HBV replication and hepatocarcinogenesis. We have reported that HBx interacts with c-FLIP-L and sensitizes cells to apoptosis upon death signals such as TNF-α and FasL. Recently, p22-FLIP has been identified as a novel isoform of c-FLIP family (c-FLIP-L, S, R, and p22) and known to induce NF-kappaB activation. Thus, we investigated whether the c-FLIP family interact with HBx and inhibit the HBV replication via sequestration of HBx.

Methods: To examine the interaction between HBx and c-FLIP family, the co-immunoprecipitation and immunofluorescence assays were performed after co-transfection of the both plasmids. To test the c-FLIP effects on HBV replication, we measured the level of replication by southern blot after transfection of c-FLIP plasmids with 1.2mer of HBV replion.

Results: All the isoforms of c-FLIP family physically interact with HBx. Among them, only the expression of p22-FLIP inhibited the HBV replication in a dose-dependent manner, suggesting different roles of complexes formed between HBx and c-FLIP isoforms. The inhibition of p22-FLIP-mediated HBV replication was restored by overexpression of HBx in a dose-dependent manner. Moreover, HBV replication was recovered by knock-down of p22-FLIP using p22-FLIP siRNA.

Conclusions: These results may provide a novel function of p22-FLIP in hepatocytes as a potential inhibitor of HBV.

615 SUPPRESSIVE EFFECT OF VIRAL PROTEIN INDUCED INTERLEUKIN-10 ON HBV AG-SPECIFIC TH17 CELLS

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Background: IL-17-secreting CD4+ T cells (Th17 cells) have been demonstrated to play an important role in host defense against viral infection. However, there has been much less information on the regulation mechanism of antigen (Ag)-specific Th17 cells in HBV infections.

Methods: In the present study, we performed investigations on peripheral blood mononuclear cells (PBMC) isolated from chronic B hepatitis (CHB) patients. The roles of IL-10 in the regulation of Th17 cells development were further analyzed through treatment PBMC with anti-IL-10 antibody or recombinant IL-10 respectively. Flow cytometry was applied to analyze the frequency of Ag-specific Th17 cells in PBMC which were stimulated with Hepatitis B core antigen (HBcAg). Production of cytokines was monitored by using Flow cytometric multiplexed bead assay.

Results: Our data indicated that HBcAg stimulation promotes the production of IL-17A, IL-22, IL-23, IL-6, TGF-β and IL-10 in CHB patients, but not in normal control. Furthermore, endogenous IL-10 inhibited HBcAg-induced production of IL-17A, IL-22, IL-6 and IL-23, but not TGF-β. Analysis of HBcAg-specific Th17 cells revealed low frequency of Th17 cells and high frequency of Th1 cells in CHB patients. Treatment with IL-10 during HBcAg stimulation inhibited the activation of Th17 cells while IL-10 blockade significantly increased the frequency of Th17 and Th1, associated with modulating the RORγt protein level in CD4+ T cells.

Conclusions: HBcAg induced IL-10 negatively regulates Ag-specific Th17 cells immune response in CHB patients and this could represent one example of an evasion strategy by the virus to subvert specific antiviral T cell responses of the host.

616 HEPATITIS B GENOMIC VARIABILITY CORRELATES WITH INTRAHEPATIC VIRAL ANTIGEN EXPRESSION

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Background and Aims: Hepatitis B virus (HBV) genotypes and mutants are shown to affect the natural course of HBV infection. The virologic differences among HBV genotype, precore and basal core promoter (BCP) mutations were thus investigated.

Methods: HBV strains were isolated from 12 hepatitis B e antigen (HBeAg)-positive patients (6 genotype B and 6 genotype C). All had precore G1896 and BCP A1762/G1764 wild-type sequences. After cloning of full-length HBV genome, the effects of viral genotype, precore and BCP mutations singly or additively on expression of viral DNA and antigens were investigated by mutagenesis and transfection assays in Huh7 cells.

Results: Significant findings included 1. the expression of intracellular core protein increased when precore mutation was introduced in genotype C strains (P < 0.01), and when BCP mutation was introduced in genotype C precore wild-type strain (P = 0.022); 2. the presence of precore mutation was associated with a lower extracellular expression level of HBV DNA in any combination of genotype and BCP sequence (P < 0.05); 3. the secretion of HBsAg in genotype C was lower than that in genotype B in any combination of precore and BCP sequence (P < 0.05); and 4. the secretion of HBeAg in genotype B was lower than that in genotype C (P < 0.01).

No additive effect was observed by combining precore and BCP mutations on expression of viral DNA or antigens.

Conclusions: HBV genotype and precore/BCP mutations correlate with intrahepatic expression of viral antigens in vitro. These findings help us understand the genotype-phenotype relationship of chronic HBV infection.

617 CHARACTERISTICS OF HEPATITIS B VIRUS QUASISPECIES EVOLUTION DURING EARLY STAGE OF ENTECAVIR TREATMENT: A PROMISING PREDICTOR OF VIROLOGICAL RESPONSE

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Objective: To investigate evolution of hepatitis B virus quasispecies within the reverse transcriptase region during the early stage of entecavir treatment and explore potential factors to identify partial responder to entecavir early.

Patients and Methods: Twenty chronic hepatitis B patients who received entecavir for at least 48 weeks were included. Ten patients acquired complete response (defined as serum HBV DNA <300 copies/mL at week 24), while the rest acquired partial response (defined as serum HBV DNA >300 copies/mL but <10^{3} copies/mL at week 24). Serial sera at baseline and week 4 were collected and HBV genomes were extracted. The entire RT region of HBV was amplified by PCR, and then was cloned and sequenced. Quasispecies complexity and diversity within the RT region were analyzed, and viral nucleotide substitution rates during the first 4 weeks were calculated. ROC curve analysis was performed to evaluate the predictors of antiviral response.
Results: Over 900 clones were sequenced in total. The quasispecies complexity and diversity were comparable between responders and partial responders at baseline ($p > 0.05$). The quasispecies complexity at nucleotide level significantly decreased at week 4 in responder group compared to at baseline (AUC = 0.95, $p < 0.001$). However, the quasispecies complexity and diversity were not different between at week 4 and at baseline in partial responders (0.824 vs. 0.758, $p = 0.150$). The viral nucleotide substitution rates in both groups were comparable ($p = 0.059$). ROC curve analysis showed that the increment of quasispecies complexity between baseline and week 4 was an excellent parameter to distinguish partial responder (AUC = 0.95, $p < 0.001$). And when zero served as cutoff value, the positive predictive value is 1 and the negative predictive value is 0.83.

Conclusions: The dynamic change patterns of HBV quasispecies within the RT region are different between responders and partial responders during early stage of entecavir treatment. The changes of quasispecies complexity during the first 4 weeks can be a promising predictor of virological response to entecavir treatment.

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EXPERIMENTAL STUDY ON BIOARTIFICIAL LIVER SUPPORT SYSTEM USING HUMAN LIVER CELL LINE EXPRESSING HUMAN AUGMENTER OF LIVER REGENERATION

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Aim: Bioartificial liver support system (BALSS) is an important measure to treat the patients suffered from liver failure. At present, the cells used in it mainly include porcine hepatocytes and C3A cell. It turns out that the clinical trial of ELAD has achieved certain effect on those patients with liver failure. But there were many adverse effects happened. In order to improve the function of liver cells, we used human augmenter of liver regeneration (hALR) in cell material of the bioartificial liver, and constructed extracorporeal BALSS to improve the therapeutic effect of it.

Methods: We extracted RNA from liver tissue, amplified the fragment of hALR, and constructed recombinant plasmid of pcDNA 3.1(−) hALR. Adapting measures of limiting dilution assay, we chose an eugenic strain of cell from HepG2 cell line, transferred it with pcDNA3.1(−)hALR. We put 10⁶ of these cells into the hollow fiber reactor, and construct BALSS system by pumps, and fluid with connection tube. During cultured 72–96 hours, the fluid were changed every day, and the cell, LDH, AFP were tested.

Results: After common cultured, we evaluated its function. Result shows that it grows well. hALR were found in endochylema through indirect immunofluorescence. The cell can synthesize human albumin better than the uninfected one, and the level of AFP in culture fluid elevated from 1 to 118.0 ± 56ng/ml.

When the cells were cultured for 72 hours or 96 hours, the number increased to 7.60 ± 10⁴ and 9.02 × 10⁴. The cells were found adhered to hollow fiber by inverted microscope and SEM. Using the BALSS, we take continuous blood purification on treatment, the level of TBil, AFP and albumin is 295.6 ± 250UI/mL, in order to expand the dynamic range of test.

Results: HBsAg levels correlated inversely to the frequency of HBV-specific immune response ($r = -0.4$, $p = 0.01$). When patients were divided into high ($>1000$UI/mL) and low ($<1000$UI/mL) HBsAg levels, the frequency and the magnitude of HBV-specific T cell responses were assessed by IFN-γ ELISpot assay after in vitro stimulation with synthetic HBV peptides. Serum HBsAg was measured at same time point with a chemiluminescent microparticle immunoassay (Architect, Abbott) and samples were diluted when the quantification resulted >250UI/mL, in order to expand the dynamic range of test.

Conclusions: The amount of HBsAg showed an inverse relationship to HBV-specific T cell response in patients with chronic HBV. Although the causative role of the two parameters need to be established, this observation suggests an immunotolerizing effect of the HBsAg on the T cell repertoire. Finally, the quantification of HBsAg may have a clinical impact in reflecting indirectly the HBV-specific immune profile in patients with chronic HBV.

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THE INCREASED EXPRESSION OF 2B4(CD244) ON NATURAL KILLER CELLS IN CHRONIC HEPATITIS B PATIENTS TREATED WITH TELBIVUDINE

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Background: Natural Killer (NK) cells, controlled by multiple activating and inhibitory receptors, are an important element of the innate immune response to viral infection including hepatitis B virus (HBV). Recent data indicate that the expression of 2B4(CD244), a member of the CD2 subset of the Ig superfamily, regulate NK functions. In this study we addressed the role of the NK cells marker 2B4 and compared the frequency of peripheral blood CD3–CD56– NK cells in CHB patients.

Methods: A total of 53 patients of chronic HBV (CHB) patients were selected, treated with Telbivudine (LDT) for 36 weeks, and...
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