (n=19) and inactive carrier phase group (n=25). 20 healthy controls were enrolled as healthy control group. In addition, another 21 HBeAg-positive patients were enrolled, and they were administrated with entecavir (0.5 mg/d) for 6 months. The peripheral bloods from all subjects were collected. The percentages of iNKT cells and the levels of IFN-γ and IL-4 expressed by iNKT cells were examined by flow cytometry. Serum HBV DNA was measured by the real-time PCR. The serum alanine transaminase levels were assayed by DXC 800 Fully-auto Bio-Chemistry Analyzer. The relationships between serum HBV DNA and ALT levels and the percentages of iNKT cells and its IFN-γ and IL-4 levels were analyzed. Results Circulating IFN-γ-producing iNKT cells gradually increased, and IL-4-producing iNKT cells gradually decreased from immune tolerance phase, immune tolerance phase to inactive carrier phase during chronic infection. The frequency of iNKT cells and its IFN-γ level were reversely correlated to viral load. The level of IL-4 expressed by iNKT cells was positively correlated to viral load and the serum alanine transaminase levels. After anti-virus therapy, the IFN-γ-producing iNKT cells were increased and IL-4-producing iNKT cells were decreased. Conclusions Circulating iNKT cells exhibit a function skewing and play dual immunoregulatory roles during chronic HBV infection. On one hand, iNKT cells contribute to the clearance of HBV by expressing IFN-γ, and on the other hand, iNKT cells induce the liver injury by expressing IL-4.

**Disclosures:**

Man Li - Employment: Shuguang Hospital Affiliated to Traditional Chinese Medicine

The following people have nothing to disclose: Zhen-Hua Zhou, Xue-Hua Sun, Yue-Qiu Gao

1730

**The dual immunoregulatory roles of circulating iNKT cells during chronic HBV infection**

Man Li, Zhen-Hua Zhou, Xue-Hua Sun, Yue-Qiu Gao; Department of Hepatology, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China

**Purpose**

The protective role of invariant Natural Killer T cells (iNKT cells) against hepatitis B virus (HBV) remains controversial. We sought to clarify the role of peripheral iNKT cells during chronic HBV infection. Methods 60 patients with chronic HBV infection were categorized into immune tolerance phase group (n=16), immune tolerance phase group (n=19) and inactive carrier phase group (n=25). 20 healthy controls were enrolled as healthy control group. In addition, another 21 HBeAg-positive patients were enrolled, and they were administrated with entecavir (0.5 mg/d) for 6 months. The peripheral bloods from all subjects were collected. The percentages of iNKT cells and the levels of IFN-γ and IL-4 expressed by iNKT cells were examined by flow cytometry. Serum HBV DNA was measured by the real-time PCR. The serum alanine transaminase levels were assayed by DXC 800 Fully-auto Bio-Chemistry Analyzer. The relationships between serum HBV DNA and ALT levels and the percentages of iNKT cells and its IFN-γ and IL-4 levels were analyzed. Results Circulating IFN-γ-producing iNKT cells gradually increased, and IL-4-producing iNKT cells gradually decreased from immune tolerance phase, immune tolerance phase to inactive carrier phase during chronic infection. The frequency of iNKT cells and its IFN-γ level were reversely correlated to viral load. The level of IL-4 expressed by iNKT cells was positively correlated to viral load and the serum alanine transaminase levels. After anti-virus therapy, the IFN-γ-producing iNKT cells were increased and IL-4-producing iNKT cells were decreased. Conclusions Circulating iNKT cells exhibit a function skewing and play dual immunoregulatory roles during chronic HBV infection. On one hand, iNKT cells contribute to the clearance of HBV by expressing IFN-γ, and on the other hand, iNKT cells induce the liver injury by expressing IL-4.

**Disclosures:**

Man Li - Employment: Shuguang Hospital Affiliated to Traditional Chinese Medicine

The following people have nothing to disclose: Zhen-Hua Zhou, Xue-Hua Sun, Yue-Qiu Gao

1731

**Pretreatment Cirrhosis and Higher Body Mass Index Predicted Alamine Aminotransferase Abnormality in Patients Achieved Undetectable Serum HBV DNA with Nucleos(t)ide Analogs Therapy**

Yukawai Wuy1,2, Yusheng Jie1,2, Xiangyong Li1,2, Guali Lin1,2, Shuru Chen1,2, Xin-Hua Li1,2, Hong Shi1,2, Fangji Yang1,2, Min Zhang1,2, Mengxing Huang1,2, Yunlong Ao1,2, Yihua Pang1,2, Yutian Chong1,2; 1Department of Infectious Diseases, the Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China; 2Key Laboratory of Tropical Disease Control (Sun Yat-Sen University), Ministry of Education, Guangzhou, China; 3Department of Infectious Diseases, the Fifth Affiliated Hospital of Sun Yat-Sen University, Zhuhai, China

**Background and objectives:** Alanine aminotransferase (ALT) is the most commonly used parameter for evaluating liver impairment. Antiviral therapy with nucleos(t)ide analogs (NAs) showed a high normalization rate of aminotransferase after suppression of hepatitis B virus (HBV) DNA in patients chronically infected with HBV. However, some patients still had abnormal serum aminotransferase levels even if they have achieved undetectable HBV DNA (or complete viral response, CVR) for a long time, the reasons of which hasn’t been studied. This research aimed to define the risk factors correlated with biochemical abnormality after CVR in patients treated with NAs. Methods: 388 chronically HBV infected patients ongoing naive NAs therapy, who achieved undetectable serum HBV DNA (<20 IU/ml) during Jan. 2006 and Feb. 2014, were retro- and prospectively followed. Patients were divided into two groups: patients with normal ALT (n=298) and with abnormal ALT (n=90) (defined as serum ALT >40 U/L in male or >35 U/L in female at least twice consecutively with a interval of 1-3 months after achieving undetectable HBV DNA). Multivariate logistic regression
Hepatitis B virus relapse after discontinuation of long-term treatment with tenofovir in chronic HBsAg-negative patients

Maria Buti1,2, Maria Homs3, Rosario Casillas1, David Tabernero1, Josep Gregori1,2, Carolina Gonzalez1, Mar Rivero-Barcia1, Maria Teresa Salcedo1, Maria Blas3, Leonardo Nieto2, Francisco Rodriguez-Frias3, Rafael Esteban1,2,1Liver Unit, Hospital Universitari Vall d’Hebron, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; 2Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain; 3Biochemistry, Hospital Universitari Vall d’Hebron, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; 4Pathology, Hospital Universitari Vall d’Hebron, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; 5Microbiology, Hospital Universitari Vall d’Hebron, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain

Background and aims: Data are limited on tenofovir (TDF) treatment discontinuation after long-term viral suppression in HBsAg-negative patients. This study investigates whether TDF discontinuation in this scenario is associated with a low rate of virologic relapse. Methods: HBsAg-negative chronic HBV patients treated with TDF for more than 8 years with complete, persistent viral suppression received HBV vaccine after TDF discontinuation and were followed-up for 1 year with monthly ALT, HBV-DNA and HBsAg determinations. In patients with viral relapse, HBV quasispecies between codons r1632-r278 was analyzed by ultra-deep pyrosequencing (GS-FLX, Roche) and compared with results prior to therapy. Results: Eight patients met these characteristics. After discontinuing TDF, only 1 patient achieved virologic remission and 7 relapsed after 4-8 weeks, but 5 of them achieved immune control (HBV-DNA<2000 IU/mL and normal ALT) over the 1-year follow-up. HBsAg levels decreased in 2 patients, but no patients lost HBsAg or developed anti-HBsAg antibodies. Changes in the HBV quasispecies after treatment discontinuation are shown in the table. Conclusions: Despite long-term complete viral suppression under TDF, most patients had initial virologic relapse and some experienced later immune control. Changes in the HBV quasispecies between baseline and after TDF discontinuation suggest continuous evolution. Further long-term follow-up studies are needed to confirm our results. Study cofinanced by Instituto de Salud Carlos III and European Regional Development Fund (grants P11/01973 and P12/01893)

Disclosures:
Yuanan Wu - Grant/Research Support: Bristol-Myers Squibb Company
The following people have nothing to disclose: Yusheng Jie, Xiangyong Li, Guoli Lin, Shuru Chen, Xinhua Li, Hong Shi, Fangji Yang, Min Zhang, Mingxing Huang, Yunlao Ao, Yihua Fang, Yulian Chong

1733 Clonal Hepatocyte expansion in the young adult HBV infected liver is at odds with the concept of a generic immune tolerant disease phase: can additional clinical parameters distinguish disease phase?

Patrick T. Kennedy1, Upkar S. Gill2, Antony Chen3, Samuel Litwin4, Antonio Bertolli5, William Mason1, 1Hepatology Unit, Centre for Digestive Diseases, Blazeard Institute, Barts and The London, School of Medicine & Dentistry, GMUL, London, United Kingdom; 2Energing Infectious Diseases Program, Duke-NUS Graduate Medical School, Singapore, Singapore; 3Fox Chase Cancer Centre, Philadelphia, PA

INTRODUCTION: Immune tolerant (IT) Chronic Hepatitis B (CHB) is a clinical definition based on normal serum ALT and high HBV DNA. We have recently challenged the precision of this disease categorisation, demonstrating immune responses in the periphery of IT patients. Here we analysed liver tissue from young adults across different disease phases for evidence of disease progression. We assayed for clonal hepatocyte repopulation; a feature of chronic liver disease and a risk factor for hepatocellular carcinoma (HCC). In addition, we studied the hepatocyte distribution of HBV core protein; which is known to vary over the viral life cycle and thus may discern disease phase. PATIENTS & METHODS: To detect clonal expansion of hepatocytes we assayed for integrated HBV DNA detectable by inverse PCR in liver biopsy specimens (n=27). Integration occurs at random sites in host DNA; quantifying copy numbers of individual integrants provides a measure of hepatocyte death and regeneration. Clone size >1,000 hepatocytes are not consistent with random regeneration events and represent hepatocytes resistant to immune killing, a risk factor for the development of HCC. Paraffin embedded sections of liver tissue from these patients were stained with HBCaAb. The percentage of hepatocytes staining positive for nuclear core were quantified and correlated with disease phase. RESULTS: Clone sizes >1,000 hepatocytes were found in 8/9 IT, 5/6 eAg+ and 7/7 eAg- immune active (IA) patients. HCC patients (5/5) all had
large clone sizes. No significant difference in the incidence of large clones was seen across disease categories (p=n.s.). We investigated for a differential clinical profile indicating the presence of large clones. Clone size tended to increase with age, eAg status and in HCC. The only significant difference noted was a high HBV DNA (>9 log) association with fewer and smaller clones (p=0.0005). We did, however, note that IT patients demonstrated increased distribution of nuclear core HBV stained hepatocytes (4.66%), compared to eAg+ (1.82%, p=0.02) and eAg- IA patients (0.88%, p=0.009). In keeping with an IT profile, patients with high levels of HBsAg (>10,000 IU/ml) displayed increased nuclear core HBV staining (4.03%), compared to those with lower HBsAg levels (0.46%, p=0.001).

CONCLUSIONS: Clonal hepatocyte expansion in patients considered IT is evidence of disease progression in the IT disease phase. However, nuclear core hepatocyte staining in addition to quantitative HBsAg may provide further data to distinguish distinct disease phase or progression. These data highlight the limitations of the current clinical definition of immune tolerance.

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Toll like Receptor Polymorphisms in Spontaneous HBsAg Seroconversion
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Introduction: Chronic hepatitis B is an immunologically driven disease. Host immunogenetic factors are determinant for eradication of the hepatitis B besides the viral factors. Toll like receptors (TLR) are pattern recognition receptors and found to be related to liver diseases. Here we aimed to genotype TLR-4, TLR-5 and TLR-9 polymorphisms in patients and spontaneous surface antigen seroconverted control group. Methods: One hundred thirty chronic hepatitis B patients who were followed up at hepatology clinic, and, age and gender matched healthy unrelated control group which consists of 168 people were enrolled. Anti Hbs and Anti Hbc IgG positivity without prior hepatitis B vaccination were the selection criteria for the control group. Local ethics committee approval was taken. Genomic DNA was extracted from peripheral blood samples. TLR4 (rs4986790), TLR5 (rs5744174) and TLR9 (rs5743836) polymorphisms were detected by polymerase chain reaction (PCR) - restriction fragment length polymorphism (RFLP) technique. The chi-square test was applied for comparing the allele frequencies between patient and healthy control group. Results: frequencies between patient and healthy control group. Results: Patient group (84 male, 47 female, mean age= 47.4±13) and age and sex matched control group (89 male, 79 female, mean age= 48.8±12.9) were recruited. In the chronic hepatitis B group, 116 patients (88%) were HBeAg negative, 29 (9.7%) had inactive disease, 43 (32.8%) had cirrhosis. The mean pretreatment ALT, AST and log DNA were 118.4±56 U/ ml, 86.8±66.5 U/ml and 5.6±2 IU/ml, respectively. Seventy patients (53.8%) had liver biopsy; the mean Ishak fibrosis score was 3.3±1.5 and the mean hepatic activity index was 7.8±3. TLR4 (rs4986790) A/G polymorphisms distribution was not statistically different between patients and the control group. TLR5 (rs5744174) TT genotype was more frequent in spontaneous seroconverted control group compared to chronic hepatitis B patients (%17.3 vs %2.3 χ² = 17.2, OR= 0.1, 95% CI= 0.03-0.38, p < 0.001). TLR9 (rs5743836) non-CC genotype (IT or CT) was more frequent in the control group compared to chronic hepatitis B patients (17.3% vs. 9.2%, χ² = 4.1, OR =2.0 95% CI= 1.01-4.2, p = 0.04) Conclusion: The ultimate treatment target for a chronic hepatitis B patient is HBsAg seroconversion. Polymorphisms in TLRs - pattern recognition receptors- are important components of host immune repertoire and also influence the outcome of hepatitis B virus infection.

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Phenotypic Characteristics of PD-1, CTLA-4 and FoxP3 Expression during Tenofovir therapy in Chronic Hepatitis B
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Background: Inhibitory molecules such as programmed death 1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) are associated with antiviral effector T-cell dysfunction, which influences on T-cell exhaustion and persistent viral infection. These PD-1 and CTLA-4 are up-regulated in chronic viral infection such as chronic hepatitis C, chronic hepatitis B and human immunodeficiency virus infection but there is few report about the role of PD-1 and CTLA-4 in patients with chronic hepatitis B during antiviral therapy with tenofovir. We investigated the expression of PD-1 and CTLA-4 during tenofovir treatment in patients with chronic hepatitis B. Methods: Nine patients with chronic hepatitis B under tenofovir treatment were enrolled for detection of intrinsic inhibitory molecules of T cell signals (PD-1, CTLA-4) and extrinsic inhibitory molecule, FoxP3. Peripheral blood mononuclear cells (PBMC) were isolated from these subjects before tenofovir treatment (T0) and 1 month (T1), 3 month (T3), 6 month (T6) during tenofovir treatment. The expressions of PD-1, CTLA-4 and FoxP3 on T cells were monitored by flow cytometry. Results: T cells from patients with chronic hepatitis B under tenofovir treatment showed decreased expression of PD-1, CTLA-4 and FoxP3 at T6 compared to T0 (%PD-1/CDB, 5.0 ± 2.2 vs. 4.0 ± 1.2, %CTLA-4/CDB, 1.7 ± 0.9 vs. 1.2 ± 0.6, %FoxP3/CDB, 7.2 ± 2.5 vs. 6.1 ± 2.6 showed as mean ± SD). During the initial phase of tenofovir treatment, FoxP3 and PD-1 fluctuate at T1 and T3 but, CTLA-4 decreased steadily even at T1 and T3. Conclusions: In chronic hepatitis B, PD-1 and CTLA-4 as inhibitory T cell molecules and FoxP3 as regulatory T cell marker are down-regulated during initial 6 month tenofovir therapy, which could restore HBV-specific T cell function during tenofovir antiviral therapy. Long-term effects of tenofovir on host immune system are needed to be elucidate.

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The following people have nothing to disclose: Hyosun Cho, Yu seung Kim, Hee Yeon Kim, Jong Young Choi, Seung Kew Yoon, Chang Don Lee
Hepatitis B virus (HBV) specifically induces CD4+CD25+CD127-veFoxP3+ with increased anti-tumor immu-
nity in HBV related HCC compared to non-HBV-HCC

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Background: Hepatocellular carcinoma is the second most common cause of cancer death worldwide. In India 50% of HCC cases are attributable to HBV infection. T regulatory cells (Tregs) increase and are likely to play a major role in HCC development. Expansion of Tregs is also induced by HBV infection. To understand their role in HCC, we investigated the expression of CD4+CD25+CD127-veFoxP3+ Tregs and their suppressor factors like PD1, IL-10 and TGF-β in HBV related HCC as compared to non-HBV-HCC. Patients and Methods: Patients with chronic hepatitis B infection (Gr. A, CHBV, n=10), HBV related HCC (Gr. B, HBV-HCC, n=17) and non-HBV-HCC (Gr. C, n=22; NASH =16, Alcohol related, n=6) were recruited. Whole blood was collected in EDTA vials for surface and intracellular immunophenotyping by flow cytometry. Using multicolour flow cytometry, expression of FoxP3, IL-10, PD-1, TGF-β, and Notch1 was observed in CD4+CD25+hi CD127-ve and also in CD8+CD25+hi T regulatory cells. Results: Alpha-fetoprotein (AFP) levels were high in Gr. B (1634.20 ± 4220) than Gr. C patients (1589 ± 456). The total lymphocyte count and CD8+ T cells were significantly lower in Gr. B compared to Gr. A (p=0.003 and p=0.04) and Gr. C (p=0.009 and p=0.05). Foxp3 expression in CD4+CD25+hi CD127-ve and CD8+CD25+hi was increased in Gr. B compared to Gr. C (p=0.007 and p=0.05; Fig 1). Low level of AFP and decreased CD4+CD25+hi population showed positive correlation (R=0.49, p=0.02) in non-HBV-HCC. While CD4+CD25+hi Tregs in Gr. B patients were secreting more of IL-10 compared to Gr. C (p=0.01) (Fig 1). The CD4+ FoxP3+ Tregs showed high TGFβ production in Gr. B patients compared to Gr. C and Gr. A, the PD1 expression on CD4+ CD25+hi cells was significantly lower in Gr. B than Gr. C patients (p=0.04) (Fig 1). Conclusions: CD4+CD25+hi Tregs from HBV-HCC show decreased expression of PD-1, resulting in increased IL-10 and TGF-β secretion. High production of immunosuppressive cytokines i.e. IL-10 and TGF-β, by Treg cells and low PD1 expression suggests that these cells are more active in immune suppression in HBV related HCC compared to non-HBV-HCC.

Figure 1. HBV HCC patients showing increased FOXP3 expression and IL-10 production (A,B) and reduced expression of PD1 on CD4+ CD25+HI (C)

Evaluation of Serum Cytokines and Chemokines in HBeAg Negative Chronic Hepatitis B Patients

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INTRODUCTION: Innate and adaptive immune responses play important roles in chronic hepatitis B infection. A recent study has shown high levels of serum CXCL-8, CXCL-9 and CXCL-10 are associated with hepatic flare. However, the pathogenesis of HBV reactivation in HBeAg negative chronic hepatitis B infection is not clear. In this study, we evaluated levels of serum cytokines and chemokines including IFN-α, IL-1β, TNF-α, IL-6, IL-8, IL-10, CCL-2, CCL-3, CXCL-9, and CXCL-10 in HBeAg negative chronic hepatitis B patients with a range of ALT values. METHODS: Eighty five serum samples of chronic hepatitis B HBeAg negative patients with different levels of abnormal ALT (1 sample/patient, all ALT>70 IU/L) were studied. In these patients/samples, 39 were during HBV reactivation while the rest 46 were not. Serum cytokines/chemokines were analyzed using Affymetrix 10-plex human cytokine kit and Bio-Plex MAGPIX system. Cytokine/chemokine concentrations were calculated using Bio-Plex Manager 6.1. HBV DNA levels were quantified using serum extracted DNA as template and real time PCR with a VQC standard panel. Statistical analyses were carried out using SPSS v17. Data were presented as mean ± SE. RESULTS: Correlation analysis of all variables showed a positive correlation between CXCL9 and ALT levels (Pearson’s r=0.37, p<0.001), and between CXCL9 and HBV DNA levels (Pearson’s r=0.33, p<0.001). Whereas, other cytokines/chemokines were not correlate with ALT and HBV DNA levels in HBeAg negative patients. In addition, the ALT and HBV DNA levels in samples of during HBV reactivation were significantly higher than those without reactivation (478.5 ± 97.4 vs. 161.6 ± 25.9 IU/L, p=0.003; and 6.2 ± 0.19 vs. 5.0 ± 0.21 Log10 IU/mL, p=0.001 respectively) as were the CXCL9 levels (249.3 ± 39.9 vs. 116.2 ± 24.4 pg/mL, p=0.005). There was no significant difference in levels of other cytokines/chemokines between samples during and non-during HBV reactivation. CONCLUSION: CXCL9 is correlated with ALT and HBV DNA levels in HBeAg negative patients. HBeAg negative patients have higher serum CXCL9 levels during HBV reactivation. CXCL9 seems to be not just important in hepatic flares but also in milder forms of abnormal ALT elevation. CXCL9 may play an important role in HBV reactivation in HBeAg negative chronic hepatitis B infection.

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The following people have nothing to disclose: Yan Cheng, Veonice Bijin Au, John E. Connolly
Hepatitis B virus (HBV) has been a marker for HCC in Thai population. Several evidences indicated that single nucleotide polymorphisms (SNPs) in STATs gene such as rs2293152 and rs1053004 were associated with HCC risk and might be used as a novel genetic marker. In the present study, we investigated the mechanism of the difference of chronicity rates in genotype A and C by using hydrodynamic injection mouse model. We investigated the association of single nucleotide polymorphism rs1053004 in signal transducer and activator of transcription 3 (STAT3) for risk of hepatocellular carcinoma in Thai patients with chronic hepatitis B.

**Background:** Signal transducers and activator of transcription (STAT) proteins, a family of transcription factors that play pivotal roles in cytokine signaling pathways. Several evidences have provided important evidence that the rs1053004 SNP at STAT4 have been associated with chronic hepatitis B (CHB) induced hepatocellular carcinoma (HCC). Objective: This study aims to describe the association between these SNPs and HCC in Thai patients with CHB. Method: Study subjects were enrolled and divided into 3 groups including CHB-related HCC (n=192), CHB without HCC (n=200) and healthy controls (n=190). The rs2293152 and rs7574865 SNPs were genotyped using polymerase chain reaction-restriction fragment length polymorphism whereas the rs1053004 SNP was genotyped using allelic discrimination assays based on TaqMan real-time PCR. Results: Data analysis revealed that the distribution of rs2293152 and rs1053004 at STAT3 and rs7574865 at STAT4 genotypes were in Hardy-Weinberg equilibrium (P > 0.05). rs2293152 SNP at STAT3 gene was not significantly associated with the risk of HCC (P < 0.05) whereas the CC genotype of rs1053004 SNP was significantly associated with an increased risk of HCC compared with the CHB without HCC (odds ratio=1.85, 95% confidence interval=1.00-3.43, P = 0.049). In addition, the genotype of rs7574865 SNP at STAT4 (GG versus TT+GT) was significantly associated with a reduced risk of HCC when compared with the healthy controls (odds ratio=1.71, 95% confidence interval=1.13-2.59, P = 0.011). Conclusion: These findings provided important evidence that the rs1053004 SNP at STAT3 and rs7574865 SNP at STAT4 were significantly associated with HCC risk and might be used as a novel genetic marker for HCC in Thai population.

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**1739 Immune system is required for HBV clearance, but not enough for explaining the difference in HBV genotype A and C clearance**


The following people have nothing to disclose: Yoshinobu Yokoyama, Hayato Hikita, Teppei Yoshioka, Kaori Mukai, Satoshi Aono, Takatoshi Nawa, Ryotaro Sakamori, Takuya Miyagi, Kazuyoshi Ohkawa, Naoki Hiramatsu, Tomohide Tatsumi

**1740 Nucleotide analogue improves interferon responsiveness in HBV-infected human hepatocytes**

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Background: It has been reported that interferon treatment could reduce HBs antigen (HBsAg) production in patients with chronic hepatitis B virus (HBV) infection. However, only limited HBsAg reduction is observed in patients treated with interferon therapy. One cause of this limitation may be that interferon responsiveness in human hepatocytes is suppressed by HBV infection, and, therefore, interferon stimulated genes (ISGs) are not induced sufficiently to promote anti-viral effects. In the present study, we analyzed whether the suppression of HBV replication using nucleotide analogues (NAs) could improve interferon responsiveness in HBV-infected human hepatocytes. Methods: Thirty-seven chronic hepatitis B patients were enrolled. Twenty patients underwent sequential interferon therapy, which included 6 months of conventional interferon
During 53 (13-172) months observation period, 15 of 146 patients developed HCC (HCC group) and 131 patients did not (non-HCC group). We conducted an univariate analysis to compare two groups and Kaplan-Meier method search for HCC risk factor. Results: According to the univariate analysis, older age (54.1±9.9 vs. 46.6±10.6 p=0.0101), liver cirrhosis (CH/LC 3/12 vs. 120/11 p=0.0001), lower platelet count (10.6±8.1 x104/μL vs. 17.4±5.7 x104/μL p=0.0001), higher AFP (0.167ng/ml (1.9-523.5) vs. 4.9ng/ml (1.4-1203.2) p=0.0233) at the beginning of NA therapy, and higher AFP (6.5ng/ml (2.7-36.2) vs. 3.3 (0.8-1.9)) one year after NA therapy were identified as risk factors associated with HCC development. Kaplan-Meier showed platelet count <10x104/μL and AFP>23.2ng/ml before NA therapy, and AFP >4.2ng/ml one year after NA therapy were significantly high risk for HCC development (p<0.0001, p<0.00186, p<0.0001, respectively). Among 70 HBeAg-negative patients, liver cirrhosis (CH/ LC 2/5 vs. 58/5 p=0.0001), lower platelet count (10.7±6.1 x104/μL vs. 16.9±6.0 x104/μL p=0.0313), higher AFP (24.6 ng/ml (3.2-523.5) vs. 3.85 ng/ml (1.4-397.3) p=0.0084) at the beginning of NA therapy, and higher AFP (5 ng/ml (4.3-12.5) vs. 2.9 ng/ml (0.8-8.4) p=0.0084)) one year after NA therapy were identified as risk factors associated with HCC development. Kaplan-Meier also showed platelet count <10x104/μL and AFP>7.0ng/ml before NA therapy, and AFP >4.2ng/ml one year after NA therapy were significantly high risk of HCC development (p<0.0034, p<0.01, p<0.0001, respectively). Conclusions: Among patients with good efficacy of NA therapy, older age, lower platelet count, and higher AFP before NA therapy, and relatively higher AFP one year after NA therapy were risk factors for HCC development.

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NKp46 Is Potentially Involved in Control of Hepatitis B Virus Replication and Modulation of Liver inflammatory response

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Background: NK cells function is regulated by the balance of multitude of activitory receptors and inhibitory receptors.However, reports on NK cell in hepatitis B are controversial. Aims: To investigate the phenotype, the expression of receptors and function of NK cells in chronic HBV infection patients, and differential surface expression of NK receptors were blocked to test the killing activity to NK target cell and hepatoma cell lines in vitro. Methods: NK-cell subsets from 86 chronic HBV-infected patients were characterized by flow cytometry. CD107a and IFN-γ secretion were studied. In vitro blockade the differential expression receptors of NK cells, the killing activity of NK-cell was studied using LDH cytotoxicity assay kit. Results: NKp46 was higher in inactive HBsAg carriers than that in other groups(p<0.05). NKp46 was negatively correlated with HBV DNA(R=-0.253, P=0.049) and ALT(R=-0.256, P=0.045). The number and the secretion of IFN-γ has no difference in chronic HBV infection patients. While, the cytotoxic activity has significant different. CD107a was higher in immune-activated group that in immune-tolerant groups(p<0.05). CD107a has relationship with viral load and HBeAg status. In vitro blockade NKp46, spontaneous cytolytic activity of NK cells against K562 cell lines and HepG2, HepG2.215 cell lines was decreased(p<0.05). Conclusion: NKp46 as a major activitory receptor...
Serum soluble CD40 is associated with liver injury in patients with chronic hepatitis B

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To clarify the role of soluble CD40 (sCD40) in chronic hepatitis B (CHB), we measured the levels of sCD40 in sera from 132 CHB patients and 33 healthy individuals, and analyzed its association with serum levels of alanine transaminase (ALT) and aspartate transaminase (AST), and liver histological characteristics. The results indicated that sCD40 concentrations in CHB patients were significantly higher than in healthy controls (82.8 pg/ml vs 32.8 pg/ml). The sCD40 level was related to serum levels of ALT (r=0.487, p<0.001) and AST (r=0.492, p<0.001), and the intrahepatic Ishak necroinflammatory score (r=0.506, p<0.001) and fibrosis score (r=0.395, p<0.001). CHB patients were distributed into four groups based on their Ishak necroinflammatory grading scores: minimal inflammation (scores 1–4), mild inflammation (scores 5–8), moderate inflammation (scores 9–12), and marked inflammation (scores 13–18), which the mean of sCD40 concentration was 61.8 pg/ml, 91.7 pg/ml, 139.0 pg/ml and 203.2 pg/ml respectively. The sCD40 concentration in CHB patients with minimal inflammation was significantly lower than that in patients with mild, moderate, and marked inflammation (p<0.01), and sCD40 concentration in CHB patients with mild inflammation was significantly lower than that in patients with moderate and marked inflammation (p<0.05). CHB patients with different fibrosis stages were distributed into four groups: normal (score 0), portal fibrotic expansion (score 1–2), bridging fibrosis (score 3–4) and cirrhosis (score 5–6), which the mean of sCD40 concentration was 59.0 pg/ml, 66.1 pg/ml, 96.2 pg/ml and 157.2 pg/ml respectively. The difference in sCD40 levels between CHB patients without fibrosis (normal group) and healthy controls was not significant (p>0.05), whereas other groups showed significantly higher sCD40 concentrations than did healthy controls (p<0.001). sCD40 concentration in CHB patients with portal fibrotic expansion was significantly lower than that in patients with bridging fibrosis or cirrhosis (p<0.01), and sCD40 concentration in CHB patients with cirrhosis was significantly higher than that in patients with bridging fibrosis (p<0.05). The area under the receiver operating characteristic curve of sCD40 for diagnosing CHB patients with marked inflammation was greater than that of ALT and AST. Multiple regression analysis showed that liver inflammation correlated with sCD40 levels, but not with ALT and AST levels. In conclusion, sCD40 levels have high diagnostic accuracy for detecting severe liver inflammation in CHB patients and could serve as an immunological marker of hepatic tissue injury.

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Association study of next-generation sequencing determined HBV heterogeneity and antiviral efficacy of Lami- vindine treatment

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We previously reported that Hepatitis B virus (HBV) heterogeneity within reverse transcriptase (RT) was a predictor of antiviral efficacy based on clone-based sequencing. Then, we compared and successfully set up a next-generation sequencing (NGS) based methodology to determine this heterogeneity. The aim of this study was to investigate the dynamic changes of HBV quasispecies within the RT region determined by NGS method during the early stage of lamivudine treatment and its correlation with antiviral efficacy. Methods: Thirty-five chronic hepatitis B patients received lamivudine treatment for at least 48 weeks. Sixteen patients responded to lamivudine, while nineteen patients were partial responders. HBV DNA was extracted from serum samples at baseline and week 4. Three sequential overlapping 400-bp segments covering the RT coding region were amplified and pyro-sequenced. Quasispecies heterogeneity characterization was conducted using bioinformatics analysis at baseline and week 4, and evolutionary patterns of quasispecies in responders and partial responders were studied. Results: The quasispecies complexity value of responders were significantly higher than that of partial responders in the first and second RT regions at baseline (P < 0.05), while in the third RT region at week 4 (P < 0.05). The quasispecies diversity value of responders were significantly higher than that of partial responders of all three RT regions at baseline (P<0.05), and in the first and third RT region at week 4 (P < 0.05). Furthermore, average changes and net changes in the mean genetic distance at amino acid level and the number of non-synonymous substitutions per non-synonymous site were significantly higher than those of partial responders in the first RT region (P < 0.05). Conclusions: Baseline heterogeneity and dynamic changes of HBV quasispecies within the RT region showed distinct patterns between responders and non-responders during early stage of lamivudine treatment. High complexity and diversity of quasispecies at baseline and the dynamic changes during the first 4 weeks were correlated with lamivudine antiviral efficacy. Thus sheds light on the future clinical application of HBV quasispecies studies.

Disclosures:
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High-throughput hepatitis B and D virus model systems for discovery of targets for viral cure

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Background: Hepatitis B virus (HBV) is a major global health problem resulting in progressive liver disease, including cirrhosis and hepatocellular carcinoma—the third leading cause of cancer death worldwide. Current therapies control viral infection and reduce progressive liver disease. However, due to the unique HBV replication cycle viral elimination is virtually absent. Around 5% of HBV infected patients are co-infected with hepatitis D virus (HDV) resulting to more rapid progression of liver disease. HBV/HDV co-infection remains very difficult to treat. Recently, the sodium taurocholate co-transporting polypeptide (NTCP) has been identified as a functional receptor of HBV and HDV. In this study we aimed to establish a high-throughput HBV and HDV infection model system to identify novel targets for viral cure. Methods: To develop high-throughput models for viral infection, we generated a panel of hepatoma cell lines stably overexpressing hNTCP. HBV infectious particles were purified from the serum of virus-infected patients and recombinant HDV and HBV infectious particles were produced in cell lines. Viral infections and their detection were optimized using various protocols including the use of automated systems. Results: Using viral protein-specific immunofluorescence, Northern Blot, HDV RNA-specific qPCR, HBV DNA-specific qPCR, we demonstrate that stable cell lines are highly susceptible to HDV and HBV infections. Screening a large series of cell culture conditions and experimental settings by qPCR, RT-qPCR, Northern blot, ELISA, Western blot and immunofluorescence. Infected cells can either be transfected with siRNAs targeting HBV or HDV transcripts or treated with direct acting antivirals (e.g. tenofovir) or antiviral cytokines (e.g. IFNs). Results: HepaRG cells support a strong, yet transient HDV mono-infection. Although HDV replication in HBV-infected cells was similar to HDV monoinfection, HDV virion secretion could only be observed in the co-infection setting as expected. Secretion of HDV particles strongly suggests co-existence of both viruses in the same cells despite the overall low numbers of infected cells. Upon HDV super-infection of HBV-infected cells, a decrease of all HBV parameters but cccDNA was observed, confirming viral interference in this model. As expected, IFN showed modest effect on both viruses, whereas tenofovir was only active on HBV. Further results will be shown with other investigational drugs (anti-HBc, farnesyltransferase inhibitors, other cytokines...). Conclusions: We established a new in vitro model to further characterize HBV/HDV interplay and confirm a suppressive role of HDV on HBV replication. HepaRG cells represent a relevant infection model to identify new and original targets and study the antiviral activity of direct-acting or immune-modulatory drugs.

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Serial Changes of Cellular, Humoral, and Innate Immune Responses following Immunosuppressive Chemotherapies Responsible for Hepatitis B Virus Reactivation

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BACKGROUND: Hepatitis B virus (HBV) reactivation is well known to be triggered by various regimens of chemotherapies and immunosuppressive therapies. The reactivation risks may be different from therapy to therapy although the frequencies and the mechanisms have not yet defined. HBV reactivation was reported to occur frequently not only in the treatments for hematological malignancy (e.g. CHOP and R-CHOP) but also in recently developed therapies including the biologic therapy to inhibit TNF-α. In the current study, we aimed to determine the incidence of reactivation in patients laterly infected with HBV when treated with regimens of six immunosuppressive chemotherapies at a single hospital, and to monitor the serum immune profiles following the treatments. METHODS: STUDY 1: Nine hundred and forty-four patients underwent six immunosuppressive chemotherapies in University of Fukui Hospital between 2006 and 2011 were enrolled in this study. The patient group comprised 392 subjects treated with steroid pulse therapy (12 patients with asymptomatic HBV infection and 18 patients with resolved HBV infection), 112 with R-CHOP (3 and 29), 50 CHOP (0 and 10), 89 with Rituximab (4 and 12), 225 with methotrexate (4 and 7), and 76 with infliximab (0 and 2), respectively. The incidences of HBV reactivation in each immunosuppressive chemotherapy were determined. STUDY 2: A total of 27 cytokines, chemokines and growth factors were measured by Bio-Plex Suspension Array System in the sera collected consecutively from patients treated with R-CHOP and imatinib. Immune profiles after the initiation of immunosuppressive chemotherapies were investigated. RESULTS: STUDY 1: Incidence of HBV reactivation was 6.9% in R-CHOP (two out of 29 resolved HBV infection) and 20% in CHOP (two out of 10). HBV was not reactivated in the other four regimens. STUDY 2: In a case of malignant lymphoma, IL-2, IL-6, IL-8, and IL-12 reduction was observed after four courses of CHOP. In a case of stomach gastrointestinal stromal tumor, IL-2, IL-6, IL-8, and IL-12 were reduced after two week administration of imatinib. CONCLUSIONS: HBV reactivation occurred only in R-CHOP and CHOP regimens, indicating that T-cell function impairment by steroid and long-lasting B-cell depletion by rituximab may enhance HBV replication and proliferation during the treatments. Furthermore, the data demonstrated that cellular, humoral, and innate immunity were inhibited rapidly after the initiation of immunosuppressive chemotherapies. These results suggest a plausible immunological basis for the reactivation of latently infected HBV after the treatments of immunomodulatory agents.

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Background and aims: Adefovir dipivoxil (ADV) is still widely used in China for treating chronic hepatitis B, either in single or in combination with nucleoside analog. The study aimed to clarify whether hepatitis B virus (HBV) mutation rtA181S was a primary ADV-resistant mutation. Methods: A total of 18,419 patients from Beijing 302 Hospital were investigated. The drug-resistant mutations and HBV genotype were analyzed by direct sequencing of the full length reverse-transcriptase/S genes. Natural replication capacity and 50% effective concentration of drug (EC50) of the rtA181S mutant and of the wild-type strain were determined by measuring intracellular HBV replicative intermediates in the HepG2 cells that had been transfected with replication-competent plasmids containing 1.1-ploid mutant or wild-type viral genome. Results: HBV rtA181S mutation was detected in 98 nucleotide analog (NA)-experienced patients by direct sequence analysis, representing 0.53% (98/18,419) across the study population and 0.86% (46/5,344) in the patients who were receiving ADV at the resistance testing. By contrast, signature ADV-resistant mutations rtA181V and/or rtN236T were detected in 1,311 patients, representing 7.12% (1,311/18,419) of the study population and 24.53% (1,311/5,344) of the patients who were receiving ADV at the resistance testing. Genotype C and genotype B HBV infection occupied 91.8% and 8.2% in rtA181S-positive patients, in contrast to 84.6% and 15.4% in rtA181S-negative patients (P < 0.01). All rtA181S-positive patients had received NA treatment, including single lamivudine (LAM) (15.3%), single ADV (20.4%), LAM switching to/and-on ADV (13.3%), LAM switching to entecavir (ETV) (9.2%), LAM switching to ADV and then switching to ETV (11.2%), and other antiviral therapy schedules (30.6%). rtA181S was detected in multiple patients with virologic breakthrough (including 7 patients with single rtA181S). Phenotypic analysis of patient-derived viral strains showed that rtA181S, rtA181S+N236T, rtN236T and rtA181V strains had 68.5%, 49.9%, 71.4% and 66.2% of 7.9- and 5.4-fold increased EC50 to ADV. The rtA181S strain remained susceptible to LAM, ETV and tenofovir, and ADV susceptibility was restored after the mutation was eliminated through site-directed mutagenesis. Consistently, rescue therapy with ETV or combination of ETV and ADV was effective for rtA181S-related ADV-refractory patients in clinical observation. Conclusion: The rtA181S mutation primarily confers moderate resistance to ADV. It could be induced by either LAM or ADV but only contribute to ADV resistance.

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