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DOES GENDER MATTER? A PROSPECTIVE EVALUATION OF RISK FACTORS FOR HCV INFECTION AND TREATMENT CANDIDACY IN WOMEN

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Background: Historically, women have comprised a small percentage of the veteran population. However, they are now the fastest growing group of veterans. Because veterans have a higher overall prevalence of HCV than non-veterans, a better understanding of gender issues and HCV treatment candidacy may be especially important. Aim: To compare risk factors for HCV infection and treatment candidacy between women and men veterans. Methods: Data were prospectively collected in HCV RNA positive veterans from 24 Veterans Affairs Medical Centers. Risk factors for HCV were assessed by questionnaire and treatment candidacy was determined by standardized criteria. Patients were excluded from treatment if at least one of thirteen standardized criteria were met. Results: Of 4,269 patients, 118 (2.8%) were women. Univariate analyses showed that women were younger, more educated, and less likely to have a history of injection drug use, incarceration, heavy alcohol use or snorting cocaine, but were more likely to have had surgery, a needle stick injury or a blood transfusion. Despite these differences, baseline laboratory results and treatment candidacy (43% vs 40%, p = ns) were similar in men and women. Reasons for exclusion from therapy were similar in men vs women, such as ongoing substance use (20% vs 13%, p = 0.05), mental health issues (18% vs 19%, p = ns) and advanced liver disease (14% vs 13%, p = ns), but, as table one shows, men were more likely to have multiple contraindications to therapy (p<0.05). Men were also almost twice as likely as women to have 3 or more reasons for exclusion from therapy (OR=1.69, 95% CI=1.07 - 4.26). Conclusions: Women veterans with HCV have fewer high risk behaviors than men, yet their overall treatment candidacy rates are similar. These seemingly contradictory results may be due to patients' exclusion from therapy based on only on a single contraindication and that women were much more likely than men to have only one reason for exclusion. These results suggest the need for gender-specific HCV risk factor screening and counseling, as well as an opportunity to improve treatment candidacy through substance use and mental health interventions, especially in women veterans who have fewer contraindications to HCV treatment.

Table 1. Exclusion criteria met for HCV treatment based on gender (maximum of 13 yes responses) N = 2,450

Exclusion Criteria	Women (n=64)	Men (n=2,386)
One exclusion criteria met	51 (79.6%)	1,665 (69.7%)
Two exclusion criteria met	12 (18.8%)	517 (21.7%)
Three or more exclusion criteria met	1 (1.6%)	204 (8.6%)

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PREVALENCE AND PREDICTORS OF OBESITY AMONG INDIVIDUALS TESTING POSITIVE FOR HEPATITIS C ANTIBODY IN A MULTICULTURAL, URBAN, TERTIARY CARE REFERRAL CLINIC

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Background: Obesity is considered an important factor contributing to hepatic steatosis and poor response to anti-viral therapy in patients with chronic hepatitis C virus (HCV) infection. Objective: To determine the prevalence of obesity and explore the possible factors associated with obesity in a HCV antibody positive, multicultural tertiary referral clinic population. Methods: Patient records describing visits to a downtown tertiary care liver clinic in Toronto, Canada between 1990 and 2006 were reviewed. We extracted data describing demography, life style, and mode of HCV transmission, as well as weight and height. The one sample proportion test was used to compare the prevalence of obesity in the clinic population testing positive for HCV antibody and the Canadian general population. Regression analyses and propensity score analysis (based on the propensity to test positive for HCV antibody) were applied to explore possible factors associated with obesity in this population. Results: The 1118 out of 3505 HCV antibody positive patients met our inclusion criteria. The included patients had a significantly higher prevalence of obesity (28.8% vs. 14.9%; P<0.001) than the Canadian general population. After stratification by age and gender, the clinic population remained with a significantly higher prevalence of obesity than the Canadian general population in every age/gender category. In the univariate analyses age was positively associated with high BMI (coefficients 0.073; P<0.001) and obesity (OR 1.020; 95% CI 1.003 to 1.038); Asian origin was negatively associated with BMI (coefficients -2.187; P<0.001); serum testing for positive HCV RNA was significantly associated with obesity (OR 1.731; 95% CI 1.075 to 2.788). In the multivariate regression analyses, age (OR 1.019; 95% CI 1.002 to 1.037) and serum positive HCV RNA (OR 1.798; 95% CI 1.109 to 2.917) were independent predictors for obesity. A total of 112 pairs of subjects qualified for matching by propensity score. The matched propensity score analysis demonstrated that the subjects with a positive serum HCV RNA test had significantly more obesity (32.1% vs. 18.8%; p=0.021) than those with a negative HCV RNA test. Conclusion: Age and serum HCV RNA (indicating chronic HCV infection) contribute to the high prevalence of obesity observed in a clinic population exposed to HCV.

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COMPARTMENTALIZATION OF HEPATITIS C VIRUS QUASISPECIES FOLLOWING LIVER TRANSPLANTATION

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Introduction. Hepatitis C virus (HCV)-related cirrhosis is the main indication for liver transplantation (LT). HCV infects the liver graft in almost all HCV-infected patients following LT. HCV compartmentalization is defined as nonrandom distribution of viral variants among the different sites of replication, and potentially reflects the presence of different HCV reservoirs including non-hepatic cells during HCV infection. Aiming to adress the impact of compartmentalization for outcome of HCV infection following LT, we analyzed the chronological evolution of HCV compartmentalization between plasma, a likely representation of hepatotropic variants, and PBMC, presumably representing lymphotropic strains. Patients and methods. Two patients undergoing LT for HCV-related cirrhosis were included in the study presented here; plasma and PBMC samples were taken before LT, 7 days, 1 month and 12 months after LT. Clinical data were collected along with biochimical, virological and histological parameters. Plasma and PBMC quasispecies were studied by cloning and sequencing in the HVR1 region of the E2 glycoprotein. The compartmentalization was analyzed using a phylogenetical approach focusing on specific amino-acid signatures. Results. The compartmentalization persisted during LT: both patients exhibited the presence of specific viral variants in each compartment. For the first patient, bootstrapped phylogenetic trees suggested a compartmental distribution of viral variants before transplantation, still maintained at day 7, month 1 and month 12 following LT (clusters of cellular variants with bootstrap values >60% at each time); interestingly, no positive selection pressure was observed in the PBMC fraction as shown by the Nei and Gojobori method. Two amino-acid signatures were observed for this patient: a lysine mutation to a serine in position 397 of HVR1 and an aspartic acid mutation to an asparagin in position 404, present in plasma at month 1, were absent in PBMCs. Compartmentalization following LT has been shown for the second patient, and absent selective pressure in the PBMC fraction was also observed. Conclusions. Immunosuppressive status of liver-transplanted patients sustains HCV compartmentalization with potential selection of hepatotropic but not lymphotropic variants following LT. Further studies are underway to analyze the impact of compartmentalization for mechanisms of HCV reinfection.

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STEATOHEPATITIS: RISK FACTORS AND IMPACT ON DISEASE SEVERITY IN HIV-HCV COINFECTION

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Hepatic steatosis (HS) has been reported in those with HIV-HCV coinfection and associated with use of antiretroviral therapy (ART) and insulin resistance (IR). However, the presence of features of steatohepatitis (SH) including cytologic ballooning (CB), its risk factors, and impact on disease severity in such patients are unknown. AIM: To prospectively define the prevalence and severity of the features of SH, its risk factors, and impact on the severity of liver disease in HIV-HCV coinfection. METHODS: Consecutive subjects with HIV-HCV coinfection underwent liver biopsy. SH was defined by the presence of HS and CB with or without Mallory hyaline or pericellular fibrosis (PF). Its individual parameters were graded according to the NASH CRN criteria (Kleiner et al, Hepatol 2005). Inflammation and fibrosis were also graded by the Ishak score; Ishak 3-6 fibrosis represented advanced fibrosis (AF). IR was measured by HOMA-IR and metabolic syndrome (MS) was defined by ATP III criteria. RESULTS: A total of 222 subjects (74% male, mean age 45, 78% African American) were studied. The mean BMI was 26 and 18% were obese (BMI > 30). Median[IQR] AST, ALT, and ALP were 62[43-102], 68[43-101], and 112[85-104], respectively. All patients were HCV RNA positive and 90% were genotype 1. Mean CD4 count was 535, 10% had a CD4 < 200, and HIV was < 400 copies in 45%. The prevalence of risk factors for HS were as follows: diabetes (31%), hypertension (15%), dyslipidemia (8%), MS (9%), and alcohol abuse (21%). ART therapy included a nucleoside reverse transcriptase inhibitor (NRTI) in 93%, non-nucleosoide reverse transcriptase inhibitor (NNRTI) in 30% and protease inhibitor (PI) in 47%. HS was present in 23% and SH was present in 17%. The steatosis was absent in 77%, mild (5-33%) in 19%, and moderate-severe (>33%) in 4% of affected subjects. CB and PF were present in 30% and 13% respectively. The mean(SD) Ishak score was 6.9(4.6) and 33% had AF. Both HS and CB were associated with BMI, MS, HOMA and presence of either was strongly associated with AF (p<.0001). MS and absence of PI use were independent predictors of HS by multiple logistic regression; alcohol use and HOMA-IR was associated with CB; NRTI use were also associated with SH, and use of d4T with AF. CONCLUSIONS: SH is commonly present in HIV-HCV coinfection. Alcohol consumption, IR and specific components of ART contribute to steatosis and SH. Both steatosis and SH increase the risk of having advanced fibrosis. These data raise the possibility that specific types of ART (NRTI, PI, and specifically d4T) may contribute to the progression of fibrosis in HIV-HCV coinfection.

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ACUTE HEPATITIS C IN HIV-INFECTED MEN WHO HAVE SEX WITH MEN IN FRANCE IN 2006 AND 2007

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BACKGROUND Recent European reports suggest that hepatitis C virus (HCV) in specific circumstances may be sexually transmitted among HIV-infected (HIV+) men who have sex with men (MSM). A prospective study is conducted during 2006 and 2007 in France to describe clinical and epidemiological characteristics of HCV-infected MSM and to evaluate the incidence of HCV among this population. Preliminary results on descriptive analysis are presented here. METHODS A random and proportional probability sample of 84 medical wards was drawn according to the number of HIV and AIDS cases in MSM reported since 2003 to the National HIV surveillance system. Acute HCV was defined by a positive anti-HCV antibody (Ab) or HCV polymerase chain reaction (PCR) within one year of a documented negative anti-HCV test. Information was collected on socio-demographics, clinical and biological status of acute HCV, HBV and HIV infections and on HCV risk factors. Two types of questionnaires were completed: one by physicians on clinical data and HCV risk factors and one (self-administered) by patients on sexual behavior, HCV risk factors and exposures. RESULTS Between January 2006 and May 2007, 43 out of the 49 patients meeting the case definition of acute HCV were included after consent, mainly (72 %) in Paris. Median age at HCV diagnosis was 38 years [min: 26; max: 58]. Median duration of HIV infection was 6.5 years [min: 0; max: 22]. HCV was suspected because of elevated ALT in 72 % of the patients (unknown: 23 %). HIV infection was asymptomatic for 66% (unknown: 16 %). HIV viral load was undetectable for 16 of the 22 treated patients. 66 % of the patients with available HCV genotyping (21/32) were infected with genotype 4 whereas this genotype represents only 10% of the prevalent cases in France. Self-administered questionnaires were available for 27 patients: in the 6-month period prior to HCV, the median number of sexual partners was 20 [min: 0; max: 170]; unprotected anal sex was reported in 81 %, having had sexually transmitted infections (STI) in 74 % and bleeding during anal sex in 41 %. Recreational drug use before sex (such as ecstasy, cocaine,...) was specified by 63 % of the patients. CONCLUSIONS In France, HIV+ MSM with frequent unprotected anal sex together with STI and mucosal trauma may be at risk of sexual HCV transmission. Therefore, it is necessary to reinforce prevention strategies targeted to this population.

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FACTORS ASSOCIATED WITH SEVERE LIVER DISEASE IN NEWLY REFERRED HEPATITIS C VIRUS-INFECTED FRENCH DRUG USERS: A MULTICENTER STUDY OF 4 373 PATIENTS, 2001-2004

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AIMS To analyze factors associated with severe liver disease in drug users infected with hepatitis C virus (HCV) at first referral to a hepatology reference center in France between 2001 and 2004. METHODS A national surveillance system was set up in 2000 to describe changes in epidemiological and clinical characteristics of HCV-infected patients at first referral in 26 hepatology reference centers. Data on risk factors, suspected year of infection, past or current alcohol consumption, HCV RNA status, ALT level, HBV and HIV status were routinely collected. The severity of the liver disease is assessed either by a "clinical" evaluation based on biochemical tests and morphological examination (mainly ultrasonography) or by a histological evaluation (Metavir scores at liver biopsy). Patients included in this analysis are those recorded in the system from 2001 to 2004 and reporting past or current injecting or nasal drug use. Severe liver disease (SLD) was defined as the presence of cirrhosis or primary liver cancer. A log-Poisson model was used to estimate prevalence ratios. RESULTS Among the 4 373 drug users, median duration of HCV infection was 18 years, 7 % were HIV-infected (HIV+) and 38 % reported past excessive alcohol consumption. Of these, 3 153 were HCV RNA+ with available clinical evaluation of SLD at first referral. Predominant reported HCV genotypes were genotype 3a (24 %), genotype 1a (23 %) and genotype 1b (14 %). SLD at "clinical" evaluation was diagnosed in 7 % and was independently associated with genotype 3 (prevalence ratio (PR) = 1.5; 95 % confidence interval (CI): 1.1-2.0), HIV+ status (PR = 2.3; 95 % CI: 1.1-4.6), past excessive alcohol consumption (PR = 2.5; 95 % CI: 1.7-3.6), duration of HCV infection ≥ 18 years (PR = 3.7; 95 % CI: 2.2-6.1) and age > 40 years (PR = 4.1; 95 % CI: 2.2-7.7). CONCLUSIONS Our findings based on surveillance data suggest that HCV genotype 3 may be associated with severe liver disease in drug users. They also underscore the role played by excessive alcohol consumption and HIV infection in the severity of HCV infection in this population. These results highlight the need for targeted management of HCV-infected drug users, especially on alcohol consumption and HIV coinfection. Moreover, the role of genotype 3 in severe liver disease needs to be further explored.

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APOLIPOPROTEIN E4 (APOE4) ALLELE PROTECTS FROM CHRONIC HEPATITIS C VIRUS (HCV) INFECTION

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BACKGROUND/AIMS: Host genetic single nucleotide polymorphisms (SNPs) are likely to influence the outcome of HCV infection. Infectious HCV particles have been shown to bind to host lipoproteins and low-density lipoprotein receptors (LDLR) have been termed candidate receptors for the hepatocellular internalization of these "lipo-viro particles" (LVP). Recent studies suggested that common SNPs in the gene encoding apolipoprotein E (apoE) - a major structural LDL component and natural ligand of LDLR and well-characterized risk factor for elevated plasma cholesterol levels - might be associated with spontaneous resolution of HCV infection, severity of histological liver damage and response to therapy in chronic HCV infection. The aim of the present study was to further investigate the role of apoE polymorphisms in a large group of well characterized patients with chronic hepatitis C. METHODS: We determined the prevalence of apoE genotypes and apoE alleles in 701 chronically HCV-infected patients, 523 healthy controls and 283 individuals with non-HCV associated chronic liver disease by PCR and RFLP analysis. We went on to assess whether apoE SNPs might be associated with the severity of histological inflammation and fibrosis of 555 liver biopsies from chronic HCV carriers. RESULTS: The comparison of apoE allele proportions in chronically HCV-infected patients revealed significant differences in comparison to healthy controls (p=0.002). This was primarily due to a substantial under-representation of the apoE4 allele (10.5% vs. 13.8% in healthy controls; p=0.011) and apoE4 allele positive genotypes (20% vs. 27% in healthy controls; p=0.002) in the group of patients with chronic HCV infection. The frequency of apoE4 alleles was increased in the group of patients with histological mild liver disease but very similar for severe hepatic fibrosis and inflammation. Patients with non HCV-associated chronic liver disease presented with an apoE allele and genotype distribution very similar to healthy controls and differed also significantly from HCV patients CONCLUSION: The significant under-representation of the apoE4 allele in chronically HCV-infected patients suggests that apoE4 allele carriers might be protected from chronic HCV infection. Common apoE SNPs affecting the host lipoprotein metabolism might therefore contribute to the genetic susceptibility to chronic HCV infection.

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IN VITRO INFECTION OF IMMORTALIZED HUMAN HEPATOCYTES BY HEPATITIS B VIRUS AND ITS APPLICATION TO ANTI-VIRAL TREATMENT

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Background: We have developed a novel three-dimensional culture system of human hepatocytes in terms of ability to metabolite ammonia and CYP3A enzyme activity as well as hepatitis B virus (HBV) infection. Recently HPV18/E6E7-immortalized human hepatocytes (HuS-E/2 cells; primary hepatocytes isolated from a 9-year-old male patient with Primary Hyperoxaluria) which could be infected by hepatitis C virus were established. Aim: To investigate the ability of HBV infection using the three-dimensional culture system of HuS-E/2 cells and to compare viral replication and sensitivity of anti-viral drugs (lamivudine, adefovir, entecavir, inferferon, etc) among HBV genotypes/subgenotypes. Methods: The three-dimensional culture system was made as follows: immortalized HuS-E/2 cells suspension (3x10^5cells) was injected into hollow fiber bundles after inoculation with HBV-infected serum samples or virions of HBV recovered from culture supernatants, that were produced by transfection with plasmids carrying 1.24-fold the HBV genome of different genotypes/subgenotypes (Ae, Ba, Bj, C) in Huh7 cells. The hollow fibers were placed in a 12-well plate and cultured in serum-free medium. Result: The hepatocytes aggregated in hollow fiber bundles formed a hepatocyte organoid and HBV DNA was detected in supernatants within 7 days. The HBV DNA increased in titer 10 days post-inoculation and continued to be detectable until 5–6 weeks. The extracellular HBV DNA levels were significantly higher in genotype C (>10^7copies/ml) than the others. Five weeks post-inoculation, intrahepatic total HBV DNA and covalently closed circular DNA were detected in hepatocytes by real-time detection PCR. Futhermore, HBV DNA levels of these genotypes were inhibited by several anti-viral drugs with different manners in this culture system. The combination of entecavir and interferon could inhibit HBV replication more efficiently. Conclusion: This in vitro system using immortalized hepatocytes could provide a simple and cheap long-term culture of human hepatocytes infected with HBV. The infection model of HBV would allow us to examine differences of viral replication capacity, changes of host factors and anti-viral efficiency of new drugs on HBV genotypes/subgenotypes.

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THE ROLE OF SERIAL MEASUREMENT OF SERUM HBV-DNA LEVELS IN PATIENTS WITH CHRONIC HBEAG(-) HEPATITIS B INFECTION; ASSOCIATION WITH LIVER DISEASE PROGRESSION. A PROSPECTIVE COHORT STUDY

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Background/Aim: The role of serum HBV-DNA levels in the natural history and management of chronic HBV infection is currently under investigation. We evaluated the fluctuating course of serum HBV-DNA levels during the natural history of chronic HBV infection in the general population of Northern-Eastern Greece, in association with liver disease progression. Patients/Methods: 257 adults(34±12.3yrs old) with chronic HBV infection, recruited and prospectively followed-up for a period up to 12yrs(1992/94-2006). Viral markers/liver biochemistry/physical examination performed every 6m. Liver biopsy/abdominal ultrasound were performed at entry and every 2-4yrs. Serum HBV-DNA levels measured by Amplicor HBV Monitor. Results: 14/257(5.5%) patients were HBeAg(+) with mean HBV-DNA levels 2.5x10(7)copies/ml (6.2x10(6)-7.1x10(7)). At entry, 195/257(76%) were HBeAg(-)/anti-HBe(+), with a history of normal ALT for at least 12 months: a) 97/195(50%) individuals had undetectable serum HBV-DNA levels at entry although 31/97(32%) had detectable in at least one occasion, always below 10(4)copies/ml, had no liver disease at entry and at follow-up period by imaging evaluation, b) 94/195(48%) had HBV-DNA levels <10(4)copies/ml, normal liver histology (97%) at entry and at follow-up period and, c) 4/195(2%) had HBV-DNA >10(4)copies/ml, nearly normal liver histology of whom 2 exhibited abnormal ALT levels after 3yrs. At entry, 48/257(19%) patients were HBeAg(-)/anti-HBe(+), with a history of elevated ALT levels for >6m; a)12/48(25%) had an erratic ALT pattern, mean HBV-DNA levels 3.5x10(5)copies/ml (2.1x10(3)-1.5x10(8)) of whom 5/12(42%) had HBV-DNA levels <10(4)copies/ml for at least one occasion and, b) 36/48(75%) had persistent elevated ALT(>1.5xUNL), mean HBV-DNA levels 1.2x10(6)copies/ml (6.7x10(3)-8.7x10(8)) of whom 8/36(22%) had HBV-DNA <10(4)copies/ml for at least one occasion. In HBeAg(-) patients with active disease, 22/48(46%) had moderate/severe histology at entry and 5/48(10%) developed liver disease progression during the follow-up period. The multivariate-adjusted OR(95% CI) of fibrosis was 604.865(67.233-999.999) for serum HBV-DNA levels >10(4) vs <10(4)copies/ml; 0.002(><0.001-0.015) for serum ALT level at study entry >40IU vs <40IU; 0.057(0.006-0.519) for age >40 vs <40 years old. Conclusions: a) 27% of patients with chronic HBeAg(-) hepatitis could be misclassified as inactive carriers if testing was performed on one occasion and not serially, b) serum HBV DNA levels >10(4)copies/ml were significantly associated with liver disease activity and c)close monitoring of serum HBV-DNA is a useful clinical tool in the management of chronic HBeAg(-) patients

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ACTIVE REPLICATION OF HEPATITIS B VIRUS (HBV) STARTS AFTER BIRTH

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Vertical transmission of Hepatitis B Virus (HBV) is thought to occur during birth, but there are only very limited data on the replicative potential of HBV in case of an earlier infection time point. Since HBV replication is discussed to depend on hepatocyte differentiation, the purpose of this study was to define the starting point and analyze the changes in HBV replication along with hepatocyte maturation during fetal and postnatal liver development in an HBV-transgenic mouse model. Methods: We analyzed different stages of fetal and postnatal liver development, starting at embryonic day (ED) 12.5 in HBV 1.3 xfs transgenic mice. Breeding pairs were selected based on high HBe antigen serum levels, indicating efficient HBV replication. To assess HBV replication in fetal, postnatal and adult livers, we quantified expression of HBV pregenomic RNA (pgRNA) and HBV DNA by real-time PCR and HBV core and envelope proteins by Western blot analysis. Additionally, expression levels of hepatocyte nuclear factors (HNF) and peroxisome proliferator-activated receptor gamma coactivator - 1 alpha (PGC-1 alpha) as main regulators of hepatocyte differentiation and HBV gene expression were quantified by Western blot analysis relative to beta-actin. Single-cell suspensions of fetal livers and adult hepatocytes were fixed on cytospins and costained for HBV core protein and epithelial-specific pancytokeratin. Results: Low levels of pgRNA were detected at all analyzed stages of liver development, but expression of pgRNA significantly increased compared to ED 12.5: 7-fold on ED 15.5, 20.8-fold on ED 18.5, 29.0-fold on 2 days postnatally and 190.0-fold in adult liver (AL). Using Western blot or immunocytochemistry, we did neither detect HBV core nor large envelope proteins before birth. Correlating with these results, HNF4alpha, PGC-1alpha and HNF1 were detected at ED 15.5 at the protein level and markedly increased until birth: 30.9-, 32.5- and 61.6-fold, 58.9-, 363- and 444.5-fold and 43.3-, 110.0- and 114.7-fold on ED 18.5, 2 days postnatally and in AL, respectively. HBV DNA increased continuously from ED 18.5 onwards. Conclusion: HBV replication starts around birth, although the transcription template is present before as a transgene. Hepatocyte differentiation and expression levels of HNF4alpha, PGC-1alpha and HNF1alpha contribute to the efficiency of HBV pgRNA transcription and protein production and determine the starting point of HBV replication.

Disclosures:

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LARGE SCALE LONGITUDINAL STUDY OF CHRONIC HEPATITIS B PATIENTS WITH HEPATITIS B SURFACE ANTIGEN SEROCLEARANCE

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Background and Aim: Seroclearance of hepatitis B surface antigen (HBsAg) is a rare event in chronic hepatitis B patients. We conducted a large scale longitudinal study investigating the virological, histological and clinical aspects, including the risk of development of hepatocellular carcinoma (HCC) in patients with HBsAg seroclearance. Patients and Methods: Two hundred and ninety-eight patients (211 male and 87 female; median age on presentation: 43.1 years) with HBsAg seroclearance were recruited and followed up every 3 – 6 months for clinical assessment. Intrahepatic HBV DNA and covalently closed circular DNA (cccDNA) were measured by real-time PCR. Serum HBV DNA was measured by the Artus HBV RG Test (QIAGEN, Germany). Liver stiffness was assessed by FibroScan (Echosens, France). Results: The median age of HBsAg seroclearance was 49.6 years. The median follow-up duration was 108.9 months and the median follow-up duration after HBsAg seroclearance was 36.4 months. Liver biopsies were performed on 29 patients (median time of biopsy: 48.6 months after HBsAg seroclearance). All have detectable intrahepatic HBV DNA (median: 1.68 copies/cell), and cccDNA were detectable in 23 patients (79.3%, median: 0.03 copies/cell). Of the 29 patients with liver biopsy, 9 and 16 patients had sera available within 1 year and between 5 – 10 years after HBsAg seroclearance, respectively, for HBV DNA analysis. All 9 patients had undetectable HBV DNA (<1.1 IU/mL) within 1 year of HBsAg seroclearance, and 4/16 patients had detectable HBV DNA levels between 5 – 10 years of HBsAg seroclearance (median: 2.37 IU/mL). Of the 26 patients with adequate liver tissues for histological examination, 4 had mild fibrosis (F1) and 5 had minimal necroinflammation. FibroScan was performed on 76 and 78 patients who had HBsAg seroclearance at age <50 and ≥50 years respectively. Significant fibrosis (liver stiffness > 8.1 kPa) was observed only in 7.9% (6/76) patients with HBsAg seroclearance at age <50 compared to 29.5% (23/78) patients with HBsAg seroclearance at age ≥50 (p = 0.001). Seven patients developed HCC (median age: 69.3). Kaplan-Meier analysis showed that the chance of HCC development in patients with HBsAg seroclearance at age <50 was significantly less than those with HBsAg seroclearance at age ≥50 (p = 0.004). Conclusion: Although serum HBV DNA was detectable in only a small proportion of patients with HBsAg seroclearance, intrahepatic HBV DNA was still present in all patients. Nevertheless, patients who cleared HBsAg at age <50 had significantly less fibrosis and lower chance of HCC development than those with HBsAg seroclearance at ≥50 years.

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FIBROTEST® (FT) AND ACTITEST® (AT) ACCURATELY PREDICT RISK OF LIVER DECOMPENSATION AND DEATH IN PATIENTS WITH CHRONIC HEPATITIS B (CHB)

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Background: FT, and AT, noninvasive methods of measuring biomarkers of liver fibrosis and necroinflammatory activity, are an alternative to liver biopsy for determining the severity of CHB. We assessed the 4-year prognostic value of FTAT staging for predicting cirrhosis decompensation and survival. Methods: 504 consecutive HBV patients with baseline FTAT and HBV DNA measurements were retrospectively analyzed. FT-AT, fibrosis stage and activity grades were assessed simultaneously in 72 patients. Disease classification at baseline was 115 patients with severe fibrosis (FibroTest >0.58), 126 with moderate fibrosis (FibroTest 0.32– 0.58), and 263 with no or minimal fibrosis (FibroTest <0.32). Results: The area under the ROC curve (AUROCs) for the diagnosis of advanced fibrosis (severe or moderate) was 0.86 (0.05) m(SE). In patients with severe fibrosis, 4-year survival without HBV complications was 63% [95% confidence interval (CI), 53%– 72%; 39 complications], and survival was 77% (95% CI, 68%–86%; 22 deaths). Survival rates were higher in patients with moderate fibrosis, [98% (95% CI, 95%–100%; 4 complications; P <0.001) and 100% (no death; P <0.001) for survival without HBV complications and overall survival, respectively], and in patients with minimal fibrosis [99.6% (95% CI, 99%–100%; 2 complications; P <0.001vs severe) and 100% (no death; P <0.001 vs severe), respectively]. FibroTest was a better predictor than HBV DNA quantification for HBV complications, with AUROC= 0.91 (95% CI, 0.89–0.96) vs 0.57 (95% CI, 0.85– 0.94; P=0.01), respectively; it was also a better predictor for deaths: AUROC= 0.95 (95% CI, 0.91– 0.97) vs 0.57 (95% CI, 0.42–0.69; P <0.001), respectively. FT was a similar predictor to biopsy staging for HBV complications and deaths, with both AUROCs= 0.99 (95% CI, 0.90– 0.998) vs 0.97 (95% CI, 0.93–0.99; P =0.32), respectively. In Cox multivariate analyses the prognostic value of FT was still significant (P <0.001) after taking into account baseline viral load (P=0.005), gender (P=0.04), age, HBeAg status, treatment, alcohol consumption, ethnic origin, AT and HIV coinfection. An algorithm enabled an AUROC=0.93 (0.89-0.96) (P<0.001) to be obtained for the prediction of death or complications. Conclusion: FibroTest has a 4-year prognostic value similar to that of liver biopsy in patients with chronic hepatitis B and can be combined with baseline viral load to improve prediction for death and complications. This study was supported by IDENIX PHARMACEUTICAL

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REDUCED HBV REPLICATION IN HBEAG-NEGATIVE PATIENTS IS DUE TO LOWER COVALENTLY CLOSED CIRCULAR DNA CONTENT AND IMPAIRED VIRION PRODUCTIVITY

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Background&Aims: Knowledge of factors regulating transcriptional activity of hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) may help understanding mechanisms of viral decay and how these processes are thwarted in chronically HBV-infected patients. Methods: Liver biopsies from 119 treatment naïve chronically infected patients (42 HBeAg-positive and 77 HBeAg-negative) were determined for transcriptional and replicative activity of HBV. Results: Significantly lower median serum HBV-DNA (-4log), intrahepatic HBV-DNA (-2log) and cccDNA (-1log) amounts were measured in HBeAg-negative vs. HBeAg-positive patients. Despite a good correlation found between intrahepatic amounts of progeny virions and serum HBV-DNA titres in all patients, cccDNA levels did not correlate with serum titres in HBeAg-negative individuals. Analysis of HBV-RNA transcripts demonstrated that impaired virion productivity in HBeAg-negative individuals was due to lower steady-state levels of pregenomic RNA produced per cccDNA molecule. Interestingly, preS/S RNAs levels and serum HBsAg concentrations did not differ between HBeAg-positive and HBeAg-negative patients when normalized for cccDNA contents, demonstrating that subviral particle production was not impaired in HBeAg-negative patients and correlated with cccDNA levels. Though the majority of HBeAg-negative individuals harboured cccDNA with common precore and/or basal core promoter (PC/BCP) mutations, occurrence of these variants was not responsible for reduced viral replication. Instead, replacement of wild type cccDNA with populations of BCP mutations re-established high virion productivity. Conclusions: Lower viremia in HBeAg-negative individuals is not only due to lower cccDNA content but also to impaired virion productivity, which can arise without emergence of HBeAg variants and without affecting HBsAg production.

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903
HIGH VIRAL LOAD AND HEPATITIS B VIRUS (HBV) SUBGENOTYPE CE HAVE INCREASED RISK OF HEPATOCELLULAR CARCINOMA (HCC) – A 7-YEAR PROSPECTIVE FOLLOW-UP STUDY

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Background: The determination of risk factors for HCC is important to design cancer surveillance strategy. Genotype C HBV is associated with a higher risk of HCC than genotype B HBV, but the effect of HBV subgenotypes on HCC is uncertain. We aimed to investigate the impact HBV DNA levels and HBV genotypes/subgenotypes on the risk of HCC. Methods: A prospective cohort of chronic HBV infected patients recruited in a surveillance program for hepatocellular carcinoma (HCC) since 1997 was studied. Ultrasound and alfa-fetoprotein were regularly performed for HCC surveillance. HBV DNA at recruitment was measured by Taqman real-time polymerase chain reaction assay. HBV subgenotyping was determined by direct sequencing of the S gene. Results: Among 1006 patients (68% male) aged 48±7 years, 86 (9%) patients developed HCC at a median follow-up of 7.7 years. 377 (38%) patients had liver cirrhosis. 146 (15%) patients had low serum albumin. 851 (85%) patients had ALT levels below 1.5 x upper limit of normal and the mean log HBV DNA was 4.72±1.84 copies/ml. 152 (15%) patients had received anti-viral treatment during the follow-up period. The hazard ratio of each log step increase in HBV DNA was 1.38 (95% CI 1.23, 1.55; p<0.0001). With referent to the low HBV DNA stratum (log HBV DNA ≤ 4.5 copies/ml), the hazard ratio for HCC of the intermediate HBV DNA stratum (log HBV DNA > 4.5 – 6.5 copies/ml) was 1.62 (95% CI 1.05, 2.48; p=0.027) and that of the high HBV DNA stratum (log HBV DNA > 6.5 copies/ml) was 2.73 (95% CI 1.76, 4.25; p<0.001). Among 769 patients with genotyping results, 330 patients had genotype B HBV (all subgenotype Ba) and 439 patients had genotype C HBV (94 subgenotype Ce and 345 subgenotype Cs). With referent to genotype B HBV, HBV subgenotype Ce has the highest risk of HCC (hazard ratio 2.75; 95% CI 1.66, 4.56; p<0.001) and HBV subgenotype Cs has intermediate risk (hazard ratio 1.70; 95% CI 1.09, 2.64; p=0.02). On multivariate analysis, HBV DNA (hazard ratio 1.79; 95% CI 1.27, 2.52; p<0.001) and HBV subgenotypes (hazard ratio 1.53; 95% CI 1.17, 2.01; p=0.002) were independent risk factors of HCC. Liver cirrhosis, male gender, older age and low serum albumin were also risk factors of HCC whereas serum bilirubin, ALT level, ascites and use of anti-viral treatment were not. Using genotype B HBV and low HBV DNA stratum as referent, the hazard ratio of HCC for HBV subgenotype Cs was 2.85 (95% CI 1.68, 4.84; p<0.001) and that for HBV subgenotype Ce was 4.00 (95% CI 2.08, 7.69; p<0.001). Conclusions: High HBV DNA level and HBV genotype C, particularly subgenotype Ce, are independent risk factors of HCC in chronic hepatitis B.

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ANALYSIS OF HBV MUTATIONS IN PATIENTS WITH EPIDEMIC FULMINANT HEPATITIS

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Background and aims: It is thought that both host and viral factors contribute to the development of fulminant hepatitis B. Especially, mutations in the core promoter region (A1762T/G1764A) and/or the precore region (G1896A) have been reported to be associated with fulminant hepatitis. We experienced five patients who died of fulminant hepatitis B from the same infectious source. The HBV isolates obtained from them were 99.8-100% identical to each other, and the genotype was revealed to belong to B2, which is known to cause fulminant hepatitis less frequently. This strain had mutations including A1762T/G1764A and G1896A. Additionally, it had a mutation of G1862T in the precore region. We focused on the high pathogenicity of this strain, and analyzed mutations that are associated with fulminant hepatitis. Methods: The full-length genome of HBV that was divided into two overlapping

regions was amplified with polymerase chain reaction (PCR). A plasmid carrying 1.3-fold the HBV genome obtained from a fulminant hepatitis patient (pBFH2) was constructed with the PCR products. Using site-directed mutagenesis, the mutations at nt 1762/1764, 1896 and 1862 in pBFH2 were converted to the wild type nucleotides with comprehensive combinations. HepG2 cells were transfected with these plasmids, and HBV-related particles that were released into the culture medium 3 days after transfection were assayed with real-time PCR (HBV DNA) and enzyme-linked immunosorbent assay (HBsAg, HBeAg). Intracellular HBV DNA in the harvested HepG2 cells was measured with southern blot analysis and real-time PCR. Results: In the analysis of HBV DNA in the culture medium, the largest single factor contributing to the replication efficiency was A1762T/G1764A. The presence of G1862T alone reduced the extracellular HBV DNA, and the production was recovered with coexisting A1762T/G1764A. When intracellular single-stranded (ss) HBV DNA was assayed by real-time PCR, it was revealed that G1862T accumulated the ss HBV DNA in cells, and coexisting A1762T/G1764A increased the accumulation further. Conclusions: Although G1862T was reported to reduce the replication of HBV, the fact that the mutation was found in this fulminant strain prompted us to investigate in more detail information about this mutation. In this study, it was suggested that the presence of G1862T impaired the release of HBV virion and the replicative intermediates were accumulated in hepatocytes. When A1762T/G1764A, which is known to enhance the viral replication, was present with G1862T, the replicative intermediate in cells was further increased, which might be associated with fulminant hepatitis.

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HBV SUPPRESSES TLR-MEDIATED INNATE IMMUNE RESPONSES IN PARENCHYMAL AND NON-PARENCHYMAL LIVER CELLS

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Background and Aims: It has been suggested that the genes reflecting the innate immune response are not detectable in the initial phase of HBV infection. Recent studies indicate that HBV may have developed strategies to suppress the initial antiviral response of the host including TLR pathways. We have previously shown that non-parenchymal liver cells (NPC; Kupffer cells (KC), liver sinusoidal endothelial cells (LSEC)) can be activated by TLR 3 and -4 to produce IFN-β and other mediators which can potently suppress HBV replication. Therefore, the aim of our study was to investigate whether HBV has the ability to suppress TLR-induced antiviral responses in parenchymal and non-parenchymal liver cells. Methods: We have isolated murine KC by counterflow elutriation as well as murine LSEC by anti-LSEC microbeads (>99% purity as controlled by FACS). These cells were cultivated in the presence or absence of supernatants from HBV-Met cells (a mouse hepatocyte line with a stably integrated 1.3 fold HBV genome) and were stimulated by TLR 1-9 ligands. The culture supernatants were added to differentiated HBV-Met cells, HBV gene expression and replication in treated cells was analyzed. In addition, primary murine hepatocytes were isolated by a two-step perfusion method. Primary hepatocytes, undifferentiated HBV-Met cells and highly differentiated HBV-Met cells were stimulated by TLR 1-9 ligands. Supernatants were collected and tested for the antiviral cytokines in viral protection assay or co-cultured with differentiated HBV-Met cells. HBV gene expression and replication in treated cells was analyzed. To assess the effects on the transcriptional level total RNA from undifferentiated HBV-Met cells and highly differentiated HBV-Met cells was isolated and analyzed by quantitative rt-PCR. Results: Pretreatment of KC and LSEC with HBV-Met cell supernatants (contain HBsAg, HBeAg and virions) strongly suppressed the antiviral activity of TLR 3 and -4 stimulated NPC which correlated with suppressed ISG induction by poly I:C and LPS on the transcriptional level. TLR 1-9 stimulation had no direct antiviral effect on differentiated HBV-Met cells in contrast to primary hepatocytes and undifferentiated cells. Conclusions: Our data indicate that HBV can suppress the antiviral activity of NPC induced by TLR 3 and -4 stimulation. Integrated HBV can downregulate the innate immune response of hepatocytes towards TLR stimulation. This is of particular relevance for the regulation of the local innate immune response against HBV in the liver and may explain why HBV appears to behave as "stealth virus" initially after infection.

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CHANGES IN SERUM HBV DNA LEVEL USING A TRAJECTORY MODEL PREDICT THE RISK OF HCC IN CHRONIC HEPATITIS B PATIENTS: THE R.E.V.E.A.L.-HBV STUDY

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Introduction: Elevated serum HBV DNA level is a strong risk predictor of developing hepatocellular carcinoma (HCC) in chronic hepatitis B (CHB) patients. Our aims were to evaluate the relationship between HBV DNA viral load changes over time and HCC risk, and the baseline factors associated with HBV DNA level changes over time. Methods: A sub-group of the R.E.V.E.A.L.-HBV cohort of HBsAg-seropositive and anti-HCV-seronegative patients, without liver cirrhosis at entry was included in this analysis. There were 1,289 participants with HBV DNA $\geq 10^4$ copies/mL at entry with ≥2 HBV DNA measurements included, and 2,020 participants with HBV DNA $<10^4$ copies/mL at entry as the reference group. Low, medium, high, and very high serum HBV DNA levels were defined as <300, 300—$<10^5$, 10^5—$<10^7$, and $\geq 10^7$ copies/mL, respectively. Low, medium, and high serum ALT levels were defined as <15, 15—44, and ≥45 U/L, respectively. A semi-parametric group-based modeling was used to determine major trajectory classes of HBV DNA and ALT. Factors associated with HBV DNA trajectories were examined using polytomous logistic regression. Cox's proportional hazards modeling was used to analyze associations between HBV DNA trajectory classes and HCC risk. Results: There were 89 new HCC cases among 3,309 participants during 40,024 person-years of follow-up. Among those with HBV DNA $\geq 10^4$ copies/mL, four HBV DNA trajectory classes were identified: class I, medium-to-low (n=148); class II, medium-to-medium (n=380); class III, high-to-high (n=542); and class IV, persistently very high (n=219). Four ALT trajectory classes were identified: class A, low-to-medium (n=1,056); class B, medium-to-medium (n=1,507); class C,

high-to-medium (n=187); and class D, medium-to-high (n=174). Gender, HBeAg status, and ALT level at entry were significantly associated with the HBV DNA trajectory. Compared with HBV DNA <10^4 copies/mL at study entry as the reference group, the multivariate-adjusted hazard ratio HR_{adj} (95%CI) of developing HCC by HBV DNA trajectory class was 0.7 (0.1—5.1) for class I; 2.6 (1.1—6.0) for class II; 8.5 (4.6—15.8) for class III; and 10.0 (4.7—20.7) for class IV after adjustment for gender, age, cigarette smoking, alcohol consumption, and ALT trajectory. The HR_{adj} (95%CI) of developing HCC by ALT trajectory class was 1.4 (0.8—2.7) for class B, 3.2 (1.5—6.9) for class C, and 2.3 (1.0—5.2) for class D compared with class A as the reference group. Conclusions: Serum HBV DNA level over time is a strong predictor of HCC independent of gender, age, cigarette smoking, alcohol consumption, and ALT trajectory.

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HBV VIRAL LOAD LESS THAN 10^4 COPIES/ML IS ASSOCIATED WITH SIGNIFICANT RISK OF HEPATOCELLULAR CARCINOMA IN CHRONIC HEPATITIS B PATIENTS: AN UPDATE FROM THE R.E.V.E.A.L.-HBV STUDY

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Introduction: Major international treatment guidelines classify CHB patients with low-level viremia (<10^4 copies/mL) as having inactive disease with little or no risk of disease progression. Risk of disease progression in these subjects has not been properly evaluated. Methods: All HBsAg-negative subjects without HCV infection (anti-HCV negative) and the subset of the R.E.V.E.A.L.-HBV study cohort without HCV infection or liver cirrhosis at baseline were included in this analysis. Ascertainment of HCC was by data linkage with computerized profiles of the National Cancer Registry and Death Certification System in Taiwan, and all HCC cases were confirmed using established criteria. The risk of HCC progression by baseline and follow-up viral load was determined (in comparison to HBsAg-negative subjects). Multivariable adjusted hazard ratios (HR_{adj}) were derived using time-dependent Cox proportional hazard modeling, which included baseline and time-dependent follow-up HBV DNA levels, baseline and time-dependent follow-up serum ALT levels, age, gender, and habits of alcohol consumption and cigarette smoking. Results: A total of 18,541 HBsAg-negative and 3,584 HBsAg-positive subjects were included in the analyses for a total of 267,809.1 person years of observation over 12.1 years, and a total of 184 HCC cases. Males made up 50.9% of the population. Compared with HBsAg-seronegative persons, the HR_{adj} (95%CI) of developing HCC by HBV DNA level in HBsAg-seropositive persons were 3.0 (1.4—6.3) for undetectable; 3.3 (1.7—6.6) for 300—9,999 copies/mL; 14.4 (8.5—24.4) for 10,000—99,999 copies/mL; 32.0 (19.9—51.6) for 100,000—999,999 copies/mL; and 30.5 (20.1—46.2) for >1 million copies/mL after adjustment for gender, age, cigarette smoking, alcohol consumption, ALT at entry and follow-up; and HBV DNA change during follow-up. Persons maintaining a HBV DNA level between 300—9,999 copies/mL during follow-up had an increased risk of HCC compared to those who were undetectable during follow-up HR_{adj} (95%CI) 2.7 (1.1—6.6). Conclusion: Results show that CHB patients with an HBV viral load of less than 10^4 copies/mL are at a significantly higher risk of developing HCC than HBsAg-seronegative persons. The risk of HCC associated with even the lowest detectable level of HBV DNA was 3-fold greater and highly significant. During follow-up, an HBV DNA level maintained between 300—9,999 copies/mL was associated with a significantly higher risk of HCC when compared to those achieving a level <300 copies/mL.

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HEPATITIS B VIRUS VACCINE ESCAPE MUTANTS HAVE IMPAIRED VIRION SECRETION EFFICIENCY IN CELL CULTURE

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Background/Aims: Vaccine failure and graft reinfection despite immunoglobulin are attributed to escape mutants with amino acid substitutions in the alpha determinant of viral envelope proteins. Recent studies revealed virion secretion defect of the prototype G145R mutant. In this study we systemically analyzed virion secretion capacity of 20 potential immune escape mutants. Methods: Huh7 cells were transiently transfected with a 1.5mer replication construct that cannot express envelope proteins and a 0.7mer expression construct of envelope proteins. Virus particles were immunoprecipitated from culture supernatant by an anti-preS2 antibody before Southern blot analysis. Results: All the mutants expressed similar levels of envelope proteins based on Western blot analysis of cell lysate. However, secreted HBsAg as measured by the Abbott kit was reduced moderately or completely for mutants affecting several cysteines or residues near G145. Those mutants showed total loss or marked reduction in virion secretion. In addition, several mutants with no impairment in HBsAg detection also displayed moderate or severe reduction in virion secretion. Co-expression of wild-type envelope proteins at 1:1 but not 1:5 (wt : mutant) ratio markedly improved virion secretion. Introduction of an M133T second-site mutation, which creates a novel N-linked glycosylation site, also restored virion secretion. Conclusions: Structural changes around the alpha determinant impair virion secretion. The detection of immune escape mutants in patient blood may result from rescue by the co-infecting wild-type virus. Immune escape mutants will not replace wild-type virus unless under conditions of anti-HBs immune pressure, or if they acquire a compensatory mutation.

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REAL-TIME PCR QUANTIFICATION OF TOTAL INTRACELLULAR HBV DNA AND CCCDNA IN HBSAG-POSITIVE PATIENTS WITH AND WITHOUT HDV CO-INFECTION AND IN PATIENTS WITH OCCULT HBV INFECTION

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Few information is available on the relation between HBVcccDNA levels and its transcriptional activity in chronic HBV infected individuals with different clinical/virological profiles. Aims of this study were (1) to quantify total HBV DNA, HBVcccDNA and pregenomic (pg)RNA in liver tissue from patients with different forms of chronic infection; (2) to evaluate serum HBV DNA and HBsAg titers in the same patients; (3) to investigate the acetylation/methylation status of HBVcccDNA-bound histones in occult HBV infected patients. We analysed serum and liver HBV extracts from 48 patients (36 HBsAg-positive and 12 occult HBV carriers). 5/36 HBsAg-positive cases were HBeAg-positive, 13 were anti-HBe positive with active HBV infection (HBV DNA>10^4 copies/ml), 14 were HDV infected with inactive HBV infection, and 4 were HDV carriers with active HBV infection. HBVcccDNA quantification method (Werle-Lapostolle, Gastroenterology 2004) was adapted to the TaqMan Cobas technology (Roche). HBsAg was quantified by Architect System kit (Abbott). HBVcccDNA chromatin immunoprecipitation (ChIP) (Pollicino, Gastroenterology 2006) was applied to study the acetylation/methylation status of HBVcccDNA from the liver tissue of 4 occult HBV carriers and 4 HBsAg-positive patients. Table reports the results concerning the median amount (range) of HBV DNA, pgRNA and HBsAg. HBVcccDNA ChIP experiments showed that in occult HBV carriers (1) the cccDNA-bound histones were hypoacetylated, and (2) histone deacetylase HDAc1, methyl CpG binding protein 2 (MeCP2) and histone methyltransferase SUV39H1 were recruited onto the HBVcccDNA. Conclusions (1) HBVcccDNA is quantifiable in all chronic carriers including occult infected individuals (2) In anti-HBe positive patients with active HBV replication, serum and total intrahepatic HBV DNA levels differ according to their HDV status whereas cccDNA levels are comparable between HDV positive and negative individuals (3) HBV DNA levels may be largely different among HDV infected patients indicating different grade of HDV interference on HBV replication (4) Serum HBsAg amount does not correlate with intracellular cccDNA levels (5) Despite the persistence of HBsAg synthesis, HBVcccDNA levels are similar in HDV patients with inactive HBV and occult HBV carriers (6) HBVcccDNA histones are hypo-acetylated and methylated in occult infection.

	HBeAg+	Anti-HBe+	HDV+/ active HBV	HDV+/ inactive HBV	Occult HBV
Total Intracellular HBV DNA (copies/cell)	652 (15-15000)	186 (0.6-549)	9.1 (4.7-9.3)	1.2 (0.01-9.4)	0.06 (0.009-1.62)
HBVcccDNA(copies/cell)	11.4 (0.4-22)	0.6 (0.02-3.8)	0.15 (0.05-0.16)	0.008 (0.002-0.3)	0.02 (0.001-0.06)
pgRNA (copies/cell)	248 (0.3-2900)	3.02 (0.1-308)	0.2 (0.1-1)	0.065 (0.004-14)	Not Detected
Serum HBV DNA (copies/ml)	$3.2x10^8$ ($1x10^6$ - $6x10^8$)	$3.7x10^6$ ($1.6x10^6$-$2.3x10^8$)	$7x10^4$ ($1.5x10^4$-$6.7x10^5$)	94 (8-$3.5x10^3$)	21.7 (2 - 98)
HBsAg (IU/ml)	12749 (5173-72000)	4905 (405-15000)	12616 (11412-51970)	6178 (515-17500)	Not Detected

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HEPATITIS B VIRUS PROTEINS INDUCE ACTIVATION OF HFGL2 TRANSCRIPTION THROUGH C-ETS-2 TRANSCRIPTION FACTOR AND MAPK SIGNAL PATHWAY

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Objective: Fibrinogen–like protein 2 (fgl2)/ fibroleukin is pivotal in the pathogenesis of both experimental and human fulminant viral hepatitis. This work was to characterize the transcription factor(s) and the upstream signal transduction pathway involved in transcription of the human fgl2 gene (hfgl2) in response to HBV proteins. Method: HBc, HBs or HBx expression plasmids were cotransfected with a hfgl2 promoter luciferase report construct into CHO cells and HepG2 cells respectively. Peripheral blood mononuclear cells (PBMC) were isolated from 10 patients with severe acute on chronic (AOC) hepatitis B, 10 patients with minimal chronic hepatitis B, 8 HBsAg carriers, and 6 health volunteers as controls. A polyclonal antibody against c-Ets-2 was used to detect the expression of c-Ets-2 protein in PBMC by flow cytometry, Western blotting and confocal microscopy. Phosphorylation of ERK, JNK and p38 MAPK in PBMC was detected by Western blotting. Results: Luciferase assay showed both HBc and HBx protein, but not HBs protein enhanced hfgl2 transcription in both CHO cells and HepG2 cells. A strong regulatory region from -712 to -568 (relative to the transcriptional starting site) was shown responsible for hfgl2 gene transcription in response to HBc or HBx proteins by a series of promoter deletion assay. By site-directed mutagenesis, EMSA and ChiP, binding of transcription factor c-Ets-2 and its cognate cis-element in the region -712/-568 was demonstrated to play an important role in hfgl2 gene transcription in response to both HBc and HBx proteins. shRNA interference of the c-Ets-2 expression was able to abolish hfgl2 gene transcription by 64.8% and 60.0% in response to HBc and HBx respectively. In human, c-Ets-2 protein was highly expressed in PBMC isolated from patients with severe AOC hepatitis B when compared with health controls. Increased phosphorylation activation of ERK, JNK and p38 MAPK were found in PBMC isolated from patients with severe AOC hepatitis B when compared with health controls. Treatment with ERK inhibitor PD098059 abolished the upregulated expression of c-Ets-2 in response to HBc protein, while JNK inhibitor SP600125 abolished the upregulated expression of c-Ets-2 in response to HBx protein. Conclusion: HBc and HBx initiated the transcription of hfgl2 gene through c-Ets-2 transcription factor, which was dependent on the activation of ERK and JNK signal pathway in corresponding to either HBc or HBx proteins. This work

provides new insights in the interaction between HBV virus and host gene hfgl2 expression. This study was supported by NSFC 30571643,30672380 and National Key Basic Research Program of China 2005CB522901.

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EFFICIENT CROSSTALK BETWEEN HBV-SPECIFIC T HELPER AND CYTOTOXIC T CELLS CHARACTERIZES SUCCESSFUL CONTROL OF HEPATITIS B VIRUS INFECTION

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Strong T helper1 (Th1) HBV-specific immune response leads to cytotoxic T lymphocyte (CTL) activation and control of viral replication. In patients with chronic hepatitis B infection, inefficient viral control may derive from impaired memory/effector CTL maturation caused by inadequate crosstalk between HBV-specific Th1 cells and CTL. Programmed cell death (PD-1) receptor, linked to functional suppression of HBV-specific T cells, may be involved in this process. Aims: To evaluate T helper and CTL HBV-specific responses in four stages of HBV infection and establish their impact on memory/effector cell maturation. Patients: 34 HLA-A2+ve children (16 males, median age 12.8 y) were divided into 4 groups according to HBeAg/HBsAg status and ALT activity (Table 1); 5 HCV-infected children (3 males, median age 13.2 y) and 5 healthy individuals (1 male, median age 31.5 y) served as controls. Methods: HBV-specific Th immune responses were determined by interferon (IFN)-γ, IL-2 and IL-10 Elispot after stimulation of peripheral blood mononuclear cells (PBMC) with HBV antigens (HBcAg, HBeAg and HBsAg). CD8+ reactivity after incubation with the HLA-A2 restricted immuno-dominant HBV-core$_{18\text{-}27}$ peptide was determined by INF-γ, granzyme B and IL-2 Elispot and staining with HLA-A2 HBV-core$_{18\text{-}27}$ pentamer. Phenotype of HBV-specific cells was determined by flow-cytometry with anti-CD27,-CD28,-CD45RA,-CD62L and-PD-1 antibodies. HBV DNA was quantified by real-time PCR ($\log_{10}$ copies/ml). Results: HBV-specific production of Th1 cytokines was higher in group D than groups A, B and C [IFN-γ HBV core: 479±145 vs. 12.6.3±4.4, 62.6±21.5 and 64.3±23.1 specific spot forming cells per 1 million PBMC (spSFC/10^6 PBMC), p=0.03]. Levels of pro-inflammatory cytokines produced by Th cells and CTL after antigen-specific stimulation correlated with the number of HBV-core$_{18\text{-}27}$ pentamer+ve cells (r= 0.569, p= 0.001) and mature memory/effector CTL (r=0.413, p=0.02). The number of PD-1+ve HBV-specific CTL was lower in group D than in groups A, B and C (76±4.8 vs.91.3±2.3, 81.6±7 and 81.8±5.4%, p=0.02). HBV DNA viral load correlated inversely with HBV-specific Th1 (r=-0.36, p=0.046) and CTL responses (r=-0.59, p=0.001) and number of pentamer+ve cells (r=-0.37, p=0.03). Conclusion: Efficient crosstalk between HBV-specific T helper and cytotoxic T cells leads to maturation of memory/effector T cells and successful control of viral replication.

Group	HBeAg	HBsAg	ALT	N	Stage
A	Positive	Positive	Normal	10	Immunotolerant
B	Positive	Positive	Elevated	10	Immunoactive
C	Negative	Positive	Normal	11	HBeAg negative hepatitis
D	Negative	Negative	Normal	3	Self-limited

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EVOLUTION OF VIRAL QUASISPECIES IN THE POLYMERASE GENE OF HEPATITIS B VIRUS DURING ANTIVIRAL TREATMENT: FROM NAIVE TO VIRAL BREAKTHROUGH

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Background/Aim: Drug resistance is emerging as the single most significant factor in treatment failure. The presence of different viral genomes in a patient (viral quasispecies), including preexisting mutations in the polymerase gene of hepatitis B virus (HBV), might be a mechanism of virus survival under selective antiviral drug pressure. We thus investigated viral quasispecies evolution and preexisting drug resistance mutations. Patients and Methods: Five chronic hepatitis B patients with lamivudine (LAM) failure were enrolled in this study. Patient sera were obtained before the treatment and at the time of viral breakthrough during LAM treatment. The polymerase chain reaction (PCR) product of the HBV polymerase gene (rt1-rt280) was cloned into pGEM T Easy Vector. After transformation, 100 clones were picked up at each time point. Cloned HBV DNA was confirmed using XbaI and PstI digestion and sequenced. The reference gene of HBV genotype C was NCBI GenBank No.AF286594, which was considered the wild type. Results: At baseline, the wild-type was present at 0–11% in five patients. Several substitutions in the HBV DNA polymerase region were observed, including reported antiviral resistant sites. Patients had 31–81 mutations of various types. Before treatment (naive), rtL80P (1%), V84F (5%), rtT128I (1%), rtV173L (2%), rtV173A (2%), rtL180P (1%), rtL180M (1%), rtA181T (1%), Q215P (1%), Q215H (1%), and N236H (7%) mutations at the LAM and adefovir (ADV) resistance sites were found. Interestingly, secondary compensatory mutations already existed without rtM204V/I mutations. Mutations were also identified at rtI169L (8%) and S202G (2%). In addition, we discovered novel unreported variations, such as rtT38A (60%), Y124H (47%), D134E (34%), I224V (98%), and R242A (67%). At the time of viral breakthrough during LAM treatment, LMV-resistant mutations were identified in most cases (94%). During LAM treatment, a proportion of the preexisting mutations changed and some disappeared. No mutations at rt169, rt181, rt184, rt202, or rt236 were identified, while rtT54S, rtH55R, rtS106C, rtY124N, rtD134G, rtP143Q, rtS223A, rtL229V, and rtP237H mutations became prevalent. Conclusion: We demonstrated the evolution of mutations in the polymerase gene of HBV during antiviral treatment. Our study may help clarify the drug resistance pathway and emphasize the early detection of drug-resistant mutants before or during

antiviral treatment to prevent antiviral therapy failure. Key Words: Hepatitis B virus, Mutations, Antivirals, Resistance, Quasispecies

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FACTORS ASSOCIATED WITH HBSAG SEROCLEARANCE IN ASYMPTOMATIC CARRIERS OF ENDEMIC AREAS DURING A LONG FOLLOW-UP PERIOD OF UP TO 17 YEARS

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Background: The seroclearance of hepatitis B s antigen (HBsAg) is a rare event and usually confers favourable outcome if there is no preexisted cirrhosis or viral superinfection, though adverse complications still can occur. However, the factors associated with HBsAg seroclearance are still under investigation. Aim:The aim of this long-term follow-up study was to reexamine the rates of HBsAg seroclearance and possible factors associated with HBsAg seroclearance. Patients/Methods: There were 1139 HBsAg-seropositive asymptomatic adult carriers, 31.9±23.3 years old, men (59%) and women enrolled in Eastern-Northern Greece between 1990 and 1999 in this analysis prospectively followed-up for a period up to 17yrs (1990-2007). Viral markers/liver biochemistry/physical examination performed at entry and every 6-24 months. Abdominal ultrasound was done at entry and every 3/4yrs. Serum HBV-DNA levels measured by Amplicor HBV Monitor kit. The HBsAg status in last follow-up samples was also tested. Logistic regression analysis was used to derive multivariate-adjusted odds ratio (OR) and 95% confidence interval (CI) for factors associated with HBsAg seroclearance. Results: Serum HBsAg cleared in 137 patients (12%) at the mean age of 41±15.3 years after the entry. The cumulative probability of HBsAg seroclearance after 17 years was only 5.3% (varied from 1.9% in low endemic areas to 21% in high endemic areas) for carriers with age at entry <20 years but around 27% for those with age at entry >20 years (varied form 11% in low to 45% in high endemic areas). Hepatitis relapse in 182 patients (16%) 0.5 to 16 years after the entry as depicted by ALT levels elevated >1.5 UNL and detectable serum HBV-DNA levels. During the follow-up period, age at entry and sustained remission of hepatitis were significantly associated with HBeAg seroclearance ($p<0.05$). The multivariate-adjusted OR (95% CI) of HBeAg seroclearance was 1.21 (1.32-2.43) for serum ALT level at study entry >40 IU versus <40 IU; 6.26 (1.87-34.47) for age <20 years versus >20 years; and 2.18 (7.25-23.91) for serum HBV DNA level <10(3) versus >10(3) copies/mL. Conclusions: The cumulative rate of HBsAg seroclearance was 30% in asymptomatic carriers after 17 years of follow-up. Longer follow-up period and sustained remission were associated with a significantly increased HBsAg seroclearance rate.

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HEPATITIS B VIRUS QUASISPECIES EVOLUTION IN HBEAG SEROCONVERTERS

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Background: We have recently demonstrated that hepatitis B virus (HBV) quasispecies diversity is higher in patients in HBeAg seroconverters (Gastroenterology, in press), while non-seroconverters have very low viral diversity. However, it is unclear how HBV viral genetic diversity transitions from a low diversity state to a high diversity state. Aim: To evaluate the long term evolution pattern of HBV quasispecies in spontaneous seroconverters that transition from low to high viral diversity state. Methods: Eight chronic hepatitis B HBeAg seroconverters, with long term stored serum and clinical follow-up of 6-15 years before seroconversion were studied. Two chronic hepatitis B patients without HBeAg seroconversion with matching length of follow-up and stored serum were included as controls. Serum samples from 7-12 time-points were used for nested PCR, cloning and sequencing of the precore/core gene (20 clones/sample). In total, 1685 sequences were analysed. Only genotype B patients were involved in this study. Sequences were aligned using ClustalX, then sUPGMA phylogenetic trees were constructed using Pebble 1.0 following which maximum likelihood estimates of pairwise distances under a GTR+I+G model was assessed. Viral diversity and substitution rates were then estimated. Results: The genetic diversity in HBeAg seroconverters was very low at baseline and similar to controls but gradually increased from 2.7X10exp-3 substitutions/site to 1.1X10exp-2 substitutions/site prior to seroconversion (Spearman's correlation analysis, R=0.67 and $p<0.0001$) followed by a striking increase post-seroconversion. The evolutionary rates in these patients showed a more complex pattern over time. Conclusions: There is a gradual transition of HBV quasispecies from a low diversity to high diversity status just prior to HBeAg seroconversion. High viral diversity appears to be a pre-requisite for HBeAg seroconversion.

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TLR9 EXPRESSION OF PLASMACYTOID DENDRITIC CELLS IN PATIENTS WITH CHRONIC HEPATIITS B INFECTION

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Backgroud and Aims: Chronic HBV infection is the result of a complex interaction between a replicating noncytopathic virus and a down-regulated antiviral immune response. Immature plasmacytoid dendritic cell (pDC) is the cell type responsible for the majority of the IFN-I production, which is the most important part of the native immunity against virus and mature plasmacytiod dendritic cell is the antigen-representing cell, which may prime naive CD4+ T cells and develop Th1 response. TLR9 functions as a pattern recognition receptors (PRRs) by interacting with CpG motif of virus. High level of cytokines such as interferon (IFN)-α,IFN-β are expressed by CpG stimulation. Previously,we have shown that TLR9 low expression and functional imparement of pDCs in patients with chronic hepatitis B.This

study aims to evaluate the frequency and function of circulating pDCs in patients at various stages of HBV infection,and to explore the role of TLR9 and pDCs function in the disease progression after HBV infection. Methods: Peripheral blood was collected from 69 hepatitis B (HB)virus-infected persons,including l4 cases of asymptomatic HBV infection,30 cases of chronic HB,25 cases of liver cirrhosis,and 21 healthy blood donors used as controls.Flow cytometry was used to analyse the frequency of circulating pDCs and the mean fluorescence intensity(MFI) of TLR9. Fresh peripheral blood mononuclear cells(PBMCs) were stimulated in vitro using CpG 2216.The supernatants were measured for IFN-a production using ELISA method. Results: Compared with healthy controls, the MFI of TLR9 from patients were significantly reduced ($p<0.0001$).In addition, the MFI of TLR9 in patients with asymptomatic HBV infection and liver cirrhosis were significantly decreased compared with that of chronic HB ($p<0.05$). The peripheral pDCs frequency in patients with chronic HB and liver cirrhosis were significantly more lower compared to the control group ($P<0.05$). Moreover, pDCs frequency inversely correlate with ALT levels ($r=-0.348$, $p<0.05$).The ability of PBMCs to secrete IFN-a also decreased significantly in patients with chronic HBV infection. Conclusions: Our results suggest that patients with chronic HBV infection have a significantly lower TLR9 expression of circulating pDC, as well as decreased number and impaired function of circulating pDC, which may be partially related to HBV disease progression in these patients.

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HEPATITIS B VIRUS X PROTEIN (HBX) TRIGGERS HEPATIC STELLATE CELLS (HSCS) ACTIVATION THROUGH TRANSFORMING GROWTH FACTOR BETA (TGF-β) SIGNALLING PATHWAY

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Activated hepatic stellate cells are the principle cell type responsible of liver fibrosis due to their great capacity to synthesize different extracellular matrix fibrilar proteins like collagen type I, and profibrotic growth factors like connective tissue growth factor (CTGF), and TGF-β. Hepatitis B virus is the main cause of hepatocarcinoma being HBx closely implicated in its development. However, HBx contribution to liver fibrosis has not been investigated. Our aim was to study if HSCs activation could be mediated by HBx and through which signalling pathways. METHODS Primary human HSCs were incubated with conditioned media from the following hepatic cell lines: CMX that expresses HBx in a dexamethasone inducible manner; 2.2.15 which bears two head to tail copies of the HBV genome. CHL and HepG2 were the control parental cell lines. To study HSCs activation α-smooth muscle actin (α-SMA) expression was analyzed by immunofluorescence (IF) and flow cytometry (FC) methods. Additionally, we studied COL-I synthesis by western blot. To discriminate the possible signalling pathways implicated, neutralization assays against the main growth factors involved in fibrosis such as VEGF, TNF-α, PDGF and TGF-β were carried out. RESULTS First of all we investigated HBx capacity to activated HSCs. IF and IC showed an α-SMA expression in HSCs conditioned with HBx expressing cell media that was not detected in those incubated with CHL and HepG2 media. Furthermore, HSCs COL-I expression was enhanced in the same cases. Interestingly, an up-regulation of TGF-β expression by HSCs themselves was observed after culturing them in the presence of culture supernatants from HBx-expressing cells compared to their respective parental cell lines CHL and HepG2. Conditioned medium from dexamethasone-stimulated CMX cells, but not CHL cells, further increased the expression of TGF-β by HSCs. Regarding to the signalling pathways we observed that only when we neutralized TGF-β effects, α-SMA and COL-I activation markers expression were reduced. Neutralization against the others growth factors implicated had no effects associated with HSCs activation. CONCLUSSION HBx expressing cells media are capable to induce HSCs activation revealed in α-SMA and COL-I enhanced expression. Neutralization assays showed that HBx influence is mediated by TGF-β signalling pathway. In conclusion, these results strongly suggest that HBx could be a promoting agent of liver fibrosis being capable of inducing the secretion of soluble mediators that result in the paracrine activation of HSCs at least in a TGF-β manner.

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INFLUENCE OF SERUM HBV DNA LEVEL ON A RECURRENCE OF HEPATOCELLULAR CARCINOMA AFTER CURATIVE TREATMENT WITH RADIO-FREQUENCY ABLATION

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Background:Elevated serum HBV DNA level is a strong risk predictor of hepatocellular carcinoma (HCC) independent of HBeAg, serum alanine aminotransferase level, and liver cirrhosis (JAMA 2006). However there are few studies investigating influence of serum HBV DNA level on recurrence of HCC. Aim:The purpose of this study was to evaluate whether serum HBV DNA level was associated with the recurrence rate after curative treatment of HBV related-HCC (HBsAg positive and HCV-Ab negative) with radio-frequency ablation (RFA) retrospectively. Methods:Consecutive patients with HBV related-HCC who were curatively treated with RFA at their first hospital admission from February 1999 to September 2006 were analyzed. Concentration of HBV DNA is measured by transcripition-mediated amplification (TMA) or polymerase chain reaction (PCR). Age, gender, albumin, alanine aminotransferase, platelet count, AFP, L3(%), DCP, tumor size, and the tumor number were also obtained for analysis. Risk factors for recurrence after RFA were evaluated using univariate and multivariate analyses. Results:A total of 66 patients (47 males, 19 females, mean age 66.4 ± 8.5 years) with HBV related-HCC were curatively treated with RFA during the study period. These patients were followed up for a mean of 3.2 years. recurrence of HCC occurred in 38 patients. 1-, 3- and 5-year recurrence rates were 30.0%, 60.4%, and 74.3%, respectively. By univariate analysis, platelet count ($<15 \times 10^4/mm^3$), the tumor number (multiple) and HBV DNA (≧ 4.0 logcopies/ml) were associated with the recurrence of HCC. A stepwise, multivariate Cox regression analysis revealed that platelet count (risk ratio, 3.74; 95% CI, 1.56-8.97, p=0.003) and concentration of HBV DNA (risk ratio, 2.87; 95% CI, 1.16-7.09, p=0.023) were independent risk factors for recurrence of HCC.

Conclusions:These data suggest that high serum concentration of HBV DNA is associated with higher risk of HCC recurrence.

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HEPATITIS B VIRUS (HBV) X PROTEIN (HBX) PROMOTES HUMAN HEPATIC STELLATE CELLS ACTIVATION AND PROLIFERATION MODULATED BY MATRIX METALLO-PROTEINASE 2 (MMP-2)

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Human Hepatic stellate cells (HHSCs) activation is crucial for liver fibrosis development. This event is characterized by α-smooth muscle actin (α-SMA) synthesis, an increased production of pro-fibrotic factors like collagen type 1 (COL-I), MMP-2, connective tissue growth factor (CTGF) and a successive enhanced proliferation that define the later perpetuation phase. HBx is a multifunctional regulator that modulates gene transcription and several signalling pathways. Also it has been shown to be essential for hepatocellular carcinoma (HCC) development; however its role in fibrosis is still unknown. OBJECTIVE: The aim of this study is to analyze the effects of HBx protein over HHSCs activation and perpetuation steps. METHODS: Primary HHSCs were incubated with conditioned media from hepatic cell lines: CMX which expresses HBx in a dexamethasone (DX)-inducible manner; 2.2.15, which bears two copies of the HBV genome. Parental cell lines CHL and HepG2 were used as controls. Human HHSCs were incubated during 24 hours with different conditioned media. Fibrosis markers expression α-SMA, COL-I, CTGF and MMP2, was analyzed by immunofluorescence, Western Blot (WB) and zymography. HHSC proliferation was spectrophotometric analyzed using a cell growth determination kit. RESULTS: HHSCs activation was demonstrated by the "de novo" expression of α-SMA as showed in immunofluorescence. WB and zymography from HHSCs exposed to conditioned media from HBx expressing cells, displayed an increased expression COL-I, CTGF and MMP-2 as compared to human HHSCs incubated with control media. In proliferation assays, conditioned media from CMX and 2.2.15 cells had a positive effect on HHSCs growth, compared to those exposed to CHL or HepG2 media. Furthermore, HHSCs incubated with presence of conditioned medium from dexamethasone-stimulated CMX cells showed a higher proliferation rate than those conditioned with unstimulated CMX or CHL media. CONCLUSION: Our results reveal that HBx could trigger HHSCs activation as demonstrated by the enhanced expression of COL-I, CTGF and α-SMA in HHSCs exposed to HBx viral protein. Additionally, an increased proliferation has been shown in HHSCs grown in the presence of conditioned medium from HBx-expressing cells. Furthermore, since a direct link between MMP-2 expression and HHSCs proliferation has been reported (Olaso E et al, 2001; Ikeda K et al, 1999), direct and paracrine induction of MMP-2 by HBx may contribute to this enhanced proliferation too. Taken together our results confirm the modulating role of HBx over HHSCs not only in the activation process but also the following perpetuation step modulated at least by the induction of MMP-2.

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EXPRESSION LEVELS OF HNF4A LINK EFFICIENT HBV REPLICATION TO HEPATOCYTE DIFFERENTIATION STATE

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The efficiency of HBV replication is discussed to depend on hepatocyte differentiation. Cellular factors that link HBV replication to hepatocyte differentiation, however, are not known so far. We hypothesized that expression levels of liver specific transcription factors determine hepatocyte differentiation as well as efficiency of HBV replication. Methods: We correlated expression levels of hepatocyte differentiation markers and transcription factors to efficiency of HBV replication following adenovirus-mediated transfer of 1.3 HBV genomes at equal levels into freshly isolated primary human hepatocytes (PHH), HepG2, HepaRG, HuH7 (hepatoma) and Pop10 (hepatocyte) cell lines. We quantified expression of albumin, ferritin, liver specific antigen (LSA), organic anion transporting polypeptide - C (OATP-C), bile salt exporting pump (BSEP), CYP1A2, 2'3'-tryptophandioxygenase (TDO), steroid regulatory element binding protein-2, apolipoprotein B100 and hepatocyte nuclear factors (HNF) HNF1α, HNF4α, HNF3α/β/γ, C/EBPα/β and LRH-1 by real-time RT-PCR and Western Blot. To monitor HBV replication, we quantified HBV pregenomic RNA (pgRNA), HBV core and large (L) envelope proteins, replicative intermediates and progeny virus. Results: The efficiency of HBV replication was 13.6-, 14.4- and 15.7-fold higher in PHH than in HepG2, HepaRG or HuH7 cells, respectively, and increased in HepaRG cells 3.3-fold upon differentiation. No HBV replication was detected in Pop10 cells. In all cells, expression levels of pgRNA determined replication efficiency and correlated closely with expression of LSA, TDO, OATP-C and BSEP as well as HNF1α, HNF3γ and HNF4α. When maintaining HBV replicating cell lines HepG2-H1.3 and HepG2.2.15 under differentiating conditions, pgRNA increased 8.6- and 8.1-, HBV core 14- and 10-, L-proteins 8.8- and 7.1- and progeny HBV 715- and 100-fold, respectively. Again, this correlated with increasing levels of HNF1α, HNF3γ and HNF4α and mRNA for OATP-C and BSEP. Knock down of HNF4α and HNF1α using specific siRNAs decreased pgRNA by 77% and 57%, expression of HBV core by 80% and 45% and L-proteins by 30% and 75%, HBV replicative intermediates by 50% each and HBV progeny release by 88% and 74%, respectively. Decrease in HBV replication was confirmed at the single-cell level after knock down of HNF4α. Levels of HNF4α closely correlated with HBV core protein and pgRNA in all analyzed tumor-peritumor samples of patients with HBV related hepatocellular carcinoma. Conclusion: High expression levels of HNF4α, maintaining a high level of hepatocyte differentiation and polarization, are required for efficient HBV replication.

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IMPACT OF CHRONICALLY ELEVATED HBV DNA VIRAL LOAD ON RISK OF HCC OCCURRENCE: AN UPDATE FROM THE R.E.V.E.A.L.-HBV STUDY

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Introduction: Previous publications from the R.E.V.E.A.L.-HBV study have demonstrated that HBV DNA at the time of cohort entry strongly predicts future risk of hepatocellular carcinoma (HCC). However, the impact of chronically elevated HBV DNA over time on the risk of HCC accounting for changes in serum ALT level has not been evaluated. The aim of this study was to assess HCC risk associated with multiple observations of serum HBV DNA and ALT levels over time. Methods: A subset of the R.E.V.E.A.L. study cohort with baseline HBV DNA $\geq 10^4$ copies/mL, no evidence of liver cirrhosis at entry, anti-HCV negative and ≥2 frozen serum samples available for HBV DNA testing were analyzed. HCC was ascertained through data linkage with computerized profiles of the National Cancer Registry and Death Certification System in Taiwan. All HCC cases were confirmed using established criteria. Multivariable adjusted hazards ratios (HR_{adj}) were derived using time-dependent Cox proportional hazard models where both HBV DNA and serum ALT were time dependent variables using multiple samples over the follow-up period. Serum ALT was categorized as 0—15, 16—44, ≥45 U/L. Results: Interim analysis of results identified 71 new HCC cases from 1,289 people over 15,508 person-years of follow-up. Males made up 68.8% of the population. In the final model adjusting for gender, age, cigarette smoking and alcohol consumption, serum ALT as a time- dependent variable, and HBeAg status, the risk of developing HCC was strongly associated with increasing HBV DNA level (P for trend <0.001) in a dose-dependent relationship. The HR_{adj} (95% confidence interval) were 6.1 (0.8—48.2), 6.2 (0.8—48.4), 7.7 (1—60.8), and 13.1 (1.7—99.6), for patients with serum HBV DNA level of 300—9,999 copies/mL, 10,000—99,999 copies/mL; 100,000—999,999 copies/mL; and $\geq 10^6$ copies/mL respectively, compared with those who spontaneously achieved an undetectable HBV DNA level (<300 copies/mL). Other important variables included increasing serum ALT level, HBeAg-positive serostatus, age, and alcohol consumption. Conclusion: The relationship between HBV DNA level and risk of HCC remained very strong in these analyses using follow-up HBV DNA levels. The risk increased as HBV DNA level increased.

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SPECTRUM OF STEATOSIS IN CHRONIC HEPATITIS B (CHB) AND ITS RELATION TO BIOCHEMICAL, METABOLIC, VIROLOGICAL AND HISTOLOGICAL PARAMETERS

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Steatosis predicts progression of histologic injury, insulin resistance and reduced response to antiviral therapy in patients with hepatitis C. However, there is limited data in patients with chronic hepatitis B (CHB). This is relevant since response to current antiviral therapies for CHB is poor. Aim: To assess the spectrum of hepatic steatosis in CHB and to determine its relationship with biochemical, metabolic, virologic and histologic parameters. Patients and Methods: Liver biopsies of 350 patients with chronic HBV infection were blindly assessed by three experienced pathologists and categorized as: Group 1- no steatosis (≤5%) and group 2- hepatosteatosis (>5%). Demographic, biochemical, metabolic, virologic and histologic parameters were compared between two groups. HBV DNA was quantified by Hybrid capture assay and HBV genotyping was done by multiplex PCR. Results: Group 1 had 232 (66.3%) and group 2 had 118 (33.7%) patients. In group 2, 65 (55.1%) patients had mild and 53 (44.9%) had moderate to severe steatosis. More than 90% cases showed macrovesicular steatosis. Group 2 patients showed significantly higher age (35.5±10.5 yrs vs 28.1±13.9 yrs, p=0.000), male preponderance (M:F ratio-8.8:1 vs. 4.8:1, p=0.02), higher BMI (26.2±9.2 Kg/m2 vs. 20.7±3.9 Kg/m2, p=0.000), triglyceride (149.4±62.2 vs. 81.9±31.4, p=0.000) and insulin levels (13.1±9.1 vs 9.1±6.0, p=0.027). Waist circumference, blood sugar, cholesterol and leptin (2.3±1.9ng/mL vs 2.4±1.3ng/mL, p=0.95) were also higher in group 2 but not statistically significant. Mean HAI and fibrosis scores in group 2 were higher than group 1 (Group 1 vs 2, HAI, 4.5±2.9 vs 4.8±2.6, p=0.28; fibrosis 1.5±1.2 vs 1.7±1.2, p=0.17). More patients in group 1 had higher HBeAg positivity (Group 1 vs 2, 57.2% vs 47.3%, p=0.039). There was no significant difference in ALT (median, 114 vs 99, p=0.36) and HBV DNA levels (2.4E08±4.9E08 vs 1.9E08±4.6E08, p=0.48). In group 2, hepatic steatosis was more frequent in patients with HBV genotype D than in genotype A (58.1% vs. 42.9%, p=0.01). Conclusions: In our study steatosis was seen in one third cases with chronic HBV infection. These patients showed higher necroinflammatory activity and fibrosis. Association of steatosis with metabolic determinants and its presence in the liver suggests the role of host factors in the etiology of steatosis in chronic hepatitis B. There is no relation to viral factors like viral load and HBeAg status although there is a possibility of relation to genotype D. The efficacy of combined "antiviral and metabolic" approaches versus standard antiviral regimes in patients with steatosis and chronic hepatitis B infection needs to be evaluated.

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PREVALENCE OF FIBROSIS AND CIRRHOSIS IN CHRONIC HEPATITIS B: IMPLICATIONS FOR TREATMENT AND MANAGEMENT

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Aim: To document the prevalence and factors associated with severe fibrosis and cirrhosis in a large population of Asian chronic hepatitis B (CHB) patients. Methods: Transient elastography was performed in unselected CHB patients using Fibroscan (Echosens, France). Liver stiffness score of >8.1 kPa was used as a cut-off for the presence of severe fibrosis or liver cirrhosis. Demographics and laboratory parameters were recorded. Results: A total of 951 patients were recruited, of which 605 (64%) were males, with median age of 43 years, and positive HBeAg in 226 (24%). Overall 319 (34%) had severe fibrosis, with higher prevalence seen in males compared with females (39% vs 24% respectively, p<0.01). Severe fibrosis was seen with increasing age from 20% in patients < 25 years to 81% in those >65 years. In patients who were ≤45 years of age, there was no significant difference in prevalence of severe fibrosis between HBeAg-positive and HBeAg-negative patients. After the age of 45 years, higher prevalence of severe fibrosis was seen in HBeAg(+) patients compared to HBeAg(-) patients (58% vs 43% respectively, p=0.03). There was also increasing prevalence of severe fibrosis with increasing ALT levels: in patients with ALT less than 0.5 x upper limit of normal (ULN) and 0.5-1 x ULN (11% vs 30% respectively, p<0.01) and 0.5-1 x ULN and 1-2 x ULN (30% vs 48% respectively, p<0.01). There was borderline significance in those with 1-2 x ULN and 2-5 x ULN (48% and 59% respectively, p=0.06) and no significant differences between those with ALT 2-5 x ULN and >5 x ULN. Higher prevalence of fibrosis was seen in patients with HBV DNA ≥4 log copies/ml compared to those below this level (41% vs 27% respectively, p<0.01). Using multivariate analysis by regression to determine factors associated with severe fibrosis, gender, age and ALT were significant factors (both p<0.001), whereas HBV DNA was not significant (p=0.25). In total there were 486 patients who had completely normal liver biochemistry with normal ALT levels, bilirubin ≤20 umol/L and albumin ≥40g/L. Of these, 89 (18%) had severe fibrosis. For those patients over the age of 45 years and with normal liver enzymes (n=211), 34% had underlying severe fibrosis. Conclusion: The overall prevalence of severe fibrosis in CHB patients was 34% with higher rates seen in older age groups, males, and in patients with higher ALT levels. There was a higher prevalence seen in male patients compared to female patients from age 36 to 55 years. Higher prevalence of fibrosis was also seen in HBeAg-positive patients compared to HBeAg-negative patients after the age of 45 years.

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ANALYSIS OF FULL HEPATITIS B VIRUS GENOME IN PATIENTS WITH AND WITHOUT HEPATOCELLULAR CARCINOMA

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Hepatitis B virus (HBV) genotype C and the basic core promoter (BCP) mutations were reported to be associated with the development of hepatocellular carcinoma (HCC). In this study the full sequences of HBV genomes were analyzed in order to find the other predictors of HCC development. Forty-four patients with HBsAg positive and HBV genotype C were studied. Among them, 24 cases developed HCC (HCC group) and the other 20 cases did not (non-HCC group) during the follow-up period. In the former 24 cases, the serum samples at the beginning of follow-up (HCC B) and at the detection of HCC (HCC A) were examined. In the latter 20 cases who did not develop HCC, the serum samples were obtained at the beginning of follow-up (non-HCC B) and at the end of observation period (non-HCC A). The two groups were matched in age, gender and follow-up time. All HBV sequences from serum samples were directly sequenced and compared with HBV X01587. HBV X promoter activity was examined using luciferase reporter assay. The nucleotide substitution rate in the full genome was 21.26±6.50×10-3/nt and 23.50±3.70×10-3/nt at the beginning of follow-up in the non-HCC group and HCC group, respectively (p=NS), and was 22.35±6.73×10-3/nt and 24.89±5.41×10-3/nt at the end of follow-up in the non-HCC group and HCC group, respectively (p=NS). More nucleotide and amino acid substitution rates were detected in the HCC group, compared with the non-HCC group. The substitution from G to A at nt 1317 in the X promoter was detected in 4 cases (20%) and 5 cases (25%) of 20 non-HCC B and non-HCC A cases, respectively, and in 14 cases (58.3%) and 15 cases (62.5%) of 24 HCC B and HCC A cases, respectively (non-HCC B vs HCC B, p=0.02; non-HCC A vs HCC A, p=0.02). The substitution from T to C/A/G at nt 1341 was detected in 3/2/1 cases (25%) and 3/3/1 cases (41.7%) of 24 HCC B and HCC A cases, while in none of 20 non-HCC B and non-HCC A cases (non-HCC B vs HCC B, p=0.03; non-HCC A vs HCC A, p=0.11). Pre-S2 deleted mutants were detected in 8 cases of 24 HCC B and 7 cases of 24 HCC A cases, respectively, while in none of the non-HCC group (HCC B vs non-HCC B, p<0.01; HCC A vs non-HCC A, p=0.01). We also examined the activity of HBV X promoter with mutations at nt 1317 and 1341 in comparison with that without mutations. The mutants at nt 1317 did not have any effect on the activity of X promoter, however, the mutants at nt 1341 from T to C/A/G resulted in 1.5, 2.3, 3.9 times increased X promoter activation, respectively. Substitutions and deletion of nucleotides of the HBV genome, especially the pre-S2 deletion and G1317A, T1341C/A/G mutations may be useful markers for predicting the development of HCC.

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HEPATITIS B VIRUS INHIBITS TLR9-MEDIATED INTERFERON ALPHA SECRETION IN PLASMACYTOID DENDRITIC CELLS

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Background and Aims: Hepatitis B virus (HBV) infection causes acute and chronic immunomediated liver injury. Viral factors responsible for immune evasion and establishment of a persistant HBV infection remain unclear but in vivo studies highlight deficient innate responses to HBV. Plasmacytoid dendritic cells (pDC) play a crucial role in innate immunity through their ability to secrete large amounts of type I interferons in response to viral pathogens in absence of active replication. Although still controversial, many studies do suggest a functional impairment of pDCs in chronic HBV infections, in both human and wood-

chucks. To investigate a direct role of HBV in this functional impairment, interactions between purified pDC and HBV particules were studied in vitro. Methods: For this purpose, HBV virions were purified using sucrose gradient from HepG2215 cells supernatants and viral load quantified by real time PCR. Interferon-alpha (IFN-α) and IL-6 secretions were determined by Elisa. Total PBMCs and purified pDCs were obtained from healthy blood donors and stimulated with oligonucleotides containing unmethylated deoxycytidyl-deoxyguanosine dinucleotides motifs (CpG) in the presence of HBV. Results/Discussion: Stimulation of pDC with HBV at MOI 10 to 1000 did not lead to any detectable levels of IFN-α and IL-6. When total PBMC and purified pDC were stimulated with CpG, secretion of IFN-α was reduced up to 40% by HBV. Inhibition was observed at 24 and 48h post-CpG stimulation and already detectable after 9h. Such an early inhibition suggest that mechanisms other than active viral replication are responsible for pDC dysfunction, consistent with studies suggesting an endocytosis mechanisms for HBV entry within DCs as opposed to an infection process. Contrary to the INF-α inhibition, the capacity of total PBMC to secrete IL6 was unchanged in presence of HBV. Conclusion: Overall, these findings do suggest that HBV alone is not recognized by pDC as a foreign "signal" and therefore fails to trigger this key component of innate immunity. This extent to which this pDC dysfunction plays a role in the outcome and immunopathogenesis of HBV infection warrants further studies.

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TRACING HEPATITIS B VIRUS DNA BACK TO THE 16TH CENTURY IN A KOREAN MUMMY

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Background and Aims: It is presumed that Hepatitis B virus (HBV) has infected humans for thousands of years, but precise evidence is lacking. Korea is a country with endemic hepatitis B. A rare find of a "naturally" mummified child from the 16th century AD, in Korea, with a relatively preserved liver, enabled testing for the presence of HBV-DNA sequences. Methods: Despite the age of the mummy (verified by C14 testing of cloths and coffin), a parencymatous organ was identified in the right upper abdominal quadrant by laparoscopy. Tissue samples were subjected to conventional microscopy and DNA extraction. A search for ancient HBV-DNA was conducted in three independent laboratories in Korea, UK and Israel. Pre-core, core and DNA polymerase sequences were analyzed In Jerusalem; DNA contamination was destroyed in liver tissue obtained by laparoscopy by incubation in a fresh bleach solution.. HBV DNA was extracted twice, using in-house guanidium thiocyanate or a commercial columns kit. PCR amplification and analysis of the pre C/C region was performed using a HBV Monitor kit (Roche). An HBV Genotyping kit (Innogenetics) was used for analysis of the polymerase gene Results: A 104 bp fragment from the preC/C region was amplified in Jerusalem and the genotype was determined by analysis of a 234 bp fragment from the polymerase gene. The genotype was confirmed by amplifying and sequencing a 239bp fragment from the core gene by the Korean team. A 98bp fragment from the S gene was amplified in London. These results confirm the presence HBV-DNA sequences, genotype C in material removed from the mummy. removed from the mummy. The tissue tested was also subjected to microscopic analysis. Although, as expected, no hepatic or lymphoid cells could be identified on H&E staining, the overall organization resembled the appearance of liver tissue. The architecture, as demonstrated by Masson trichrome and reticulin stains, appeared normal overall, with minimal fibrotic expansion of the portal tracts and condensation of the sinusoidal walls, as expected after the lysis of hepatocytes.Conclusion: Extraction of DNA from the a liver remnant of a 500 year old Korean mummy revealed evidence for presence of intrahepatic HBV-DNA. No statement can be made regarding the stage of HBV infection in this child presumed to be an HBsAg carrier. Other evidence obtained suggest that the child died of tuberculosis based on identification of mycobacterium tuberculosis sequences in nodules removed from intra abdominal tissue.

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EFFECT OF AINTIVIRAL THERAPY ON THE IMMUNOHISTOCHEMICAL EXPRESSION OF BCL-XL AND BAX PROTEIN IN PATIENTS WITH HBEAG-NEGATIVE CHRONIC HEPATITIS B

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BACKGROUND: Although increasing evidence suggest that apoptosis of hepatocytes plays an important role in the initiation of liver injury/carcinogenesis, the exact role of antiviral therapy in apoptosis has never been investigated. AIMS: To evaluate the immunohistochemical expression of anti-apoptotic bcl-xL and pro-apoptotic bax proteins in hepatocytes of patients with HBeAg-negative chronic hepatitis B (CHBe-) before and after treatment. MATERIAL AND METHODS: Thirty six paired paraffin-embedded liver biopsies obtained from CHBe- patients: between the 1st and 2nd biopsy, 29 patients received 12-month courses of antiviral therapy (interferon-alpha: 17, adefovir: 12) and 7 untreated patients were used as controls. In each pair of biopsies, changes in expression of apoptotic proteins (D-bcl-xL and D-bax), total apoptotic trend (D-apoptosis: difference between D-bcl-xL and D-bax), necroinflammation (D-grade) and fibrosis (D-stage) (using the Ishak score) were evaluated. RESULTS: Bax-positive compared to Bax-negative biopsies had significantly worse necroinflammation (8.2 vs 6.7, p=0.05) and fibrosis score (3.9 vs 3, p=0.036). Bcl-xL-positive compared to bcl-xL-negative biopsies had significantly lower intralobular inflammation (1.6 vs 2.2, p=0.03). Negative, compared to stable/positive D-bax from 1st to 2nd biopsy, was associated with significantly greater improvement in necroinflammation only in treated patients (mean D-grade : -4.6 vs -1.6, p=0.06) and improvement in fibrosis in the interferon treated patients (D-stage: -0.4 vs 0.55, p=0.05). Stable/decreased compared to increased D-apoptosis was associated with significantly worsen fibrosis, particularly in patients treated with adefovir (D-stage= 2.3 vs 0, p=0.004). In the 11 patients without significant changes from 1st to 2nd biopsy (D-stage ≤1 and D-grade ≤2), positive D-apoptosis was significant more frequent in treated than untreated control

patients (p=0.046). In multivariate analysis, D-bax was independently associated with D-grade (p=0.038) and antiviral therapy (p=0.015). CONCLUSION: In CHBe- patients, improvement in liver histology (grade/stage) after antiviral therapy was associated with a decrease in the apoptosis of hepatocytes. On the other hand, in patients without substantial changes in necroinflammation and fibrosis, antiviral therapy was found to increase the apoptosis of hepatocytes, thus having a possible protective effect on hepatocarcinogenesis.

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DIFFERENCES OF EARLY DYNAMICS AND LIVER DAMAGE AMONG HEPATITIS B VIRUS GENOTYPES IN UPA/SCID MICE WITH HUMAN HEPATOCYTES

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Background: Hepatitis B virus (HBV) genotypes/subgenotypes indicate different clinical features, i.e. genotype C is associated with hepatocellular carcinoma and Bj with precore mutation (PCm) is associated with fulminant hepatitis. We previously showed the difference of each genotype in vitro and partially in vivo. Aim: Our purpose of this study is to investigate the differences of viral replication, protein production and liver damage among HBV genotypes in vivo. Methods: Sera of six HBV carriers [three each for Bj_wild-type (from acute HB patients) and Bj_PCm (fulminant HB patients)] were prepared. The sera or virions of HBV recovered from culture media, that were produced by transfection with plasmids (3 strains each for Ae, Ce, Bj_wild-type and Bj_PCm) carrying 1.24-fold the HBV genome in Huh7 cells, were inoculated into uPA/SCID mice with the liver replaced by human hepatocytes (chimeric mice). HBV DNA in mice serum was measured by real-time detection PCR weekly and then histopathologic changes of liver were examined with H.E, Masson trichrome, Orcein stainings, and immunostaining using anti alpha-smooth muscle actin (alpha-SMA) antibody. Results: The replication efficiency in chimeric mice was the highest in HBV/C and Bj_PCm, followed by Ae (P<.05). HBV DNA levels of Bj_wild-type were extremely lower than the others by 4 log levels. To exclude the possibility that immature virus particles were produced in culture media, sera of 6 HBV carriers with Bj_wild-type or Bj_PCm were inoculated with chimeric mice. The HBV DNA levels in mice serum were similar to those by injection of culture media. HBcrAg in serum were expressed in a trend similar to HBV DNA levels among HBV genotypes, while HBsAg was expressed at the highest level in HBV/Ae. The protein production of Bj_PCm transiently increased and peaked in earlier phase. Based on histopathologic examinations, chimeric mice inoculated with HBV/C or Bj_PCm for 6 months showed that human hepatocytes had somewhat ground-glass appearance and hepatic fibrosis, while chimeric mice inoculated with HBV/Ae or Bj_wild-type were not induced. Conclusion: Virological differences among HBV genotypes demonstrated in chimeric mice may influence HBV infections with distinct genotypes in clinical and epidemiological settings. Precore mutation of Bj could induce high replication and liver damage in vivo, which might be associated with development of fulminant hepatitis.

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GENDER DIFFERENCE IN THE NATURAL COURSE OF HBEAG-POSITIVE CHRONIC HEPATITIS B

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PURPOSE: Cross-sectional age-specific seroprevalence studies from Taiwan have provided evidence to suggest that male patients have earlier spontaneous hepatitis B e antigen (HBeAg) seroconversion but, paradoxically, bear a worse prognosis in disease progression when compared to their female counterparts. On the other hand, recent studies have shown that patients infected with genotype B hepatitis B virus (HBV) have earlier HBeAg seroconversion and better prognosis than genotype C infected patients. This longitudinal study was conducted to clarify this gender-related paradox while taking account the HBV genotype factor. METHODS: HBeAg seropositive patients from 1977 to 1998 were recruited if they met the following criteria: (1) Histologic and biochemical confirmation of chronic hepatitis B; (2) Follow-up every 3-6 months and ≥ 2 years; (3) No concomitant hepatitis C or D virus infection; (4) No drug treatment during follow-up. The clinical course and biochemical/serological changes were compared between male and female patients using t test, X^2 and Kaplan-Meir survival analysis. RESULTS: There were 454 males and 110 females who met our entry criteria. The age, baseline serum alanine aminotransferase (ALT) level and HBV genotype distribution were comparable between male and female patients. During a mean follow-up period of 12 years, there was no significant difference in the timing and the cumulative incidence of spontaneous HBeAg seroconversion between male and female patients (97.6 vs 100%; P=0.915). To minimize the influence of other confounding factors, the 110 female patients were compared with a 1:1 group of male patients well matched for age (± 2 years), baseline ALT (± 20 U/L) and duration of follow-up (± 1 year). The HBV genotype distribution in the female and the matched male patients was comparable (P=0.864). There was no significant difference in the incidence of hepatitis flare with ALT over 5 times the upper limit of normal (66.4 vs 72.5%; P=0.326) and in the 12-year cumulative incidence of HBeAg seroconversion (85.5 vs 74.4 %; P=0.129). However, 47.7% of the male patients showed persistent ALT elevation, with or without ALT flare, in contrast to 14.5% of the female patients (P<0.0001). CONCLUSION: This longitudinal study showed that there was no difference in the occurrence of hepatitis flare and spontaneous HBeAg seroconversion between males and females with active chronic hepatitis B. Male patients were found to have a higher propensity for persistent ALT elevation, which may be responsible for a more progressive course.

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BOW BODY MASS INDEX (BMI) PREDICTS DISCORDANCE BETWEEN TRANSIENT ELASTOGRAPHY (TE) AND LIVER BIOPSY (LB) ASSESSMENT OF LIVER FIBROSIS

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Background and aim. In patients with chronic liver disease (CLD) liver fibrosis stage can be accurately predicted by TE. Variables associated with discordant results between TE and LB need to be defined. Methods. All consecutive patients with CLD who underwent percutaneous LB for diagnostic/therapeutic purposes were concurrently examined by TE (FibroScan®; Echosens, Paris, France). Grading (A) and staging (F) were assessed by METAVIR score. Steatosis was arbitrarily graded from 0 to 3 (0<5%, 1=5-24%; 2=25-50%; 3≥50% of fatty hepatocytes). Only TE examinations with at least 10 validated measurements and a success rate of at least 60% were considered adequate. Statistical analysis. Determinants of TE stiffness value (log transformed) were investigated by the multiple linear regression model and variables possibly influencing discordance between TE and LB by a logistic regression model. The variables included in the models were: gender; age; body mass index (BMI, kg/m2) (<19 vs 19-25 vs 26-30 vs >30 kg/m2); etiology; AST, ALT and GGT levels (<1.5 vs 2-3 vs >3 ULN), APRI score (<0.5 vs ≥0.5); steatosis (0-1 vs 2; 2 vs 3); A (0-1 vs 1-4) and F; LB specimen length (≤20 vs >20 mm). Discordance was the lack of correspondence between the METAVIR F and TE diagnostic cut off (7.9, 10.3 and 11.9 kPa for F≥2, F≥3 and F=4, respectively) identified in a previous cohort. Results: 303 males and 257 females (50±12 years) were studied. TE examination failed in 30 (5%) mainly due to high BMI. In four cases (0.7%) LB was inadequate as shorter than 15 mm or contained <11 portal spaces. By multivariate analysis, fibrosis stage (p=0.0001), BMI (p=0.0004), etiology (p=0.02), GGT (p=0.0001), APRI score (p=0.0001) and A (p=0.0001) were significantly related to TE values. TE and LB were discordant in 116 cases (22%) for the diagnosis of F≥2 and in 85 (16%) for the diagnosis of F≥3. The probability of discordance was positively associated with BMI (BMI<19 vs 19-25, OR=3.2, 95%CI 1.3-7.8 for F>2; OR=4.9, 95%CI 2.0-11.8 for F>3). Conclusion: In the face of many factors other than fibrosis influencing TE results, low BMI was the only predictor of discordance between TE results and LB.

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RAPID IMPROVEMENT OF INNATE IMMUNE RESPONSES AND MONOCYTE TOLL-LIKE RECEPTOR-2 (TLR2) EXPRESSION DURING LAMIVUDINE AND PEGYLATED INTERFERON THERAPY FOR CHRONIC HEPATITIS B (CHB)

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Background/Aims: Toll-like receptors (TLR's) are critical receptors that promote innate immune responses to pathogen-associated molecular patterns. Activation of TLR's leads to production of pro-inflammatory cytokines. We have previously demonstrated that patients with HBeAg positive CHB have suppressed TLR2 expression on peripheral monocytes and hepatocytes associated with decreased pro-inflammatory cytokine expression. Methods and Patients: The TLR2 and TLR4 expression on peripheral blood monocytes was measured from 21 patients with untreated HBeAg-positive and HBeAg-negative CHB and 10 uninfected control patients. These 21 patients were treated with lamivudine 100mg daily. Individual patient blood was stimulated with specific TLR ligands and the resultant supernatant was assayed for TNF-α and IL-6 by ELISA. In addition serum HBeAg was measured by enzyme immunoassay . Similar data was also obtained for 20 HBeAg positve patients treated with pegylated interferon Results: Expression of TLR2 on peripheral monocytes was significantly reduced in patients with HBeAg-positive CHB in comparison to HBeAg-negative CHB and to controls whilst it was significantly increased in HBeAg-negative CHB compared to controls (P=0.001). TLR2 levels increased within four weeks of effective therapy in HBeAg-positive patients (P<0.05) whilst remaining stable in HBeAg negative patients. The level of TLR4 expression did not differ significantly between the groups. The functional relevance of these findings was established by the demonstration in HBeAg positive patients of initial reduced TNF-α and IL-6 and phospho-p38 kinase production after stimulation of monocytes with specific TLR ligands. This immunosuppresion improved rapidly during treatment (P<0.05). In HBeAg negative patients innate immune responses were initially increased but normalized with therapy. In the HBeAg positve patients treated with pegylated interferon TLR2 expression was further suppressed at 4 weeks (P<0.05). before improving over subsequent months. Cytokine production secondary to stimulation with Pam-3-Cys and LPS were markedly increased at week 4, dropping dramatically thereafter (P<0.05). Correlations of these results with HBV viral load and eAg levels (eAg positive patients) will also be presented. Conclusions: This is the first study to investigate how peripheral innate immune responses and TLR expression changes with different therapies. It demonstrates a potentially important interaction between HBeAg, HBV and the innate immune response and suggests that TLR2 expression and activation could represent a sensitive new biomarker in the treatment of HBeAg-positive CHB.

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DIFFERENTIAL EXPRESSION OF HEPATIC APURINIC/APYRIMIDINIC ENDONUCLEASE 1, A DNA REPAIR ENZYME, IN CHRONIC HEPATITIS B VIRUS AND HEPATITIS C VIRUS INFECTION

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Background: Hepatocellular carcinoma (HCC) is frequently encountered in chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, while HCC rarely develops in autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC). However, the mechanisms responsible for HBV- or HCV-mediated hepatocarcinogenesis have not been fully clarified, yet. Cellular DNA damage can lead to mutation induction and subsequent carcinogenesis if DNA repair processes are not completely effective. Apurinic/apyrimidinic endonuclease 1 (APE-1; also designated Ref-1) is one of the major DNA repair enzymes, and its altered expression is associated with tumorigenesis. Therefore, APE-1/Ref-1 may be involved in HBV- or HCV-mediated hepatocarcinogenesis. The aim of the present study was to examine hepatic expression of APE-1/Ref-1 in patients with chronic HBV or HCV infection, compared with those with AIH or PBC. Methods: Liver biopsies were obtained from 14 patients with chronic hepatitis B, 10 patients with chronic hepatitis C, 5 patients with AIH and 5 patients with PBC. APE-1/Ref-1 expression was assayed by Western blot or real time-PCR, and its cellular distribution was determined by using immunohistochemistry. Results: APE-1/Ref-1 protein levels were reduced by 64% in HBV-infected livers compared with AIH and PBC livers, while HCV livers had no significant difference in APE-1/Ref-1 protein levels compared with those of autoimmune chronic liver diseases. APE-1/Ref-1 mRNA levels were reduced by 58% in HBV livers compared with HCV livers. APE-1/Ref-1 had a predominant nuclear localization in the majority of hepatocytes with a panacinar distribution in all of the liver specimens. The hepatocytic nuclear APE-1/Ref-1 expression was also reduced in the HBV group. Conclusions: Patients with chronic HBV infection, not chronic HCV infection, showed reduced expression of hepatic APE-1/Ref-1 compared with those with autoimmune chronic liver diseases. Hepatic APE-1/Ref-1 expression may undergo differential regulation in chronic HBV and HCV infection. Downregulation of hepatic APE-1/Ref-1 expression may contribute to the development of HCC in chronic HBV infection.

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GENETIC POLYMORPHISMS AT THE APOLIPOPROTEIN E LOCUS AFFECT THE OUTCOME OF CHRONIC HEPATITIS B

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Introduction. Genetic variations at the apolipoprotein E (ApoE) locus are known to affect plasma lipoprotein concentrations; therefore, by modulating transport into the blood circulation and entry into the hepatocytes of hepatitis B virus (HBV), they may influence the course of HBV infection. The present study aimed to verify this hypothesis. Methods. One-hundred five HBV positive, HCV negative patients (74 males, 31 females, median age 51 years, range 22-84) were studied. Among them, a cohort of 62 patients (42 males, 20 females, median age 51 years, range 28-81) had underwent periodic clinical monitoring for a median time of approximately 20 years since first identified as HBsAg carriers. ApoE genotypes were determined by a previously described polymerase chain reaction/restriction length fragment polymorphism method. Results. Twenty-seven patients out of 105 (26%) fulfilled criteria for being inactive HBV carriers, whereas 61 had chronic hepatitis B (58%; 15 with cirrhosis) and 17 had underwent liver transplantation for hepatic failure and/or hepatocellular carcinoma (16%). The allelic frequencies of the ApoE gene were the following: ApoE2 = 15%; ApoE3 = 78%; ApoE4 = 7%. There was a strong linear trend for E3 homozygotes to be progressively overrepresented among patients with liver disease of advancing severity: E3/E3 were 13/27 (48%) among inactive HBV carriers, 36/61 (59%) among patients with chronic hepatitis B, and 16/17 (94%) among patients transplanted for hepatitis B-related sequelae ($p<0.005$). In the longitudinal cohort, 12/62 patients (19%) became HBsAg negative after a median follow-up time of 14.6 years (range, 1.5-32.3). Being an E3/* carrier was associated with significantly lower probability of loosing HBsAg (9/56, 16% vs. 3/6, 50%, $p<0.05$), as well as significantly longer time to HBsAg loss ($p<0.03$ by Mantel-Cox test). Conclusions. The probability of undergoing disease progression is higher, and that of remaining inactive carriers or of loosing HBsAg is lower among possessors of the ApoE3 allelic variant. Down-regulation and/or reduced binding involving the LDL-receptor might explain the more benign course of hepatitis B among ApoE2-E4 carriers.

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PREDICTIVE MODEL FOR FIBROSIS AND CIRRHOSIS IN CHRONIC HEPATITIS B USING LIVER STIFFNESS MEASUREMENT

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Aim: To correlate liver stiffness measurements with demographic and laboratory parameters, and assess the predictive value of these parameters as non-invasive markers of fibrosis and cirrhosis in chronic hepatitis B (CHB). Methods: Transient elastography was performed in 265 CHB patients using Fibroscan (Echosens, France). Liver stiffness scores of >8.1 and >10.3 kPa were used as cut-off values for significant fibrosis and cirrhosis respectively. To derive a new index using commonly measured laboratory markers, the study sample was randomly split into a training set and a validation set. Results: Of the 265 patients, 170(64%) were male. Ninety-six patients (36%) were HBeAg-positive, and 90 patients (34%) had severe fibrosis or cirrhosis. The median liver stiffness score was 6.3 kPa (range, 3.0 to 56.3). Liver stiffness correlated positively with ALT, bilirubin and AFP, and negatively with albumin levels. Using 13 parameters (age, sex, platelet, AST, ALT, GGT, AFP, albumin, globulin, bilirubin, ALP, HBV DNA, and HBeAg), the sequence of variables in order of their associations with liver stiffness (co-effi-

cient path) was determined using L1 regularized regression. The AUROC were calculated for each number of variables used for the prediction of fibrosis and cirrhosis, and the number of variables used was determined as the one that additional variables would not give a relatively higher accuracy. Four best predictive parameters (AST, Platelet, GGT and AFP) were used to derive a model for fibrosis (the APGA index). Additional variables did not improve accuracy significantly. The model using log(index)=1.44 + 0.1490(GGT) + 0.3308 log(AST) – 0.5846 log(platelets) + 0.1148 log (AFP+1) to predict significant fibrosis had an AUROC of 0.85 in both training and validation groups. An optimal cutoff value of 6.9 had sensitivity of 82%, specificity of 69%, with negative predictive value of 91%. The AUROC for predicting cirrhosis were 0.89 and 0.85 in training and validation groups respectively. Using an optimal cutoff of 8.9 was associated with sensitivity of 64% and specificity of 89% with a negative predictive value of 92%. The AUROC for the APGA index was higher compared to that of the AST-to-platelet ratio index, AST/ALT ratio, and age-platelet index in the training group (0.85, 0.81, 0.50, and 0.77 respectively) and the validation group (0.85, 0.80. 0.38 and 0.68 respectively) Conclusion: Measurement of liver stiffness with transient elastography correlates well with known factors associated with the severity of disease in Asian CHB patients. The APGA index is a reliable non-invasive method for predicting significant fibrosis and cirrhosis in CHB.

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INHIBITION OF VIRAL REPLICATION WITH PEGYLATED INTERFERON ALFA-2A INCREASES IL-17 IN CHRONIC HEPATITIS B

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Background and Aim: Interleukin (IL)-17 is produced mainly by activated CD4+ T cells, currently known as Th17. Chronic hepatitis B (CHB) infection is associated with weak and narrowly focused immune response with low CD4+ and CD8+ activity. This is the first report of IL-17 in CHB infection. We assessed IL-17 expression in the CD4+ T cells (Th17) of 8 consecutive CHB patients treated with pegylated interferon alfa-2a for 48 weeks. Methods: Peripheral blood mononuclear cells (PBMC) were stained with CD45, CD4, IL-17 and analyzed by three-color flow cytometry. PBMC of 8 hepatitis B e antigen (HBeAg) positive patients; 4 with sustained virological response (SVR) and 4 without SVR; were collected every 4 weekly during treatment until end of therapy (week 48) and every 12 weekly until the end of follow-up (week 72). Serum HBV DNA was quantified by quantitative PCR with a linear range of 100 to 109 copies/ml. SVR was defined as HBeAg seroconversion with serum HBV DNA < 104 copies/ml at the end of follow-up. HBeAg seroconversion was defined as HBeAg negativity with hepatitis B e antibody on 2 consecutive occasions 3 months apart. IL-17 was defined as CD4+IL-17+ cells/CD45+ cells. Results: In parallel with decline in serum HBV DNA, we found an increase in IL-17 at the end of follow-up when compared with baseline [mean±standard error of mean (SEM) 2.46±0.37 vs. 1.44±0.23 % respectively, p=0.03]. There was a trend that IL-17 at the end of therapy was also higher than at baseline [mean±SEM 1.93±0.23 % respectively, p=0.05]. The serum HBV DNA at the end of therapy [mean±SEM 3.10±0.59 vs. 5.41±0.49 log10 copies/ml respectively, p=0.04] and end of follow-up [mean±SEM 4.31±1.25 vs. 8.31±0.87 log10 copies/ml respectively, p=0.04] in patients with SVR was lower when compared with those without SVR. Similarly, those with SVR had a higher, though insignificant IL-17 at the end of therapy [mean±SEM 2.13±0.08 vs. 1.73±0.48 % respectively, p=0.47] and end of follow-up [mean±SEM 2.57±0.28 vs. 2.35±0.74 % respectively, p=0.79] when compared with patients without SVR. Conclusion: Pegylated interferon alfa-2a induced viral load reduction results in an increase in IL-17. SVR may be associated with a higher IL-17. The role of IL-17 in CHB infection and immunopathogenesis requires further investigation.

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INTRACELLULAR LEVELS OF HEPATITIS B VIRUS DNA AND PREGENOMIC RNA IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF CHRONICALLY INFECTED PATIENTS

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Background & Aims: Several previous studies have demonstrated the presence of HBV genomes in peripheral blood mononuclear cells (PBMC) from patients with chronic hepatitis B (CHB) infection. Recently, there are additional reports on the detection and quantitation of HBV cccDNA and pgRNA in the serum. The aims of this study are: (1) to clarify whether HBV cccDNA and pgRNA can be detected in the serum and (2) to examine the effect of antiviral treatment on the HBV DNA viral loads in serum and PBMC of CHB patients. Methods: Paired serum and PBMC samples from 50 treatment naive CHB patients [Group I: HBeAg+, n=12, Median ALT:28.5 (8-49)U/L; Group II: HBeAg+, n=13, Median ALT:78 (56-250)U/L; Group III: HBeAg-, n=12, Median ALT:39 (17-48)U/L; Group IV: HBeAg-, n=13, Median ALT:110 (48-424)]were assayed for HBV total DNA, cccDNA and pgRNA. Real time PCR was used for the quantification of HBV total DNA in serum and PBMC. For HBV pgRNA detection, the levels of pgRNA were calculated by subtracting precore mRNA from total core promotor-directed transcripts. For HBV cccDNA quantification, two primers were used which crossed the nick on plus strand and the DR2 region on minus strand in order to avoid the rcDNA and dsDNA contamination. In the longitudinal study, 30 patients [median ALT: 124 (42-642) U/L; HBeAg+, n= 26; HBeAg-, n= 4] treated with lamivudine therapy were followed-up for a median of 34 (12-48) weeks. Serially collected serum and PBMC samples were analyzed for HBV total DNA, cccDNA and pgRNA. Results: HBV cccDNA and pgRNA were undetectable in all serum samples, and HBV cccDNA was under the lower detection limit in most PBMC samples (84%). For all 50 patients, both the quantity of HBV DNA (r=0.889, p<0.001) and pgRNA (r=0.696, p<0.001) in PBMC were significantly correlated with the quantity of HBV DNA in serum. The PBMC and serum viral loads in different groups were related with the HBeAg status and the ALT levels. In the longitudinal study, the medians of HBV DNA and pgRNA reduction in PBMC before and after treatment were 1.318 (-0.471-3.846) log units and 1.010 (-0.717-5.232) log units respectively. And the median of serum HBV DNA reduction was 3.371 (-0.883-9.454) log units. The reduction levels of both HBV DNA and pgRNA in PBMC were significantly lower than that of HBV

DNA in serum (P<0.005). Conclusion: (1) HBV cccDNA and pgRNA were undetectable in the serum of CHB patients; (2) HBV viral loads in PBMC were associated with the serum HBV DNA;(3) Lamivudine therapy had less effect on the HBV viral loads in PBMC compared with the serum viral loads. The inefficiency of antiviral therapy on PBMC viral loads may relate with the HBV persistence.

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PREDICTORS OF SIGNIFICANT HISTOLOGICAL FINDINGS IN CHRONIC HEPATITIS B PATIENTS WITH PERSISTENTLY NORMAL ALT LEVELS

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Background/aim: Current guidelines recommend antiviral therapy for chronic hepatitis B (CHB) patients with elevated ALT and high viral load. Little histological data exists for CHB patients with persistently normal ALT levels (PNAL) and low viral load since disease progression is thought to be rare. The aim of the study is to find the predictors of significant histological findings (significant necroinflammation with a score of HAI 4 or greater and/or significant fibrosis Ishak's staged at 3 or greater) in such patients. Methods: The study of all patients with CHB who underwent percutaneous liver biopsy and who had detectable viral load (Taqman Real Time PCR, lower limit of detection is 10^3copies/ml) was performed. 139 patients who had PNAL (defined as normal ALT measured on at least 3 occasions in the intervals of more than two months over a period of 12 or more months apart prior to biopsy) were identified (group A). These patients were compared with 135 patients with abnormal ALT during the same period and who had no prior antiviral treatment. The comparisons included age, ALT, viral load, HBeAg status, and histological finding. Results: The ALT values of all 139 patients with PNAL had been normal from one year to thirty years, and the median was six years. Even though 66 (47.5%) patients with PNAL had normal liver histology, 33 (23.7%) patients were found to have significant histological findings, even 13 (9.4%) had established cirrhosis. When compared to patients within 0~0.75 $\times$ ULN ALT, patients within 0.75~1 $\times$ ULN ALT had higher rate of significant histological findings (43.5% vs. 19.8%, p=0.029). In the subgroup of patients with PNAL, significant histological findings increased sharply after the age of 40 yrs as seen in other cohorts (p=0.005). However, the subgroup of patients with PNAL and significant histological findings was not identified by viral load or HBeAg status. Conclusions: 23.7% of CHB patients with PNAL regardless of HBeAg status or viral load levels had significant histological findings including inflammation and fibrosis. Liver biopsy should be considered in CHB patients with PNAL and detectable viral load, even LVL, especially in those older than age 40 yrs and higher ALT within 0.75~1 $\times$ ULN.

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TREATMENT-INDUCED HBEAG SEROCONVERSION IS A POOR THERAPEUTIC ENDPOINT

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INTRODUCTION HBeAg seroconversion is considered a key therapeutic endpoint of Chronic Hepatitis B treatment, but its effectiveness as an endpoint is compromised by HBeAg negative reactivation and its durability, consequently we sought to examine these events in patients with well documented spontaneous or treatment induced HBeAg seroconversion. METHODS All patients with documented HBeAg seroconversion were followed prospectively at 3-6monthly intervals. Persistent reactivation was defined as ALT> ULN, for >2 time points, with detectable HBV DNA. Seroreversion was defined as return of HBeAg with or without loss of anti-HBe, after loss of HBeAg and development of anti-HBe. The outcome events of reactivation and seroreversion were compared in spontaneous and treatment induced seroconversion using Kaplan-Meier survival analysis and Cox Regression. Data is presented as median (range). RESULTS There were 298 patients with documented seroconversion, of which 116 patients were treatment-induced and 182 were spontaneous. The patients were mainly male (71.81%), with age of 34.81 (68.7) years, with follow up duration of 39.29 (188.03) months post-seroconversion. Persistent reactivation occurred in 71 patients (Cumulative proportion=39.27%), with older age (P=0.001), male gender (P=0.002), higher ALT at seroconversion (P=0.006) being more likely to develop reactivation. No significant difference rate of reactivation was observed among different treatment groups (IFN, lamivudine & adefovir) (P=0.598). Treatment-induced seroconversion was less robust and remission of ALT was of shorter duration than spontaneous seroconverters (median 14.13 months versus 22.44 months, P=0.037). They were also more likely to develop HBeAg negative reactivation at 48 months (37.76% versus 24.96%, P= 0.0482). Treatment responders are more likely to develop reactivation after adjusting for age, gender and ALT with Cox regression (Adjusted cumulative proportion 48.5% versus 34.0% at 96 months, P=0.045). HBeAg reversion occurred in 39 patients (19.64%). Again, treatment induced seroconverters are more likely to get HBeAg reversion than spontaneous seroconverters: 24.09% versus 11.29% at 48 months (P=0.0094). After adjusting for age, gender, and ALT at seroconversion by Cox regression, the difference is consistent (P=0.010). CONCLUSION The effectiveness of treatment induced seroconversion is significantly worse than that of spontaneous seroconverters with significantly higher and faster HBeAg negative reactivation rate with less durable seroconversion over time. Treatment induced seroconversion may not be a good therapeutic endpoint in the treatment of chronic hepatitis B.

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FOUR-YEAR ENTECAVIR TREATMENT IN NUCLEOSIDE-NAïVE HBEAG(+) PATIENTS: RESULTS FROM STUDIES ETV-022 AND -901

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Background: Entecavir (ETV) 0.5 mg demonstrated superior virologic suppression compared to lamivudine (LVD) 100 mg in study ETV-022[1]. One hundred and eighty-three entecavir-treated patients from ETV-022 enrolled in rollover study ETV-901. We present efficacy and safety results in a cohort of patients from ETV studies -022 and -901 who received a total of 4 years of therapy with ETV. **Methods:** The nucleoside-naïve HBeAg(+) 4-year cohort consists of ETV patients who completed ETV-022 and enrolled into ETV-901 with a treatment gap ≤35 days. In ETV-901, patients were treated with 1 mg of ETV. The proportions of patients with HBV DNA <300 copies/mL by PCR assay, ALT normalization, HBeAg loss and HBeAg seroconversion were evaluated among patients with available samples at week-192. **Results:** The nucleoside-naïve HBeAg(+) 4-year ETV treatment cohort consists of 146 patients. Efficacy parameters through 4 years of ETV therapy are presented below. The safety profile of ETV was consistent with previously reported experience. **Conclusions:** At Week 192, 91% of patients who received ETV treatment during 4 years achieved undetectable HBV-DNA and 86 % had ALT normalization, with patients continuing to experience HBeAg loss and HBeAg seroconversion during Years 3 and 4. The safety profile was consistent with previously reported experience. [1] Chang TT, et al, N Engl J Med 2006;354:1001-1010.

Endpoint	Week 192*
HBV DNA <300 copies/mL, n (%)	98/108 (91)
ALT ≤ 1 x ULN, n (%)	96/112 (86)
HBeAg loss**, n (%)	39/96 (41)
HBeAg seroconversion**, n (%)	15/96 (16)

* Denominator represent patients with available samples

** Numbers/proportions represent additional patients achieving HBeAg loss or HBeAg seroconversion during treatment in ETV-901; serology tests were performed by local laboratories

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THE MULTI-DRUG RESISTANT HBV RTA181T HAS A SECRETION DEFECT AND SIGNIFICANTLY REDUCES THE KINETICS OF VIRAL REBOUND

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Background/Aims: Lamivudine (LMV) [rtM204V/I] or adefovir (ADV) [rtN236T] resistance results in rebound of serum HBV DNA of at least a 1.0 log10 IU/ml increase over 3-6 months (Locarnini et al. 2004. AVT;9:679). The rtA181T mutant is also associated with both LMV and ADV resistance but occurs as a mixed population with wild-type (wt) HBV. The rtA181T also encodes a stop codon in the overlapping surface gene at aa172 (sW172*) resulting in truncation and loss of the last 54 amino acids of the C-terminal hydrophobic region. The aims of this study were to examine the in vitro effects of this mutation on HBV replication, and its effect on HBV DNA levels in patients following its selection Methods: To measure HBV replication, Huh7 cells were transfected with infectious clones encoding wt HBV, rtA181T or a mixture of both. HBV replication products were detected by Western blotting (WB), Southern blotting (SB), Immunohistochemistry (IHC) and electron microscopy (EM). The clinical occurrence and accompanying viral load data from patients with rtA181T was determined using SeqHepB (Yuen et al. 2007. AVR;75:64). Results: The rtA181T change resulted in a severe secretion defect preventing egress of surface proteins and virions from the cell as demonstrated by SB, WB and IHC. WB also revealed that the surface proteins from the rtA181T transfected cells were truncated and displayed differential glycosylation and stability. When rtA181T was transfected and expressed with wt to mimic a mixed infection as found in vivo, secretion of subviral particles was restored, but virion secretion was still greatly impaired. There was also increased intracellular accumulation by IHC, although not as extensive as with rtA181T alone. EM of cell supernatant detected viral-like structures secreted from doubly-infected cells that comprised mainly of spherical particles, with few virions or filaments compared to wt. Thus, rtA181T is dependant on wt HBV for secretion but acts as a dominant negative mutant on HBV virion secretion resulting in its retention within the cell. Examination of sequential HBV DNA levels from the blood of patients where only the rtA181T change was detected by direct PCR-sequencing, revealed that viral load rebound did not occur at all or increased in incremental amounts of less than 1.0 log10 IU/ml over 12-18 months. Conclusion: The HBV rtA181T mutation causes a secretory defect and exerts a dominant negative effect on wt HBV virion secretion. The rtA181T masks viral rebound, which in the clinical situation can result in mis-diagnosis of drug resistance if only viral load is used (> 1.0 log10 IU/ml increase over 3-6 months) as criterion for breakthrough failure.

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CREATINE KINASE (CK) ELEVATIONS AND MUSCLE TOXICITIES ASSOCIATED WITH CHRONIC TELBIVUDINE (LDT) USE IN PROSPECTIVE CLINICAL TRIALS

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Background: Nucleoside-associated myopathy has been well described in treatment of the Human Immunodeficiency Virus; however CK elevations and myopathy have been a more unusual adverse event with the use of nucleosides for treatment of Hepatitis B Virus (HBV). We reviewed the LdT approval database for CK elevations and adverse event patterns consistent with muscle toxicity. Methods: Data were reviewed from trials that prospectively measured CK levels and recorded clinical adverse events, and in which LdT was used in at least one arm in New Drug Application 22-011. We examined the time to onset and frequency of CK elevations from the pivotal Phase 3 trial (52 week data) using Kaplan-Meier plots and Fisher's Exact Analysis (LdT vs. lamivudine (LAM)). We also reviewed detailed event narratives of clinical adverse events (AE) for CK elevations or events suggestive of myopathy, the Food and Drug Administration Adverse Event Reporting System for reports of muscle toxicity since LdT market approval (10/25/06) and preclinical evaluations for mitochondrial toxicity submitted with the Application. Results: The pivotal Phase 3 trial safety database included 680(LdT) and 687(LAM) subjects with HBeAg-positive and HBeAg-negative chronic HBV. More LdT subjects developed new-onset CK elevations compared to LAM subjects (Grade 1-4: 72% LdT, 42% LAM) after several months on study drug. After reviewing narratives for serious adverse events and study drug discontinuations/interruptions, there were a total of 16 LdT and 1 LAM subject with CK elevations or muscle symptoms; 6 of whom had a convincing picture of myopathy with muscle weakness, 2 of whom had biopsy confirmation, and 1 of whom was on a concomitant drug associated with myopathy. All presented after more than 300 days on study drug. History, degree and timing of CK elevations, and demographic characteristics were variable. No reports of LdT-associated CK elevations, myopathy or muscle weakness have been reported to our Adverse Event Reporting System since approval. No evidence of mitochondrial toxicity was found in review of preclinical studies. Conclusions: We found an emerging pattern of a cumulative toxicity resulting in myopathy with chronic LdT that may or may not be associated with mitochondrial toxicity. The imperfect relationship between the timing and severity of CK elevations and myopathy, coupled with the variable demography and symptom presentation in these clinical trial settings suggest that further characterization of this toxicity, its mechanism and its predisposing factors is warranted. These toxicities may occur more frequently in the general population with longer-term use of LdT.

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MUTATIONS IN IMMUNODOMINANT EPITOPES OF HEPATITIS B VIRUS (HBV) CORE REGION: ENHANCEMENT OF VIRAL EVOLUTION BY THE EFFECT OF INTERFERON THERAPY

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Background and aims In HBV infection, the host immune system attacks core peptides as the main target. Amino acid (aa) substitutions in immunodominant epitopes of the HBcAg sequence may allow HBV to escape immune surveillance, thereby maintaining infection. The aim of this study was to analyze aa substitution rates in HBcAg immunodominant epitopes in a group of chronic hepatitis B (CHB) patients, throughout IFNa therapy and during a period without this therapy. Patients and methods A cohort of 44 CHB patients (19 HBeAg+) (BASELINE group) was studied retrospectively, all cases with a baseline serum sample. Sixteen (1 HBeAg+) of these patients who did not respond to 24 weeks of IFNa therapy (IFN group) were longitudinally studied. Eleven of the 16 patients (1 HBeAg+) and 10 other CHB cases (5 HBeAg+) (untreated group) were longitudinally studied in the absence of antiviral treatment. In the IFN and untreated groups, serum samples were analyzed during therapy and every six months over the non-treatment period. The complete sequence of HBV core region (precore and HBcAg) was determined by direct sequencing. The viral genotype was established by comparison of obtained sequences with consensus sequences deduced from the alignement of 185 complete sequences of the eight HBV genotypes obtained from GenBank. Yearly rates of aa changes in the immunodominant HBcAg epitopes (B cell HBc/HBe1-aa 74 to 84, and Th cell-aa 50 and 69), were calculated in the IFN and untreated groups. In the BASELINE group this changes were recorded in relation to the consensus sequences. Results: Rates of aa changes in the immunodominant HBcAg epitopes are shown in table. Conclusion The higher rates of aa substitutions suggest enhancement of the evolutive pressure due to IFN therapy, which may allow HBV to escape from immunosurveillance, resulting in persistent infection. This does not seem to be related to viral genotype or precore variants. In baseline samples the highest variability in Th cell epitope (aa 50- 69) could be related with the absence of the immunotolerogen effect of HBeAg. This study was supported by a grant from the Spanish Ministery of Health (FISS PI061512).

GROUP		Th cell epitope (aa 50-69)	B cell (HBc/HBe1 epitope(aa 74-84)
BASELINE (N=44)	Genotype A (N=22)	1.45	1.23
	Genotype D (N=22)	1.36	1.27
	Precore variant: WT (N=20)	1.05 (e)	1.15
	Precore variant: MT (N=24)	1.71 (f)	1.33
IFN (N=16)		4.5 (a)	3.38 (c)
Non-treated (N=21)		0.66 (b)	0.86 (d)

Statistical results: a vs. b P<0.001, c vs. d P<0.001, e vs. f P=0.087

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A HIGH PREVALENCE OF SIGNIFICANT LIVER DISEASE IN ASYMPTOMATIC HEPATITIS B PATIENTS WITH HIGH VIRAL LOAD; CANDIDATES FOR ANTIVIRAL THERAPY

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Background/Aim: Current treatment guidelines recommend that antiviral therapy should be considered in chronic hepatitis B (CHB) patients with high viral load if a biopsy shows significant liver disease despite ALT levels two times or less than the upper limit of normal (ULN). We evaluated the histological findings in CHB patients with high viral load and persistently normal or slightly elevated serum ALT levels. Methods: Between January 2003 and June 2006, all consecutive treatment-naive patients with CHB who underwent ultrasonography-guided percutaneous liver biopsy, expressed HBsAg for at least 6 months, and had detectable serum HBV DNA on a direct hybridization assay, and normal or slightly elevated serum ALT levels (≤ 2×ULN) for 12 months were included in this study. One hundred and five patients were enrolled. Serum ALT levels were tested in at least three consecutive samples within a 12-month period, and all values were less than two times normal. Histological assessment was based on the METAVIR classification (significant histology was defined as ≥ grade A2 or ≥ stage F2). Results: All 105 patients had HBV DNA titers above 105 copies/mL. The mean (± SD) ALT level at biopsy was 42 ± 19 (range, 8-75) and the mean age was 37 ± 14 years (range, 18-68). 36 patients had persistently normal ALT levels. Significant fibrosis was observed in 63 patients (60.0%) and significant histology was found in 69 patients (65.7%). On multivariate analysis, the serum ALT levels and age at study entry were independent meaningful factors associated with significant histology. Compared to the lowest ALT levels (< 0.5× ULN), 0.5-1× ULN, 1-1.5× ULN and 1.5-2× ULN had odds ratios (95% CI) for significant histology of 1.1 (0.2-5.1), 3.9 (0.7-21.6) and 8.0 (1.1-60.5), respectively. Compared to patients younger than 20 years, the odds ratios for patients in their 20s, 30s, 40s, and over 50 years old were 7.1 (1.4–36.9), 14.2 (2.1–94.2), 24.4 (4.2–141.3), and 38.8 (4.0–309.8), respectively. Conclusion: A large proportion of CHB patients with high viral load and ALT two times or less than the ULN had significant liver disease on liver biopsy. Our findings warrant a more accurate evaluation in a practical setting and, if confirmed, would have a great impact on future treatment approaches for patients awaiting potential antiviral therapy.

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AN UPDATED LINE PROBE ASSAY (HBV DR V.3) HAS HIGH CONCORDANCE WITH DIRECT SEQUENCING IN DETECTING MUTATIONS ASSOCIATED WITH RESISTANCE TO LAMIVUDINE, ADEFOVIR AND ENTECAVIR

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Background/Aims: Early detection of antiviral drug-resistant (DR) mutations enables prompt initiation of rescue therapy. Direct sequencing is necessary in characterizing mutations to new therapies but it is not sensitive in detecting minor variants. Mutations associated with resistance to entecavir (ETV) had been reported at positions: 184, 202 and 250. We compared the concordance and sensitivity of direct sequencing, sequencing of multiple clones, and a line probe assay in the detection of ETV-resistant mutations. Methods: Serial serum samples from patients who received ETV, lamivudine (LAM) or tenofovir (TDF) were tested for DR mutations in the HBV P gene by direct sequencing, sequencing of multiple clones (mean of 20/sample), and an updated line probe assay (Innogenetics, HBV DR LiPA v.2 and v.3) that incorporates probes to detect mutations at 11 positions: 80, 173, 180, 181, 184, 194, 202, 204, 223, 236 and 250 of the reverse transcriptase region of the HBV P gene. Results: 55 samples from 13 patients were studied: 7 received ETV and 6 received TDF, 12 had prior LAM. Results of direct sequencing and LiPA were concordant in 580/605 (96%) positions analyzed. Complete concordance between results of direct sequencing and LiPA was observed in 34 (62%) samples. In 6 samples, direct sequencing did not detect any mutation while LiPA detected 1 (n=5) or 2 (n=1) mutations. In the remaining 15 samples, LiPA identified 1 or 2 more mutations than direct sequencing. Concordance between results of direct sequencing and LiPA was only 36% when mutations were present in <10% of clones and increased to 98% when mutations were present in >10% of clones. LiPA DR v.3 detected ETV-R mutations in 2/13 patients who had received LAM but not ETV: 1 had S202G+M250I and the other had T184A+M250L mutations. In both patients, these mutations were not detected by direct sequencing but present in 10% of clones sequenced. Three other patients developed virologic breakthrough after switching to ETV for LAM resistance. All 3 patients were confirmed to have ETV-resistant mutations, LiPA DR v.3 detected these mutations earlier than direct sequencing by 6, 9, and 21 months, respectively. Conclusions: The updated version of the line probe assay (LiPA HBV DR v.3) has high concordance with direct sequencing in detecting mutations associated with resistance to LAM and ETV. The improved sensitivity of LiPA permitted earlier detection of genotypic resistance in patients who had virologic breakthrough on ETV. Screening of patients for ETV-resistant mutations using LiPA may help to determine the optimal therapy for patients with LAM resistance.

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ONCOLOGISTS AND HEPATITIS B: RESULTS OF A QUESTIONAIRE SURVEY TO DETERMINE THEIR CURRENT LEVEL OF AWARENESS AND CLINICAL PRACTICE OF ANTIVIRAL PROPHYLAXIS TO PREVENT REACTIVATION

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The risk of chronic hepatitis B virus (CHBV) reactivation under the influence of chemotherapy or other immunosuppression is being increasingly recognized. However, many oncologists either have not observed this complication, and/or are not aware of current recommendations for Hep B prophylaxis. METHODS: In an effort to determine the degree of awareness of the potential reactivation risk and to understand the current practice of screening and prevention among oncologists, we prepared a questionaire that was administered to 131 Hematology-Oncology physicians in the Washington, DC area during March and April 2007. Questions related to their awareness of the risk of hepatitis B reactivation, screening patients for HBV, awareness of preventative treatment for HBV and how they would administer prophylaxis and follow their patients. RESULTS: Responses to 10 questions are as follows: 1) Are you aware that reactivation of HBV can occur: Yes 78% ; No 22%. 2) Are you aware that prophylactic antiviral therapy is available: Yes 56%; No 44%. 3) Have you ever seen reactivation of HBV in your practice: Yes 30%; No 70%. 4) Do you screen for HBV prior to chemotherapy: Yes 38% (86% use selective criteria vs 14% use universal screening); No 62%. 5) Which pts would you prophylax: chronic carrier 46%; active HBV 76%; resolved HBV infection 52%. 6) Which antiviral would you give: lamivudine 46%; adefovir 14%; not sure 48%. 7) How often would you monitor: q2wk 4%; q4wk 18%; q6wk 14%; q8wk 16%; q12wk 12%; not sure 36%. 8)How long would you continue prophylaxis after completing chemotherapy: 1 mo. 8%; 2 mo. 15%; 3 mo. 71%; 4 mo. 8%. 9) Would you monitor for reactivation even if you did not give prophylaxis: Yes 66%; No 6%; Not sure 28%. 10) Would you want a gastroenterologist or hepatologist to follow the pt with you: Yes 88%; No 12%. DISCUSSION: While most oncologists are aware of the potential risk of HBV recativation, only 30% have ever seen a reactivation and most are not screening pts for HBV prior to chemotherapy. Fewer than half of our respondents were aware that prophylactic antiviral agents are available, and even fewer were aware that prophylaxis has been shown to reduce HBV flares, and most were not sure what agent to select. A majority would, however, seek the recommendations of a gastroenterologist or hepatologist and most felt that prophylactic treatment should continue for at least 3 months after chemotherapy was completed. CONCLUSIONS: Improving awareness of HBV reactivation and antiviral prophylaxis in the Oncology community seems warranted .

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PRE-EMPTIVE LAMIVUDINE REDUCES THE RISK OF CHEMOTHERAPY-INDUCED HBV-RELATED MORBIDITY AND MORTALITY IN HBSAG-POSITIVE CANCER PATIENTS: META-ANALYSIS

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Background: Lamivudine (LAM) has been used for preventing HBV-reactivation in hepatitis B surface antigen (HBsAg)-positive cancer patients undergoing chemotherapy but its beneficial effects have not been assessed quantitatively. Purpose: To estimate the degree of beneficial effect of pre-emptive LAM in reducing chemotherapy-induced HBV-related morbidity and mortality in HBsAg-positive cancer patients. Methods: Databases searched (until February 2007) included Medline, Ovid, Toxnet, Scopus, and Web of Science. Studies with data suitable for risk estimation of cancer chemotherapy-induced HBV reactivation in HBsAg-positive patients receiving pre-emptive LAM vs. controls that met the pre-specified criteria on outcomes and sample size. 2 investigators independently performed the literature search and data extraction, and 2 different investigators independently reviewed whether the studies met pre-specified criteria. A two-tailed p-value <0.05 was considered statistically significant. Comprehensive Meta-Analysis Software was used for the analysis. Results: Twelve studies met predefined criteria (2 RCT, 6 prospective, and 4 retrospective cohort studies). Of 12 trials in the meta-analysis, 10 were conducted in East Asia, and one each in Turkey and Israel. All studies were in English except for one in Chinese. 214 patients received pre-emptive LAM, and 399 patients served as controls receiving either no (3 studies) or deferred LAM (9 studies). The log odds ratios demonstrating risk reduction with pre-emptive LAM vs. no pre-emptive LAM in HBsAg- positive individuals undergoing cancer chemotherapy was 0.06 (95% confidence interval [CI], 0.03-0.13) for HBV reactivation, 0.07 (95% CI, 0.04-0.14) for HBV-related hepatitis, 0.20 (95% CI, 0.06-0.64) for liver failure, and 0.21 (95% CI, 0.08-0.58) for mortality. Combining results from all the studies indicated that HBV reactivation was decreased from 35.6% to 2.8% ($p<0.0001$) with pre-emptive LAM therapy, while HBV-related hepatitis was decreased from 32.3% to 2.8% ($p < 0.0001$), HBV-related liver failure from 5.7% to 0.5 % ($p = 0.0002$) and HBV-related mortality from 7.0% to 0.5 % ($p < 0.0001$). Based upon this analysis, 1 death can be prevented by treating 15 patients with pre-emptive LAM in this clinical setting. Limitations: Small studies; only two involved a randomized-controlled design. Discussion: This meta-analysis demonstrated that pre-emptive LAM reduces the risk of HBV-reactivation, HBV-related hepatitis, HBV-related acute liver failure, and HBV-related mortality by 16, 14, 5, and 5-fold, respectively. Conclusions: HBsAg-positive patients undergoing cancer-chemotherapy warrant pre-emptive therapy with LAM.

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CHARACTERIZATION OF HEPATITIS B VIRUS POLYMERASE MUTATIONS DETECTED DURING ANTIVIRAL TREATMENT WITH ADEFOVIR DIPIVOXIL (ADV)

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Background/Aim: Recent studies have shown that, in contrast to lamivudine, adefovir dipivoxil (ADV) therapy is associated with delayed and infrequent selection of drug resistant hepatitis B virus reverse transcriptase (RT) mutations. To date, apart from rtN236T and rtA181V ADV-resistant mutations, the impact of other mutations in the HBV RT region on the susceptibility to ADV is unknown. The aim of this study is to genotypically and phenotypically analyse emerging mutations in the HBV RT during ADV therapy. Patients/Methods: 68 patients (44 ±14 years) with chronic HBV (CHB)-infection treated with ADV (18 ± 13 months) demonstrating persistent viraemia (5.5x10E+7 ± 1.4x10E+6 HBV IE/ml) were included in this analysis. The RT-region was amplified by PCR after extraction of viral DNA from patient sera. Mutational analysis of the RT-region was done by sequencing followed by genotyping. Selected mutations were introduced into HBV replication competent constructs using site-directed mutagenesis and analyzed for sensitivity to ADV (IC50) in HepG2 cell culture experiments. Results: The ADV resistant mutations rtA181V and/or rtN236T were detected in 11 patients. Sequence exchanges at positions rtL122(F), rtN124(H), rtP130(Q), rtD131(N) in the RT-fingers domain were detected in 60% of the patients. However, these changes could be also detected in treatment naïve patients and represent polymorphisms. Additionally, the exchange rtN248H in the E-domain was frequent (26/72; 36%). Statistical analysis revealed that rtN248H positively correlated with ALT (r=0.65, p<0.0001), rtP130Q (r=0.5, p<0.0001) and rtN124H (r=0.42, p<0.0001), and negatively correlated with rtL180M (r=0.45, p=0.001). The phenotypic cell culture analysis of rtN248H showed a moderate reduction of susceptibility to ADV (IC50 <6μM ADV) compared to wild type HBV (IC50 <3μM ADV) and rtN236T (IC50 >10μM). Notably, the recently published mutation rtI233V could not be detected in any of our ADV treated patients. Conclusion: Apart from the previously described rtN236T and rtA181V ADV resistant mutants, our analysis shows no evidence for further distinct ADV resistant mutants.. However, antiviral treatment of CHB patients with ADV can select further exchanges in the HBV polymerase. Exchanges in the RT-fingers domain, e.g. rtL122F, rtN124H, rtP130Q, and rtD131N, may represent polymorphisms and have little or no impact on antiviral susceptibility. Phenotypic analysis of exchanges in the RT E-domain, rtN248H, showed only a slight reduction in sensitivity to ADV. Suspected ADV resistant mutations, e.g., rtQ215S and rtI233V, were extremely rare or not detectable in our patients.

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DURABILITY OF ANTIVIRAL RESPONSE IN LAMIVUIDNE TREATED HBEAG POSITIVE CHRONIC HEPATITIS B PATIENTS WHO DID NOT RELAPSE WITHIN A YEAR OF POST TREATMENT

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Introduction: On the contrary to Western reports, relapse rate after lamivudine (LMV) discontinuation in HBeAg positive chronic hepatitis B (CHB) patients is higher in Asian patients and most reported relapses occur within 1 year after LMV discontinuation. The aims of this study to investigate the long-term relapse rate and associated risk factors in HBeAg positive CHB patients who had kept a sustained virologic response (VR) for 1 year after LMV discontinuation. Methods: 376 treatment naïve HBeAg positive CHB patients who took LMV were enrolled. Among them, 113 patients were achieved VR and then discontinued LMV. During the post treatment follow up, 58 of 113 patients (51.3%) showed relapse within 1 year. For the remaining 55 patients who maintained the VR within a year of treatment discontinuation, cumulative rate and risk factor for long term post treatment relapse were analyzed. VR was defined as loss of HBeAg and undetectable serum DNA in hybridization assay or serum HBV DNA level less than 10^5 copies/mL. Relapse was defined as reappearance of HBV DNA or increase of HBV DNA more than 10^5 copies/mL. Delayed relapse was defined as relapse that occurred after sustained VR for 1 year after LMV discontinuation. Results: The baseline characteristics of 55 patients were follows as: Mean age, serum ALT, HBV DNA, duration of LMV treatment, duration of LMV consolidation, and follow up duration after LMV discontinuation was 39.2 years, 374.5 IU/L, 7.5 $\log_{10}$ copies/mL, 22.5 months, 10.5 months (range, 0-28), and 38.8 months (range, 14-95), respectively. During follow up period (mean 26.7 months; range, 2-83), 16 of 55 patients (29%) showed delayed relapse. Cumulative rate of delayed relapse from 1 year after LMV discontinuation was 16%, 29%, 39%, 44% at 1, 2, 3, 4 years, respectively. At relapse, 2 patients showed decompensation and three patients showed flare of hepatitis. In univariate analysis, age, pretreatment albumin, serum HBV DNA > 2000 copies/ml at 3 months after LMV discontinuation predicted the delayed relapse significantly. In multivariate analysis, age (P=0.029) and serum HBV DNA > 2000 copies/mL after 3 months of LMV discontinuation (P=0.047) were significant predictors of delayed relapse. Conclusion: Even in patients who had kept sustained VR for 1 year after LMV discontinuation, delayed relapse are not infrequent. Therefore, a LMV maintenance therapy might be considered in HBeAg positive CHB patients who achieved VR weighing the risk and benefit.

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ANALYSIS OF DETERMINANTS FOR SUSTAINED VIROLOGIC RESPONSE TO LAMIVUDINE MONOTHERAPY IN PATIENTS WITH HBEAG POSITIVE CHRONIC HEPATITIS B: A MULTI-CENTER TRIAL

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Backgrounds/Aims: Several reports showed inconsistent results on the durability of virologic response after successful lamivudine monotherapy. Especially, the question remains whether virologic responses can be maintained over extended follow-up period. The aim of our study was to evaluate post-treatment relapse rates for 5 years after virologic responses and to elucidate the predictive factors for sustained virologic response in patients who experienced HBeAg loss to lamivudine monotherapy. Methods: 748 patients (Male : Female = 570 : 178) with HBeAg positive chronic hepatitis B were treated with lamivudine from 7 medical institutions in Korea between January 1999 and August 2004. The mean duration of lamivudine monotherapy was 34 months (range, 12-88). Among 178 patients, who discontinued lamivudine monotherapy after complete response (HBeAg loss, undetectable HBV DNA and ALT normalization), 138 patients (77.5%) maintained sustained virologic response. Both host and viral factors were compared between 138 patients with sustained HBeAg response and 40 patients whose response was not sustained. Results: The cumulative relapse rates at 1, 3, and 5 years were 15.9%, 26.4%, and 30.2%, respectively. The mean time to relapse after cessation of lamivudine was 12 months (range, 2-42). Most relapses were occurred within 2 years after discontinuation of lamivudine (35/40, 87.5%). In multivariate analysis, age ≤ 40 years, additional treatment duration over 12 months after HBeAg loss were independent factors for sustained virologic response. In conclusion, only 22.5% of patients who discontinued lamivudine after HBeAg loss experienced a relapse for 5 years follow-up. Age and additional treatment are major predictive factors for sustained HBeAg loss. Key words: chronic hepatitis B, lamivudine, treatment

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MULTIDRUG RESISTANCE AND CROSS-RESISTANCE PATHWAYS IN HBV AS A CONSEQUENCE OF TREATMENT FAILURE

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Background/Aims: Antiviral resistance is now the single most important factor in treatment failure using nucleos(t)ide analogues (NA). Primary drug resistance mutations refer to amino acid change(s) that result in reduced susceptibility to an antiviral agent. Secondary compensatory mutations restore replication defects associated with primary drug resistance, and may be associated with low level reduced susceptibility. Several evolutionary pathways of drug resistant HBV have been observed in patients treated with NAs. It is possible that the drug resistance mutations selected with one agent may affect the efficacy of other NAs. The aim of this study was to elucidate mutation pathways associated with use of NAs and to determine the potential cross-resistance profiles selected under a particular NA. Methods: The HBV reverse transcriptase (rt) gene was amplified by PCR and sequenced from patients pre- and during-treatment. A software program (SeqHepB) was used to analyse the treatment-associated mutations (Yuen L et al. 2007. AVR;75:64). Associations between drug resistance and rt mutations found in the most recent samples from 159 patients pre- and 215 patients during failing monotherapy with either lamivudine (LMV) or adefovir (ADV) were evaluated using the Fisher's Exact method and a pattern discovery program Magnum Opus (Rule Quest Research, Australia). Results and Discussion: Therapy with LMV or Telbivudine (LdT) or entecavir (ETV) resulted in the selection of rtM204I/V. Statistical analysis identified several secondary compensatory mutations (rtL80I/V, rtV173L, rtL180M, rtT184G and rtS202I) during LMV monotherapy that can also affect response to ETV or LdT. Therapy with ADV resulted in rtN236T and/or rtA181T/V selection as well as the maintenance of rtL180M and rtM204V in a subset of patients with add-on ADV. The loss of rtL180M and/or rtM204I/V was also statistically significant among LMV resistant patients who subsequently switched to ADV monotherapy. The rtA181T/V mutation, which has been implicated with reduced sensitivity to LMV and ADV, could be either common with or separate to the "204 and 236 pathways". Conclusion: Three major HBV evolutionary NA-resistance pathways (rtM204I/V, rtN236T and rtA181T/V) have been characterised. The rtM204V/I pathway is responsible for resistance to the L-nucleosides such as LMV, LdT and also ETV whilst the rtN236T pathway is responsible for adefovir resistance. Both are associated with clusters of secondary mutations that can affect subsequent treatment with NAs (rtT184G, rtS202I). The third pathway, rtA181T/V, is associated with resistance to LMV and ADV and is a potential multi-drug resistance pathway.

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VARIABILITY OF THE HBV POL GENE REVERSE-TRANSCRIPTASE DOMAIN IN VIRAL ISOLATES FROM UNTREATED AND LAMIVUDINE-RESISTANT CHRONIC HEPATITIS B PATIENTS

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Different HBV variants carrying point mutations at level of the reverse transcriptase (rt) domain of the viral Pol gene had been detected in patients under treatment with nucleos(t)ide analogues. At present, few information is available about the occurrence of these drug-resistant mutants in untreated patients. Aim of this study was the genomic characterization of HBV isolates from individuals (1) naive for antiviral therapy or (2) who had developed lamivudine resistance. We analysed - by amplification, cloning and sequencing procedures - the entire rt-domain in viral isolates from 84 untreated (naive-group) and 49 lamivudine-resistant (Lam-group) consecutive HBsAg carriers with chronic liver disease. All the cases had serum HBV DNA levels > 20,000 UI/mL. Thirty-two of the 84 naive-group cases (38.1%) carried single or multiple mutations potentially predisposing to antiviral resistance. In particular, we found the following mutations: rtV173M in 2 cases (2.4%), rtA181D in 1 case (1.2%), rtV214A/E in 3 cases (3.6%), rtQ215S/P/H in 12 cases (14.3%), rtL217R in 8 cases (9.5%), rtS219S in 4 cases (4.8%), rtF221Y/L in 15 cases (17.8%), rtI233V in 4 cases (4.8%), rtP237T in 3 cases (3.6%), rtN238T/H/D in 4 cases (4.8%). In the Lam-group – besides the mutations typically inducing Lamivudine resistance – 28/49 (57.1%) carried single or multiple mutations conferring resistance to other antivirals. In particular, 4 cases (8.2%) had either the rtT184S or the rtM250V/L mutations favouring resistance to Entecavir, whereas various combined mutations predisposing to Adefovir resistance were detected in 26 cases: rtV84M in 1 case (2%), rtA181S/T in 2 cases (4.1%), rtV214A/T in 2 cases (4.1%), rtQ215S/E/P in 12 cases (24.5%), rtL217R in 3 cases (6.1%), rtS219A in 3 cases (6.1%), rtF221Y in 6 cases (12.2%), rtI233V in 1 case (2%), rtP237HT in 2 cases (4.1%), rtN238T/H/D in 12 cases (24.5%). In conclusion, our study shows that HBV mutants predisposing to drug resistance may be present as major infecting population in untreated patients, and that variants emerging during Lamivudine treatment may also carry mutations potentially inducing resistance to either Adefovir or Entecavir. Thus, the genetic characterization of HBVs infecting patients undergoing treatment with nucleos(t)ide analogues may be a helpful tool for tailoring the most proper therapeutic strategy in each individual case.

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ENTECAVIR: A RESCUE THERAPY FOR CHRONIC HEPATITIS B PATIENTS WITH A LIMITED VIROLOGICAL RESPONSE TO ADEFOVIR?

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Background The frequency of detection of drug resistant HBV mutants correlates with the rapidity of initial decline of viral load. In treatment-naive patients with HBeAg-positive hepatitis B entecavir (ETV) achieves approximately 6$\log_{10}$ HBV DNA decline and PCR-negativity in 45% of the patients after 24 weeks of treatment. In lamivudine (LAM)-refractory patients who are subsequently switched to ETV, it has shown to be less effective. Here we describe 12 patients with chronic HBV infection and a persistently high viral replication after one year of treatment with ADV, who were switched to ETV as rescue therapy. **Methods** Limited ADV response was defined as presence of HBV DNA levels greater than 5$\log_{10}$ copies/mL after 48 weeks of treatment. Patients were directly switched from ADV to ETV at a daily dose of 1 mg. ALT and HBV DNA levels were assessed at baseline and after 24 weeks of treatment (detection limit 400 copies/ mL). All subjects were screened for resistance-associated mutations within the HBV polymerase gene using Inno Lipa HBV DR v2 and v3 (Innogenetics). **Results** The baseline characteristics of the 12 patients with limited ADV response were: median age 44 [range 23-73]; m/f: 9/3; 11 HBeAg+; 3 cirrhosis; genotype A: 3, B: 3, C:1, D: 5. Six patients had a prior history of LAM-resistance. Median period of ADV administration was 79 weeks [range 49-135]. HBV DNA levels ranged between 5.2 to 10.1 $\log_{10}$ copies/mL (median 7.7 $\log_{10}$ copies/mL). At baseline three patients had ADV-resistance substitutions (N236T ± A181V/T), and in only one patient also LAM-resistance was detected (M204I). After 24 weeks of treatment the median decrease of HBV DNA was 3.7 $\log_{10}$ copies/mL [range 1.9-5.9]. There was no difference in decline between patients with or without a prior history of LAM-resistance (3.7 vs. 3.7). Median HBV DNA level at 24 weeks was 3.8 $\log_{10}$ copies/mL. None of the patients reached HBV DNA levels below the detection limit. The proportion of patients with ≤ 3 $\log_{10}$, 3.1-5 $\log_{10}$, > 5 $\log_{10}$ copies/mL was 33%, 42%, and 25%, respectively. HBeAg-seroconversion occurred in one patient after 12 weeks of treatment. After 24 weeks of treatment two patients showed LAM-resistance (M204I), no ETV-resistance substitutions were detected (I169, T184, S202, M250). **Conclusion** Entecavir treatment leads to a suboptimal viral decline in patients with a limited response to adefovir, even in the absence of ADV or LAM resistance. Our finding that none of these patients achieved HBV DNA negativity by 24 weeks necessitates close monitoring for potential development of ETV resistance in ADV nonresponders.

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COMBINATION ANTIVIRAL EFFECTS OF 2′, 3′-DIDEOXY-3′FLUOROGUANOSINE WITH NUCLEOS(T)IDE ANALOGS AGAINST HEPATITIS B VIRUS IN VITRO

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Background & Aims: 2′, 3′-dideoxy-3′fluoroguanosine (FLG) is a nucleoside analogue which acts as an inhibitor of viral DNA polymerase and a chain terminator of viral reverse transcription. FLG has been shown to be active against wild-type and drug-resistant HBV in the in vitro models (Jacquard et al 2006). In this study, we investigated the in vitro antiviral efficacy of combinations of FLG with L-nucleoside analogs (lamivudine and clevudine), acyclic phosphate nucleotide analogues (PMEA, adefovir and PMPA, tenofovir) and carbocyclic analogues (entecavir). Methods: Using the HepAD38 cell line that expresses high levels of wild-type HBV as the in vitro cell model, we assayed the antiviral activities and cytotoxities of each drug alone and in combination with FLG (Tibotec Pharmaceuticals Ltd). Real time PCR was used for the measurement of HBV DNA generated by HepAD38, and the cytotoxicity was determined by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. Each combination experiment included 24 samples, 13 of which were controls (five doses of each drug alone plus three untreated wells) and 11 of which were drug combinations. Of the five concentrations tested for each drug, the middle dose was equal to the IC_{50}; two higher doses (corresponding to three and six times the IC_{50}) and two lower doses (corresponding to 0.16 and 0.33 times the IC_{50}) were also tested. The combination data were analyzed through the Bliss independence model which is defined by the equation $E_{xy}= E_x+E_y-(E_xE_y)$, where E_xE_y is the additive effect of drugs x and y as predicted by their individual effects E_x and E_y. The MacSynergy II program, version 1.0 (M. N. Prichard, University of Alabama) was used to evaluate antiviral data according to the Bliss independence model. MacSynergy II uses a nonparametric three-dimensional approach to quantify areas where observed effects are significantly greater (synergy) or less (antagonism) than those predicted from single-drug control data. Results: Analysis using the Bliss independence model indicated that FLG exerted significant synergistic antiviral effects when combined with lamivudine (V=41.51 μM^2) or tenofovir (V=95.07μM^2) and additive effects when combined with adefovir (V<25 μM^2). However, minor antagonism effects were displayed when FLG combined with entecavir (V=-32.12 μM^2) or clevudine (V=-60.25 μM^2). There was no evidence of cytotoxicity with any of the drugs when used alone or in combination at the tested doses. Conclusion: FLG exerted different kinds of combination antiviral effects when it was used with other nucleos(t)ide analogs. Future clinical study on FLG-related combination therapy is warranted.

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DEMONSTRATION OF AN ASSOCIATION BETWEEN DETECTION OF IGG ANTIBODY REACTIVITY TOWARDS THE C-TERMINAL REGION OF THE PRES1 PROTEIN OF HEPATITIS B VIRUS AND THE CAPACITY TO RESPOND TO INTERFERON THERAPY IN CHRONIC HEPATITIS B

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Background: The aim of this study was to determine the predictive value of the pre-treatment presence of circulatory antibodies towards a synthetic peptide mimicking the amino acids 94-117 of the preS1 protein of hepatitis B virus (HBV) and the capacity to respond to interferon–alpha (IFN-alpha) treatment. Methods: The anti-preS1 (94-117) antibodies were measured by a peptide-based enzyme-linked immunosorbent assay (ELISA) and the response to INF-alpha therapy was judged by the effect on the viral kinetics as measured by a quantitative polymerase chain reaction (PCR) based assay during the treatment and follow-up. Results: We found a significant ($p<0.001$) correlation between the pre-treatment presence of anti-preS1 (94-117) antibodies and a decrease in viral levels on follow-up after the end of IFN-alpha therapy. The combined response of HBV DNA suppression ($p<0.001$), HBeAg loss ($p<0.0001$) anti-HBe seroconversion ($p<0.005$) and ALT normalisation ($p<0.01$), was also highly associated with the pre-treatment presence of anti-preS1(94-117) antibodies. Conclusions: The positive predictive value (PPV) of anti-preS1 (94-117) in determining a virological response was 83% and the negative predictive value (NPV) was 100%, indicating that in the absence of pre-treatment anti-preS1 reactivity virtually no patient has the capacity to respond to IFN-alpha therapy. Our findings may help to improve the efficacy of INF-alpha therapy for chronic hepatitis B (CHB) by guiding the selection of patients to treatment and optimising the clinical management of the individual patient.

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INCREASING ADEFOVIR AND ENTECAVIR UTILIZATION DRIVE HEPATITIS B RESISTANCE PATTERNS IN A US NATIONAL REFERENCE LABORATORY DATABASE

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INTRODUCTION An increasing number of antivirals are available for treatment of chronic hepatitis B virus (HBV) infection. Increasing use of antivirals can affect the frequency of resistance mutations in the treated population. We surveyed a large clinical database of HBV polymerase gene sequences to assess changes in the prevalence of resistance-associated mutations for lamivudine, adefovir, and entecavir over a 5-year period. METHODS HBV pol sequences for 10,800 clinical samples obtained between June 2002 and May 2007 were compiled in a database. Genotypes were determined by phylogenetic analysis of a 348-nt pol fragment. Antiviral drug prescription utilization data were obtained from NDCHealth (http://www.ndchealth.com). RESULTS The HBV sequences submitted for resistance analysis comprised genotypes C (37%), B

(31%), A (18%), D (8%), and E–H (6%). The frequency of predicted lamivudine resistance among clinical samples declined during the 5-year period: from 18.2% in 2002-3 and 15.6% in 2004, to 10% in 2006 and 2007. Adefovir prescriptions increased 2.8-fold since approval in 2003, to 129,000 in 2006. Correspondingly, the frequency of samples with predicted adefovir resistance increased from < 0.5% in 2002-3 to 3.4% in the first 5 months of 2007. In 2007, adefovir-associated mutations A181V/T and N236T were found in 2.7% and 1.3% of samples, respectively (2002-3: 0.4% and 0%, respectively). Predicted adefovir resistance was highest in genotype C (3.8% C vs 2.0% other, for 2004-2007; Fisher exact: p<.0001). Utilization of entecavir, approved in 2005, increased 4.6-fold in 2006, to 45,217 prescriptions. Twenty-one samples in 2006 (0.47%) and 15 samples in 2007 (0.71%) had predicted resistance to entecavir, constituting 4.7% (2006) and 7.1% (2007) of the samples with predicted lamivudine resistance. The increase from the pre-entecavir period (3 samples in 2002-2005) was significant (Fisher exact: p<.0001). The frequency of 2 entecavir resistance mutations increased significantly in 2006-7 (n=6,543) relative to 2004-5 (n=3,799): S202G (0 vs 17; Fisher exact: p=.0008) and T184S/A/I/L (3 vs 29; p<.0001). DISCUSSION While lamivudine resistance among clinical HBV specimens has declined, variants with predicted resistance to the newer antivirals adefovir and entecavir are emerging in the clinical population, following steeply increased utilization. The percentage of clinical samples with predicted entecavir-resistance in 2006 and 2007 is consistent with findings of a recent study showing a rebound rate of 9% in entecavir-treated, lamivudine-refractory patients. Continued surveillance of emerging antiviral resistance in the clinical population is warranted.

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DRUG SUSCEPTIBILITY TESTING OF A NOVEL MUTATION PATTERN (A181S+M204I) CONFERRING RESISTANCE TO LAMIVUDINE AND ADEFOVIR DIPIVOXIL

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Background and aim: We recently described a cross-resistance pattern to adefovir (ADV) in a patient with lamivudine (3TC) resistance (EASL 2005). We aimed to further analyse the drug susceptibility of this novel mutation pattern (A181S+M204I) against 3TC, ADV, tenofovir (PMPA), clevudine (L-FMAU) and embricitabine (FTC). Methods: Successful suppression of HBV replication by sequential therapy of 9 MU/TIW interferon (IFN) and 3TC was followed by the genotypic resistance detected at the 19th month of treatment. ADV was added to 3TC therapy on the 44th month of antiviral treatment. However, neither ALT normalization nor a stable decrease in the HBV viral load was observed although ADV was used for more than 40 months. HBV pol region was amplified from 8 serum samples obtained before and after ADV treatment. Out of 8, complete genome was amplified in 3 serum samples and was cloned into a TA vector. DNA sequencing was performed for 8 PCR products of HBV pol gene and for 9-10 clones from 3 cloned constructs. Full genome of A181S+M204I variant was also cloned into an expression vector and its in vitro susceptibility to 3TC, ADV, PMPA, L-FMAU and FTC was determined in transiently transfected Huh7 cells. Results: Of the 29 clones sequenced, A181S + M204I pattern was detected in 14. The novel A181S mutation in the reverse transcriptase gene was associated with W172C mutation in the overlapping surface antigen gene. The results of the in vitro drug susceptibility assay revealed that this mutation pattern displays more than 1000 fold resistance for 3TC and FTC, and 28.2 fold resistance to ADV. This novel mutation pattern was relatively sensitive to the effect of PMPA and L-FMAU, displaying 5.9 and 5.6 fold resistance, respectively. Conclusion: In conclusion, A181S+M204I mutation pattern is associated with reduction in the susceptibility of all antiviral agents tested. While resistance is very high to 3TC and FTC, it is moderate to ADV and relatively mild to L-FMAU and PMPA This study underlines the potential clinical aid of drug susceptibility assays in the area of multi-resistant viral variants.

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ADEFOVIR DIPIVOXIL PLUS LAMIVUDINE COMBINATION TREATMENT IS SUPERIOR TO ADEFOVIR DIPIVOXIL MONOTHERAPY IN LAMIVUDINE-RESISTANT HEPATITIS B E ANTIGEN-NEGATIVE CHRONIC HEPATITIS B PATIENTS

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The aim of this study was to evaluate the long-term efficacy of adefovir dipivoxil (ADV) in patients with hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) who have developed resistance to lamivudine (LAM) treatment. In addition, we aimed to assess whether LAM should be discontinued in these patients. Sixty patients with lamivudine-resistant HBeAg-negative CHB were randomized in a 3:1 ratio to receive either ADV + LAM combination treatment (Group A; n = 45) or ADV monotherapy (Group B; n = 15). Baseline characteristics did not differ between groups. After a median follow-up time of 43 months (range, 10 to 63 months), hepatitis B virus (HBV) DNA became undetectable (< 400 copies/ml; virological response) in 37/45 patients in Group A (82.2%) and in 11/15 patients in Group B (73.3%)(p = 0.709). During follow-up, transaminases' levels normalized in 40/44 patients in Group A (90.9%) and in 8/14 patients in Group B (57.1%)(p = 0.012). ADV-resistant mutations were identified in 2/45 patients in Group A (4.4%) and in 5/15 patients in Group B (33.3%)(p = 0.011). The 2 patients with ADV-resistance (group A) were suboptimal responders and in one of two (50%) the duplication of ADV dose to 20 mg per day resulted in disappearance of HBV DNA (< 6 IU/ml) six months later. Virological breakthrough (reappearance of HBV DNA after its initial disappearance) did not develop in any of the 37 Group A patients with virological response. In contrast, 3/11 Group B patients with virological response (27.3%) developed virological breakthrough (p=0.011). Treatment was well-tolerated and no adverse effects

occurred. In conclusion, ADV + LAM combination treatment is superior to ADV monotherapy in patients with lamivudine-resistant HBeAg-negative CHB, in terms of both efficacy and development of resistance to ADV. Increase of ADV dose to 20mg/day may be effective in suboptimal response patients or even ADV-resistant patients.

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PRETREATMENT ALANINE TRANSAMINASE LEVEL MIGHT NOT BE THE MOST IMPORTANT FACTOR IN PREDICTING HBEAG LOSS IN OLDER PATIENTS WITH MORE PROLONGED HBV INFECTION

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Background/Aims:Pretreatment ALT level, especially over 2 times the upper limit of the normal (ULN) reference range, has been accepted as the relevant indicator of initiating the antiviral therapy. Prolonged abnormal ALT level less than 2XULN has been frequently noticed especially in older patients.This analysis was to determine the usefulness of pretreatment ALT level in predicting HBe Ag loss in older patients with lamivudine therapy. Methods: We retrospectively analyzed 820 HBeAg positive chronic hepatitis patients treated with lamivudine. The patients with either hepatocellular carcinoma, or with overt liver cirrhosis recognized by imaging studies were excluded from the analysis. Patients were diagnosed as having persistent HBeAg if they had serum HBeAg positive after 3 years of antiviral therapy. Patients were determined as having loss of HBeAg when they had HBeAg negative serum within 3 years of the therapy. Lamivudine was switched into adefovir when lamivudine resistance was detected. 406 patients met the criteria and were recruited for the analysis. Age, sex, ALT, and HBV DNA level were used in multivariate analysis of 406 patients (Group 1). The same analysis was performed in 152 patients with age over 45 (Group 2). Results: Baseline characteristics Group 1 patients were: median age 40 years, 75.1% male, median serum HBV DNA 403 pg/ml and median ALT 4.5 X ULN. Baseline characteristics of Group 2 patients were: median age 50 years, 72.4% male, median serum HBV DNA 470 pg/ml and median ALT 4.0 X ULN. HBe Ag loss was noticed in 47.5% of Group 1, and 45.4% in Group 2. Although HBe Ag loss correlated with increased pretreatment ALT levels in both groups, multivariate modeling indicated that elevated pretreatment ALT levels was a predictor of HBe Ag loss in Group 1 (P<0.05), but not in Group 2. HBV DNA level was not a significant predictor in either groups. Conclusion: Elevated pretreatment ALT levels might not be a powerful predictor of lamivudine-induced HBeAg loss in the patients aged over 45 years. Another marker predicting HBeAg loss might be needed in patients with more prolonged HBV infection

Odds ratios of HBeAg loss for potential prognostic factors; multivariate analysis

	Factor	Odd ratio	P value
All Patients (Group1)	ALT level	1.03	<0.05
All Patients (Group1)	HBV DNA level	1.00	0.24
Patients≥45 (Group2)	ALT level	1.03	0.10
Patients≥45 (Group2)	HBV DNA level	1.00	0.30

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IN VITRO ANTI-HBV ACTIVITY OF TELBIVUDINE/TENOFOVIR AND TELBIVUDINE/ENTECAVIR COMBINATIONS

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Background: Several nucleoside (entecavir, lamivudine and telbivudine) and nucleotide (adefovir) analogs are approved for the treatment of hepatitis B virus (HBV) infection and several others, including tenofovir, are in clinical development. These drugs are effective against HBV but all have been associated with the emergence of drug-resistant mutants. Combinations of antiviral agents with complementary resistance profiles may offer the potential for enhanced suppression of HBV replication and reduced drug resistance. In this study, the L-nucleoside telbivudine was combined with either tenofovir, a nucleotide analog, or entecavir, a nucleoside analog. Methods: Drug combinations were evaluated in stably transfected human liver cells expressing replication-competent HBV nucleocapsids and virions. The anti-HBV efficacy of each drug alone and in combination was determined using in vitro endogenous HBV polymerase assays after 10 days of drug treatment. In vitro cytotoxicity was determined by standard cell viability staining. MacSynergy II, CombiTool, and isobologram plots were used to analyze the data. Results: Telbivudine/tenofovir and telbivudine/entecavir combinations exerted greater anti-HBV in vitro activity than telbivudine, tenofovir or entecavir alone. Bliss independence analysis (MacSynergy) revealed that the interaction volumes of all 2-drug combinations were <25 μM2 suggesting that the antiviral in vitro interactions between the 2 drugs in each combination are additive. Similar results were obtained with CombiTool (Loewe additivity model); two-drug combination points were clustered around the zero-interaction plane consistent with an interpretation of additivity. Telbivudine/tenofovir exhibited an additive interaction in isobolograms, as implied by D values fluctuating around 0, however an average D value of -0.16 was calculated for telbivudine/entecavir suggesting weak but statistically significant synergy (P = 0.0012). There was no evidence of in vitro cytotoxicity with either of these combinations. Conclusions: Telbivudine demonstrated additive to weakly synergistic anti-HBV activity in vitro when combined with tenofovir or entecavir. These data support the investigation of telbivudine as a component in multi-drug therapy of chronic HBV infection, particularly combinations with drugs exhibiting different resistance patterns such as nucleotide analogs.

Disclosures:

April Patty - Employee: Indenix

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DOES THE GENOTOXIC EFFECT OF LAMIVUDINE TREATMENT IN CHRONIC HEPATITIS B TO THE HOST DNA?

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Chronic viral hepatitis B is the main cause of chronic liver disease, cirrhosis and hepatocellular carcinoma in the world. Lamividune, a nucleoside analog, is an inhibitor of viral DNA replication. Patients are treated with lamividune show normalization of serum alanine aminotransferase (ALT) level, and histological improvement. However, discontinuation of therapy often leads to reactivation of HBV so long-term therapy is necessary for many patients with chronic HBV infection. The aim of this study is to detect the genotoxic effects of the long-term lamividune treatment to the host DNA. Material and Metod: Fourteen chronic hepatitis B (CHB) patients (4 women, 10 men, mean age; 44 ranges; 29-64 yrs) who were treated with lamividune were included in this study. Blood samples were collected before the treatment and 6 months and at least sixteen months (mean 27 months) of the treatment. Peripheral blood lymphocytes of these patients were cultured to make cytogenetic evaluation by observing chromosome breakage and cytologic evaluation by micronucleus (MN) test. For each individual 100 metaphase chromosome spreads were analysed. 190-1091 binucleated cells were observed and for each individual MN were scored. Their results were compared with baseline, six months and at least one year of the treatment. Mann-Whitney U test was used for statistical analysis. Results: Our results showed a significantly increase of chromosome breaks after one year of the treatment (p=0,025) Chromosome breaks of the six months of treatment were slightly higher than before the treatment however the difference was not statistically significant (p>0.05) Moreover we did not find any difference at MN scores between each period of the treatment (p>0.05). Based on this data, we concluded that lamividune treatment in patients with chronic HBV may be chromosomal instability and this instability may increases during long-term therapy especially after one year of the treatment.

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TENOFOVIR SHOWS LIMITED EFFICACY IN TREATMENT OF HBV INFECTIONS RESISTANT AGAINST ADEFOVIR

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Background: Genotypic HBV resistance against adefovir dipivoxil (ADV) develops rarely during the first two years of treatment. However with increasing treatment duration ADV resistant HBV mutants are selected in up to 28% of the patients after 5 years which is often associated with viral rebound. In vitro, these mutations cause a 3-14-fold decrease of ADV efficacy. Tenofovir (TDF), a nucleotide analogue approved for HIV therapy, is closely related to ADV, shows equipotent antiviral activity against HBV on molar basis and is cross resistant to ADV in vitro. However, due to the 25-fold higher dosage of TDF (300 mg/d) as compared to ADV (10 mg/d), TDF might still be effective in ADV resistant HBV infections. Methods: The efficacy of TDF monotherapy was retrospectively evaluated in 10 HBV monoinfected patients (m/f 9/1, mean age 47±11[27-67] years, 6 HBeAg positive) with initial lamivudine resistance who had developed genotypic ADV resistance (rtN236T and/or rtA181V/T) after consecutive 11-45 (mean 24±9) months of ADV monotherapy. HBV DNA and ALT values were measured every 3 months (HBV Monitor, Roche, detection limit 400 copies/mL). In six patients, during TDF treatment the HBV polymerase gene was sequenced from codon rt88 to rt282 after PCR products were cloned in E. coli (TOPO-TA cloning system, Invitrogen; minimum of 10 clones sequenced each). Results: At the begin of TDF treatment (mean duration 16±3[12-21] months), the mean HBV DNA was 7.5±1.3[4.6-9.4] log copies/mL and nine patients had elevated ALT levels (mean 151±118 [34-358] U/mL). The mutations associated with ADV resistance N236T, A181T and combination of both were detected in 3, 2 and 5 patients. During TDF treatment, HBV DNA decreased by a mean of 3.6±1 [2-5] and 4.3±1.2[2-6.2] log copies/mL at week 24 and week 48, respectively. HBV DNA was still detectable in 9, 8 and 6 patients while ALT levels remained elevated in 8, 8 and 6 patients at week 24, week 48 and at the end of observation. Subspecies analysis in six patients showed that ADV resistance mutations remained detectable throughout the whole observation period in increasing ratio as compared to HBV wild type. Combination therapy with TDF and lamivudine was started in two patients at month 12 and lead to undetectable HBV DNA and normal ALT levels after two months. Conclusion: Although TDF shows significant antiviral efficacy in patients with genotypic ADV resistance, HBV DNA can be completely suppressed only in a minority of the patients and the selection of ADV resistant mutations is not prevented. Therefore, patients with genotypic ADV resistance should receive combination treatment with TDF or ADV plus lamivudine or entecavir.

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THE EFFECT OF LAMIVUDINE AND ADEFOVIR DIPIVOXIL ON PREVENTING HEPATOCELLULAR CARCINOMA IN HEPATITIS B VIRUS-RELATED LIVER CIRRHOSIS

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Background/Aims: We evaluated the effect of lamivudine and adefovir dipivoxil on preventing HCC in hepatitis B virus(HBV)-related liver cirrhosis. Patients and Methods: We reviewed the medical records of the patients who were diagnosed with HBV-related liver disease at Yeungnam University hospital from Jan 1983 to Dec 2005. The number of patients treated with lamivudine over 12 months was 570 and the number of patients treated with adefovir dipivoxil after lamivudine resistance was

284 from Mar 1997 to Dec 2005. The number of patients who could not be treated with oral antiviral agent from 1983 to 1999, followed over 12 months, was 1592. In our results using Cox regression model, 4 factors were related with HCC; gender(male, OR=1.81, p<0.001), age(≥40 years, OR=4.95, p<0.001), platelet count(<150x10^3/mm3, OR=3.48, p<0.001), and ascites(yes, OR=1.69, p=0.009). Using SPSS program, 111 patients of the oral antiviral agent-treated group and the untreated group were randomly selected, respectively, matching an age variable, a gender variable, liver cirrhosis with Child-Pugh class A and positive HBe antigen. Results: The mean follow-up period was 4.4 years in the oral antiviral agent-treated group and 5.4 years in the control group. In oral antiviral agent-treated group, HCC occurred in 5 patients (4.5%) with an annual incidnce rate of 1.02% patients/year, whereas in the control group, HCC occurred in 36 patients (32.4%) with an annual incidence rate of 6.0% patients/year. The cumulative incidence of HCC in the oral antiviral agent-treated group was lower than that in the control group(Log-rank, p=0.003). Conclusion: Oral antiviral agents, such as lamivudine and adefovir dipivoxil may reduce the incidence of HCC in patients with HBV-related liver cirrhosis.

Cumulative Incidence of HCC Between the Treated Group and the Control Group in HBV-Related Child A Liver Cirrhosis with Positive HBe Antigen



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THE INDICATION AND LIMITATION OF INTERFERON THERAPY AS THE FIRST LINE THERAPY OF CHRONIC HEPATITIS B- FROM THE HISTOLOGICAL ANALYSIS OF 800 CHRONIC HEPATITIS B PATIENTS

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[AIM] Current therapy of chronic hepatitis B does not eradicate HBV and has limited long-term efficacy. Thus, careful consideration of the patient's age, severity of liver disease, likelihood of response, and potential adverse events is needed before treatment is initiated. The role of interferon in the first line therapy is not clearly defined. The degree of liver histology might be related to interferon's efficacy and limitation. To prove this, we investigated the relationship between the staging score and the interferon response and durability in patients with chronic hepatitis B. [Materials and Methods] A total of 800 Japanese genotype C treatment naive chronic hepatitis B were studied. All of them had liver biopsy before the treatment. Seven hundred patients were HBeAg positive and the remaining 100 were HBeAg negative. All patients had elevated ALT levels (>2x UNL) and increased HBVDNA (>106) levels. Among these 800 patients, 269 patients with HBeAg positive chronic hepatitis B were treated with interferon alfa therapy (Sumiferon, 6 MU/d, TIW, 4 mo). The patients were stratified by age into 5 year groups beginning with 15-20 y/o until 70-75 y/o. All liver tissue specimens were obtained by needle biopsy. The grade and stage were based on international standard criteria and METAVIR. The relapse after seroconversion was defined as that HBeAg became positive again or that HBeAg remained negative but HBVDNA and ALT elevated for one year after the terapy. [RESULT] 1) The staging scores for each group were generally increased with age from 1.51 (15-20) to 2.75 (61-65), and the degree of fibrosis increased significantly especially after 30 y/o (mean, 2.2) every year at the rate of 0.04-0.06 until 45 y/o (mean, 2.54). 2) Among 269 HBeAg positive patients treated with interferon, 89 (30%) seroconverted to anti-HBe, and among them 74 (83%) had staging score either 1 or 2, and durability was 80-90%. While those with stage 3 or 4 had 50% durability. In nonresponders, the staging score was significantly higher than seroconverters (p=0.0001). [Conclusions] 1) The current study showed that progression of fibrosis appeared to increase significantly especially after 30 year old until 45 year old at the rate of 0.04 to 0.06 every year. 2) Interferon appeared to be effective in patients who had less than staging score 2 with 80-90% durability, while in patients with staging score 3 or 4, interferon did not seem to be similarly effective and the durability appeared to be poor. Thus the degree of fibrosis may be one of the important factors to indicate and limit interferon efficacy for the treatment of chronic hepatitis B.

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EMERGENCE OF HEPATITIS B VIRUS GENE MUTATION RELATED TO ENTECAVIR-RESISTANCE IN CHRONIC HEPATITIS B PATIENTS PARTICIPATED IN THE PHASE 2 CLINICAL STUDIES OF ENTECAVIR IN JAPAN

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[Background/Objective] The objective of this study was to elucidate the emergence rate of HBV DNA gene mutation related to entecavir-resistance (ETVr) in chronic hepatitis B patients participated in the phase 2 entecavir (ETV) clinical studies performed in Japan. [Method] The subjects of this study were 287 patients in total, consisted of (1) 137 nucleoside-naive patients in the ETV-047 study administered with 0.01, 0.1, 0.5 mg/day of ETV or 100mg/day of lamivudine (LVD) for 24 weeks, (2) 66 nucleotide-naive patients in the ETV-053 study administered with 0.1 or 0.5mg/day of ETV for 52 weeks, and (3) 84 LVD-refractory patients in the ETV-052 study administered with 0.5 or 1.0 mg/day of ETV for 52 weeks. After the studies, a total of 282 patients were enrolled in ETV-060 roll over study, and subsequently administered with ETV for another 2 years. Single-nucleotide-polymorphism(SNP)-PCR analysis for HBV polymerase was performed to detect the ETVr mutation for the patients who experienced viral rebound, defined as elevation in serum HBV DNA by greater than 1 log copy/mL. [Results] (Result-1: Nucleoside-naive patients). Five out of 169 nucleoside-naive patients with ETV administration (comprising 103 from ETV-047 and 66 from ETV-053), experienced viral rebound before week 148. ETVr-related mutation (rtS202S/G) along with LVDr-related mutation (rtL180M and rtM204V) was detected in two patients (1.2%) between week 100 and 148. The two patients who developed ETVr never experienced a disappearance of serum HBV DNA. The 34 patients in ETV-047 study, who were administered with LVD for 24 weeks followed by administration with ETV 0.5mg, did not develop LVDr- or ETVr-related mutation throughout the study duration. (Result-2: LVD-refractory patients). Viral rebound developed in 21 patients out of 84 in ETV-052 study, before week 148, and LVDr-related mutation (rtM204I/V and/or rtL180M) along with ETVr-related mutation (rtS202 and/or rtT184), was detected in 19 patients (22.6%). [Conclusions] The cumulative number of patients who developed ETVr-related mutation were 0/0/2 at years 1/2/3 in nucleoside-naive group (total n=169 at baseline), and 0/10/19 at years 1/2/3 in LVD-refractory group (total n=84 at baseline), respectively. Development of ETVr-related gene mutation was rare in nucleoside-naive patients.

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MUTATIONS OF THE HBV-POLYMERASE GENE ASSOCIATED WITH ADV DRUG RESISTANCE IN PATIENTS UNDERGOING A FIRST ADV THERAPY

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Background : HBV resistance mutations have been identified for all oral antiviral agents used to treat chronic hepatitis B. Whereas the rtA181V and rtN236T mutations are associated with long-term adefovir dipivoxil (ADV) treatment, the newly discovered mutation I233V is associated with primary resistance to ADV. Aim : To assess the association between existing HBV polymerase mutations and failure of antiviral therapy in patients given ADV for the first time. Patients and methods : 86 patients began ADV therapy during 2001 and 2005 for more than 12 months, of these 6 (7%) showed no significant virus suppression (less than 1 log IU/ml) and their ALAT was elevated when therapy was stopped (NR group). The HBV rt amino-acid sequences from these 6 patients were compared to those of 5 patients who had virus sensitive to ADV (R group). The HBV rt gene was sequenced with in-house protocols (ABI 3100) using sera collected at baseline and at the end of treatment. Results : Phylogenetic analysis of the rt gene HBV gave genotypes A (6/11), D (4/11) and E (1/11). The rtA181T/V and rtN236T mutations were never detected at the beginning of ADV therapy. rtM204I/V and/or rtL180M were detected in 5/11 patients (45%) because most of them had been treated with Lamivudine (LMV). Two of them had lost these LMV-resistance-associated mutations at the end of ADV therapy. Only one patient in the NR group developed rtA181T/V and rtN236T mutations (17%) during the ADV treatment. The rtI233V mutation, recently associated with primary resistance to ADV was not detected in any patient and did not exist in patients whose virus was resistant to ADV. But, rtL217R was detected at baseline and at the end of treatment in 3/6 of the NR patients (50%) and in 1/5 R group patients (20%). This mutation, which is more prevalent in HBV strains with genotype A2, was found in 3 patients infected with genotype A. Conclusion : HBV strains that are naturally resistant to adefovir can occur, but are not frequent. The rtI233V mutation cannot explain the failure of ADV therapy in our patients, whereas rtL217R mutation seemed to be more frequent. Further studies are needed to clarify the influence of the rtI233V and the rtL217R mutations on ADV therapy.

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SUSTAINED HBEAG AND HBSAG LOSS AFTER LONG-TERM FOLLOW-UP OF HBEAG POSITIVE PATIENTS TREATED WITH PEGINTERFERON ALPHA-2B

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We evaluated the long-term sustainability of response and clinical outcome in patients treated with PEG-IFN α-2b alone or in combination with lamivudine for 52 weeks. Methods: All 266 HBeAg positive chronic hepatitis B virus (HBV) infected patients from 41 centers enrolled in the randomized HBV99-01 study were offered participation in a long-term follow-up (LTFU) study. Initial response was defined as HBeAg negativity at the end of the initial study (26 weeks post-treatment; week 78). For the LTFU study, patients had one additional visit after the initial study (mean interval 3.0 ± 0.8 years). For analysis, retreatment was considered as non-response. Results: 172 patients (65%) from 28 centers (68%) were enrolled in the LTFU study. 91 patients (53%) received PEG-IFN α-2b alone and 81 (47%) its combination with lamivudine. Response rates at different time points are shown in the table. At LTFU, HBeAg loss was durable in 52 of 64 initial responders (81%), of whom 67% had HBV DNA <10,000 copies/ml and 65% had normal ALT. 14 initial responders (21%) and 67 of 108 (62%) non-responders were retreated (p<0.001). HBsAg was negative in 19 initial responders (30%). Sustained HBeAg negativity was observed in 96%, 86%, 67% and 77% of initial responders with genotype A, B, C and D infection, respectively (A vs. C, p=0.04; A vs. D, p=0.07). Serum HBV DNA <10,000 copies/ml was observed in 81%, 29%, 44% and 35% of initial responders with genotype A-D, respectively (A vs. B or D, p<0.02). HBsAg negativity was observed in 58% of genotype A infected initial responders compared to 14%, 0% and 6% of those with genotype B, C or D (A vs. C or D, p<0.004). At LTFU, 3 non-responders had died and 1 developed hepatocellular carcinoma; decompensated liver disease was observed in 1 responder and none underwent liver transplantation. Conclusions: HBeAg response to PEG-IFN α-2b ± lamivudine is durable in the majority of patients and is associated with an increase in HBsAg loss. This study further emphasizes the importance of HBV genotype in PEG-IFN therapy, with high rates of sustained virological response in genotype A infected patients. PEG-IFN should therefore particularly be considered as first line therapy in genotype A infected HBeAg positive patients.

	Week 32		Week 52		Week 78		LTFU	
	PEG	PEG + LAM	PEG	PEG + LAM	PEG	PEG + LAM	PEG	PEG + LAM
HBeAg negative	21%	35%*	34%	47%*	39%	36%	37%	36%
HBV DNA <10,000	14%	69%*	19%	70%*	17%	20%	25%	31%
HBV DNA <400	9%	32%*	9%	40%*	6%	11%	13%	26%*
Normal ALT	25%	40%*	30%	52%*	34%	37%	28%	33%
HBsAg negative	ND	ND	7%	9%	6%	9%	8%	15%

*p<0.05 PEG-IFN α-2b + lamivudine (PEG LAM) vs. PEG-IFN α-2b (PEG); ND = not done

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PREDICTION OF LONG-TERM MAINTENANCE OF VIROLOGIC RESPONSE DURING LAMIVUDINE TREATMENT IN HBEAG NEGATIVE CHRONIC HEPATITIS B

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Background and Aims: It has recently been claimed that non-detectability of serum HBV-DNA by PCR after six months of treatment with telbivudine or lamivudine in chronic hepatitis B (CHB) predicts maintenance of the response after two years of treatment with a positive predictive value (PPV) for HBeAg-negative patients of > 80% The aim of the present study was to investigate in such CHB patients the relation between HBV-DNA suppression achieved during the early phases of LAM treatment with the long-term outcome of therapy after 4 and more years. Patients and Methods: The study included 156 patients with 56 of them (37%) in maintained virologic and biochemical responses for more than 4 years. Their virologic data was compared to those of patients who experienced a breakthrough 3 months to 5 years after starting treatment. HBV-DNA data assessed by sensitive real time PCR assays was available every 3 to 6 months and all samples negative or <1000 cp/ml were retested with the COBAS TaqMan Test (Roche Molecular Systems, CA, USA, sensitivity 50 cp/ml) Results: The positive predictive value of negative HBV-DNA after 6 months of treatment was 93% for maintenance of the response for two and 71,5% for maintenance of response for four or years more. Similar percentages were found for the results after 3 months of treatment. On the other hand, a positive result even >10,000 cp/ml at month 6 could not exclude the possibility that LAM therapy would be successful and was associated with a possibility of maintenance of response for 2 years of 36,5% and for 4 or more years of 8%, those percentages being 43,7 and 19,5% for the month three results. Overall, patients who maintained response had lower levels of HBV-DNA both at month 3 and 6 compared to patients who experienced a breakthrough. Conclusion: HBV-DNA non-detectable by sensitive PCR assays during the "early" phases of antiviral treatment of HBeAg(-) CHB are very useful in predicting maintenance of the response up to year 3 of Rx but their positive predictive value decreases to approximately 70% for response maintained longer than 4 years. Moreover their low negative predictive value cannot provide reliable information for deciding treatment discontinuation or modification.

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AMINO ACID VARIABILITY WITHIN HEPATITIS B SURFACE ANTIGEN AND THE OVERLAPPING REVERSE TRANSCRIPTASE REGION IN HBSAG NEGATIVE/HBCAB POSITIVE PATIENTS PRESENTING HBV REACTIVATION WHILE UNDERGOING CHEMOTHERAPY AND/OR STEM CELL TRANSPLANTATION FOR CANCER

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Background/Aim: Hepatitis B virus (HBV) reactivation has been described in HBsAg-/anti-HBc+ patients (pts) undergoing immunosuppressive chemotherapy (CT). We previously reported HBV reactivation in 8.3% of pts (n=7) with HBV serological profile indicating past infected who underwent CT and/or hematopoietic cell transplantation (HCT) for cancer. Baseline anti-HBs titer <100 IU/L was significantly associated with HBV reactivation in these pts. In the present study, we aimed to analyse the amino acid (aa) variability and mutations patterns within HBsAg and the overlapping HBV reverse transcriptase (rt) region of HBV strains from these pts to assess whether HBV reactivation might be associated with specific virological features. Patients/Methods: 7 male pts (mean age, 60 years), HBsAg-/anti-HBc+ (HBsAb+/-) prior to CT and/or HCT who presented HBV reactivation between 11/2003 and 12/2005. 4/7 pts received anti-CD20. HBV HBsAg and rt genes were amplified/sequenced using in-house protocols. Newly-diagnosed HBsAg-positive pts (n=51) served as controls. Results: HBV genotype was D and C in 5 and 2 cases, respectively. Mean number of substitutions/100 aa was significantly higher vs controls within HBsAg major hydrophilic region (MHR) (8.,6 vs 2.1; p<0,01), its "a" determinant (1*5.7* vs 2.5; p=0.003), and the HBsAg C-terminal region (*7.5* vs 3.8; p=0,054). Substitutions D144A/V and G145R/A previously associated with altered S antigenicity were more frequent (from 2 pts each) than in controls (p=0.001 and p=0.004, respectively). Among genotype D sequences, substitutions at positions s116, s126, s134, and s144 within the MHR were significantly more frequent than in controls (p<0.05). No rt drug resistance mutation was detected at time of reactivation. However, within rt sub-domains B-C-D that overlap HBsAg C-terminal region and where drug resistance mutations occur, the mean number of substitution/100 aa showed a tendency to be higher in the reactivation group (3.0 vs 1.3; p=0.081), and a significantly higher proportion of sequences harboured >1 substitutions (P<0.05). Mutations rtR/W153Q, known to restore HBV replication of lamivudine (LAM) resistant strains and corresponding to substitutions sG145R, were found in HBV from 1 pt. In 1 pt, LAM-resistance was detected 1 month after LAM introduction. Conclusions: Our data show an increased aa variability within HBsAg and HBV RT associated with HBV reactivation in AgHBs-/anti-HBc-positive pts undergoing CT and/or HCT. Whether or not these virological patterns may play a role in the pathogenesis and frequency of HBV reactivation or might impact the response to antiviral treatments needs further investigations.

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ESTIMATING THE IMPACT OF CHRONIC HEPATITIS B ON FUTURE LIVER-RELATED MORBILITY, MORTALITY AND COST IN SPAIN

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Background and aims: Chronic hepatitis B (CHB) is a common infection often producing progressive disease. Some studies suggest that HBV-related complications will increase in the future, impacting future disease costs. Our aim was to estimate future morbidity, mortality and costs associated with CHB infection in a cohort of patients in Spain. Methods: A Markov model was used to project HBV-related complications and costs over the next 20 years in a cohort of 132,028 patients representing the HBV-infected population in Spain. This cohort was stratified according to age (25-34, 35-44, 45-54, 55-64, 65-74 years), HBeAg status (13% HBeAg-positive and 87% HBeAg-negative), and the degree of liver disease. The probabilities of disease progression in the model were obtained from data published in the literature, and the cost of the disease and its complications were based on actual variable costs from the Spanish Health Care System. An annual 3.5% discount rate was applied to the costs. Results: A greater disease progression and morbidity will be observed in HBeAg-negative patients compared with HBeAg-positive patients, with a higher proportion of HBeAg-negative patients requiring liver transplant (9% vs. 5%) and a higher proportion of deaths (59% vs. 45%). The total cost of the HBV-infected cohort during the next 20 years will be US$5,007 million. The disease progression in HBeAg-negative patients requires an additional cost of 65% in relation to HBeAg-positive patients. The mean estimated cost in US$ per patient and the different stage of the disease distribution over the next 20 years are shown in the table below. Conclusions: In the future, HBV mortality, morbidity, and associated cost will increase. Treatment of the CHB-infected population potentially may control the infection, reduce progression, increase patient survival and reduce the need for liver transplantation.

	HBeAg+		HBeAg-		Total	
	Cost per patient (%)	Patients (%)	Cost per patient (%)	Patients (%)	Cost per patient (%)	Patients (%)
CHB	$1,485 (5)	20,224 (45)	$1,664 (4)	19,606 (22)	$1,604 (4)	39,830 (30)
Cirrhosis	$636 (2)	1,457 (3)	$1,195 (3)	6,203 (7)	$1,006 (3)	7,661 (6)
Decompensated Cirrhosis	$341 (1)	323 (1)	$674 (2)	1,290 (1)	$561 (1)	1,613 (1)
Hepatocellular Carcinoma	$1,395 (5)	180 (1)	$1,917 (4)	699 (1)	$1,740 (5)	879 (1)
Liver Transplant	$22,430 (81)	2,416 (5)	$35,882 (83)	7,993 (9)	$31,336 (83)	10,409 (8)
Death	$1,236 (4)	20,012 (45)	$1,900 (4)	51,624 (59)	$1,676 (4)	71,636 (54)
Total	$27,523 (100)	44,613 (100)	$43,231 (100)	87,415 (100)	$37,923 (100)	132,028 (100)

Disclosures:

Magdalena Rueda - Employee: Gilead

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NEUTRALIZING ANTIBODIES TO INTERFERON ALPHA IN CHRONIC HEPATITIS C PATIENTS NON-RESPONDING TO PEGYLATED INTERFERON PLUS RIBAVIRIN RETREATED TO PEG-INTERFERON α-2A AND RIBAVIRIN (ANRS GAMMATRI)

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Background. Neutralizing antibodies to interferon α-2a (NAb to IFN-α) may explain a lack of antiviral response (Van der Eijk and al. NEJM 2006). We aimed at assessing NAb to IFN-α and IFN levels in non responders (NR) patients retreated by PEG-interferon α-2a (PEG-IFN α-2a) + ribavirin (RIBA). Methods. NR to a first-line treatment of PEG-IFN+RIBA were included to receive PEG-IFN α-2a (180µg/week) + RIBA (weight-based dosage) for 48 weeks. HCV RNA was measured at week 12 using Taq Man Roche (lower limit of detection 15 IU/ml) in a central laboratory. IFN levels and Nab to IFN-α were retrospectively measured on stored sera at baseline, weeks 4 and 12, using a quantitative sandwich ELISA for NAb to IFN-α (Bender Med-Systems Diagnostics GmbH) with a lower limit of detection of 1.38 ng/ml (similar to Van der Eijk study). Interferon α-2a levels were measured using a quantitative sandwich ELISA for interferon α (Bender Med-Systems Diagnostics GmbH) with a lower limit of detection of 3.3 pg/ml. Results. Among 43 NR patients with baseline mean HCV RNA 6.1 log10 IU /ml (Inter-Quartile Range 4.7 to 7.4), 85% genotype 1, 53% F3-F4 Fibrosis, HCV RNA decreased greater than 2 log10 in 20 patients at week 12. Nab titers against IFN-α and IFN α titers are shown in the table. No significant association was observed between the presence nor the titers of NAb and HCV RNA levels. Median IFN levels (interquartile range) at weeks 0, 4 and 12 (pg/ml) were < 3.3 (<3.3-to-371.4); 1532 (106.8-to-3284.8); 1677 (90.8-to-5000) in the 20 responders and 84.5 (3.3-to-277.4); 1407.4 (120.2-to-2443.4); 1620.1 (120.1-to-2287.1) in the 23 non responders. Conclusion. This study, performed in non selected consecutive NR patients, do not confirm recently published case reports. Re-treatment with PEG-IFN + RIBA is able to be associated with virological response in the presence of antibody-mediated resistance to conventional IFN treatment.

		HCV RNA decrease greater than 2 Log10		Undetectable HCV RNA at week 12		Undetectable HCV RNA at week 28	
		yes	no	yes	no	yes	no
Nab to IFN-alpha	<1.38	11(58%)	17(73%)	1(33%)	26(64%)	5(56%)	18(72%)
(ng/ml)	>=1.38	8(42%)	6(26%)	2(67%)	12(36%)	4(44%)	7(28%)
P-value		0.27		0.25		0.42	

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LIVER BIOPSY: STILL ESSENTIAL IN THE MANAGEMENT OF CHRONIC HEPATITIS B

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BACKGROUND & AIMS: In the absence of an established role for non-invasive markers of fibrosis, liver biopsy remains the gold standard for the assessment and staging of disease in chronic hepatitis B virus (HBV). With growing numbers of chronic HBV patients attending hepatology clinics, the indications and timing of liver biopsy needs to be clearly defined. The aim of this study was to formally review all biopsies undertaken for a primary diagnosis of HBV in a tertiary referral centre and to assess how histology correlates with disease profile, in particular HBV DNA & ALT. Factors associated with more advanced disease or progression of disease on repeat biopsy were sought. PATIENTS & METHODS: Between 1995 and 2007, 212 chronic HBV patients underwent liver biopsy. This comprised of 76 HBeAg+ patients (Group 1), 9 of whom underwent repeat biopsy and 136 anti-HBe+ patients (Group 2), 31 of whom underwent repeat biopsy. Two specialist liver histopathologists reviewed all biopsy material and determined Ishak fibrosis scores. A fibrosis score of 0-2 was designated mild disease, 3-4 moderate and 5-6 severe. Quantitative HBV DNA and ALT values [> or < twice upper limit of normal (2ULN)] at the time of biopsy were collected. RESULTS:Fibrosis score correlated with age at biopsy ($p<0.01$). In Group 1, 43 patients (57%) (M:F 29:14) had mild disease on histology: median age at biopsy was 30 years (range 16-64); 33 patients (43%) (M:F 27:6) had moderate/severe disease on biopsy with a median age at biopsy 37 years (range 16-73); 4/14 (29%) patients with severe disease had HBV DNA >107 and normal ALT were, therefore, considered immuno-tolerant. In Group 2, 71 patients (52%) had mild disease on biopsy, (M:F 50:21) median age at biopsy 36 years (range 18-70). The remaining 48% (65/136) had moderate/severe disease (M:F 50:15), median age at biopsy 47 years (range 23-73); 27/65 (42%) with moderate/severe fibrosis had both HBV DNA<105 and ALT <2ULN. Repeat biopsy group Rate of fibrosis change in untreated patients undergoing serial biopsies was highly variable, ranging between improvements of fibrosis score of 0.44 points/year versus progression of one point per year (mean 0.22 points/year). Rate of progression did not correlate with eAg status, HBV DNA or ALT level. However, there was a trend towards faster progression in those with a BMI>25. CONCLUSIONS: This study highlights the importance of liver biopsy in the diagnosis and management of chronic HBV. It emphasizes the inadequacy of quantitative HBV DNA and ALT values in reflecting underlying liver damage. We conclude that any decision not to offer antiviral therapy should be based on histological findings.

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PREDICTORS OF LIVER DECOMPENSATION IN HBEAG– PATIENTS WITH CHRONIC HEPATITIS B

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Objective: To determine factors affecting liver decompensation in patients with chronic hepatitis B. Methods: Predictors of liver

decompensation (ascitis, liver insufficiency, encephalopathy, gastro-intestinal bleeding) were retrospectively evaluated in a cohort of 336 HBeAg- patients referred to the Hepatology Departement of Pitié Salpêtrière Hospital (Paris France) since 1980. Patients co-infected with HIV, HDV and HCV were excluded. Age, race, oral anti-HBV therapy (lamivudine, adefovir, tenofovir, entecavir), IFN based therapy, baseline ALT and serum HBV DNA were analyzed using Kaplan Meyer (KM) survival curves. A Cox regression analysis was performed to assess independent predictors of liver decompensation. Results: Main baseline characteristics of included patients are summarized in the table. Age, sex, race, oral anti HBV therapy, serum ALT, serum HBV DNA were significantly associated with liver decompensation in univariate analysis. Age (>35 years, RR 25.72 (3.08-214.80), p= 0.003) and high baseline serum HBVDNA (>6 log copies/mL, RR 11.97 (2.75-52.13), p<0.001) remained independently associated with the risk of liver decompensation in the Cox analysis (n=252). Conclusion: High HBV DNA (> 6 log copies/mL) and age older than 35 years strongly predict the risk of liver decompensation in HBeAg- patients. These patients should be actively considered for anti-HBV therapy independently of serum ALT level. This study was supported by IDENIX PHARMACEUTICAL

Main baseline characteristics in HBeAg- (n=336)

Median (95 CI) follow up (months)	68.37 (61.42-72.52)
Mean (95% CI) Age (years)	38.12 (36.74-38.49)
Male (%)	237 (70.54)
Race - African n (%) - Asian n (%) - Cocasian n (%)	180 (53.57) 87 (25.89) 69 (20.54)
Alcool consumption ≥50 g/day n (%)	16 (4.88)
Median (95% CI) ALT (UI/L) ALT ≥2 x ULN*	34 (31-38) 90 (26.79)
HBV DNA n (%) - ≥6 log copies/mL - 4≤ <6 log copies/mL - <4 log copies/mL - Undetermined	25 (7.44) 99 (29.46) 128 (38.10) 80 (23.81)
Anti HBV therapy - IFN based n (%) - Oral n (%) - Untreated	20 (5.95) 214 (63.69) 118 (35.12)
HBsAg seroconversion n (%)	4 (1.83)
Liver decompensation n (%)	41 (12.20)

ULN time the upper limit of normal

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LONG TERM FOLLOW-UP OF CHINESE PATIENTS WITH HBEAG-NEGATIVE CHRONIC HEPATITIS WHO DISCONTINUED TREATMENT AFTER 2 YEARS OF LAMIVUDINE MONOTHERAPY

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Background: The optimal duration of treatment of HBeAg-negative chronic hepatitis B is unknown. Treatment withdrawal after one year has been associated with a high rate of relapse, while long-term treatment is associated with risks of drug resistance. We previously reported a clinical relapse rate of 18% and 30% at 12 and 18 months, respectively, after withdrawal of a 2-year course of lamivudine. Aims: To determine the long-term durability of response to lamivudine following withdrawal of a 2-year course of lamivudine therapy in HBeAg-negative patients. Methods: Consecutive HBeAg-negative patients attending the Toronto General Hospital Liver Clinic from 07/98- 05/03 were treated with lamivudine 100 mg daily for 2 years. Those who achieved undetectable HBV DNA by PCR assay (COBAS Amplicor, LLOD 200 copies/ml) on 3 consecutive occasions during the second year of therapy were eligible for treatment withdrawal after 2 years of lamivudine. Thereafter, patients were monitored every 6 months. Clinical relapse defined as HBV DNA >3.3 log10 IU/ml [>4 log10 copies/ml] and ALT > 1.5 X ULN [ULN, 40 U/L] and viral relapse (redetection of HBV DNA by PCR) on 2 consecutive occasions were estimated by Kaplan Meier analysis. Antiviral therapy was restarted in those who met criteria for clinical relapse. Results: A total of 50 HBeAg-negative patients, mean age 52+/-11 years, 80% men and 52% cirrhosis were included. Treatment was withdrawn in 45 patients and relapse occurred in 30 with a mean time to relapse of 16 months. The probability of viral and clinical relapse at 24, 36 and 48 months post treatment was 50% and 40%, 62% and 56%, 74% and 60%, respectively. No baseline factor including cirrhosis, gender, age, ALT, or HBV genotype was associated with clinical relapse. During a mean follow-up of 56+/-15 months, no patient underwent reversion to HBeAg-positivity and 3 patients lost HBsAg. All patients who relapsed responded promptly to retreatment using lamivudine with or without adefovir or tenofovir. No patient developed hepatic decompensation or died as a result of clinical relapse. Conclusions: Approximately 60% patients with HBeAg-negative chronic hepatitis had clinical relapse within 4 years of withdrawal of a 2-year course of lamivudine monotherapy. Our data suggest that a longer duration (>2 years) of treatment is needed for patients with HBeAg-negative chronic hepatitis and a long duration of follow-up (>5 years) is needed to determine the post-treatment relapse rate.

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ASSOCIATION OF PRETREATMENT SERUM INTERFERON-GAMMA INDUCIBLE PROTEIN 10 (IP-10) LEVELS WITH VIROLOGICAL RESPONSE TO LAMIVUDINE MONOTHERAPY AND LAMIVUDINE INTERFERON SEQUENTIAL TREATMENT IN CHRONIC HEPATITIS B

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Background and aim: Recently, several studies reported that pretreatment serum IP-10 levels predict viral response and therapeutic outcome to pegylated interferon plus ribavirin therapy in genotype 1-infected patients with chronic hepatitis C (CHC). Other groups reported that patients with CHC express higher levels of IP-10 in serum and liver tissue compared with chronic hepatitis B (CHB). It remains unclear whether pretreatment serum IP-10 levels associate with response to antiviral therapy for CHB. We aimed to evaluate association of pretreatment serum IP-10 levels with virological response to lamivudine (LMV) monotherapy and LMV interferon (IFN) sequential treatment in CHB. Patients and Methods: 83 patients (55 HBe Ag +ve and 28 HBe Ag – ve) were treated with LMV. 22 HBe Ag positive patients were treated with LMV IFN sequential treatment (Serfaty et al; Hepatology.2001). Serum HBV-DNA was quantified with quantitative PCR (detection limit is 400copies/ml). Early virological response (EVR) in LMV treatment group was defined as serum HBV-DNA undetectable within 24 weeks from start of treatment. Sustained virological response (SVR) in sequential

treatment group was defined as HBe seroconversion and HBV-DNA<105 copies/ml at 24 weeks after end of treatment. Age, sex, HBe Ag, pretreatment ALT, IP-10 and HBV-DNA levels and stage of fibrosis were examined by multivariate logistic regression analysis. Results: All patients had HBV genotype C. In LMV group, 53 patients (63.9%) (29 HBe Ag +ve and 24 HBe Ag –ve) achieved EVR. Pretreatment median serum IP-10 levels had no significant difference EVR between non-EVR (381 v 339 pg/ml, respectively; p=0.89). From multivariate analysis, pretreatment ALT levels (p<0.001) and negativity for HBe Ag (p=0.01) were independent predictor for EVR. Meanwhile, in sequential treatment group, 11 patients (50%) achieved SVR. Pretreatment median serum IP-10 levels had significant difference SVR between non-SVR (665 v 431 pg/ml, respectively; p=0.03). From multivariate analysis, only pretreatment IP-10 levels (p=0.002) was independent predictor for SVR.During sequential treatment, IP-10 levels in treated all patients showed dramatic changes (median levels:pre LMV(460pg/ml), pre IFN(160pg/ml), IFN 1week(665pg/ml), end of IFN(299pg/ml), post IFN 24weeks(159pg/ml). Conclusion: Our data suggest that pretreatment serum IP-10 levels predict SVR to LMV- IFN sequential treatment in CHB. The difference of significance of pretreatment IP-10 levels between LMV mono therapy and LMV- IFN sequential treatment may be derived from that the CHB patients with high IP10 levels will show good IFN response which is contrary to response for IFN in CHC.

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NOT HBEAG-STATUS BUT HBV GENOTYPE PREDICTS RESPONSE TO IFN IN CHRONIC HEPATITIS B

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Background and Aims: In contrast to HBeAg-positive hepatitis B, HBeAg-negative hepatitis B is considered to be associated with a poor sustained virological response towards interferon therapy. The present study investigated the influence of HBV genotype on the presumed HBeAg-dependence of IFN response. **Patients and Methods:** 399 patients with chronic replicative hepatitis B infected by HBV genotype A (n=141) or HBV genotype D (n=258) were treated with standard interferon-alfa or pegylated interferon-alfa for 4-12 months. HBV genotype was determined by direct sequencing of the HBV X or S gene. 44.4% of the patients had HBeAg-negative hepatitis B and 55.6% HBeAg-positive hepatitis B. **Results:** Sustained virologic response to standard interferon-alfa therapy was lower in HBeAg negative compared to HBeAg-positive patients (22.1% vs 35.6%; p<0.003). Prevalence of HBV genotype A was higher in HBeAg–positive hepatitis compared to HBeAg-negative hepatitis (59.3% vs.16.2%; p<0.0001) and opposite to the prevalence of HBV Genotype D (40.7% in HBeAg-positive hepatitis vs. 83.8% in HBeAg-negative hepatitis). SVR was lower for HBV genotype D patients (19.8%) compared to HBV genotype A patients (43.3%; p<0.001). However, there was no difference in SVR between HBeAg-positive and HBeAg-negative patients of HBV genotype A (44.8% vs 38.9%;p=n.s.) neither for HBeAg-positive and HBeAg-negative patients of HBV genotype D (18.5% vs. 21.1%: p=n.s.). Multivariate logistic regression performed for a representative cohort of patients (n=208) identified HBV genotype A vs. D (p<0.002; 95%CI:1.48-5,43) and HBV DNA (<or ≥200pg/ml; p<0.04; 95%CI: 1.01-3.82) but not pretreatment ALT levels (>2 x ULN) nor HBeAg-status as independent predictive parameters of IFN response. **Conclusions:** The present data demonstrate that SVR to IFN in hepatitis B was not affected by HBeAg status but by HBV genotype. Therefore genotype dependence of IFN response and geographic variability of HBV genotype prevalence might explain the divergent SR to IFN between HBeAg-positive and HBeAg-negative patients. This should be taken into account for delineation of antiviral therapy in chronic hepatitis B.

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INTRAHEPATIC AND SERUM MARKERS OF HBV REPLICATION AND THEIR RELATIONSHIP TO SERUM HBEAG TITRES: IMPLICATIONS FOR THE USE OF QUANTITATIVE HBEAG TESTING AS A PREDICTIVE TOOL FOR TREATMENT OUTCOME

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Background: HBeAg seroconversion is one of the major therapeutic endpoints used when treating HBeAg-positive chronic hepatitis B (CH-B). Data from clinical trials suggests a potential role for quantitative HBeAg titres in guiding therapy. However, a commercial assay is not available and HBeAg titres have not been well-characterized in vivo. The relationship of HBeAg titre to viral load (VL) and cccDNA in vivo remains unresolved; interpretation may be confounded by the presence of the basal core promoter (BCP) or precore (PC) variants in the quasispecies pool. Although shown to reduce/abolish HBeAg production in vitro, their impact on HBeAg titre in vivo has not been defined. Aims: 1) To define the normal range of HBeAg titres seen in patients with HBeAg-positive CH-B in the immunoelimination phase of disease. 2) To correlate HBeAg titres with VL, liver cccDNA, viral genotype and the presence of PC/BCP mutations Methods: A high throughput commercial HBeAg ELISA kit (Architect, Abbott Laboratories, Ill) was optimised for use as a quantitative assay using the Paul-Ehrlich (PE) reference standard. HBeAg titre was correlated with VL in all patients. Detailed virological characterization was performed in a subset; this included genotype, BCP/PC sequence, and intrahepatic cccDNA (Werle-Lapostolle, Gastro, 2004). Results: The assay was validated using a cohort of 85 untreated HBeAg-positive patients in the immunoelimination phase of disease. Sequencing was performed in 30 patients. Intrahepatic cccDNA was measured in 17 patients. The linear range of the assay was 0.5 – 90 PE IU/ml. The observed HBeAg titres followed a log-normal distribution (median 1509, range 1–14236 PE IU/ml). Median VL was 3.43x108 IU/ml. Median cccDNA was 3.65 copies/Geq. VL correlated strongly with

cccDNA (r =.81, p <.05). HBeAg titre correlated modestly with VL (r =.50, p-value <.001) and cccDNA (r =.48, p-value =.05). The lack of a stronger correlation was attributed to the presence of BCP and PC variants in the quasipspecies pool (dominant virus WT/WT in 17/30, BCP/WT in 9/30, and WT/PC in 4/30; median HBeAg titres 3095, 209 and 149 respectively, p <.05 vs WT). The effect of the BCP/PC variants was independent of VL. HBeAg titre did not differ between genotypes A - D. Conclusion: We have optimised a commercial HBeAg assay for use as a sensitive and accurate quantitative tool. HBeAg titres were log-normal distributed and modestly correlated with VL and cccDNA, which was due to the presence of BCP/PC variants reducing HBeAg titre independent of VL. BCP/PC sequencing should be incorporated into research protocols evaluating the utility of HBeAg titres in predicting treatment outcome

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DRUG RESISTANCE MUTATION ANALYSIS BY DIFFERENT METHODS IN A HIGHLY TREATMENT-EXPERIENCED CHRONIC HEPATITIS B PATIENT POPULATION

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Background: Antiviral drug resistance in patients with chronic hepatitis B has become a major drawback, ultimately leading to antiviral treatment failure, subsequent biochemical breakthrough, and hepatic decompensation. To optimally monitor treatment of HBV patients, a highly sensitive assay is required that can detect resistance mutations early. Patients and Methods: 133 adult HBV patients attending the liver clinics of University Health Network (Toronto, Canada) were prospectively monitored for antiviral resistance mutations using INNO-LiPA HBV DR assays (Innogenetics, Belgium) and sequencing. The majority was Asian (78%); HBeAg positive (57%), and had mean HBV DNA upon resistance testing 5.4±2.1 log10 IU/ml. Ninety percent had received lamivudine therapy, 40% adefovir, and a few tenofovir or entecavir. INNO-LiPA HBV DR v2* probes cover mutations at codons 80, 173, 180, 181, 204 and 236; a prototype INNO-LiPA HBV DR v3 strip covers codons 184, 194, 202, 233 and 250. Sequencing and LiPA were performed on the same amplicon. Concordance between the LiPA and sequencing was calculated. Results: Viral loads ranged from 1.2-9.7 log10 IU/ml. All genotypes except F were found, with the majority being genotype C (51.1%). Interpretable LiPA patterns were obtained for 133/133 samples (100%); sequence information for 131/133 samples (98.5%). Thirty-four of the 131 samples (26%) showed no mutations at any one of the 11 codon sites with LiPA or sequencing, whereas 97 samples (74 %) displayed at least one mutation with one or both methods. Of resistance mutations detected by sequencing, concordance with LiPA occurred in 99.2% of cases, while LiPA provided additional information (wild type [wt]/mutant mix) for at least one codon in 66.7% of cases. In more than 1/3 cases, LiPA showed a drug resistance mutation in a wt/mutant mixture versus only wt virus for sequencing. Mutations at codons 180 and 204, associated with lamivudine resistance, were most frequently encountered. Fifty-two patients carried a mutation at codon 180, whereas 76 patients had a known mutation at codon 204 after LiPA analysis. With sequencing, 45 patients (86%) and 70 patients (92%) showed these respective mutations. Conclusions: Both LiPA and sequencing are useful and accurate methods for drug resistance mutation detection. However, LiPA enables detection of minor mutant populations as mixtures earlier than sequencing. Earlier detection of resistance mutations using LiPA followed by prompt salvage therapy could help optimize the management of patients with antiviral-resistant HBV. * In US: For research use only. Not for use in diagnostic procedures.

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RESTORATION OF HBV-SPECIFIC T CELL RESPONSES IN LAMIVUDINE LONG TERM RESPONDER

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Background: Hepatitis B virus (HBV)-specific T cell responses appear to play a pivotal role in infection control and disease outcome. The benefit of Lamivudine (LAM), a nucleoside analogue used for HBV treatment, is compromised by progressively increasing emergence of drug-resistant strains (up to 70% after 5 years). However, 20-30% of patients do not develop resistance despite long-term therapy. While LAM has been shown to induce early but only transient restoration of CD4 and CD8-specific T cell responses, the immunological profile of LAM long-term responders (LAM-LTR) has not yet been analyzed. Aim: To assess the HBV-specific T cell responses in LAM-LTR compared to LAM resistant (LAM-R) and to evaluate if successful long-term LAM therapy restores HBV-specific immunity. Methods: Seven LAM-LTR (male/female: 5/2; median age: 60, range: 42-67), defined by sustained HBV suppression during prolonged LAM administration (median years of treatment: 9, range: 7-11) and 13 LAM-R patients (male/female: 12/1; median age: 52, range: 30-60), defined by occurrence of viral breakthrough, were tested with a panel of overlapping peptides (18-20 mers) spanning the entire HBV sequence. The frequency and magnitude of HBV-specific T cell responses was assessed by an IFN-γ ELISPOT assay after in vitro stimulation with the synthetic HBV peptides. Results: HBV-specific immune responses were detected in all LAM-LTR (100%) and in eight of the 13 subjects with LAM-R (61%). The frequency of HBV-specific T cell responses were significantly higher in LAM-LTR compared to LAM-R (median frequency: 5 vs 1, respectively, p=0.001). Moreover, LAM-LTR presented significantly stronger IFN-γ responses (median: 2'793 vs 1'740 SFC/1,000,000 PBMC, p=0.03). Polymerase-specific immune responses dominated the HBV-specific T cell repertoire in both groups of patients, although LAM-LTR presented multiple responses to all four HBV proteins. Interestingly, two regions, in the core protein and in polymerase, were targeted by 3 LAM-LTR (42%), suggesting two new regions of immunogenicity. Conclusions: LAM-LTR are characterized by a multispecific pattern of HBV specific T cell responses. Moreover, the immune repertoire of responses is stronger in terms of both frequency and magnitude in long term responders than resistant ones. These results can contribute to

better characterize HBV patients with LTR to antiviral therapy and to help for their clinical management.

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TWENTY-FOUR WEEKS THERAPY WITH PEGINTERFERON ALFA-2A IS SIMILAR TO 48 WEEKS THERAPY IN PATIENTS WITH HBEAG POSITIVE CHRONIC HEPATITIS B AND GOOD PREDICTORS OF RESPONSE

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Background: 48 week therapy with Peginterferon alfa-2a has demonstrated to be effective in about one third of patients with HBeAg-positive chronic hepatitis B. Although the recommended treatment duration for these patients is 48 weeks, there is no enough data supporting 48 weeks of therapy over 24 weeks of therapy. Treatment might be shortened particularly in patients with good predictors of response. Aim: To compare the efficacy of 48 weeks vs 24 weeks of therapy with Peginterferon alfa-2a, in patients with chronic hepatitis B who had good predictors of response. Patients and Methods: 19 patients with high baseline ALT levels (> 3 ULN) and low viral load (HBV DNA < 109 cp/ml) were treated with Peginterferon alfa-2a 180 mcg/week, during 48 weeks. Virological, biochemical and serological responses were compared with those obtained in 16 patients with similar baseline characteristics treated with Peginterferon alfa-2a for 24 weeks. All patients had a follow-up period of 24 weeks after end of therapy. Results: At end of follow-up, HBeAg seroconversion was observed in 7/19 (36.8 %) of patients treated for 48 weeks and in 6/16 (37.5 %) of patients treated for 24 weeks (p=ns). Patients treated for 48 weeks evidenced a significantly higher decrease in HBV DNA at the end of therapy than patients treated for 24 weeks (-4.8 logs vs -3.6 logs respectively, p<0.05). However, the % of patients with HBV DNA < 100.000 cp/ml was similar in both groups at the end of follow up (42.1 % vs 43.7 %, ns). No significant differences between both groups were observed regarding ALT normalization, HBsAg loss or seroconversion. Serious adverse events occurred in 1 patient from each group. Conclusion: The results from this study indicate that 24 weeks of therapy with Peginterferon alfa-2a is similar to 48 weeks therapy in patients with HBeAg positive chronic hepatitis B who have good predictors of response.

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LONG-TERM FOLLOW-UP OF HBSAG CLEARANCE IN PATIENTS WITH HBEAG-NEGATIVE CHB TREATED WITH PEGINTERFERON ALFA-2A: INCREASE IN HBSAG CLEARANCE RATE FROM 3% 6 MONTHS POST-TREATMENT TO 8% AFTER 3 YEARS

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Clearance of HBsAg in patients with CHB is associated with long-term clinical outcome and improved survival. It can be induced by treatment with interferon-based therapy but is extremely rare following treatment with direct antivirals. The HBsAg clearance rates 24 weeks post-treatment in patients with HBeAg-negative CHB who were treated for 48 weeks treatment with peginterferon alfa-2a, peginterferon alfa-2a plus lamiviudine or lamivudine alone in a multinational randomized trial were 3%, 4% and 0%, respectively. All patients included in this trial were offered entry into an observational study of long-term response 3 years post-treatment. The proportion of peginterferon alfa-2a-treated patients losing HBsAg increased from 3% 24 weeks post-treatment to 8% at 3 years; 9 patients treated with peginterferon alfa-2a monotherapy cleared HBsAg and 9 in the combination therapy group. Loss of HBsAg was observed across all the major HBV genotypes. Eight of the 18 patients developed anti-HBs antibodies. We analyzed the quantitative on-treatment HBsAg levels using the Architect assay (Abbott Diagnostics) for 198 patients treated with peginterferon alfa-2a +/- lamivudine who were included in the long-term follow-up study for whom 3-year post-treatment follow-up data were available. An HBsAg level <10 IU at week 48 was signficantly associated with HBsAg loss 3 years post-treatment (relative risk [RR] 22.8; 95% CI 8–64.9; p<0.0001). Of the 23 patients with HBsAg <10 IU/mL at week 48, 12 (52%) had cleared HBsAg by year 3 vs 4/171 patients (2.3%) with HBsAg >10 IU/mL at the end of treatment. An on-treatment reduction of >2 log IU/mL from pre-treatment level to week 48 was significantly associated with HBsAg clearance at year 3 (RR 14.6; 95% CI 5.5–38.5; p<0.0001). Of the 26 patients with an on-treatment HBsAg decline of >2 log IU/mL, 11 (42.3%) had HBsAg at year 3 compared with only 5 of 172 patients (2.9%) with an on-treatment HBsAg decline <2 log IU/mL. Despite the fact that at the end of the 48-week treatment period patients who cleared HBsAg had HBV DNA suppressed to levels <400 cp/mL, an HBV DNA level <400 cp/mL was not predictive of HBsAg clearance 3 years post-treatment. In conclusion, the ability to induce HBsAg clearance, an outcome that is associated with long-term improvement in survival, supports the use of peginterferon alfa-2a as a first-line treatment for patients with HBeAg-negative CHB. Potent HBV DNA suppression appears to be required, but is not sufficient, for subsequent clearance of HBsAg. The ability of interferon-based therapy to induce HBsAg clearance may be associated with its dual immunomodulatory and antiviral mode of action.

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VERY HIGH PREVALENCE OF PRECORE (PREC) AND/OR BASAL CORE PROMOTER (BCP) MUTATIONS (MUT) IN HBEAG-POSITIVE (EAG+) AND NEGATIVE (EAG-) CHRONIC HEPATITIS B (CHB) ESPECIALLY AMONG OLDER PATIENTS

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PURPOSE: Relapse is common following anti-HBe seroconversion and discontinuation of therapy for eAg+ CHB. Presence of an eAg- HBV with preC/BCP Mut among patients who "appear" to have eAg+ CHB may be responsible for such treatment failure. Our goal is to examine the prevalence of preC and BCP Mut in eAg+ CHB patients. METHODS: We performed a cross-sectional study of 330 CHB patients with genotypic Mut analysis done between 11/05-5/07 at a U.S. clinic. Mut analysis was performed by Quest Diagnostics, San Juan, CA using a direct sequencing test (test code: 10529x) with reported sensitivity of 25%. RESULTS: A total of 330 cases were identified and included. All but 4 were Asians. Mean age=46±14,61%=male, median ALT=35 (range:7-1070 U/L). Treatment history was as follows: naïve=242 (73.3%), adefovir only=27 (8.2%), lamivudine±adefovir=57 (17.3%), and entecavir=4 (1.2%). Mean HBV DNA was lower in patients with preC/BCP Mut (3.6x107±2.7x108 vs. 1.9x108 ±4.2x108 IU/L, p=0.0002). Distribution of HBV genotypes was as follows: A=4%, B=67%, and C=28%. eAg+ was seen in 38% and eAg- in 62%. Overall prevalence of preC/BCP Mut was 77%: 95% in eAg- and 49% in eAg+. Mut prevalences increase with age (Figure). The majority of preC Mut detected were G1698A (87%) with G1896A/G, G1896G/A, 1839, 1841, 1845, 1874, or mixed in the remaining. The majority of BCP Mut detected were A1762T(A)+G1764A(G,A/G)(90%) with the remaining having single Mut. There were no significant differences PreC/BCP Mut prevalence by gender or treatment groups. CONCLUSIONS: The prevalence of PreC/BCP Mut is 77% and increases with age. PreC/BCP Mut prevalence among eAg+ patients is 49% which also increases with age. Patients with mixed wild-type and preC-BCP Mut may be at higher risk for relapse after anti-HBe seroconversion. Baseline Mut analysis may help identify pts who would benefit from long-term therapy despite anti-HBe seroconversion.

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CLINICAL COVARIATES ASSOCIATED WITH VIROLOGICAL RESPONSE AND ADEFOVIR RESISTANCE IN CHRONIC HEPATITIS B HBEAG NEGATIVE LAMIVUDINE-RESISTANT PATIENTS TREATED WITH ADEFOVIR ALONE OR IN COMBINATION WITH LAMIVUDINE FOR 28 MONTHS

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BACKGROUND. In lamivudine resistance patients (LAM-R) adefovir dipivoxil (ADV) seems to be effective in term of virological (VR) and biochemical response (BR) both in mono or combined therapy with lamivudine (LAM). Clinical covariates of virological response are usually low baseline HBVDNA and alanino amino transferase (ALT) levels. Adefovir associated to LAM in LAM-R patients seems to prevent ADV resistance over time. AIMS. To evaluate the efficacy of ADV administered either alone or in combination with LAM, in HbeAg negative patients with chronic hepatitis B or cirrhosis with clinical LAM-R. To evaluate the clinical covariates associated with a sustained virological response (SVR). To determine the occurrence rates of viral resistance to ADV in patients with ADV monotherapy compared to ADV associated to LAM. METHODS. Seventy HbeAg-negative patients with clinical LAM-R were treated for a median of 28 months (range: 27 to 36 months) with ADV alone [34 patients (Group A)] or ADV in combination with LAM [36 patients (Group B)]. The study endpoints were: undetectable serum HBV-DNA, normalization of ALT levels, and occurence of resistance to adefovir. RESULTS. By month 3, serum HBV-DNA became undetectable in 30 patients (83%) of Group B and in 26 patients (76%) of Group A and in 5 additional patients (14%) of Group B and in 2 additional patients (6%) of Group A by month 12. One patient of Group B (3%) and 6 patients of Group A (17%) maintained detectable HBV-DNA levels and high ALT levels up to the end of the study period. In Group B and in Group A the rates of ALT normalization were, respectively: 56% and 53% at month 3, 83% and 72% at month 6 and 97% and 82% at month 12, persisting up to the end of the study period. Logistic regression analysis showed that pretreatment levels of HBV-DNA <5 log10 UI/mL, ALT levels >150 U.I./L and a fibrosis score < 2 were the strongest covariates independently associated with a SVR in both groups of patients. A primary non response (basal rtA181V) was identified in 2 patients of the group A who maintained detectable HBVDNA and high ALT levels up to the end of the study period. No adefovir resistance was noted in the remain 5 patients. CONCLUSIONS. Adefovir administered either alone or in combination with lamivudine is efficacy in suppressing HBVDNA in HbeAg-negative patients resistant to lamivudine, especially in those with pretreatment low HBV-DNA levels, high ALT levels and low fibrosis score. rtA181V/T resistance to ADV was observed in

clinical isolates from 2 patients of group A undergoing long term LAM treatment. In the other 5 patients with detecteble HBVDNA no ADV major resistant mutations were disclosed

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FACTORS AFFECTING RESPONSE TO ADEFOVIR TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS B AND LAMIVUDINE RESISTANCE

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Background & Aims: Adefovir (ADV) may not always gain control of lamivudine resistant (LAM-R) chronic hepatitis B (CHB). To identify factors that predict adequate ie. undetectable HBV DNA (<60 IU/mL) response to ADV in the treatment of LAM-R CHB. **Methods:** A retrospective study of CHB with genotypic or phenotypic LAM-R treated with ADV 10mg daily in an *Add To* or *Switch To/Add Back Lam* treatment strategy. Adequate response to ADV was defined as undetectable HBV DNA (<200copies/mL or <60IU/mL) at 1 yr. Factors evaluated included eAg status, treatment strategy, pre-treatment (just prior to starting ADV) HBV DNA level (PreTxDNA), whether ADV was added at phenotypic or genotypic resistance, and rapid HBV DNA decline at 6 months, defined as >50% logarithmic decline from PreTxDNA. A multivariate analysis was performed both for all patients and for the subset with cirrhosis. **Results:** Only 45 of 95 (47%) patients achieved undetectable HBV DNA at 1 yr. In multivariate analysis, only PreTxDNA level and >50% decline in HBV DNA at 6 months predicted achieving undetectable HBV DNA at 1 yr. Though patients with a PreTxDNA >7 log IU/mL were only 12% (3% to 43%) as likely to achieve an undetectable HBV DNA at 1 yr compared to patients with HBV DNA <7 log IU/mL, this effect continued below this cutoff. For each 1 log IU/mL increase in PreTxDNA a patient was only 40% (25% to 64%) as likely to attain an undetectable HBV DNA at 1 yr. Patients who at 6 months had >50% logarithmic decline in HBV DNA compared to pre-treatment were 25.4 (3.1 to 206.1) times more likely to reach undetectability at 1 yr. These same factors significantly predicted response at 1 yr when only patients with cirrhosis were evaluated. Three of 21 patients with a <50% decline in HBV DNA at 6 months developed ADV resistance in 1 yr while on ADV. **Conclusions:** ADV should be started at the first emergence of LAM-R when HBV DNA is lowest to maximize the probability of achieving an adequate response. Assessing the 6 month rate of decline in HBV DNA may identify those who should have anti-viral therapy changed earlier.

Predictive Factors For Effective ADV Therapy In LAM-R CHB (Multivariate Analysis)

Predictive Factor	1 Yr All Patients OR (95% CI)	1 Yr Cirrhotics OR (95% CI)
eAg Status	ns	ns
Treatment Strategy	ns	ns
Pheno/Genotypic Resistance	ns	ns
PreTxDNA (>7 vs <7 logIU/mL)	0.12 (0.03-0.43)[1]	0.23 (0.05-1.0)[4]
PreTxDNA (per 1 logIU/mL rise)	0.40 (0.25-0.64)[2]	0.45 (0.26-0.76)[1]
>50% decline at 6 months	25.4 (3.1-206.1)[1]	24.4 (2.4-249.3)[3]

p value: [1]<0.005, [2]0.0001, [3]<0.01, [4]0.05, ns=not significant

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PREDICTORS OF VIROLOGIC RESPONSE AND RESISTANCE TO ADEFOVIR IN PATIENTS WITH LAMIVUDINE-RESISTANT CHRONIC HEPATITIS B VIRUS INFECTION

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Background: Adefovir (ADV) can be used as initial therapy in patients with HBeAg positive or HBeAg-negative chronic hepatitis B or as additional therapy in those with lamivudine (LAM)-resistant HBV. ADV resistance was common in LAM-resistant patients than in treatment-naive patients. We evaluated the virologic response to ADV in patients with LAM-resistant chronic hepatitis B virus infection, and the potential role of primary non-response in prediction of virologic response and resistance. Methods: Liver panel, HBeAg/anti-HBe, HBV DNA PCR (COBAS Amplicor HBV Monitor assay) were measured every 2 or 3 months during ADV treatment. Genotypic mutation was detected using mass spectrometry-based genotyping assay (RFMP). Primary non-response was defined as a decrease in serum HBV DNA by < 2 log10 copies/ml at 24 weeks of therapy. Virologic breakthrough (VBT) was defined as a >1 log10 copies/ml increase in serum HBV DNA from nadir after achieving virologic response. Virologic response was defined as a decrease in serum HBV DNA to undetectable levels by PCR, and loss of HBeAg. Results: 146 HBeAg positive CHB patients with LAM resistance were received ADV for at least 12 months. Mean treatment duration was 25 months (range 12-42). Mean pretreatment HBV DNA and ALT levels were 7.7 log10 copies/ml and 357 IU/L, respectively. 115 patients were chronic hepatitis, 31 were liver cirrhosis. Serum HBV DNA became undetectable and ALT normalized in 59 (44%) and 121 (82.9%), respectively. Thirty-four (23.3%) patients represented primary non-response. Virologic response was archived in 34 (23.3%) patients. The cumulative rates of virologic response were 9, 22, and 40% at 12, 24, and 36 months, respectively. In multivariate analysis, the absence of primary non-response (OR, 3.34; 95% CI, 1.017-10.965; P<0.001), higher pretreatment ALT (OR, 1001; 95% CI, 1.000-1.002; P<0.05), and lower pretreatment HBV DNA (OR, 1000; 95% CI, 1.000-1.000; P<0.05) were independently associated with virologic response. VBT and genotypic ADV resistance were observed in 24 (16.9%) and 18 (12.3%), respectively. The rtA181T, rtN236T, and rtA181V and rtN236T were detected

in 6, 3, and 9, respectively. The cumulative rates of genotypic ADV resistance at 12, 24, and 36 months were 3, 13, and 26%, respectively. In multivariate analysis, the predictive factor associated with VBT was only the presence of primary non-response (OR, 2.46; 95% CI, 1.089-5.547; P<0.05). Conclusions: Primary non-responder to ADV for LAM rescue therapy showed not only lower rate of virologic response but also higher rate of virologic breakthrough than those of primary responder.

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MANAGEMENT OF CHRONIC HEPATITIS B VIRUS (HBV)INFECTION BY PRIMARY CARE PHYSICIANS IN URBAN HOSPITALS AND CLINICS IN NEW YORK CITY

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Current primary care physician practices with respect to the management of persons with chronic HBV infection are largely unknown and comprehensive recommendations directed to primary care physicians are lacking. In this pilot study we examined chronic hepatitis B disease management practices in primary care settings in New York City HHC South Manhattan Network Hospitals and clinics. Analysis was conducted, retrospectively, through electronic medical record review. We present preliminary results for patients 19-49 years of age who first visited primary care clinics in affiliated facilities during January 2005-December 2006. For the purposes of this analysis, chronic HBV infection was defined as a positive test for hepatitis B surface antigen (HBsAg). The minimum adequate evaluation for chronic HBV infection was defined as testing for HBeAg, VL and ALT. Among the 18,457 patients, 42.4% (7,826) were screened for HBsAg. 893 were HBsAg+. The mean age of HBV-infected patients was 31.8 ±7.4 years, 57.8% were men. The HBV-infected patients were overwhelmingly APIs (83.9%), followed by Blacks (7.8%), Hispanics (3.6%), and Whites (0.9%). 97.9% of HBV-infected persons were tested for ALT, 71.0% for HBeAg, 68.2% for viral load, and 63.6% for AFP. The rates of testing for HCVAb, HAVAb, HDVAb and HIV were 52.0%, 47.3%, 3.8% and 22.8%, respectively. Approximately one third of the patients (37.8%) received an abdominal ultrasound exam, 9.9% had a CT scan and 0.6% had a MRI. 564 patients (63.2%) had HBeAg, VL and ALT measurements and 306 patients (34.3%) had all three measurements and an ultrasound exam. 83.1% of those with abnormal ALTs had HBeAg and VL tests, higher than 58.5% among those with normal ALTs, p<0.01. Management practices did not differ by patient sex (p=0.51), race/ethnicity (p=0.07), age (p=0.18) or country of birth (p=0.82). Patients at hospital facilities were more likely to receive the minimum adequate evaluation (68.6%, and 64.9%, respectively), compared to patients at community clinics (45.5%, and 29.3%, respectively), p<0.01. In conclusion, the management of patients with chronic HBV infection by primary care physicians in this study was suboptimal according to practice guidelines established by the AASLD. Additional education and training of primary care physicians in the proper management of these patients needs to be implemented and evaluated in order to decrease the long-term morbidity and mortality of persons chronically infected with HBV.

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HEPATITIS B SURFACE ANTIGEN TITER WAS DECREASED DURING CLEVUDINE THERAPY

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Background: Clevudine therapy in chronic HBV patients resulted in a significant reduction of serum HBV DNA level. The purpose of this abstract is to determine if reduction of HBV DNA with clevudine therapy may lead to decrease of serum hepatitis B surface antigen (HBsAg). Methods: A total of 50 naïve patients with chronic hepatitis B received clevudine 30 mg daily for 24 weeks followed by clevudine 10 mg daily for another 24 weeks. Serum HBV DNA was determined by Digene hybrid capture II assay (limit of detection of 4700 copies/mL) and Amplicor PCR assay (limit of detection of 300 copies/ml). Serum HBsAg was quantified by the ARCHITECT HBsAg assay. Results: Based on the level of HBsAg titer at baseline, patients were stratified into four groups: group I (n = 18), < 10,000 IU/mL; group II (n = 15), 10,000 – 20,000; group III (n = 9), 20,000 – 40,000; group IV (n = 8), > 40,000). Median baseline HBV DNA were 6.1, 7.1, 8.1 and 9.0 log10 copies/mL and baseline HBsAg levels were 2922, 14577, 25200 and 102605 IU/mL in the group I, II, III and IV, respectively. During first 24 weeks of clevudine treatment, HBsAg titers of group II, III and IV except group I showed a rapid decrease which showed the similar pattern of HBV DNA suppression. At the end of 1-year treatment, median HBV DNA reduction from baseline was 3.6, 4.6, 5.6 and 4.9 log10 copies/mL and the change of HBsAg level from baseline of 1061, -2887, -5573, and –83909 IU/mL in the group I, II, III, and IV, respectively. Particularly, median serum HBsAg level in group IV at week 48 dropped to 18.2% of pretreatment level. Combined data of HBsAg reduction from group II, group III and group IV at week 48 showed statistically significant decrease (P = 0.002). Con-

clusion: Strong viral suppression with clevudine therapy led to a significant decrease in serum HBsAg titer in patients who had more than 10,000 IU/mL of HBsAg titer before treatment.

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A HEPATITIS B VIRUS MUTANT IMPLICATED IN NON-RESPONSE TO SEQUENTIAL TREATMENT WITH ADEFOVIR, LAMIVUDINE AND ENTECAVIR SHOWS CROSS-RESISTANCE TO MULTIPLE ANTIVIRAL AGENTS

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Background: Development of safe and effective nucleos(t)ide analogue reverse transcriptase inhibitors (NRTI) has revolutionised chemotherapy for chronic hepatitis B (CHB), but prolonged treatment engenders viral resistance and sequential treatment with different NRTI can promote the emergence of multi-drug resistant mutants, which are refractory to continuing therapy and may be cross-resistant to new drugs. Consequently, control of multi-drug resistant HBV is becoming a major challenge. We describe the phenotype of an HBV mutant isolated from a patient with CHB who responded poorly to sequential treatments with adefovir, lamivudine, and entecavir. Isolates from this patient were genotype D and HBeAg negative due to the presence of the precore [pc] G1896A mutation and encoded multiple substitutions in the polymerase region including rtA181T, rtI233V, rtN236T and rtM250L. Cloning established that these were encoded on a single genome. Aims: To determine antiviral drug resistance phenotype of the putative multi-drug resistant rtA181T/I233V/N236T/M250L mutant, and determine how rtI233V and rtM250L influence phenotype. Methods: Mutant HBV clones encoding rtI233V, rtM250L, rtA181T/N236T/M250L and rtA181T/I233V/N236T/M250L were generated by site-directed mutagenesis from a wild type (WT) genotype D HBV isolate. WT and derived clones harboured the [pc] G1896A mutation, which may increase replication efficiency but does not significantly affect drug resistance. The phenotypes were determined by comparing viral replication in transfected Huh-7 cells. Virus replication and its inhibition were monitored by measuring changes in the amounts of intracellular core-associated viral DNA in cell lysates using a commercial assay kit (Bayer Versant HBV 3.0). Results: The rtA181T/I233V/N236T/M250L substitutions in combination imparted a severe replication defect but conferred high level resistance to all L-nucleosides (lamivudine, telbivudine, clevudine and emtricitabine) as well as high level resistance to the deoxyguanosine analogues diaminopurine, 2′,3′-dideoxy-3′-fluoroguanosine and abacavir, and conferred lower level, but still significant, resistance to adefovir and tenofovir. The rtI233V and M250L substitutions in isolation did not confer significant drug resistance and significantly reduced replication capacity in the absence of selection pressure, but presumably act to compensate for the replication defects associated with acquisition of drug resistance. Conclusions: Sequential treatment with NRTI promotes multi-drug resistance. It is likely that development of multi-drug resistance could be reduced by combination therapy optimised to individual viral phenotypes.

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COST-EFFECTIVENESS OF NEW TREATMENT PARADIGMS FOR E-AG NEGATIVE CHRONIC HEPATITIS B

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BACKGROUND: A number of new drugs for chronic hepatitis B have become available in recent years, and several studies show that treatment with either interferon or the oral nucleos(t)ide analogs is cost-effective compared with no treatment. However, the cost-effectiveness of recent treatment algorithms has not been evaluated. METHODS: We considered the base case of a forty year old patient with newly-diagnosed, treatment-naïve, e-Ag negative chronic hepatitis B. Using a decision analysis model, we examined seven treatment strategies: (1) no treatment (natural history arm), (2) Adefovir (ADV) with switch to entecavir (ETV) upon emergence of resistance (3) ETV with switch to ADV upon emergence of resistance, (4) ADV with addition of lamivudine (LAM) upon emergence of resistance, (5) ETV with addition of ADV upon emergence of resistance, (6) ADV with addition of ETV upon emergence of resistance, and (7) Peginterferon alfa-2a for one year. We examined the cost-effectiveness of each option from a third-party payor perspective, using a sixty year time horizon and an annual discount rate of 3%. We then separately considered the case of a similar patient with pre-existing LAM resistance. RESULTS: All treatment options yielded more quality-adjusted life years (QALY's) than the "no treatment" arm. The oral agents demonstrated extended dominance over pegylated interferon. For treatment-naïve patients, the most cost-effective option was initial ADV with switch to ETV upon emergence of resistance, yielding an incremental cost effectiveness ratio (ICER) of $6,697 over the next most cost-effective strategy. Among the "add-on" regimens, initial ETV with addition of ADV was a dominant strategy, giving the highest number of QALY's at the lowest cost. For patients with pre-existing LAM resistance, adding ADV was preferred over switching to ADV or ETV, with an ICER of $37,118 per QALY gained. CONCLUSION: New treatment recommendations for the treatment of e-Ag negative chronic hepatitis B are cost-effective. Initial ADV with switch to ETV upon emergence of ADV resistance is the most cost-effective treatment strategy. Initial ETV with ADV add-on is superior to other "add-on" strategies due to a relatively longer allowable period of monotherapy. In patients who have already developed LAM resistance, adding ADV is cost-effective compared with switching to ADV or ETV, due to a lower rate of subsequent resistance.

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SCREENING FOR HEPATITIS B IN CHEMOTHERAPY PATIENTS: SURVEY OF CURRENT ONCOLOGY PRACTICES

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Background: Hepatitis B virus (HBV) reactivation occurs in up to 78% of patients receiving cytotoxic chemotherapy for non-hepatic malignancies. Consequences of reactivation include hepatic dysfunction, jaundice, and fulminant hepatic failure. Patients with hepatic failure due to reactivation are generally precluded from liver transplantation secondary to underlying malignancy and antiviral therapy may be ineffective in reducing liver injury during chemotherapy induced flares. Current recommendations to prevent chemotherapy induced flares include screening patients at risk for HBV prior to immunosuppressive therapy and providing a short course of lamivudine in patients with chronic HBV as pre-emptive therapy against reactivation. Current practice among oncologists is unknown in regard to screening and prophylaxis strategies for HBV. Aims: To assess the practice of oncologists in screening and management of HBV prior to initiating chemotherapy. Methods: A survey was sent to AMA registered oncologists in the United States assessing practice demographics and HBV screening practices. Fischer's exact test was used for analysis. Results: Responses were obtained from 265 individuals: 38% (n=101) from an office-based practice, 55% (n=145) from a university/academic/research institution, and 6% (n=17) from other practice setting. Office-based physicians were less likely to screen for HBV prior to chemotherapy (p<0.001). Years in practice varied: 51% with <5 years (n=136), 29% with 5-15 years (n=77), and 18% with >15 years (n=49). No difference was found in screening practices based on years of experience (p=NS). Surveyors screen for HBV as follows: never- 20% (n=54), abnormal liver tests- 30% (n=80), risk factors or history of hepatitis- 38% (n=101), randomly- 11% (n=28), always- 13% (n=35). Most oncologists used HB surface antigen as a screening test (76%), in conjunction with surface antibody (64%), and core antibody (46%). In pts with HBV, 75% (n= 200) of oncologists refer to specialists and 7% (n=18) initiate therapy themselves, while 15% (n=39) do not refer or initiate therapy, most of whom are in an office setting (p=0.02). Conclusions: 20% of oncologists never screen for HBV prior to initiating chemotherapy, and fewer than 40% screen even if patients have risk factors or a history of hepatitis. Screening practices were not influenced by years in practice, however office-based physicians were less likely to screen, treat, or refer to a specialist prior to chemotherapy. Greater education and awareness regarding risk of HBV reactivation is needed for clinicians treating patients with immunosuppressive therapies.

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PROFILES OF HBV DNA, ALT AND HBEAG STATUS AFTER STOPPING LAMIVUDINE IN PATIENTS WITH HBEAG SEROCONVERSION

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Aim: To determine the virological and biochemical relapse rates in Chinese CHB patients with lamivudine-induced HBeAg seroconversion after stopping lamivudine therapy, and to identify significant viral or biochemical factors predictive of subsequent relapse. Methods: All patients from all Hepatology Clinic with lamivudine-induced HBeAg seroconversion with subsequent cessation of lamivudine therapy were recruited. Liver biochemistry, HBeAg, anti-HBe, and serum HBV DNA by PCR (COBAS Taqman) was determined at the time of stopping lamivudine, at 3 months, 6 months, 1 year, and yearly thereafter. Results: Twenty-two patients were included, of which 16(73%) were male with a median age of 32 years (range, 21-55). The median duration of follow-up was 104 months (range, 31-150). Six (27%) patients had undetectable HBV DNA by PCR at the time of stopping lamivudine treatment. In total, 7(32%) patients had subsequent flare (as defined by ALT >2x upper limit of normal), with associated virological rebound of HBV DNA (as defined by 1 log increase from time of stopping therapy). The cumulative incidence of flare at 5 years was 44%, all occurring before 25 months. There was no significant difference in the cumulative incidence of flares between those patients that continued lamivudine for 6-12 months, 1-2 years and over 2 years after HBeAg seroconversion (p=0.58). In patients who had normal ALT and undetectable HBV DNA, their cumulative incidence of flare was not significantly different to those that had detectable HBV DNA or abnormal ALT at time of stopping lamivudine (p=0.73). Two patients underwent HBeAg seroreversion at 5 and 10 months, with resulting flares. The cumulative incidence of HBeAg seroreversion after stopping lamivudine was 9% at 5 years. Fourteen (64%) patients underwent virological rebound, with a cumulative incidence of 82% at 4 years. There was no significant difference in the cumulative incidence of virological rebound in patients with undetectable HBV DNA at the time of stopping lamivudine therapy compared to those with detectable HBV DNA. Of those with virological rebound, 8(57%) had HBV DNA $>10^5$ copies/ml. Neither patient age or gender, genotype, presence of precore or core promoter mutations were significant factors in subsequent flare or virological rebound after stopping lamivudine therapy. Conclusion: Despite the high sustained HBeAg seroconversion (81%) after stopping lamivudine, HBV DNA rebound occurred in 64%, of which 57% had HBV DNA $>10^5$ copies/ml and 32% had ALT flares. Patients who had both normal ALT and undetectable HBV DNA by PCR at the time of stopping lamivudine therapy did not have a lower cumulative incidence of flares.

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REDUCTION IN SERUM HBSAG LEVEL IN PATIENTS WITH CHRONIC HEPATITIS B INFECTED WITH GENOTYPE D INDUCED BY (PEGYLATED)INTERFERON ALFA-2A ALONE OR IN COMBINATION WITH NUCLEOS(T)IDE ANALOGS: A LONG-TERM SINGLE CENTRE COHORT STUDY

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In patients with CHB undergoing antiviral therapy, clearance of serum HBsAg appears to be associated with better long-term clinical outcome. In clinical practice quantification of serum HBsAg might provide a valuable tool to identify the best therapeutic strategy for individual patients. We quantified serum HBsAg (Architect, Abbott Diagnostics) in a total of 80 consecutive HBV genotype D patients treated at our chronic viral hepatitis reference center. The patients received interferon [IFN] alfa-2a (recombinant or pegylated) monotherapy (11 patients), PEG-IFNalfa-2a + lamivudine [LMV] (24 patients), LMV or adefovir [ADV] monotherapy (6 patients) or LMV + ADV (39 patients). Of the 80 patients, 45 were resistant to LMV; we treated 39 of these with LMV + ADV and 6 with with LMV + PEG-IFNalfa-2a). Baseline characteristics such as gender, HBeAg, viral load and ALT level were not significantly different between the 2 groups of patients receiving (PEG)-IFN +/- nucleos(t)ide analogs (NA) or NAs alone. Patients treated with NAs had lower HBsAg baseline levels (mean 3.39 vs 3.77 log IU/mL, p=0.025) and were older (mean 57 vs 45 years, p<0.001). Patients were followed up for a mean duration of 40 months (24–84 months). The overall serum HBsAg delta variations (log decline from baseline to last observation) according to treatment group are reported in the Table. Almost all patients treated with NAs alone had no or minimal reduction in HBsAg level and none of these cleared HBsAg during follow-up. In contrast, the majority of patients who received interferon-based therapy showed a reduction in HBsAg level of at least 0.5 log and 4 patients achieved HBsAg clearance. In conclusion, IFNalfa-2a (conventional or pegylated) appears to be most effective in suppressing serum HBsAg independently of baseline host and virologic features, also in patients who are resistant to LMV therapy. Quantification of serum HBsAg could provide a useful tool to predict the long-term outcome of interferon-based therapy and its ability to induce HBsAg clearance in CHB patients infected with genotype D.

Reduction in HBsAg level (log)	<0.5 n (%)	>=0.5 n (%)	>=1 n (%)	>=2 n (%)	>=3 n (%)	HBsAg loss n (%)
(PEG)-IFN +/- NA n=35	17 (48.6%)	18 (51.4%)	8 (22.9%)	8 (22.9%)	5 (14.3%)	4* (11.4%)
NA alone n=45	33 (73.3%)	12 (26.7%)	4 (8.9%)	1 (2.2%)	0	0
Total n=80	50	30	12	9	5	4

*1 patient cleared HBsAg during PEG-IFNalfa-2a rescue therapy for LMV resistance.

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HIGH RATES OF HBSAG SEROCONVERSION IN CHRONIC HEPATITIS B PATIENTS RESPONDING TO INTERFERON THERAPY: A LONG TERM FOLLOW-UP STUDY

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Background and Aim Loss of HBsAg with seroconversion to anti-HBs is the most desired treatment endpoint in chronic hepatitis B (CHB), and can be considered a complete response indicating resolution of hepatitis B. The difficulty is that HBsAg loss is rare, occurring spontaneously in less than 1% per year in inactive carriers and in patients receiving a one-year course of nucleos(t)ide analogue therapy. HBsAg loss is more frequent with interferon based therapy, occurring in up to 8% of patients three years post-treatment. The aim of this study was to assess the long term HBsAg response in a cohort of HBeAg-positive CHB patients treated with interferon. Patients and Methods All consecutive patients with HBeAg-positive CHB treated with conventional interferon between 1987 and 2000 were retrospectively evaluated. There were 97 patients. Sustained virological response (SVR) was defined as persistent HBeAg seroconversion and undetectable HBV DNA in serum 24 months after treatment discontinuation and during the follow-up period. Cumulative incidence of HBsAg seroconversion was assessed during the follow-up period and compared between patients with and without SVR. Results The baseline characteristics of the study population were: male gender (88%), mean age (39±13 years), mean ALT level 4.5±3.5 ULN, mean serum HBV DNA level 7.3±0.9 log10 copies/ml. Liver histology showed moderate-severe necroinflammation (METAVIR A2-A3) in 42%, and severe fibrosis (METAVIR F3-F4) in 44 % of patients. SVR was achieved in 25 patients (26%). At multivariate analysis, SVR was independently associated with age > 45 years (p= 0.008, OR=4.6) and serum HBV DNA level < 6 log10 copies/ml (p=0.002, OR=9.4). During a median follow-up period of 11 years (4-20), HBsAg seroconversion developed in 28 patients (29%), and was significantly more frequent in patients who achieved SVR than in those who failed interferon treatment (64% vs. 17%, p<0.0001). The cumulative incidence of HBsAg seroconversion was significantly higher in patients who developed SVR compared with those who failed interferon treatment during the whole follow-up period (40% vs. 8% at year 5, and 60% vs. 18% at year 10 and 80% vs. 30% at year 15). Five patients developed hepatocellular carcinoma (HCC) after a median period of 7 years (5-12) post interferon treatment. None of these patients had developed HBsAg seroconversion (p=0.02). Conclusion Patients with HBeAg-positive CHB achieved a 26% SVR rate following treatment with conventional interferon. Long-term follow-up showed that SVR was associated with a very high rate of HBsAg seroconversion (60% at 10 years) and a significant decrease in the incidence of HCC.

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HBSAG SEROCONVERSION: DIFFERENT KINETICS OF SERUM HBSAG LEVEL DECREASE IN INACTIVE CARRIERS AND CHRONIC HEPATITIS B PATIENTS TREATED WITH INTERFERON

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Background and Aim HBsAg is used typically as a qualitative serological marker for diagnosing an ongoing HBV infection. HBsAg clearance is the most desired treatment endpoint in hepatitis B and considered as resolution of disease. Recently, a quantitative, fully automated chemiluminescent microparticle immunoassay (Abbott Architect HBsAg QT) has become available for the quantitation of HBsAg at a wide range of concentrations. The aim of this study was to assess the kinetics of HBsAg level decrease in serum of patients who have cleared HBsAg spontaneously or following interferon therapy. Patients and Methods 22 HBV-infected patients who have cleared HBsAg have been retrospectively studied. Five patients were inactive carriers while 17 have been successfully treated with interferon. HBsAg clearance occurred after a median period of 6 years (2-14) after treatment discontinuation. Serum samples collected at yearly interval up to four years prior to HBsAg clearance and adequately kept frozen were retrospectively analysed. Samples were diluted 1:20 and 1:500 with the Architect HBsAg diluent in order to expand the upper limit of the dynamic range up to 125000 IU/ml. Results Inactive carriers had significantly lower HBsAg levels than interferon-treated patients ($p<0.05$),4 years (1.6 ± 0.4 vs. 2.7 ± 0.8 log10 IU/ml), 3 years (1.3 ± 0.4 vs. 2.4 ± 0.9 log10 IU/ml), 2 years (0.8 ± 0.7 vs. 1.9 ± 0.9 log10 IU/ml) and 1 year (0.3 ± 0.5 vs. 0.9 ± 0.8 log10 IU/ml)before HBsAg clearance. Inactive carriers and interferon-treated patients had similar kinetics of decrease of HBsAg level in serum 4 years (0.5 ± 0.2 vs. 0.4 ± 0.3 log10 IU/ml) and 3 years (0.7 ± 0.3 vs. 0.7 ± 0.6 log10 IU/ml) prior to HBsAg clearance. However, interferon-treated patients showed a more rapid decline in HBsAg serum level compared to inactive carriers in the last 2 years prior to HBsAg clearance ($p<0.05$): year 2 (0.8 ± 0.5 vs. 0.5 ± 0.2 log10 IU/ml)and year 1 (0.9 ± 0.8 vs. 0.3 ± 0.5 log10 IU/ml)(figure). Conclusion These preliminary data suggest that Abbott Architect HBsAg QT is an effective assay for HBsAg quantitation in serum at wide range of concentrations. These data also suggest that interferon treatment accelerates HBsAg clearance. Further studies are ongoing to explore the clinical relevance of HBsAg quantitation for the monitoring of patients treated either with interferon or analogues.

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HIGH PREVALENCE OF SIGNIFICANT HISTOLOGIC DISEASE IN PATIENTS WITH CHRONIC HEPATITIS B (CHB) AND NORMAL ALT

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PURPOSE: Current guidelines recommend therapy for CHB with elevated ALT. However, many patients presenting with "normal" ALT may not have persistently normal levels on f/u. Our goal was to define the spectrum of histologic findings in such patients. METHODS: We performed a retrospective cohort study of all patients with active CHB (HBV DNA≥10000 copies/mL) & ALT<UNL during liver biopsy at a GI clinic. Significant histology was stage II or grade 2+stage I or higher. RESULTS: Of the 136 potential cases, we excluded 4 with +anti-HCV and 3 with significant EtOH use and included 129 cases. Mean age=45±11, mean weight =62±14 kg, 65%=male, 28%=smokers. 6% had DM, 40% had hyperlipidemia, 18% had FH of liver CA/liver-related death. All were Asian: 58% were HBeAg-. At biopsy, mean HBV DNA=6.2±1.9 log10 copies/mL and mean ALT=45±18 U/L. Average number of ALT levels per patient =4.1±2.5 over a median f/u of 17 months (range:1-113), when 38% had at least one ALT>UNL. Mild steatosis was seen in 28% and moderate in 11% but this finding was not a significant predictor. The overall prevalence of significant histology was 43.4% & increased with age (Figure): 31% in those with persistently normal ALT and 63% in those without. On univariate analysis, predictors of significant histology were age, weight, HBeAg negativity, & abnormal ALT on f/u. On multivariate analysis, predictors were elevated ALT (OR=5.1, $p<0.0001$) and age (OR=5.2, $p=0.037$ for age 35-49; OR=11.2, $p=0.005$ for age 50-64; & OR=28.0, $p=0.007$ for age≥65). CONCLUSION: Only 62% of patients with normal ALT at evaluation have persistently normal levels on follow-up, a third of whom can still have significant histology. Older age starting at 35 is the strongest independent predictors of significant histology. Asian patients with normal ALT should be followed closely and histologic evaluation should be considered if patients are ≥ 35 or if ALT becomes elevated.

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BASELINE PARAMETERS PREDICT BOTH EARLY VIROLOGIC RESPONSE AND LONGER TERM OUTCOMES FOR TELBIVUDINE-TREATED PATIENTS WITH CHRONIC HEPATITIS B (THE GLOBE STUDY)

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Background: Viral load at 24 weeks of telbivudine treatment has been linked to efficacy outcomes after 1 and 2 years. Studies have shown that pretreatment, elevated ALT and low HBV DNA levels predict higher responses to interferon, however few corresponding data are available for nucleos(t)ide therapies for hepatitis B. Here we report that baseline disease variables predict virologic responses to telbivudine at 24 weeks, which in turn predict efficacy outcomes at 2 years. Methods: The GLOBE trial enrolled 1,367 HBeAg+ and HBeAg- hepatitis B patients with HBV DNA >6 log10 copies/mL, ALT 1.3-10 xULN, and compensated liver disease, randomized to receive telbivudine or lamivudine for 2 years. The influence of baseline parameters and early HBV DNA suppression on subsequent efficacy outcomes and resistance were evaluated by univariate and multivariate regression analyses. Results: Pretreatment serum HBV DNA and ALT levels were significant predictors of virologic response at Week 24 and of efficacy outcomes and viral breakthrough at Week 104. Among HBeAg+ telbivudine recipients with ALT ≥2 xULN and HBV DNA <9 log10 copies/mL at baseline, 47% and 14% experienced HBeAg seroconversion and viral breakthrough at Week 104, respectively, vs. 30% and 29% among all HBeAg+ telbivudine recipients. 71% of patients in this subset were PCR-negative after 24 weeks of telbivudine, vs. 44% of HBeAg+ telbivudine recipients overall. HBV genotype had minor, generally nonsignificant effects on outcomes. Similar relationships were observed in HBeAg- patients with elevated ALT and serum HBV DNA <7 log10 copies/mL at baseline, however due to the higher overall rate of viral clearance in this group, baseline parameters had a less pronounced effect on virologic responses at Weeks 24 and 104, compared with HBeAg+ patients. After initiation of treatment, multivariate analyses showed a diminished impact of baseline parameters on subsequent outcomes, and confirmed viral load at Week 24 as the most significant predictor of outcomes at Week 104. Among patients with HBV DNA <9 log10 copies/mL and elevated ALT at baseline, PCR negativity at Week 24 resulted in 52% HBeAg seroconversion and 3.6% viral breakthrough at 2 years. Conclusions: High rates of efficacy responses after 2 years of telbivudine, and low resistance, occurred in patients with elevated ALT and HBV DNA <9 log10 (HBeAg+ patients) or <7 log10 (HBeAg patients) copies/mL pretreatment, coupled with optimal viral load reduction at Week 24. These results define patient characteristics associated with optimal responses to telbivudine and may contribute to on-treatment patient management strategies.

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ON-TREATMENT VIROLOGIC SUPPRESSION AT WEEK 24 DECREASES THE RISK OF HISTOLOGIC PROGRESSION AT 1 YEAR; DATA FROM THE GLOBE TRIAL

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Background: Serum HBV DNA and ALT level have been correlated with active liver disease in patients with chronic hepatitis B (CHB). However, on-treatment predictors of liver disease progression have been inadequately studied. Objective: To identify on-treatment predictors of worsening of HBV-related liver disease. Methods: The GLOBE trial enrolled 1367 compensated CHB patients with baseline HBV DNA >6 log10 copies/mL, ALT 1.3-10 xULN. Patients were randomized 1:1 to treatment [telbivudine (600 mg/d PO) or lamivudine (100 mg/d PO)] and stratified by HBeAg status and baseline ALT level. Predictors of histologic progression at Week 52 (W52) were evaluated using baseline (BL) and 24-week (W24) data from the GLOBE trial. Progressors (P) exhibited [1-unit increase in Ishak Fibrosis Score (IFS)] OR [2-unit increase in Knodell Necroinflammatory Score (KNS) with no decrease in IFS] at W52. All other patients were deemed non-progressors (NP). Stepwise logistic regression analysis of data from 549 telbivudine patients and 535 lamivudine patients compared P (n=142) to NP (n=942) after 52 weeks of treatment; p-values and odds ratios of being P at W52 were calculated. Factors were entered into the model using α=0.25, and retained in the model using α=0.1. BL factors included IFS, KNS, viral load, e-antigen status, genotype, ALT, BMI, age, gender, and treatment assignment. On-treatment factors were viral load reduction, ALT decline, and PCR negativity (all at week 24). Results: BL IFS (OR=5.0) and KNS (OR=2.7) were the only significant factors predicting P (p <0.0001 for both), with lower scores being more predictive of P than higher scores, possibly due to higher IFS and KNS responding better to antiviral treatment. The only significant on-treatment predictor of P ($p \leq 0.05$) was failing to achieve a decline in HBV DNA of >5 log10 copies/mL by W24 (p=0.0058), which increased the likelihood of P by 57%. Genotype, baseline BMI and treatment arm were retained in the model but were not significant at p =0.05. However, significantly more telbivudine-treated patients achieved ≥ 5 log10 decline in HBV DNA compared with lamivudine (69% vs. 55%, p<0.05). Conclusion: Although baseline histology status was the most important predictor of disease progression at 1 year, the only significant on-treatment factor found to reduce the likelihood of progression was attaining HBV DNA decline >5 log by W24. These data suggest that using a more potent antiviral agent can result in histologic improvements over periods as short as one year. Longer term studies are needed to evaluate the effects over time. Acknowledgment: D Walczak, Cayuga Consulting & V Mats, Quartesian LLC

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IMPACT OF ADEFOVIR DIPIVOXIL ON LIVER FIBROSIS AND ACTIVITY ASSESSED WITH FIBROTEST-ACTITEST IN PATIENTS INFECTED BY HEPATITIS B VIRUS

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Background: The aim was to assess the usefulness FibroTest-ActiTest (FT-AT) as surrogate markers of histological features in patients with chronic hepatitis B based on a correlation analysis with biopsy data in pivotal studies with adefovir dipivoxil. Methods: Patients with chronic hepatitis B randomized in two pivotal trials of adefovir versus placebo, with available paired liver biopsy and FT-AT at baseline and after 48 weeks of treatment were included. Fibrosis and Activity were assessed blindly according to ISHAK scoring system. Diagnostic value of FT-AT was assessed using the area under the receiver operating characteristics curves (AUROCs) for the diagnosis of bridging fibrosis, cirrhosis, and moderate-severe necro-inflammatory activity; sensitivity analyses take into account race, length of biopsy, HBeAg status and sample date. The impact of treatment versus placebo was assessed on liver injury according to baseline stage, and virological response at 48 weeks. For baseline discordant cases, 48 weeks repeated estimates in adefovir virological responders and placebo non-responders permitted to estimates the ratio due to FT-AT or biopsy failures. Findings: 462 patients were included, 304 treated with adefovir and 158 received placebo with paired FT-AT and biopsies. The analysis of 924 estimates showed for the diagnosis of bridging fibrosis, cirrhosis and moderate or severe necro-inflammatory activity very significant FT-AT AUROCs: 0.76±0.02, 0.81±0.02 and 0.80±0.01 respectively. Similar impacts on liver fibrosis and activity were observed both with biopsy and FT-AT, with greater efficacy in patients with baseline advanced fibrosis, treated with adefovir and virological responders. The discordances analyses suggested that 66% of discordances were attributable to biopsy failure and 34% to FT-AT failure. Conclusion: In patients with chronic hepatitis B, FibroTest-ActiTest is a simple and non-invasive quantitative estimate of liver fibrosis and necrotico-inflammatory activity, which could be used as a surrogate marker to reduce the need for liver biopsy.

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LONG-TERM FOLLOW-UP OF ENTECAVIR TREATED PROTOCOL-DEFINED NON-RESPONDERS IN ROLLOVER STUDY ETV-901

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Background: Entecavir's (ETV) efficacy in nucleoside naïve HBeAg(+) [ETV-022] and HBeAg(-) [ETV-027] patients with chronic hepatitis B (CHB) has been demonstrated.[1,2] Protocol-defined patient management criteria dictated that *'Non-Responders'* in ETV-022 and ETV-027 discontinued study therapy. However, *'Non-Responders'* who enrolled in rollover study ETV-901 continued to be monitored and are reported here. **Methods:** 679 nucleoside-naïve HBeAg(+) and HBeAg(-) patients were enrolled and treated with ETV in studies ETV-022 and ETV-027. Patients who failed to achieve HBV DNA <0.7 MEq/mL by bDNA at week 48 or during year-2 were classified as *'Non-Responders'*, discontinued study and were offered continued entecavir (1 mg) treatment in rollover study ETV-901 or at the discretion of the investigator, alternative off-study anti-HBV therapy. The proportions of patients with HBV DNA <300 copies/mL by PCR assay, ALT normalization and HBeAg seroconversion were assessed during long-term follow-up in ETV-901. **Results:** At 48 weeks, 19 HBeAg(+) and 3 HBeAg(-) ETV-treated patients were classified as 'Non-Responders' (3%). Among patients treated during year-2, 8 additional HBeAg(+) patients became *'Non-Responders'* (8/269 [3%]). Twenty-one HBeAg(+) patients from ETV-022 enrolled in ETV-901. Median HBV DNA and ALT at entry into ETV-901 were 3.3 $\log_{10}$ copies/mL and 37 IU/L respectively. The table below shows proportions of patients who achieved HBV DNA <300 copies/mL by PCR, ALT normalization and HBeAg seroconversion as well as the proportions of patients who maintained these endpoints at their last observation. Four patients experienced a virologic breakthrough. However, genotypic and phenotypic analysis failed to show any evidence of resistance. No patients discontinued due to adverse events. **Conclusions:** Fewer than 5% (30/679) of nucleoside-naïve HBeAg(+) and HBeAg(-) patients treated in ETV-022 and ETV-027 met protocol-defined criteria of *'Non-Response'*. Substantial proportions of *'Non-Responders'* subsequently achieved undetectable HBV DNA and ALT normalization in ETV-901. Approximately one third of patients also experienced HBeAg seroconversion. **References** 1. Chang TT, et al. NEJM 2006;354:1001-1010. 2. Lai CL, et al. NEJM 2006; 354:1011-1020

Endpoint	Achieved endpoint in ETV-901	Maintained endpoint at last observation
HBV DNA < 300 copies/mL, n (%)	15/21 (71%)	12/21 (57%)
ALT normalization, n (%)	20/21 (95%)	13/21 (62%)
HBeAg seroconversion*, n (%)	7/21 (33%)	7/21 (33%)

*serology tests were performed by local laboratories

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FACTORS AFFECTING INITIAL VIROLOGIC RESPONSE AND EMERGENCE OF RESISTANT MUTANTS AFTER ADEFOVIR TREATMENT IN LAMIVUDINE RESISTANT CHRONIC HEPATITIS B PATIENTS

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Background : Adefovir dipivoxil effectively inhibits both wild-type and lamivudine resistant hepatitis B virus (HBV) replication. The development of adefovir resistance is delayed and infrequent compared with lamivudine resistance. Aims : The aim of this study was to characterize the serologic, biochemical and virologic response to adefovir, and to explore the factors affecting initial virologic response (IVR) and adefovir resistance in lamivudine resistant HBV infected patients. Methods : Between March 2004 and December 2006, 76 patients with lamivudine resistance who received adefovir for more than 12 months were included. Assay of adefovir resistant mutant was performed at 6 months and 12 months during adefovir administration. Restriction fragment mass polymorphism analysis was used for YMDD mutant and adefovir mutant detection. Results : After adefovir administration, IVR was observed in 31% patients with lamivudine resistance. Factors associated with IVR were HBeAg negativity(p=0.04), higher pretreatment ALT(p=0.05) / AST (p=0.05) and presence of liver cirrhosis(p=0.04). Age, sex, pretreatment HBV DNA levels, presence of precore mutation and types of YMDD mutants were not related to IVR after adefovir treatment. The prevalence of adefovir resistance was 5% at 6 months and 13% at 12 months after therapy (rtA181T in 6 cases, rtA181V in 3 cases). Mixed infection of precore mutant (TGG+TAG) were risk factors for emergence of adefovir resistance(p=0.01). Conclusion : Patients having HBeAg negativity, high ALT/AST and liver cirrhosis were more likely to achieve initial virologic response after adefovir therapy in lamivudine resistant HBV patients. Adefovir resistance was associated with mixed infection of precore mutant.

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PROGNOSTIC FACTORS THAT ASSOCIATED WITH HEPATITIS B VIRUS DNA BREAKTHROUGH IN CHRONIC HEPATITIS B PATIENTS WITH LAMIVUDINE TREATMENT

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Objective: To early determine the prognostic factors associated with hepatitis B virus DNA breakthrough in chronic hepatitis B patients with lamivudine treatment. Methods: Clinical data, YMDD genotyping, HBV DNA loads, ALT level, HBeAg titer and lamivudine-resistant HBV mutant ratio, before and during therapy collected from 64 patients throughout lamivudine treatment 48 weeks were retrospectively analyzed by using non-parametric test and logistic regression model test, HBV DNA viral loads were determined by real-time PCR, ALT level were determined by automatic biochemistry analysis system, HBeAg titer were measured by microparticle enzyme immunoassay, and the lamivudine-resistant HBV ratio were determined by Pyrosequencing. Results: the ratio of YMDD and rtL180M mutants were significantly higher in patients who developed HBV DNA breakthrough after lamivudine therapy than before, although they were not significantly associated with HBV DNA breakthrough, for 24 weeks data, multivariate logistic regression analysis revealed that HBeAg titer (24 weeks) was the independent factor for HBV DNA breakthrough (p=0.004, 48w), and was correlated with HBV DNA loads(r=0.493). Conclusions: Pre-existing lamivudine-resistant HBV ratio was not the prognostic factors associated with hepatitis B virus DNA breakthrough, while HBeAg titer after 24 weeks of lamivudine treatment was the independent factor that influenced HBV DNA breakthrough after 48 weeks of lamivudine treatment.

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ADEFOVIR DEPIVOXIL FOR DECOMPENSATED LIVER CIRRHOSIS PATIENTS WITH LAMIVUDINE-RESISTANT HEPATITIS B: MULTI-CENTER LONG-TERM RESULTS

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Background/Aim: The effect and safety of adefovir dipivoxil for patients with decompensated cirrhosis were not well known. Long-term efficacy and safety of adefovir dipivoxil in decompensated cirrhosis patients with lamivudine-resistant hepatitis B (HBV) were studied. Methods: One hundred nineteen cirrhotic patients who developed hepatic decompensation (any cirrhosis complication or Child-Pugh (CP) score ≥ 7) after lamivudine treatment were enrolled from eleven university hospitals and treated with 10 mg of adefovir dipivoxil for a median 33 months between January 2003 and October 2006. Lamivudine was switched to adefovir dipivoxil with or without overlap period (median 2 months). We evaluated the overall survival

rate and the improvement of Child-Pugh score, virologic response (HBV DNA <0.5 pg/ml) (Digene Hybrid Capture II assay, Detection limit 0.5 pg/ml), and virologic breakthrough after long-term adefovir dipivoxil treatment. Results: 1) At the baseline, median serum HBV DNA was 7.3 log10 copies/mL, median MELD was 15 (9-37). Child B was 63%, and Child C was 37%. 2) Overall patient survival rate was 90% for a median 33 months. Seven (5.8%) patients died within 6 months. Clinical factor associated with early mortality (≤ 6 months) was initial high MELD score (>25) by multivariate analysis (p=0.020, HR1.270 95% C.I. [1.038-1.553]). 3) The mean change from baseline CP score was -2.9±2.1 points (p<0.001) and 76.4% (84/110) of patients had a decrease of at least 2 points in CP score. 4) The mean change from baseline serum HBV DNA was -2.8 log10 copies/mL. After 48 weeks, serum HBV DNA became undetectable in 71% (78/109). Virologic breakthrough occurred in 33.7% (37/110). 5) Six (5.5%) patients confirmed an increase of at least 0.5 mg/dl in serum creatine concentration. Conclusions: Long-term treatment of adefovir dipivoxil was effective and safe in decompensated cirrhosis associated with lamivudine-resistant HBV. High MELD score at the baseline was associated with early mortality after adefovir dipivoxil treatment.

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CLINICAL FEATURES OF LYMPHOMA CHEMOTHERAPY ASSOCIATED HEPATITIS VIRUS B REACTIVATION

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Objective Investigate the incidence of Hepatitis Virus B reactivation and its related severity of hepatitis after the Non-Hodgson's Lymphoma (NHL) patients had been administered with chemotherapy, recognize the significance of detecting HBV-DNA and employing prophylactic antiviral therapy. Methods Retrospective study was conducted on 252 NHL patients who had received chemotherapeutics, among which 166cases with HBV serologic test negative, 86 cases with HBV positive (60 with HBsAg positive, 26 with HBsAg negative and anti HBc-IgG positive). Results After these 252 NHL patients received chemotherapy, 56 cases occurred hepatic biochemical abnormality, among which 11 with HBV negative (6.6%), 45 with HBV positive (52.3%), there was a statistically significant difference between these 2 groups (P<0.01). In addition, within the HBV positive group, 39 cases with HBsAg positive (65.0%) and 6 cases with HBsAg negative and anti HBc-IgG positive (23.1%) . There was also a statistical difference between these 2 groups (P<0.05). The prevalence of HBV reactivation in patients with HBsAg positive was much higher when compared with those with HBsAg negative and anti HBc-IgG positive (53.3% vs 7.7%, P<0.01). 32 among 39 patients with HBsAg positive occurred liver function abnormal within three cycles of chemotherapy, nevertheless 4 among 6 patients with HBsAg negative and anti HBc-IgG positive occurred liver function abnormal following five cycles of chemotherapy. Conclusions NHL patients with HBV positive have a higher incidence of hepatic biochemical abnormality than those with HBV negative. Patients with HBsAg positive developed abnormal liver function easily than those with HBsAg negative and anti HBc-IgG positive. HBV reactivation usually occurs on patients with HBsAg positive and especially those with higher viral load. HBV reactivation delayed on the NHL patients with HBsAg negative and anti HBc-IgG positive or even with anti HBsAb positive. Our results suggest that the importance of HBV viral load and liver function surveillance routinely during chemotherapy and necessity of prophylactic antiviral therapy to patients with hepatitis B carriers at the onset of chemotherapy.

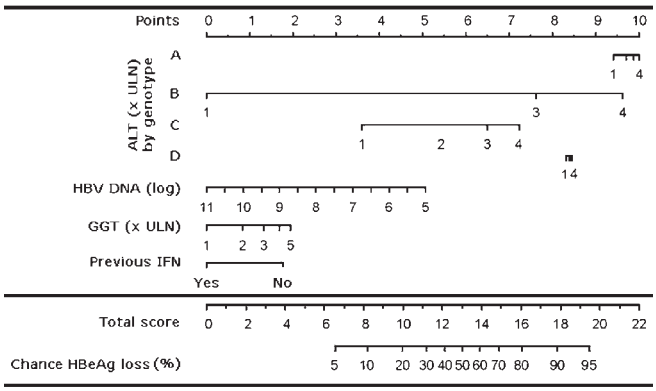
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The following people have nothing to disclose: Dongmei Shi, Qing Xie, Honglian Gui, Hui Wang, Nina Jia

1002
PREDICTING SUSTAINED HBEAG LOSS AFTER TREATMENT WITH PEGINTERFERON ALPHA-2B: DEVELOPMENT AND VALIDATION OF A PRACTICAL MODEL

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We developed and validated a model based on readily available factors for the prediction of response to peginterferon in individual patients. Methods: Patients randomized to PEG-IFN α-2b 100μg alone (n=136) or in combination with lamivudine 100mg (n=130) for 52 weeks were analyzed. Univariate logistic regression analysis, followed by backward stepwise selection was used to identify predictors of HBeAg loss at 26 weeks post-treatment among the variables age, sex, HBV genotype (A-D), serum HBV DNA, ALT, GGT, treatment allocation and previous antiviral therapy. Interactions between variables and nonlinear relationships were investigated. Discrimination was quantified by the area under the receiver-operating characteristic curve (AUC). Bootstrap sampling was used for internal validation to reduce overfit bias. Results: 23 patients were excluded because of missing values or infection with HBV genotype other than A-D, leaving 233 patients for analysis. HBV genotype, serum HBV DNA, GGT and previous IFN therapy were found to be independent predictors of HBeAg loss (p<0.05). Since the influence of ALT was dependent on HBV genotype, an interaction between these variables was included (p=0.03). A multivariable model based on the above mentioned variables had adequate discriminative ability (AUC 0.73). After bootstrapping, the discriminative ability of the model was found to be somewhat lower (AUC 0.69). A nomogram was generated from the logistic regression formula (figure). The probability of HBeAg loss is calculated by drawing a vertical line from each of the 4 variable axis to the top Points axis. The sum of these 4 points is put on the Total score axis, from which a vertical line is drawn to the Chance of HBeAg loss axis. Conclusion: A multivariable model based on HBV genotype, serum HBV DNA, ALT, GGT and previous IFN therapy provides an adequate prediction of PEG-IFN induced HBeAg loss and will be a useful tool to guide treatment choice of PEG-IFN vs. nucleos(t)ide analogues in individual patients.



logit(p) = 3.3861 -4.2854 *(geno=B) -2.6463 *(geno=C) -0.4907 *(geno=D) +0.4497 *log ALT -0.8043 *previous IFN -0.3849 *log HBV DNA +1.2544 *log GGT +6.8256 *log ALT *(geno=B) +2.3102 *log ALT *(geno=C) -0.3586 *log ALT *(geno=D).

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1003
THREE-YEAR ASSESSMENT OF ENTECAVIR RESISTANCE IN GENOTYPE C CHRONIC HEPATITIS B PATIENTS IN JAPAN REVEALS DIFFERENT CLINICAL OUTCOMES REGARDING BREAKTHROUGH HEPATITIS PREDICTED BY THE RESISTANCE SUBSTITUTIONS USING RECENTLY DEVELOPED INNO-LIPA HBV ASSAY

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Background/Aim: Currently, entecavir (ETV) is being considered as s first- or second-choice treatment for nucleoside-naive or lamivudine (LVD)-refractory chronic hepatitis B patients. To assess frequency of ETV-resistance (ETVr) and the specific substitutions associated with breakthrough hepatitis (BTH) during 3-years ETV therapy, recently developed INNO-LiPA HBV DR v2 and ETV TDF assay was applied. Methods: Forty-five patients including 25 chronic LVD-refractory genotype C patients (m/f: 22/23, mean age: 41.6 yrs, ALT: 129.1 HBeAg: 36 and HBV-DNA (TMA): 6.98) and 20 naïve patients were enrolled. Drug resistance was assessed by detection L80VI, V173GL, L180M/A181TV, M204VID, N236T for LVD (LVDr) and T184SCGAILMF, A194T, S202GCI, M250VL for ETVr. Results: In 25 LVD-refractory patients, the ETVr substitutions (SCGA184, ILMF184, G202, C202, VL250) were present in 3/25 (12.5%) at baseline and emerged in 5/25 (20.8%), and 7/25 (29.4%) and 5/17 (29.2%) at weeks 48, 96, and 144, respectively (Table1). No patient was found with S202I during the 3 years. There was no evidence of ETVr substitutions in LVD naïve patients during 3 years. Percentage of typical LVD substitutions (M180, V204, I204) observed in LVD-refractory patients was 92%, 95%, 94%, and 100% at baseline, weeks 48, 96, and 144, respectively, versus 8%, 8%, 0%, and 13% in naïve patients, respectively. Five of the 25 LVD-refractory (20%) patients treated with 1.0 mg ETV had evidence of BTH after 72 to 136 weeks of the therapy. Of the all BTH patients, LDVr substitutions (M180 and V204) were detected at baselines, and an additional G202 ETVr substitution occurred (0-96 weeks), resulting in BTH 40-72 weeks later. Other substitutions emerged during ETV therapy, but none of them seemed to affect susceptibility to ETV or associated to BTH. Conclusions: INNO-LiPA is useful in early ETVr detection. In LVD-refractory, genotype C, Japanese patients, LVDr (M180 and V204) plus G202 ETVr substitutions resulted in BTH. High and long-term efficacy of ETV was demonstrated in naïve patients, whereas adefovir add-on LVD should be considered as a first-, or second-line treatment option for LVD-refractory chronic hepatitis B patients.

Table1. EDVr associated substitutions emerged in LVDr cohort during follow up

LVD-refractory patients						
weeks	No.	SCGA184	ILMF184	G202	C202	IL250
0	25	1(4%)	0	1(4%)	0	1(4%)
48	25	1(4%)	0	4(16%)	1(4%)	0
96	25	2(8%)	2(8%)	6(24%)	0	0
144	17	2(11.8%)	1(5.9%)	3(17.6%)	0	0

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1004
THE EFFECTS OF α-GALACTOSYLCERAMIDE ON CHRONIC HEPATITIS B INFECTION IN A RANDOMIZED PLACEBO CONTROLLED PHASE I/II TRIAL

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Background: The glycosphingolipid alpha-galactosylceramide (α-GalCer) has been shown to have an immunomodulatory effect mediated via the activation of natural killer T (NKT) cells and is able to induce a powerful antiviral immune response through the production of pro-inflammatory cytokines, such as IL-6. α-GalCer activated NKT cells are able to inhibit hepatitis B virus replication in HBV transgenic mice. ***Aim***: The aim of this dose-escalating randomized placebo-controlled phase I/II trial was to investigate the antiviral activity and safety of α-GalCer as a novel class of treatment for chronic hepatitis B patients. ***Methods***: Twenty-seven patients were allocated to a α-GalCer dose of 0.1 μg/kg (n=8), 1 μg/kg (n=6), 10 μg/kg (n=6) or to placebo (n=7). All patients had an HBV DNA level above 10^5 copies/ml at screening and ALT values >1.2 times the upper limit of normal. The treatment period consisted of intravenous injections with α-GalCer at 0, 4 and 8 weeks. Thereafter, patients were monitored for an additional 16 weeks. ***Results***: Although some patients showed an HBV-DNA decrease after administration, no clinically relevant suppression of viral replication was observed. Four patients exhibited a more than 0.5 $\log_{10}$ copies/mL decrease in HBV DNA fol-

lowing the first administration (1 μg/kg n=2, 10 μg/kg n=2) with a median decline in the first week of 1.1 $\log_{10}$ copies/mL (range 0.5 – 3.0) from baseline, but this decline was not sustained and also not observed after the second and third administration of α-GalCer. There were no clear changes in ALT levels. In three patients of the highest dose level, a rise in IL-6 production in serum was observed four hours after drug administration. In four patients (1 μg/kg n=1, 10 μg/kg n=3) α-GalCer was discontinued prematurely because of an episode of fever and severe rigors shortly after drug administration. Otherwise there were no significant side effects. ***Conclusion***: α-GalCer used as monotherapy for chronic hepatitis B infection at the doses (0.1-10 μg/kg) used in this trial had no clear effect on HBV DNA and ALT levels, was poorly tolerated, and is unlikely to provide an alternative as monotherapy for the current treatment of PEG-IFN and nucleos(t)ide analogs.

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1005
CROSS-RESISTANCE CHARACTERIZATION OF THE MAIN HBV DRUG RESISTANT MUTANTS

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Introduction - Currently, four nucleoside analogues are approved for the treatment of chronic hepatitis B: lamivudine, adefovir, entecavir and telbivudine. They suppress viral replication through inhibition of HBV DNA polymerase and chain termination. However, prolonged treatments with these analogues result in the emergence of resistant virus harbouring mutations within the HBV polymerase gene which are associated with resistance to treatment. Objectives - We isolated and cloned HBV DNA genomes from patients who developed viral resistance following various therapies. We determined in vitro the antiviral susceptibility of the main resistance mutants to nucleoside analogues approved for chronic HBV treatment, and tested new nucleoside analogues on these mutants. Results - A bank of mutants, constructed by site directed mutagenesis or from patients serum samples were intensively analyzed. We could observed that all the mutants harbouring the rtM204V substitution, except the rtM204V mutation alone, showed a >1000 fold decrease in susceptibility to lamivudine as compared to wild-type (wt) HBV. We also characterized different mutants harbouring the rtA181V mutation and all these mutants were >2.7 fold less sensitive to adefovir than wt HBV. This in vitro decrease in susceptibility to adefovir seems to be sufficient to induce in vivo viral breakthrough. Concerning entecavir, all the mutants harbouring the rtS202G substitution, but only in addition to rtL180M+M204V main lamivudine resistance mutations showed a >200 fold resistance to this drug in comparison with wt HBV. Tenofovir seems to be one of the most efficient drugs on these mutants, since only two of them (rtN236T and rtL180M+M204V+N236T) showed a decrease in susceptibility to tenofovir as compared to wt HBV. We also tested new nucleoside analogues for their capacity to inhibit replication of drug resistant mutants and demonstrated that PMEO, a novel pyrimidine analogue, showed a better activity than adefovir on all the tested mutants except the rtN236T mutant. Phenotypic characteristics of additional complex mutants were also determined. Conclusion - These data on antiviral susceptibility of the main HBV polymerase mutants observed in patients provide useful information to guide anti-HBV therapy.

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DETECTION OF HEPATITIS B VIRUS YMDD MUTANTS BY MLTIPLEX-PCR USING DUAL PRIMING OLIGONUCLEOTIDE (DPO)

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Background:The mutants of hepatitis B virus (HBV) emerge selectively during long-term lamivudine therapy. Since lamivudine resistance has cross-resistance with other antiviral agents, initial choice of antiviral agents for prevention of multi-drugs resistance is very important. According to recent studies, early detection of HBV mutants at 3 months is useful to predict the long-term outcomes of lamivudine therapy. In addition, predominance of mutants is significantly associated with viral DNA breakthrough. Therefore, early detection and periodical testing of HBV mutants is needed. However current methods of detecting mutants are time-consuming and labor intensive which require multiple steps and expensive instruments. Aims:To determine the effect of recently developed YMDD genetype method using Multiplex-PCR technology, we compared this method with restriction fragment mass polymorphism (RFMP) and direct sequencing method. Patients and Methods: Total eighty chronic hepatitis B patients treated with lamivudine more than 6 months participated in this study. YMDD mutants were genotyped by RFMP using MALDI-TOF mass spectrometry, sequencing and Multiplex-PCR using dual priming oligonucleotide (DPO). Results:The Multiplex-PCR method with DPO was available for sensitive, specific and simultaneous detection of 4 types of HBV mutants (rtM204V/I/S, rtL180M) by only one time PCR. Additionally, this assay enabled to estimate relative abundance of mutants in variant/variant mixed or variants/wild type mixed serum by agarose gel electrophoresis. The Multiplex-PCR assay detected as few as 1000 copies/ml of HBV genome plasmids, also detected as little as 2% mutant plasmids. The total protocol was carried out easily within 4 hours. The patients with breakthrough (virologic or biochemical), early viral response to lamivudine therapy, HbeAg positivity, HBV-DNA >100,000 copies/ml and elevated ALT (>2 times upper limit of normal) had higher mutation rate. For these patients, Multiplex-PCR with DPO was the most sensitive method for detection of the mutants (especially mixed variants). Interestingly, 18 of 80 patients (22.5%) had single or mixed variants, these mutants only detected by the Multiplex-PCR, but not by RFMP or sequencing. These results indicate that Multiplex-PCR with DPO is more sensitive than RFMP and sequencing to detect variants. Conclusions:Multiplex-PCR with DPO is rapid, sensitive and cost-effective method for detection of the YMDD mutants and relative quantitation as well. Therefore, this method can be useful diagnostic tool for early detection of HBV mutants and estimate their predominance, thereby preventing drug resistance and early rescue therapy.

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1007
FIVE-YEAR RETROSPECTIVE SURVEY OF HBV GENOTYPIC DRUG RESISTANCE PATTERNS IN A DATABASE OF 381 HBV REVERSE TRANSCRIPTASE SEQUENCES IN SOUTHERN FRANCE

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Background/Aims: The growing number of HBV nucleos(t)ide inhibitors, the widening of anti-HBV sequential and/or combination therapies, and the viro-clinical impact of drug resistance mutations warrant surveys of HBV genotypic drug resistance. Therefore, we performed a 5-year retrospective survey during the 2002-04/2007 period aiming to analyse the prevalence and dynamic of HBV reverse transcriptase (rt) drug resistance mutations patterns in patients (pts) chronically-infected with HBV followed-up in Marseilles public hospitals. Patients/Methods: 381 HBV rt sequences from 312 pts (70% male; mean age, 47) were analysed. 39 pts have been tested twice, and 12 pts >2 times. For each year from 2002 to 2007, only 1 sequence per >18 year-old pt was selected. Serum HBV DNA direct sequencing was performed using in house protocols. 25 amino acid (aa) positions within domains A-E of HBV rt and previously associated with drug resistance were analysed, which included all positions at which association of aa substitution with drug resistance is clearly established. Results: A nearly 1.7-time increase of the number of HBV sequences was observed in 2006 (n=96) as compared to 2002-2003 (55 and 57, respectively). The mean number of aa substitutions per sequence ranged between 2.1 in 2003 and 1.7 in 2007, without significant change. The proportion of non-A HBV genotypes increased from 53-54% in 2002-2003 to >60% in 2006-04/2007 (p=0.24). The proportion of sequences harbouring >3 aa substitutions was significantly higher for genotypes A vs genotypes D or non-A-non-D (23% vs 11 and 9%, respectively; p<0.05). In contrast, the proportion of aa substitutions was significantly higher at positions 80, 181, 215, and 237 for genotypes D vs genotypes A (p<0.02). The most variable aa positions were rt204 and rt180. Substitution rtM204I/V was found in 71 sequences (19%), associated with L180M in all but one case for M204V and with A181V/T in two cases. Substitution rtA181V/T was found in 4.7% of sequences (n=17) and 6.4% in 2007. The proportion of mutated sequences significantly increased between 2002 and 2007 at positions 236, 237, and 238 (p<0.05). Substitution rtN236T was observed in 6 sequences (1.8%), always associated with rt204 wild-type aa, and was significantly associated with rtA181V and rtI169L (in 2 sequences each; p<10-3). Conclusions: Our data underline the changing pattern of HBV drug resistance mutations and the association or exclusion of rt aa mutations in the setting of emerging nucleos(t)ide inhibitors and combination and/or sequential anti-HBV therapies. They also suggest that genotypic drug resistance patterns might differ according to the HBV genotype.

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1008
INFLAMMATION AND FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS B DESPITE LOW VIRAL LOAD AT THE TIME OF LIVER BIOPSY

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Background: Three hundred and fifty million people worldwide have chronic hepatitis B (CHB). Current treatment guidelines recommend starting treatment for patients with elevated liver enzymes and hepatitis B (HBV) DNA ≥100,000 copies/ml in HBeAg+ or ≥10,000 copies/ml in HBeAg- patients. However, the threshold viral load (VL) level associated with significant liver fibrosis is unknown. Objective: To compare the degree of fibrosis and inflammation among CHB patients with low-level vs. high-level viremia. Methods: CHB patients with a liver biopsy from January 1995 to May 2007 and contemporaneous ALT and HBV DNA levels were identified by electronic chart review. Patients with co-existing liver disease (other than NAFLD) or HIV infection were excluded. Normal ALT was defined as ≤30 U/L in men and ≤19 U/L in women. Low-level viremia (LLV) was defined as HBV DNA <100,000 copies/mL in HBeAg+ and <10,000 copies/mL in HBeAg– patients. Liver biopsies were evaluated for fibrosis and necroinflammation according to the modified Scheuer scoring system. Results: Of 53 patients (mean age 40.3y; range 23-69y; 58% male) who met our inclusion criteria, 34 (64%) were HBeAg-. Of these, 9 (26%) had LLV (78% female), including 4 with HBV DNA <1,000 copies/ml (range 185-799 copies/ml). HBV DNA was ≥100,000 copies/mL and ALT was elevated in all HBeAg+ patients. Inflammation was mild in 89% (8/9) of the HBeAg– patients with LLV, 84% (21/25) of HBeAg- patients with high-level viremia (HLV), and 79% (15/19) of HBeAg+ patients. Of 9 HBeAg- patients with LLV, 5(56%) had at least stage 2 fibrosis (including one patient with incomplete cirrhosis), as compared with 52% (13/25) of HBeAg- patients with HBV DNA ≥10,000 copies/ml and 53% (10/19) of HBeAg+ patients. Of the 4 patients with HBV DNA <1,000 copies/mL, 2 (50%) had stage 2 fibrosis. Even among the 5 patients with LLV and normal ALT, 2 (40%) had at least stage 2 fibrosis, including one with incomplete cirrhosis. Stage 2 fibrosis was no more common among patients with elevated ALT and high VL [47% (9/19) of HBeAg- and 53% (10/19) of HBeAg+ patients] for whom treatment is recommended than in patients with normal ALT or low VL [60%(9/15) (all HBeAg-)]. Conclusion: Neither HBeAg status nor viral load predicted more severe fibrosis or necroinflammation in our patients. In HBeAg- patients with low-level viremia, a significant proportion had at least stage 2/4 fibrosis, including one with incomplete cirrhosis. More data are needed on the natural history of CHB in patients with low viral loads. For now, liver biopsy should be considered in the evaluation of HBeAg- patients with low-level viremia including those with normal ALT.

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The following people have nothing to disclose: Evan B. Grossman, Maya Gambarin-Gelwan, Thomas A. Hahambis, Rhonda K. Yantiss, Brian R. Edlin, Ira M. Jacobson

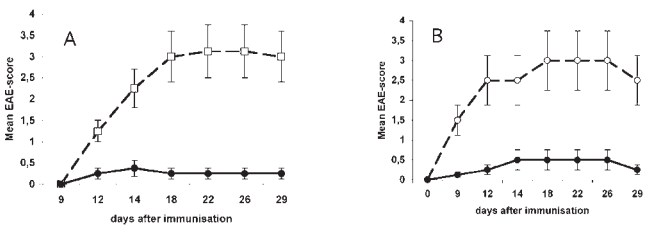
1009
ECTOPIC EXPRESSION OF NEURAL AUTOANTIGEN IN LIVER SUPPRESSES AUTOIMMUNE NEUROINFLAMMATION BY INDUCING ANTIGEN-SPECIFIC CD4+ CD25+ FOXP3+ REGULATORY T CELLS

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CD4+ CD25+ Foxp3+ regulatory T cells (Treg) are important mediators of immune tolerance to self-antigens, and their inactivation may cause autoimmune disease. Therefore, immunotherapy for autoimmune diseases may aim at restoring or expanding autoantigen-specific Treg activity. Here we report that Treg-mediated suppression of autoimmune disease could be achieved in vivo by taking advantage of the liver's ability to promote immune tolerance. Ectopic expression of the neural autoantigen myelin basic protein (MBP) in the liver, which was accomplished constitutively in liver-specific MBP-transgenic mice, or transiently after hydrodynamic gene transfer to liver cells in vivo, induced protection from experimental autoimmune encephalomyelitis (EAE), a mouse model for human multiple sclerosis. A role of central tolerance in the observed resistance to EAE was excluded by neonatal thymectomy. Protection from EAE was mediated by MBP-specific CD4+CD25+Foxp3+ Treg cells, as demonstrated by adoptive transfer to non-transgenic mice and by their ability to suppress conventional CD4+CD25- T cells after antigen-specific stimulation with MBP in vitro. Our findings indicate that autoantigen expression in the liver may generate autoantigen-specific Treg cells. Thus, targeting of autoantigens to hepatocytes may be a novel therapeutic approach to prevention or treatment of autoimmune diseases.

A) Transient hepatic MBP expression facilitated by hydrodynamics-based gene transfer to hepatocytes with MBP-encoding pcDNA3.1 expression vector (black circles, unbroken line), but not by empty control vector (white circles, broken line) suppresses EAE.

B) Protection of wild-type mice from autoimmune encephalomyelitis by adoptive transfer of 10^5 splenic CD25+ CD4+ T cells from MBP-transgenic mice (black circles, unbroken line), but not by transfer of 10^5 splenic CD25- CD4+ T cells from MBP-transgenic mice (white squares, broken line).



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1010
CRITICAL ROLE FOR CD1D-RESTRICTED Vα14 INKT CELLS IN STIMULATING INTRAHEPATIC CD8 T CELL RESPONSES TO LIVER-EXPRESSED ANTIGEN

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Background and aims. Activation of CD1d-restricted Vα14 invariant (i) NKT cells rapidly induces their secretion of effector cytokines. As Vα14 iNKT cells are localized in peripheral tissues such as the liver rather than lymphoid tissues, their role in modulating the stimulation of conventional, MHC-restricted T cell responses has remained ambiguous. We here describe a role for Vα14 iNKT cells in modulating conventional T cell responses to antigen expressed in liver, using transferrin-mOVA (Tf-mOVA) mice. Methods. Naïve ovalbumin-specific Class I MHC-restricted T cells (OTI) were adoptively transferred into Tf-mOVA mice in the presence or absence of CD1d-ligand α-galactosylceramide, after which their priming, antigen-specific cytokine production and liver damage were analyzed. Results. Transfer of OTI cells resulted in robust intrahepatic, antigen-specific proliferation of T cells. OTI T cells were activated in liver, and IFNα production by OTI T was stimulated by co-activation of Vα14 iNKT cells using a=α-galactosylceramide. This stimulation was absent in $CD1d^{-/-}$ Tf-mOVA mice, which lack Vα14 iNKT cells. OTI T cells induced hepatitis and liver damage was corroborated by α-galactosylceramide injection. Conclusion. CD1d-restricted Vα14 iNKT cells stimulate intrahepatic antigen-specific effector CD8 T cell responses. Our findings elucidate a previously unknown intervention point for targeted immunotherapy to autoimmune and possibly infectious liver diseases.

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The following people have nothing to disclose: Dave Sprengers, Fenna C. Sillé, Gurdyal S. Besra, Harry L. Janssen, Eckart Schott, Marianne Boes

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CTS-1027, A POTENT MMP INHIBITOR, PROTECTS AGAINST TNFα- AND α-FAS-INDUCED LIVER INJURY

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Background: CTS-1027 is an orally bioavailable small molecule that is a potent inhibitor of MMP-2, 3, 8, 9, 12, 13 and 14, but not MMP-1. In the liver, in response to a variety of insults, excessive MMP activity may play a role in destruction of the extracellular matrix, and recruitment of inflammatory cells. CTS-1027 was previously evaluated in an extensive phase 2 clinical trial in which a well tolerated chronically administered dose was identified. Thus, it was of interest to evaluate a MMP inhibitor whose safety profile was known in models of acute liver injury in mice. **Methods**: CTS-1027 (0.001-30 mg/kg) was administered PO to male mice (C57Bl/6), 30 minutes prior to treatment with TNFα/D-Galactosamine (D-Gln) or the Fas activating antibody (α-Fas). Six hours later, animals were anesthetized with Nembutal and blood taken by cardiac puncture. Plasma ALT activity was determined using a commercially available kit. For survival studies, mice were observed up to 24 hours post-insult. Pharmacokinetic analysis of CTS-1027 was

conducted in treatment-naive mice to determine plasma and liver levels. **Results**: CTS-1027 dose-dependently decreased plasma ALT activity in the TNFα model. The average ED50 from 4 studies was 0.26 ± 0.08 mg/kg. Twenty-four hour survival was also increased by CTS-1027 (10 mg/kg). The average 24 hour survival from 3 studies was 27 ± 7.3% and 55 ± 7.6% (p=0.03) in the TNFα/D-Gln control mice and CTS-1027-treated mice, respectively. CTS-1027 (10 mg/kg; PO) was also protective against α-Fas, significantly (p<0.05) reducing the elevation in plasma ALT activity in 2 independent studies by an average of 49%. In a separate group of mice, PK analysis of CTS-1027 in plasma vs. liver indicated that CTS-1027 was rapidly absorbed with Tmax of 0.25-0.5 hour in liver and plasma. AUCs increased in a dose-dependent manner and were 1.7-fold greater in liver than plasma. The AUC in plasma at the ED50 dose in the TNFα/D-Gln model (~0.3 mg/kg) was 13-fold lower than the AUC at a dose previously determined to be well tolerated in man. **Conclusions**: CTS-1027 potently antagonized the liver toxicity induced by treatment with TNFα/D-Gln or α-Fas as indicated by reductions in plasma ALT and/or lethality/morbidity at doses lower than the well tolerated dose in man. CTS-1027 is an attractive candidate for further development and is expected to enter clinical trials in patients with liver disease by the end of 2007.

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Patricia C. Contreras - Employee: Other

Karen Valentino - Employee: Other

1012
INVESTIGATING THE ROLE OF AUTOREACTIVE CD8+ AND CD4+ T CELLS IN THE PATHOGENESIS OF AUTOIMMUNE HEPATITIS IN A TRANSGENIC MOUSE MODEL

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Autoimmune hepatitis (AIH) is a chronic active liver disease of unknown cause. Autoreactive lymphocytes are thought to mediate the liver injury but the pathogenesis of AIH remains poorly understood. To investigate liver-specific CD4+ and CD8+ T cell responses and the role of autoreactive T lymphocytes in the pathogenesis of AIH, we have generated transgenic mice that express the influenza virus hemagglutinin (HA) neoantigen in the liver under the control of albumin regulatory elements (Alb-HA mice). Adoptive transfer of HA-specific naive CD8+ T cells into Alb-HA mice leads to an activation of autoreactive CD8+ T cells in the liver or in liver-draining lymph nodes and elicits a transient hepatitis characterized by portal and lobular inflammation and elevated serum level of alanine aminotransferase (ALT). Adoptive transfer of HA-specific naive CD4+ T cells into Alb-HA mice leads to an activation of autoreactive CD4+ T cells in liver-draining lymph nodes or in the spleen and causes mild transient necroinflammatory lesions of the liver. These data suggest that both, CD8+ and CD4+ T cells play an active role in the pathogenesis of AIH and that tolerance mechanisms keep autoreactive T lymphocytes specific for a liver antigen normally in check.

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1013
DELETION OF SOCS3 GENE IN THE LIVER PARENCHYMAL CELLS PROTECTS FROM T CELL-MEDIATED LIVER INJURY

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Introduction:T cell-mediated fulminant hepatitis is a life-threatening event. The IL-6 level elevates in T cell-mediated liver injury. The suppressors of cytokine signaling (SOCS) are inhibitors of cytokine signaling that function via the JAK/STAT pathway. The role of SOCS3, that suppress the STAT3 activation, in T cell-mediated liver injury had not been determined. In this study, we assessed the effect of the deletion of SOCS3 on hepatocyte in T cell-mediated liver injury. **Methods**:(1)Liver-specific SOCS3 conditional knockout (cKO) mice were generated by crossing Albumin-Cre mice to floxed-SOCS3 mice. T cell-mediated liver injury was induced by intravenous injection of Concanavalin A (ConA) (15 μg/gBW). The mice were analysed in 9 or 20 hours after the ConA treatment by Western Blot and Real-time RT-PCR analyses. (2) Hepatic mononuclear cells were prepared from cKO and control mice that were treated by ConA. The activation of lymphocytes in the liver was examined by FACS analysis of the early activation marker $CD69^+$. **Results**:(1)The ConA-induced increase of ALT levels was marked and prolonged in wild-type mice compared with cKO mice. An examination of the liver histology showed massive necrosis in wild-type mice, whereas cKO mice appeared to be largely protected from hepatic injury. TANEL staining revealed that less apoptosis occurred in cKO mice liver than in wild-type mice liver. In Western blot analysis, the activation of STAT3 was stronger and more sustained in cKO mice than in wild-type mice, whereas that of STAT1 was weaker in cKO mice than in wild-type mice. Consistent with the reduced up-regulation of STAT1 activation, the expression of the IRF-1 gene was diminished in cKO mice. Bcl-XL protein and mRNA levels also were higher in cKO mice than in wild-type mice. The strong activation of caspase 3 was detected in the wild-type mice, whereas the activation of caspase 3 was reduced greatly in cKO mice. (2)The administration of ConA caused a significant increase in $CD4^+CD69^+$ double-positive cells in wild-type and cKO mice. Also, we observed that natural killer T cells were activated rapidly and consequently depleted after administration of ConA. These changes were not significantly different between wild-type and cKO mice. **Conclusion**:In our liver-specific SOCS3-deficient mouse model, there was increased activation of STAT3 and reduced activation of STAT1. These findings suggest that our hepatitis model might be controlled tightly by the mutual functional antagonism of IFN-γ/STAT1 and IL-6/STAT3, which negatively regulate one another. Our study showed that deletion of the SOCS3 gene in mouse liver correlate with increased antiapoptotic effects.

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The following people have nothing to disclose: Hisanobu Ogata, Koichi Azuma, Jacquelyn J. Maher

1014
DELETION OF CD39 INHIBITS DEVELOPMENT OF METASTATIC LIVER CANCER

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New vessel formation and tumor infiltrating lymphocytes (TIL) influence host responses to malignant tissues. Extracellular adenosine-mediated pathways promote both vascular endothelial cell proliferation and inhibit cytotoxic T cells, thereby potentiating cancer growth. CD39 is the dominant ectonucleotidase of vascular and T regulatory cells and has the potential to generate high levels of adenosine locally. We have previously shown that deletion of Cd39 results in angiogenic failure and T regulatory cell dysfunction with loss of immune suppressive functions. Aim: Investigate impact of CD39 upon development of hepatic metastases. Methods and Results: We studied the development of metastatic liver deposits following portal vein infusion of 1.5x105 melanoma B16/F10 cells, with luciferase expression, in wild type and Cd39-null C57BL/6 mice (n=24). Tumor formation in liver was directly examined and animals imaged at days 7-17 after tumor cell implantation. As predicted, the formation of hepatic malignant foci was markedly suppressed in Cd39-null mice, at all time points examined. To test whether the major impact of Cd39-deletion was upon neovasculature formation or immune responsiveness, adoptive transfer experiments were conducted. Bone marrow transplants (BMT) from Cd39-null or wild type BL/6 mice were placed in lethally irradiated control and/or null mice, in a crossover manner (total n=24 for each group, respectively). Eight weeks post-adoptive transfer, melanoma cells were infused via portal vein as before and tumor growth studied. The Cd39-null mice that received wild type BMT mirrored the wild type phenotype with progressive tumor growth observed (n=8 per time point; p=0.015). In contrast, metastases were significantly inhibited in both number and size and ultimately became necrotic in the wild type mice that had received Cd39-null BMT. Conclusions: Bone marrow derived cells mediate the major inhibitory effects of CD39 deletion on tumor growth. Pharmacological inhibition of CD39 may find utility as an adjunct therapy in the management of hepatic malignancy.

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1015
EVIDENCE FOR ALTERNATIVE- AND CLASSICAL-PATHWAY INDEPENDENT ACTION OF COMPLEMENT FACTOR C3 DURING LIVER REGENERATION

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The liver regenerates itself following injury. Recently, the complement pathway has been implicated as important for the regenerative response. For example, both complement factor C3- and C5-null mice show impaired hepatic regeneration that can be rescued by exogenous supplementation of the respective factors. However, the specific mechanism by which complement pathway signaling is activated during liver regeneration (classical, alternative, or lectin-dependent) has not been defined. The classical and lectin dependent pathways of complement activation are absolutely dependent on complement factor C4 while the alternative pathway of activation requires factor B. In the studies reported here we investigated whether either C4 or factor B is required for normal liver regeneration using the mouse partial hepatectomy model. **Methods:** 2-3 month old wildtype (WT) C57Bl/6J and C3-, Factor B-, and C4-null mice on C57Bl/6J backgrounds were subjected to partial hepatectomy (PH) or sham surgery, allowed to recover, and then sacrificed for plasma and tissue harvest. 3-6 animals were examined per time point and genotype. Liver tissue was formalin-fixated, paraffin-embedded, and stained with H&E or for bromo-deoxiuridine (BrdU). Cyclin D1 protein expression was assessed by protein immunoblot. **Results:** Compared to WT mice, C3-null mice had reduced hepatocellular proliferation and delayed peak proliferation, as well as suppressed induction of hepatic cyclin D1 protein expression. At 48h after PH, hepatocellular mitotic body frequency was significantly reduced in C3-null mice, compared to WT mice. In contrast, both C4-null and Factor B-null mice showed normal hepatocellular BrdU incorporation, cyclin D1 expression, and mitotic body frequency. Neither C3-, C4- nor Factor B-null mice had increased mortality or increased liver tissue injury. **Conclusion:** Our results suggest that neither the C4-dependent classical or the lectin-dependent pathways nor the factor B-dependent alternative pathway of complement cascade activation is absolutely required for normal liver regeneration. These data raise the possibility that other mechanisms exist by which C3 and C5 can be activated during the regenerative response.

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HEPATOCYTE SURVIVAL IN ACUTE HEPATITIS IS DUE TO C-JUN/AP-1-DEPENDENT EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE

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Analysis of the molecular factors determining hepatocyte survival or death in response to inflammatory stimuli is essential for understanding the pathogenesis of inflammatory liver disease and for identifying novel therapeutic approaches. Jun N-terminal kinase (JNK) is a major mediator of cytokine-induced cell death during hepatitis, but the signaling pathways downstream of JNK remain less well defined. Here we show that the transcription factor c-Jun/AP-1, a prototypic target of JNK, is strongly expressed in the liver of patients with acute liver injury of viral or toxic etiology. The contribution of c-Jun to cell death was further analyzed in mice using the Concanavalin A (ConA) model of TNFα-dependent hepatitis. Mice lacking c-Jun in hepatocytes display increased liver cell death and mortality upon ConA injection. This phenotype is due to impaired expression of inducible nitric oxide synthase (*nos2*, also known as *iNOS*), which we identified as direct transcriptional target of c-Jun during hepatitis, and reduced production of hepatoprotective nitric oxide (NO). The increased hepatotoxicity and hepatocyte necrosis in mutant mice are likely caused by hypoxia and oxidative stress and can be rescued pharmacologically by liver-specific delivery of NO or by the antioxidant N-Acetylcysteine. These findings demonstrate that c-Jun/AP-1 mediates hepatocyte survival during acute hepatitis by regulating *nos2* expression and thus functionally antagonizes the cell death-promoting functions of JNK. Moreover, our data provide a strong rationale for further exploring NO-based therapies for the treatment of severe liver injury.

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EFFECTS OF COPY NUMBER VARIANTS (CNVS) OF THE CHEMOKINE GENE CCL3L1 ON SUSCEPTIBILITY TO HCV INFECTION

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Recently a novel class of genomic diversity, copy numbers variants (CNVs), of the *CCL3L1* gene have been associated with susceptibility to HIV infection (Gonzales et al. *Science* 2005;307:1434-40). The chemokine CCL3L1 is a potent ligand for CCR5, which plays a central role in anti-viral lymphocyte activity. Of note, CCR5 deficiency has been associated with differences in susceptibility to hepatitis C virus (HCV) infection and the hepatic fibro-inflammatory reaction. Hence, variation in the gene dose of *CCL3L1* due to CNVs may also contribute to differences in the overall susceptibility to HCV infection and/or the inflammatory response. Thus, our aims now were to compare the *CCL3L1* CNV distributions between patients with chronic HCV infection, HCV/HIV co-infection and non-infected controls, and to test for association with histological grades of inflammation and stages of fibrosis in patients with chronic HCV infection. **Patients and methods:** Patients with chronic HCV infection (n=254), HCV/HIV co-infection (n=144) and non-infected controls (n=210) were recruited. CNVs of the *CCL3L1* gene were determined using a real-time fluorescent-dye labelled PCR adapted from Gonzales et al. (*Science* 2005;307:1434-40), and CNVs were compared between groups using t-tests and non-parametric Mann-Whitney-U tests. **Results:** Copy numbers of the *CCL3L1* gene are in line with previously published data for non-Africans, ranging from 0 to 12 copies (mean 2.5 ± 1.4 copies). Consistent with previous studies, *CCL3L1* copy numbers of HCV/HIV co-infected patients are significantly lower compared to HCV patients (2.2 vs. 2.6; $P < 0.01$) and controls (2.2 vs. 2.9; $P < 0.001$). Of note, *CCL3L1* copy numbers of HCV mono-infected patients are also significantly lower compared to controls (2.6 vs. 2.9; $P = 0.011$). Accordingly, CNV distributions are shifted to lower copy numbers in HCV mono-infected patients compared to controls ($P = 0.036$) and in HCV/HIV co-infected patients compared to HCV mono-infected patients or controls (both $P < 0.01$). Patients with two or less copies of the *CCL3L1* gene are over-represented in the HCV cohort compared to controls (OR = 1.54; 95% CI 1.07 - 2.55; $P = 0.02$). HCV/HIV patients show a similar over-representation of less than two or two *CCL3L1* copies (OR = 3.42; 95% CI 2.18 - 5.38; $P < 0.001$). However, no association is found between *CCL3L1* CNVs and grades of inflammation or stages of fibrosis in HCV mono-infected patients. **Conclusions:** HCV patients carry lower *CCL3L1* gene copy numbers than non-infected controls. Our findings indicate for the first time that susceptibility to HCV infection might be influenced by copy number variation within the host's genome.

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INHIBITION OF NKT CELL ACTIVATION AND THE DEVELOPMENT OF HEPATITIS BY A GLYCOSAMINOGLYCAN-BINDING DEFICIENT RANTES MUTANT

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Background: Hepatitis is associated clinically with an increase in hepatic chemokine expression, including RANTES/CCL5, as well as the activation of NKT cells within, and recruitment of lymphocytes to, the liver. Recently, a glycosaminoglycan-binding deficient mutated version of CCL5 has been developed (GAG-CCL5) and demonstrates anti-inflammatory properties, via a mechanism which remains unclear. Methods/Results: Concanavalin A-induced hepatitis is a well characterized murine model of T cell mediated hepatitis which is critically dependant upon hepatic NKT cell activation. Using this model we have documented a significant inhibition of hepatitis in mice treated with GAG-CCL5 prior to concanavalin A (con A) administration, as reflected by serum ALT levels and histology (serum ALT 8 hrs post-Con A: veh 8186 + 489[SEM] U/L vs GAG-CCL5 1514 + 230; $p<0.01$;n=5/group). This inhibition of hepatitis was associated with a significant suppression of hepatic NKT cell activation as reflected by a > 50% reduction in the number of hepatic NKT cells producing either IL-4 or IFNγ in GAG-CCL5 vs vehicle treated mice receiving Con A ($p<0.05$ vs respective controls for both; NKT cell cytokine production determined by flow cytometry). We then used the specific in vivo NKT cell activator α galactosylceramide (given as an intraperitoneal injection) to confirm that GAG-CCL5 was directly inhibiting the activation of hepatic NKT cells (as reflected by a > 65% reduction in hepatic NKT cell IFNγ production in GAG-CCL5 treated vs vehicle treated mice, as determined by flow cytometry; $p<0.05$). We next examined the effects of GAG-CCL5 in acetaminophen (APAP)-induced hepatitis which is known to be dependant upon both hepatic NKT cell activation and NK cell recruitment into the liver. In this model, GAG-CCL5 again significantly suppressed hepatitis (serum ALT 24 hrs post-APAP: veh 2378+275 vs GAG-CCL5 1094+145; $p<0.05$;n=4/group) and inhibited hepatic NKT cell activation (reflected by a >50% reduction in the numbers of IFNγ-producing hepatic NKT cells in GAG-CCL5 vs vehicle treated mice which received APAP; $p<0.05$) and also significantly inhibited the recruitment of NK cells, and IFNγ-producing NK cells, into the liver (>50% reduction in number of NK cells/liver in GAG-CCL5 treated mice vs vehicle treated mice receiving APAP; $p<0.05$). Conclusion: In summary, GAG-CCL5 inhibits the development of experimental hepatitis by preventing hepatic NKT cell activation, indicating that GAG-CCL5 may represent a novel therapeutic approach for the treatment of patients with hepatitis by targeting the innate immune system within the liver.

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CONTRIBUTION OF TRIF TO SECRETION OF MATURE IL-1β AND IL-18 VIA THE TLR-MEDIATED CASPASE-1 ACTIVATION DURING EXTRACELLULAR BACTERIAL INFECTION IN MICE

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Innate immune cells (such as macrophages) participate in early host defense against pathogens by production of proinflammatory cytokines. We previously reported that *Propionibacterium acnes*-primed mice are highly susceptible to LPS and develop endotoxin shock or liver failure by their hyper-production of and hyper-responsiveness to TNF, IL-1β and IL-18. Both IL-1β and IL-18 are synthesized as precursor form and become active after being cleaved with caspase-1. Caspase-1 is also produced as precursor form and become enzymatically active when innate cells are stimulated with LPS or other appropriate stimuli. Two distinct pathways are activated for caspase-1-dependent release of IL-18 and IL-1β by stimulation with LPS. LPS alone induces caspase-1-dependent release of IL-1β and IL-18 via activating extracellular sensor, TLR4 but independently of MyD88. On the other hand, LPS plus ATP can induce those responses through activating intracellular sensor, NOD-like receptors (NLRs), but not TLR4, which is due to intracellular influx of LPS by ATP. Since TRIF and TRAM are responsible for transducing MyD88-independent signaling, we investigated whether these common signal adaptors are involved in LPS-induced caspase-1 activation. LPS stimulation of wild type Kupffer cells accelerates capase-1 activation, while TRIF or TRAM deficient Kupffer cells fail to do it, indicating that both adaptors are essentially required. However, LPS plus ATP could induce activation of caspase-1 in TRIF-deficient Kupffer cells, indicating the dispensability of TRIF. After infection with *Escherichia coli*, extracellular bacterium, wild-type mice, but not *tlr4* $^{-/-}$, *trif* $^{-/-}$ or *caspase1* $^{-/-}$ mice, showed elevated serum levels of IL-18, indicating the importance of TLR/TRIF-mediated caspase-1 activation in vivo. These results demonstrate that mammalian host equips two caspase-1 activation machineries, i.e. TLR-mediated TRIF-dependent and NLR-mediated TRIF-independent pathways, and that TRIF links extracellular bacterial pathogen with caspase-1 activation would provide new regimen for the treatment of inflammatory liver diseases associated with aberrant activation of casp1.

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ALCOHOL CONSUMPTION INDUCES EXPRESSION OF SOCS3 AND SOCS1 AND INHIBITS SIGNALING VIA STAT3 AND STAT1 PATHWAYS IN HUMAN MONOCYTES

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Introduction: Acute alcohol consumption is associated with downregulation of pro-inflammatory responses to various pathogens, induction of immunoinhibitory cytokines, and poor responses to IFNα treatment in HCV infection. We previously reported that alcohol activates JAK1-STAT3 signaling leading to IL-10 induction. The JAK-STAT pathway also activates its own negative regulators, suppressors of cytokine signaling, SOCS1 and SOCS3. SOCS proteins are inducible inhibitors that negatively regulate STAT3/STAT1 signaling pathways induced by cytokine, IL6 or IFNα. Aim: To explore the effect of alcohol on induction of SOCS1/SOCS3 and regulation of STAT3/STAT1 pathways induced by IL6 or IFNα in human monocytes in vitro and in vivo. Methods: Monocytes were isolated from blood samples of treatment-naïve patients with chronic hepatitis C infection. Blood samples from normal volunteers were collected before and 24 hours after consumption of 2 ml vodka/kg body weight. In in vitro experiments human monocytes were treated with 25-50mM of alcohol with or without cytokines for 45 min-5 h. Whole cell lysates were analyzed by western blot, nuclear proteins by EMSA, mRNA by real time RT-PCR. Results: Acute in vivo or in vitro alcohol increased both SOCS1 and SOCS3 mRNA expression in monocytes. Alcohol treatment resulted in increased phosphorylation of STAT3 at tyr705 and ser727 residues and increased STAT3 DNA binding. Activation of STAT3 has been shown to induce expression of SOCS1 and SOCS3. We hypothesized that induction of SOCS proteins by alcohol may also lead to modulation of cytokine signaling through STAT1 and STAT3. Indeed, we observed significant downregulation of both IL6- and IFNα-induced STAT3 and STAT1 DNA binding when alcohol was added to monocytes 3 hours prior to the cytokine stimulation. To address whether inhibition of IL6-induced STAT3 DNA binding had downstream effects, we measured mRNA levels of IL6-dependent genes, MCP-1 and ICAM-1. We detected significant inhibition of IL6-induced expression of MCP-1 and ICAM mRNA in monocytes after acute alcohol exposure. Finally, we found increased baseline as well as inducible STAT1 and STAT3 DNA binding in monocytes of patients with chronic HCV infection compared to normal controls. Importantly, acute alcohol inhibited both IL6- and IFNα-induced STAT1/3 activation in monocytes of HCV patients. Conclusion: Acute alcohol activates STAT3 signaling pathway and induces SOCS3 and SOCS1 levels in monocytes, thus leading to downregulation of IL6- and IFNα-induced signaling via STAT1/STAT3 pathways. These mechanisms may be involved in attenuated responses to IFNα therapy after alcohol use in HCV infection.

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ACTIVATION OF CYCLIC-AMP RESPONSE ELEMENT BINDING PROTEIN CONTRIBUTES TO ADIPONECTIN-STIMULATED INTERLEUKIN-10 EXPRESSION IN RAW 264.7 MACROPHAGES

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Adiponectin, an adipokine predominantly secreted from adipose tissue, has potent insulin-sensitizing effects in liver and muscle, as well as potent anti-inflammatory properties in macrophages. Adiponectin concentrations in the circulation are decreased in response to chronic ethanol feeding; treatment with adiponectin during chronic ethanol exposure protects mice from the development of ethanol-induced liver injury (Xu, et al., 2003). The mechanisms for the protective effects of adiponectin are likely complex. However, recent data suggests that adiponectin's anti-inflammatory properties play a critical role in its protective effects against ethanol-induced liver injury, since adiponectin treatment normalizes LPS-stimulated TNF-α produc-

tion by isolated Kupffer cells after chronic ethanol exposure. We have recently reported that increased production of interleukin-10 (IL-10), a potent immune-modulatory cytokine, is involved in the anti-inflammatory actions of adiponectin in macrophages (Park, et al., 2007). Here we have further investigated the molecular mechanisms by which adiponectin increases IL-10 production in macrophages. Globular adiponectin (gAcrp) increased IL-10 promoter activity and IL-10 mRNA accumulation in RAW 264.7 macrophages. Deletion of the sequences from –416 and –369 in the IL-10 promoter, containing a cyclic AMP-response element binding protein (CREB) binding site, prevented gAcrp-induced IL-10 promoter activation. Treatment of RAW 264.7 macrophages with gAcrp increased the phosphorylation of CREB at Ser133, as well as enhanced the DNA binding activity of CREB. Further, overexpression of a dominant negative form of CREB prevented gAcrp-induced transcriptional activation of IL-10. gAcrp-stimulated CREB phosphorylation was mediated by the activation of both ERK1/2- and PKA-dependent pathways. Inhibition of either ERK1/2 or PKA activity prevented gAcrp-stimulated CREB phosphorylation, as well as gAcrp-stimulated IL10 promoter activation. Taken together, these data identify gAcrp-stimulated phospho-CREB as a key transcription factor responsible for gAcrp-induced IL-10 promoter activation. Supported by NIH AA11975 and AA013868.

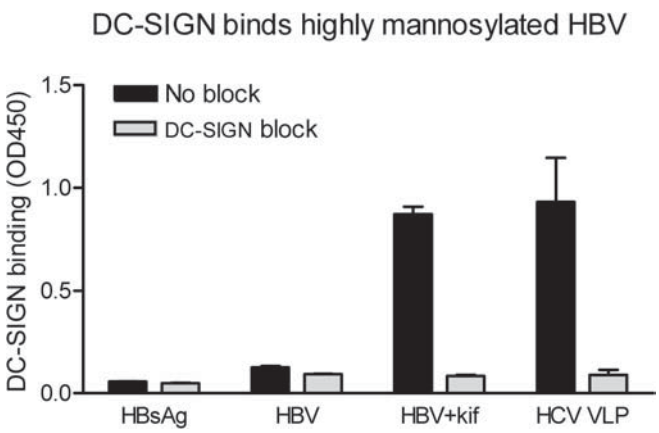
Disclosures:

The following people have nothing to disclose: Pil-Hoon Park, Honglian Huang, Kathryn Bryan, Megan R. McMullen, Laura Nagy

1022
TRIMMING OF BRANCHED OLIGOSACCHARIDE STRUCTURES ON HBV INHIBITS DC-SIGN BINDING

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Background Currently, a high affinity receptor for Hepatitis B virus (HBV) is still unknown. For HCV, both DC-SIGN, a mannose recognizing lectin expressed on dendritic cells, and L-SIGN, a liver expressed homologue, are known to bind glycoprotein E2. The oligosaccharide structures on HBV surface antigen (HBsAg) may also allow DC-SIGN/L-SIGN interaction. Therefore, we aimed to investigate the role of both DC-SIGN and L-SIGN in binding of HBV. **Methods** Interaction between HBV or HBsAg and DC-SIGN/L-SIGN was studied by ELISA, using recombinant DC-SIGN-Fc and lysates of DC-SIGN/L-SIGN transfected Raji cells. Specificity was determined by blocking with mannan (100 μg/ml) or EGTA (10 mM). As a positive control, HCV virus like particles (VLP) were used. Highly mannosylated HBV was generated by treating HBV producing HepG2.215 cells with the mannosidase inhibitor kifunensin (5 days, 20 μg/ml). Uptake of HBsAg by DC-SIGN/L-SIGN transfected Raji cells was studied by flow cytometry and compared to lectin mediated uptake of dextran-FITC. **Results** DC-SIGN-Fc specifically interacted with HCV VLP, but no interaction with either HBsAg or HepG2.215 derived HBV was detected. HBV binding to DC-SIGN was observed after kifunensin treatment, indicating that HBV oligosaccharide trimming inhibits interaction with DC-SIGN. In theory, the recombinant DC-SIGN-Fc protein might be misfolded, therefore binding of HBsAg to native DC-SIGN/L-SIGN was also studied with lysates of transfected Raji cells. No interaction was observed between HBsAg and either DC-SIGN or L-SIGN. Also, cultured transfected Raji cells did not bind HBsAg. In contrast, both Raji DC-SIGN/L-SIGN showed mannan sensitive binding of dextran-FITC. **Conclusions** Although both DC-SIGN and L-SIGN are known receptors for HCV, they do not seem to play a role in recognition of HBV. Inhibition of mannose trimming during HBV replication renders the virus susceptible to recognition by DC-SIGN. Whether HBV might use mannose trimming as a way to escape recognition by DC-SIGN and L-SIGN remains to be elucidated.



Disclosures:

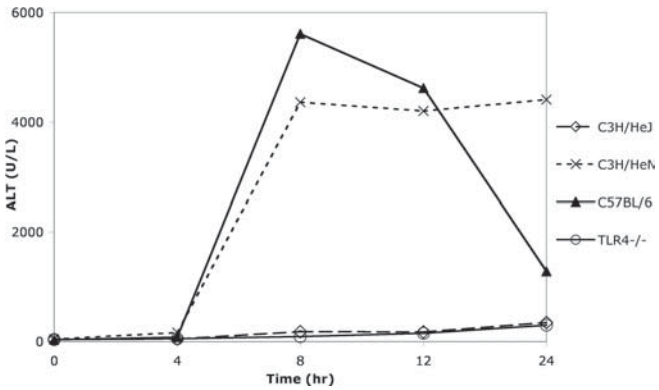
The following people have nothing to disclose: Marjoleine L. Op den Brouw, Marein A. de Jong, Renate G. van der Molen, Theo B. Geijtenbeek, Harry L. Janssen, Andrea M. Woltman

1023
CONCANAVALIN A-INDUCED HEPATITIS IS DEPENDENT ON TOLL-LIKE RECEPTOR 4 (TLR4) SIGNALING PATHWAYS

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Background: Concanavalin A (Con A) administration in mice leads to T-cell mediated hepatitis, which involves cytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Lipopolysaccharide (LPS)/TLR4 signaling pathway has been shown to be involved in underlying mechanism in a number of liver pathologies (I/R-induced injury, alcohol-induced hepatitis). Involvement of TLR4 in T-cell mediated hepatitis has not been elucidated. In the current study the impact of TLR4 gene deletion (TLR4-/- mice) and a defective TLR4 (C3H/HeJ mice) was investigated. Methods: Mice with normal TLR4 (C3H/HeN and C57BL/6), C3H/HeJ and TLR4-/- were injected with Con A (20 mg/kg). Plasma ALT was measured to assess liver injury. Plasma TNF-α and IFN-γ were measured. Results: Following Con A administration, C3H/HeN and C57BL/6 mice developed a severe liver injury indicated by increased plasma ALT (Fig). However, no ALT increase was seen in C3H/HeJ or TLR4-/- mice. Histological examination confirmed signs of necrosis following Con A in the wildtype animals that was absent in the C3H/HeJ and TLR4-/- mice. TNF-α was significantly elevated at 2 hours post-Con A in C3H/HeN (1478±715 pg/mL) and C57BL/6 mice (7302±3500 pg/mL) but not in C3H/HeJ mice (347±199 pg/mL). IFN-γ levels were significantly higher in C3H/HeN (770±160 pg/mL) and C57BL/6 mice (1113±415 pg/mL) compared to C3H/HeJ mice (330 ± 30 pg/mL). Conclusions: Our results show that Con A causes liver injury and proinflammatory cytokine production in mice with functional TLR4. In the absence of TLR4 signaling pathway, cytokine production is attenuated and injury is prevented (C3H/HeJ and TLR4-/-

mice). Thus we have shown for the first time the involvement of LPS/TLR4 signaling may play a role in immune-mediated liver injury, probably mediated by proinflammatory cytokines produced by T cells and Kupffer cells.



Disclosures:

The following people have nothing to disclose: Beth L. Mallard, Jacquie Harper, Ayako Mabuchi, Antony M. Wheatley

1024
INHIBITION OF RIG-I/CARDIF-INDUCED INTERFERON RESPONSES BY N TERMINUS OF HCV NS4B PROTEIN

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Background and aim: Viral infection to cell activates expression of type-I interferons (IFN). These responses are partly triggered by cytoplasmic double-strand RNA (dsRNA) sensor molecules such as RIG-I and MDA5, controlled by LGP2, mediated by Cardif, TBK1, Ikkε, and IRF-3. We analyzed mechanisms of the dsRNA-induced IFN responses in various cell lines that supported subgenomic Hepatitis C virus replication or in JFH-1 infected Huh7.5.1 cells. Methods: Poly I-C (dsRNA) was transfected into naive and replicon-expressing Huh7, HeLa and HEK293 cell lines, and activities of the ISRE-regulated and IFN-β gene expression were measured. The dsRNA-induced dimerization of IRF-3 was detected. Plasmids used were full-length RIG-I, delta RIG-I, full length Cardif, TBK1, IKKε and plasmids expressing HCV-NS345, NS3, NS3/4A, NS4A, NS4B, NS5A, NS5B and truncated plasmids of NS4B. IFN expression in HCV-JFH1 cell culture was analyzed. Results: Transfection of poly I-C into Huh7, HeLa and HEK293 cells induced IRF-3 dimerization, and it completely abolished in respective cell lines that expressed HCV replicon. Overexpression of full-length RIG-I in Huh7 and HEK293 acquired dsRNA-induced ISRE activation. These effects were substantially suppressed in HCV replicon cells and restored in IFN-treated cells. The RIG-I dependent IFN responses were suppressed in HCV-JFH1 infected cells and also restored in IFN-treated cells. Interestingly, overexpression analysis revealed that NS3/4A and NS4B significantly inhibited the ISRE activation mediated by expression of RIG-I and Cardif, but not by TBK1 and IKKε. Moreover, co-transfection of truncated NS4B expression plasmids showed that N-terminus as well as C-terminus of NS4B was responsible for the inhibition. Conclusions: The dsRNA-triggered IFN responses were suppressed in HCV-replicating cells. These effects were partly mediated by HCV-NS4B as well as NS 3/4A. Furthermore, N terminus and C terminus of NS4B were revealed to be responsible for the suppression. These mechanisms may contribute to the clinical persistency of HCV infection and might constitute a novel antiviral therapeutic target.

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1025
EXPRESSION OF HEPATITIS C VIRUS PROTEINS IS SUFFICIENT TO INDUCE RECOGNITION BY RESIDENT HEPATIC AND INVARIANT CD1D-RESTRICTED T CELLS

Kazuhiko Yanagisawa[1], RouJie Wang[1], Wenyu Lin[2], Rachel Baden[1], Khadija Iken[1], Stanley M. Lemon[3], Raymond T. Chung[2], Lucy Golden-Mason[4], Hugo R. Rosen[4], Margaret J. Koziel[1], Mark A. Exley[1]; [1]Hematology / Oncology, Beth Israel Deaconess Medical Center, Boston, MA; [2]Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA; [3]University of Texas Medical Branch at Galveston, Galverston, TX; [4]Colorado Health Sciences University, Denver, CO

BACKGROUND: The liver is selectively enriched for innate immune cells, including natural killer T cells ('NKT'), defined by co-expression of NK markers and T cell antigen receptor. The corresponding presenting molecule for a diverse subset of NKT, CD1d, is expressed at very low levels in healthy liver, but is specifically up-regulated in certain inflammatory conditions, including chronic HCV infection. The CD1d:NKT system is highly conserved across mammalian species. CD1d-restricted NKT are a major rodent hepatic subset and we identified analogous but distinct pro-inflammatory Th1 human populations. NKT cells positively or negatively regulate immune responses, partly through cytokine production. Their activation can be protective against certain viral and other infections, but excessive stimulation causes liver damage. METHODS: Human intrahepatic lymphocytes (IHL) and hepatocytes were isolated from subjects with HCV or other conditions along with hepatocytes from HCV transgenic mice. Blood-derived 'invariant' NKT served as control effectors and hepatocytes and other CD1d+ cells were used as targets. Responses of hepatic NKT were measured ex vivo and in vitro by cytokine production. An HCV-expressing Huh-7 hepatocyte line was also used as stimulator. CD1d expression was monitored on potential target cells. RESULTS: IHL CD1d-reactive NKT could be readily detected ex vivo, particularly in chronic hepatitis C, confirming their presence as a major population. Hepatocyte surface CD1d was specifically up-regulated in chronic HCV infection. Expression of HCV proteins in a hepatocyte cell line was sufficient for CD1d-dependent recognition by human IHL as well as blood invariant NKT. Furthermore, human and murine peripheral as well hepatic NKT responded efficiently to HCV transgene-expressing murine hepatocytes, suggesting a direct role for HCV protein expression in activating these cells in disease. CONCLUSIONS: HCV protein expression by hepatocytes can induce CD1d for surveillance by resident Th1 hepatic CD1d-restricted NKT. While potentially of benefit in acute hepatotropic infections, HCV appears to have evolved to exploit this system, thereby facilitating chronic inflammation.

Disclosures:

The following people have nothing to disclose: Kazuhiko Yanagisawa, RouJie Wang, Wenyu Lin, Rachel Baden, Khadija Iken, Stanley M. Lemon, Raymond T. Chung, Lucy Golden-Mason, Hugo R. Rosen, Margaret J. Koziel, Mark A. Exley

1026
PROSPECTIVE ANALYSIS OF HEPATIC CD1D-RESTRICTED T CELLS IN ADVANCED HCV LIVER DISEASE

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BACKGROUND: The liver is selectively enriched for innate immune cells, including natural killer T cells ('NKT'), defined by co-expression of NK markers and T cell antigen receptor. The corresponding presenting molecule for a diverse subset of NKT, CD1d, is expressed at low levels in healthy liver, but is specifically up-regulated in certain inflammatory conditions including chronic hepatitis C. The CD1d:NKT system is highly conserved across mammalian species. CD1d-restricted NKT are a major rodent hepatic subset with analogous but distinct pro-inflammatory Th1 human populations. NKT cells can control immune responses, partly through cytokine production, and their excessive stimulation causes liver damage. To test for a role in HCV-mediated liver disease progression, we performed prospective analysis of hepatic CD1d-restricted NKT in advanced HCV-mediated liver disease from samples obtained within the HALT-C trial. METHODS: Human intrahepatic lymphocytes (IHL) were expanded from up to 3 biopsies over 48 months from HALT-C subjects and tested for CD1d responses. Blood-derived 'invariant' NKT served as control effectors. Responses of hepatic NKT were measured by cytokine production. RESULTS: There was an increase in hepatic NKT cell Th2 pro-fibrotic IL-4 production (5 / 6; 6th to be tested at Month 48) but not IFNg from hepatic CD1d-restricted NKT. Cross-sectional analysis of samples from a total of 19 subjects to date with 1 or more time point analyzed for IL-4 also support an increase in this prototypical Th2 cytokine over time. Notably, in the non-serial samples also, there was at least as much IFNg at Month 48. Whether treatment (in this by definition treatment refractory cohort) selectively impacts hepatic CD1d-restricted NKT IFNg responses is under study. However, unlike the steady (likely treatment refractory) IL-4 rise for 5 / 6 subjects, IFNg dropped at Month 24 (4 / 4 informative subjects; most proximal point post-therapy in those who actually received ribavirin / IFNa) and then rebounded to baseline biopsy level at the Month 48 biopsy. Other pro-and anti-fibrotic cytokines are being analyzed. CONCLUSIONS: HCV infection of treatment refractory patients was associated with a switch from Th1 hepatic CD1d-restricted NKT toward mixed Th1 and Th2 cytokine production. While potentially of benefit in control of HCV infection, NKT cells may facilitate HCV-mediated liver disease progression.

Disclosures:

The following people have nothing to disclose: RouJie Wang, Lyly Tran, Kristin K. Snow, Karen L. Lindsay, Margaret J. Koziel, Mark A. Exley

1027
BLYS/BAFF RECEPTOR-LIGAND SYSTEM IN HCV INDUCED B-CELL CLONAL DISORDERS

Dan Landau[1,2], David Saadoun[1,2], David Klatzmann[2], Michelle Rosenzwajg[2], Patrice Cacoub[1,2]; [1]Service de Médecine Interne, Hôpital Pitié-Salpêtrière, Paris, France; [2]Laboratoire de Biologie et Thérapeutique des Pathologies Immunitaires, CNRS/UPMC UMR 7087, Hôpital Pitié-Salpêtrière, Paris, France

Background & Aims: Chronic hepatitis C virus (HCV) infection induces B lymphocytes clonal disorders, including mixed cryoglobulinemia (MC)-vasculitis and B-cell non-Hodgkin's lymphoma (B-NHL). The aim of this study was to examine the activity of the B-Lymphocytes Stimulator (BLyS) receptor-ligand system in HCV-induced B-cell disorders. Methods: A group of 94 patients with chronic HCV infection (28 HCV+ without MC, 23 HCV+ with asymptomatic MC, 35 HCV+ MC-vasculitis and 9 HCV+ B-NHL) and 15 healthy volunteers participated in this study. BLyS studies included serum BLyS measurement, membrane bound BLyS (mBLyS) staining on PBMC, BLyS binding capacity on CD19+ cells, BLyS receptors staining on CD19+cells, CD3+ cells and clonal Vh1-69+ population and BLyS anti-apoptotic and proliferative culture studies. Finally, we evaluated BLyS measurement following treatment with Interferon-alpha and Ribavirin (64 patients) or Rituximab (14 patients). Results: Serum BLyS concentration was elevated in HCV induced MC-vasculitis and B-NHL compared with HCV+ patients without vasculitis or healthy controls ($p<0.05$). Healthy controls and HCV patients without MC-vasculitis had similar levels of mBLyS expression with a trend towards lower expression in HCV-induced MC vasculitis. Compared with healthy controls BLyS binding to CD19+ cells was decreased in HCV patients without vasculitis, with a further decrease in patients with HCV-induced MC vasculitis (MFI, 41.6 ± 7, 20.8 ± 1.9, and 7.9 ± 4.3, respectively, $p<0.01$ and $p<0.05$, respectively). BR3 staining showed a step wise decrease with highest values in healthy controls and HCV+ without MC, and lowest in HCV-induced B-NHL ($p<0.0001$). BR3 staining levels were further decreased in Vh1-69+ clonal B-cells. BLyS anti-apoptotic effects were maintained in culture despite this decrease in BR3 staining. Complete clinical remission in HCV-induced MC-vasculitis or B-NHL was associated with a decrease in serum BLyS concentration, as well as with a marked increase in BR3 staining on CD19+ cells. Rituximab treatment in HCV induced MC-vasculitis or B-NHL was associated with a remarkable five-fold increase in serum BLyS concentration ($p<0.001$). This increase mirrored the depletion of CD19+ cells. BR3 staining in repopulating B-cell sub-populations was significantly decreased from 19.8 ± 2.5 to 6.9 ± 2.5 ($p<0.005$) Conclusion: The results of this study suggest that BLyS is significantly involved in HCV-induced B-cell clonal disorders.

Disclosures:

The following people have nothing to disclose: Dan Landau, David Saadoun, David Klatzmann, Michelle Rosenzwajg, Patrice Cacoub

1028
HCV-SPECIFIC CELLULAR RESPONSES IN ANTIBODY NEGATIVE, HCV RNA NEGATIVE INJECTION DRUG USERS

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Background & Aims: Hepatitis C virus (HCV) infection resolves spontaneously in 15-50% of adult patients, while the remainder becomes persistently viremic. However, growing evidence suggests that some people exposed to HCV may develop adaptive cellular immune responses without becoming viremic or developing antibodies. The aim of this study was to examine the prevalence of HCV-specific interferon (IFN)-γ cellular responses in high-risk individuals that are both HCV RNA and HCV antibody negative. Methods: We evaluated HCV-specific T-cell responses in 13 HCV RNA and HCV antibody negative young injection drug users (IDUs, aged 18-35 years) by IFN-γ enzyme-linked immunospot (ELISPOT) assay using 429 overlapping HCV peptides that cover the entire HCV genome. Peptides were pooled in 21 mixes before incubation with patients' peripheral blood mononuclear cells. Nine IDUs who were HCV antibody

positive and HCV RNA negative (who had presumably spontaneously resolved HCV infection) were used as positive controls. Fourteen people at low-risk for HCV acquisition were used as negative controls. Responses >2 standard deviation above the mean of the 14 controls for each respective mix were considered positive. *Results*: HCV-specific cellular responses in IDUs negative for both HCV RNA and HCV antibodies were similar to those measured in spontaneous resolvers. While we detected very strong responses to nearly all peptide mixes in 3/13 (23%) patients, 4/13 (31%) additional patients had moderately strong responses to at least 7 antigenic combinations. *Conclusions*: Intermediate to strong IFN-γ^+ responses to multiple HCV epitopes were detected in approximately half of analyzed patients, suggesting their prior exposure to HCV despite the lack of detectable HCV antibodies. The high frequency of cellular responses against HCV in seronegative IDUs supports the hypothesis that multiple low-level exposures to HCV may promote the development of cellular immunity without the development of viremia as has been shown previously in older IDUs, and suggests that this immunity may develop after a shorter duration of exposure. The potential protective role of these immune responses remains to be determined. *Drs. Edlin and Talal have contributed equally to the project.

Disclosures:

The following people have nothing to disclose: Marija Zeremski, Queenie B. Brown, Marla A. Shu, Brian R. Edlin, Andrew Talal

1029
DETECTION OF CD4+ T CELL RESPONSES IN PATIENTS WITH ACUTE HCV INFECTION IRRESPECTIVE OF CLINICAL OUTCOME

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Background and aims: HCV specific proliferative CD4+ responses have been demonstrated to be associated with the outcome of acute HCV infection. In contrast, little is known about the frequency and phenotypic properties of the HCV-specific CD4+ T cell response. Direct characterization of circulating CD4+ T cells has represented a significant challenge in human immunology, despite the recent development of MHC Class II tetramers. The purpose of this study was to longitudinally study CD4+ responses directly ex vivo by class II tetramer staining and by expanded CD4+ T cell lines. **Methods:** Multicolor ex vivo class II tetramer analysis was performed in a large cohort of 39 subjects with acute HCV infection. In a subset we were able to track the HCV specific CD4+ responses longitudinally. Additionally, we assessed the breadth of the response using expanded HCV specific short term lines of these subjects. **Results:** Using novel class II tetramers we could analyze HCV-specific CD4+ T cells directly ex vivo. We were able to track up to 3 different specificities by class II tetramers in a single patient. The frequency of tetramer positive cells reached up to 1/250 of all CD4+ cells during acute infection and declined significantly after resolution of HCV. Surprisingly, we found specific CD4+ cells by ex vivo Tetramer stainings or after short-term stimulation with Il-2 also in most subjects with chronically evolving HCV, despite the absence of a proliferative response. These responses disappeared over time, but were occasionally preserved in patients in whom treatment with Interferon-alpha was started early. Preliminary phenotypic studies also did not reveal a T-cell marker clearly associated with the outcome of infection. **Discussion:** HCV specific CD4+ T cells are detectable at early timepoints of HCV infection in the vast majority of subjects, irrespective of clinical outcome and independent of the results in standard proliferation assays. Direct ex vivo analysis of those cells using class II tetramers will be a powerful tool to understand the mechanisms by which CD4+ T cells fail to control HCV infection and to elucidate the fate of the T cell response when HCV persists.

Disclosures:

The following people have nothing to disclose: Julian C. Schulze zur Wiesch, Victoria Kasprowicz, Arthur Y. Kim, Steve Longworth, Brian Nolan, Thomas Kuntzen, Barbara McGovern, Lia Lewis-Ximenes, Raymond T. Chung, William Kwok, Bruce D. Walker, Georg M. Lauer

1030
CHRONIC EVOLUTION OF ACUTE HEPATITIS C IS ASSOCIATED WITH LOWER FREQUENCIES OF HCV-SPECIFIC CD4+ T-CELLS BUT UNRELATED TO HCV-SPECIFIC FOXP3+ REGULATORY CD4+ T-CELLS

Malte H. Heeg[1,2], Axel Ulsenheimer[1,2], Norbert H. Gruener[1,2], Maria C. Jung[1,2], Reinhart Zachoval[1], Winfried Schraut[2], Martin Waechtler[3], Gerd R. Pape[1,2], Helmut M. Diepolder[1,2]; [1]Dept. of Medicine II, LMU-Munich, Munich, Germany; [2]Dept. of Immunology, LMU Munich, Munich, Germany; [3]Hospital Munich-Schwabing, Munich, Germany

Spontaneous viral clearance in acute hepatitis C infection is associated with a strong HCV-specific CD4+ T-cell response. So far, studies of HCV-specific CD4+ T-cell response were limited to functional assays which are unable to identify dysfunctional T-cells or characterize the phenotype of HCV-specific CD4+ T-cells directly ex-vivo. These limitations can be overcome by the use of HLA-class-II-tetramers which allow direct enumeration and phenotyping of specific CD4+ T-cells independent of functional characteristics. Using two different novel HCV-NS3 specific HLA-DRB1*1501 tetramers we investigated the HCV-specific CD4+ T cell response longitudinally in 14 patients during the course of acute hepatitis C (mean 9.3 time points per patient). Patients with self limited disease showed considerable higher initial and peak frequencies of tetramer positive CD4+ T-cells than patients who developed chronic hepatitis C (mean 3600/million (m) vs. 800/m for Tet1 and 2900/m vs. 1200/m for Tet2 during peak phase). Likewise patients with self limited disease showed higher frequencies of IFN-γ secreting cells than patients who developed cHCV. IL7R and CD62L expression on HCV-specific CD4+ T-cells increased following viral elimination in patients with self limited acute hepatitis C but also in patients who achieved only transient viral control. Relapse and chronic hepatitis C were associated with reduced expression of IL7R and low expression of CD62L. CCR7 expression was low during acute hepatitis C and was only slowly up regulated in both resolved and chronic patients. Importantly, a significant number of Foxp3/CD25 expressing HCV-specific regulatory T-cells were detected only transiently in two patients and were accompanied by a loss of functional T-cell responses at the same time points. In both patients, however, functional T-cell responses recovered and spontaneous viral clearance was achieved. In conclusion, spontaneous viral clearance in acute hepatitis C is associated with a higher early frequency of HCV-specific CD4+ T-cells compared to patients with chronic disease evolution. Moreover, viral clearance is associated with the rapid up regulation of IL-7R and CD62L corresponding to

the ability to develop an (intermediate) effector memory phenotype. While, in a subgroup of patients, we were able to demonstrate antigen specific Foxp3+ regulatory T-cells, which were associated with the down regulation of HCV-specific CD4+ T-cell responses, their occurrence was short lived and not related to the evolution of chronic hepatitis C.

Disclosures:

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1031
HIGHER PERCENTAGES OF INTRA-HEPATIC REGULATORY T CELLS ARE PRESENT IN CHRONIC HEPATITIS B PATIENTS WITH A HIGH VIRAL LOAD

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CD4+CD25+FoxP3+ regulatory T cells (Treg) play a key role in the impaired immune response observed in patients with a chronic hepatitis B virus (HBV) infection. Most studies concerning Treg and HBV are performed with cells isolated from peripheral blood, while the liver is the main site of infection. To gain more insight in the role of Treg in chronic HBV we have monitored intra-hepatic Treg. Peripheral blood and liver biopsy samples were obtained from 40 patients with a chronic HBV infection. The liver tissue was collected in culture medium and was digested with collagenase for 15 minutes to create a single cell suspension. The percentage of Treg was determined by flowcytometry using antibodies to CD4, CD25 and FoxP3. The results show that patients with a high viral load of $>1 \times 10^5$ geq/ml have a significantly higher percentage of intra-hepatic Treg as compared to patients with low viral load $<1 \times 10^5$ geq/ml (8.20 % ± 0.50% vs. 6.18% ± 0.54% respectively; $p< 0.05$), while the percentage of peripheral blood Treg was similar in both groups (9.38% ± 0.39% vs. 8.94% ± 0.43% respectively). When patients were divided into two groups based on liver inflammation, indicated by their serum ALT levels (ALT < 2 x ULN and ALT > 2 x ULN) no differences in percentages were observed between the two patient groups for intra-hepatic Treg (7.61% ± 0.56% vs. 7.03% ± 0.52% respectively) as well as for peripheral blood Treg (9.34% ± 0.31% vs. 8.89 ± 0.60% respectively). There was also no relation observed between the proportion of Treg and the Metavir Fibrosis score. Next to CD4+CD25+FoxP3+ Treg the liver also contained a large population of CD4+CD25-FoxP3+ cells (2.40% ± 0.21% (intra-hepatic) vs. 0.89 % ± 0.06% (peripheral blood); $p < 0.05$). Additionally, approximately 40% of liver Treg expressed the immunoregulatory marker programmed death-1 (PD-1) while peripheral blood Treg hardly express PD-1. PD-1 expression does not seem to be specific for Treg, since the liver contains more PD-1+CD4+ T cells compared to peripheral blood. In conclusion: Liver CD4+ T cells have a higher expression of the immunoregulatory receptor PD-1. There was no relation between previous liver damage or liver inflammation and the presence of Treg. On the other hand, an increased proportion of intra-hepatic Treg was observed in patients with a high viral load, suggesting the importance of Treg in HBV tolerance and persistence of viral replication.

Disclosures:

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ANTIGEN-PULSED DENDRITIC CELLS ARE CAPABLE OF INDUCING BOTH INNATE AND ADAPTIVE IMMUNITY: A LESION LEARNT FROM CROSS TALK BETWEEN NATURAL KILLER CELLS AND DENDRITIC CELLS AND THEIR APPLICATION FOR IMMUNE THERAPY OF CHRONIC HEPATITIS B VIRUS INFECTION

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Purpose: The magnitudes of both innate and adaptive immunities are decreased in chronic infections and cancers. Accordingly, immune therapy against these diseases have been formulated to activate innate immunity targeting natural killer (NK) cells and adaptive immunity targeting dendritic cells (DC). This study was conducted to develop insights about crosstalk between NK cells and DCs and to assess if antigen-pulsed DCs can activate both innate and adaptive immunity. Methods: Both normal C57BL/6 mice and hepatitis B virus transgenic mice (that express hepatitis B surface antigen (HBsAg), HBV DNA, and hepatitis B e antigen) were used. NK cells were depleted by administrating anti-asialo GM1 antibody, 100 microliter/mouse, intraperitoneal, twice at an interval of 48 hours. Normal C57BL/6 mice and NK-depleted mice were immunized with hepatitis B vaccine containing HBsAg, intraperitoneally and the levels of antibody to HBsAg (anti-HBs) and HBsAg-specific T cells were estimated 28 days after immunization. Spleen DCs from normal C57BL/6 mice and hepatitis B virus transgenic mice were cultured with HBsAg to prepare HBsAg-pulsed DCs and administered to NK-depleted mice, twice, at an interval of 2 weeks. We assessed if HBsAg-pulsed DCs activated innate immunity in NK-depleted mice by inducing proinflammatory cytokines (interleukin-12p70, interferon-gamma, tumor necrosis factor-alpha, and interleukin-6), 3 days after administration of HBsAg-pulsed DCs. Also, we evaluated if HBsAg-pulsed DCs induced anti-HBs and HBsAg-specific lymphocytes in NK-depleted mice. Results: Flow cytometric analyses revealed that injection with anti-asialo GM1 in mice resulted in depletion of >90% NK cells from liver, spleen and bone marrow. Compared to normal C57BL/6 mice, DCs from NK-depleted normal C57BL/6 mice produced significantly lower levels of all proinflammatory cytokines ($p<005$) and could not activate HBsAg-specific lymphocytes in vitro indicating that NK cells are essential for proper functioning of DCs. High levels of anti-HBs were detected in the sera of all normal mice (10 of 10, 100%), but very low levels of anti-HBs were found in only 2 of 10 NK-depleted mice due to immunization with HBsAg ($p<0.05$). Also, the levels of HBsAg-specific T cells were significantly lower in NK-depleted mice compared to those in normal C57BL/6 mice ($p<0.001$). Administration of HBsAg-pulsed DCs induced higher levels of all proinflammatory cytokines, anti-HBs and HBsAg-specific lymphocytes in NK-depleted mice. Conclusions: This study provides the rationale for using antigen-pulsed DCs for treatment of chronic infections when both innate and adaptive immunities are impaired.

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1033
HLA CLASS II AND RACE-DEPENDENT DIFFERENCES IN IMMUNE RESPONSE TO HCV, SUSCEPTIBILITY TO CHRONIC INFECTION, AND RESPONSE TO THERAPY

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A hallmark of HCV infection is its tendency to develop into chronic disease. The response to therapy is more effective for Caucasian (CAU) than African American (AA) patients. The goals of the present study within the University of Tennessee Cooperative Hepatitis C Research Center have been to identify HLA associated genotypic differences among chronically infected AA and CAU patients that are 1) correlated with susceptibility to chronic infection, 2) correlated with response to combination therapy, and 3) determine class II-dependent immune responses by patient PBMC. HLA genotypes have been accumulated for 57 CAU and 85 AA patients. All patients had genotype 1a or 1b and received pegylated interferon-alpha2b and weight-based ribavirin. The difference in inheritance of HLA-DRB1*11 among African Americans (AA) chronically infected with HCV and the inheritance of DRB1*11 among AA in the Southeast is highly significant (Binomial Probability p = 0.000097). There is no DRB1*11-dependent significant difference in response to therapy among AA enrolled in our study. Among chronically infected Caucasians (CAU), we have observed a highly significant difference in the frequency of inheritance of HLA-DRB1*03 (Binomial Probability p = 0.00022). Both associations have been reported. What has not been reported is the difference in response to therapy among CAU with DRB1*03. Among CAU patients, 34% initially responded to therapy with elimination of circulating virus to below detectable levels for at least 24 consecutive weeks only to relapse to pre-therapy levels of viremia. More than half (53%) of relapsing CAU patients were DRB1*03 (p = 0.027, Fisher). Prior to therapy most chronically infected patients had relatively poor in vitro T cell proliferative and interferon-gamma responses to HCV, compared to mitogen or tetanus toxoid (TT). Immune responses to HCV were significantly higher (p = 0.007) in patients that remained virus free 24 weeks after the end of therapy compared to patients in which virus was not eliminated or who had viral relapse. A very surprising finding was that T cell responses were significantly higher to tetanus toxoid (TT) than HCV proteins (p = 0.011) for non-DRB1*11 AA but not DRB1*11 AA (p = 0.307). This result suggests that HLA-DRB1 inheritance may have a remarkable impact on immune potential to HCV and may explain in part why DRB1*11 AA are more prone to chronic infection. This result may also implicate an influence of Treg in susceptibility to chronic HCV, a hypothesis currently under investigation. This research was supported by NIH grants NIAID U19AI066316 and NCRR RR016949, RR022465, and M01 RR000211.

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1034
ADHESION OF *IN VITRO* GENERATED DENDRITIC CELLS TO HUMAN HEPATIC ENDOTHELIUM UNDER CONDITIONS OF SHEAR STRESS

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The incidence of hepatocellular carcinoma (HCC) is rising as a consequence of the increased prevalence of cirrhosis. Despite the presence of large numbers of tumour-infiltrating lymphocytes in HCC, effective killing of tumour cells is not achieved. Tumour-pulsed dendritic cells (DCs) can induce effective T cell responses in animals and early clinical trials are promising. However little is known about the mechanisms that regulate the recruitment of adoptively transferred DCs to the tumour. **Aims:** To determine the molecular regulation of DC trafficking to the human liver and hepatocellular carcinoma **Methods:** Liver-derived DCs (LDC) were isolated from patients with HCC. Monocyte derived DCs (MoDC) were generated in vitro in the presence of GM-CSF and IL-4, before being matured with macrophage conditioned media. Primary hepatic sinusoidal endothelial cells (HSEC) isolated from human liver were cultured in glass capillary tubes before being perfused with DCs at physiologically relevant shear stress. **Results:** LDCs from livers containing HCC expressed maturation markers including CD86 and Class II but expressed higher levels of CCR5 than CCR7 suggesting only partial activation. Under conditions of fluid flow anti-ICAM-1/VCAM-1 antibodies reduced adhesion of immature MoDCs to TNF-α stimulated HSEC by around 40%, though no significant variation was seen with mature MoDCs. Inhibition of CX_3CL1 on HSEC reduced total adhesion of both immature and mature MoDC by 60% and 40% respectively and transmigration by up to 70% in both cases. Similar inhibition of adhesion and transmigration was seen when pertussis toxin was used to block G_i protein linked signalling. **Conclusion:** These data demonstrate that MoDCs use a unique combination of adhesion molecules comprising CX_3CL1, ICAM-1 and VCAM-1 to bind HSEC from flow. This data has allowed us to set up a small clinical study to visualise MoDC homing to the human liver following administration peripherally or via the hepatic artery, the results of which we will present at this meeting.

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INTRAHEPATIC REGULATORY T CELLS DIFFER PHENOTYPICALLY FROM THEIR CIRCULATING COUNTERPARTS IN CHRONIC THERAPY NAïVE HCV PATIENTS

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BACKGROUND: Regulatory T cells (Treg) play a significant role in the hampered immune response against HBV, as we have shown previously. Similar findings have been suggested for Treg in chronic HCV infection. This may explain the weak HCV specific T cell response in these patients. The immune response at the primary site of infection, the liver, is likely to play a critical role in HCV persistence. METHOD: Minimally invasive aspiration biopsies enabled us to repeatedly study intrahepatic and peripheral Treg in parallel. RESULTS: In 20 chronic HCV therapy naïve patients, CD8+ lymphocytes were more frequent in the liver than in blood (22.8% and 13.6% respectively, p<0.001). In contrast, the proportion of CD4+ lymphocytes was lower intrahepatically than peripherally (26.34% and 31.57% respectively, p<0.05). Careful analysis of CD4+CD25hi cells and FoxP3+CD4+CD25+ cells revealed a smaller proportion of Treg in the liver (8.1% against 9.6%). Preliminary data suggest that Treg in liver and in blood predominantly consist of a memory phenotype (a.o. CD45RO). Our findings indicate that a significant proportion of CD4+ lymphocytes infiltrating the liver are Treg. However, the ratio Treg to total CD4+ lymphocytes and total CD8+ lymphocytes was lower than in peripheral blood. CONCLUSIONS: Our data

indicate that the chronicity of HCV can not be solely explained by an increased ratio of Treg to effector T cells in the liver. We are currently further investigating phenotypical and functional differences between liver and blood Treg. This could delineate the contribution and possible mechanism of intrahepatic Treg to the attenuated HCV immunity.

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1036
THE ROLE OF NANOBACTERIA IN CHOLECYSTITIS AND CHOLELITHIASIS

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Background: Recently discovered sub-microscopic blood particles known as nanobacteria, have a special characteristic no other blood particles have been known to possess: they form calcium phosphate shells. They have been found in human blood, arterial wall plaques, psammoma bodies of ovarian tumors and nephrolithiasis. The clinical trials demonstrated that the eradication of nanobacteria reduced the calcifications in coronary arteries. The aim of this study is to access the relation between nanobacteria and cholecystitis. ***Materials & Methods:*** Tewenty-six surgically resected gallbladders with cholecystitis were tested for the presence of nanobacteria by immunohistochemical staining using primary antibody (NanoVision Antibody 8D10, Nanobac OY, Finland) and super sensitive link-label immunohistochemical detection system (QA000-5L, BioGenex, San Ramos, CA). The formalin fixed tissues were imbedded in paraffin, sectioned at 50 microns and stained. The control slides (provided by Nanobac OY, Finland) included cell cultures with and without nanobacteria inoculation. The stained tissue slides were reviewed blindly and graded from 0 to 4+ in staining intensity and location of staining. The results were then compared to the clinical, gross pathology and histological findings. ***Results:*** The initial evaluation reveled that 25 out of 26 cases were stained positively for nanobacteria. Following intensity of staining was observed: 0 (1 case), 1+ (5 cases), 2+ (13 cases), 3+ (6 cases) and 4+ (1 case). The one case that did not stain was a gangrenous gallbladder with no normal epithelium remaining for evaluation. No staining was observed when the primary antibody was omitted. Six out of 13 cases with gross lithiasis, have staining intensity 3+ or more compared to one out of 13 without lithiases, suggesting a strong association between nanobacteria and cholelithiasis. Nanobacteria were detected within the gallbladder epithelium, inflammatory debris, calculi, surface sludge and focal arterioles. The stain was present in the arteriole intima and was not observed in the venules within the same slide. ***Conclusions:*** Nanobacteria were found in 25 out of 26 gallbladders with cholecystitis. It was present within the epithelium, inflammatory debris, stones and sludge of cholecystitis with and without lithiasis. The presence and intensity of staining were associated with well-formed gross lithiasis. This observation suggests the contributing role of nanobacteria in gallstones formation. It is conceivable that a specific therapy for nanobacteria may prevent cholecystitis and reduce the need for surgical interventions. Further assessment of additional specimens is underway.

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CRITICAL ROLE OF CD44 IN HEPATOTOXIN-MEDIATED LIVER INJURY

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Purpose. Blocking of adhesion molecules is considered to be one of the therapeutic strategies for inflammatory diseases, although it remains unclear whether this strategy is beneficial. Here, we evaluated role of CD44, which is one of the adhesion molecules against liver injury using carbon tetrachloride (CCl4). Methods. We injected CCl4 into CD44 deficient mice (CD44KO) or wild type mice and sacrificed mice at several time points after injection and analyzed serum ALT activity and cell number in intrahepatic leukocytes by FACS and cytokine, chemokine expression in the liver by RNAse protection assay. To determine which cell subpopulation produce cytokine we analyzed intrahepatic leukocytes by intracellular cytokine staining. In addition, we stained with H.E. and TUNEL using liver and performed EMSA for analyzing NF-kappaB activity. Furthermore, we injected CCl4 into CD44KO mice with neutralizing antibody for anti-CD11b and CD44NKT double knock out (DKO) mice to determine which cells population contribute to liver injury. Next, we injected CCl4 into CD44KO mice with neutralizing antibody for MIP-2 and TNF-a to clarify which cytokine or chemokine is responsible for liver injury. Results. We found that CD44 KO mice exhibited suppressed liver inflammation during the early phase after CCl4 injection due to reduced infiltration of inflammatory cells and cytokine production, but showed severe liver inflammation with increased numbers of apoptotic hepatocytes at the late phase. The induction of hepatocyte apoptosis was triggered by reduced NF-kappaB activity, which was induced by the low inflammatory cytokine concentrations. During the inflammatory response, the kinetics of natural killer T (NKT) cells were distinct. Specifically, these cells showed increased infiltration into the liver with greater proliferation and migration, which this phenomenon were CD44-independent. Furthermore, NKT cells and macrophages contributed to the induction of hepatocyte apoptosis, since CD44NKT DKO or neutralization by an anti-CD11b antibody significantly protected against hepatocyte apoptosis. Finally, we found that blocking of MIP-2 and TNF-a reduced hepatocyte apoptosis with decreased numbers of intrahepatic leukocytes and reduced inflammatory cytokine production. Conclusion. These findings suggest that targeting of CD44 as a therapeutic approach for inflammatory liver diseases may require caution for particular immune systems in the liver.

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INCREASED ACETYLATION OF ALPHA-TUBULIN AND DECREASED EXPRESSION OF FIBROCYSTIN ARE SENSITIVE MARKERS OF CELLULAR DAMAGE AND ACTIVATION IN INFLAMMATORY LIVER DISEASE

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Background. Primary cilia are highly conserved chemosensory organelles present in normal renal and hepatic ductular epithelium that determine cell polarisation, proliferation and activation. Alpha-tubulin is a constitutive structural component of the cytoskeleton, basal body and axoneme of primary cilia. Acetylation of alpha-tubulin (acAT) is necessary for microtubule stabilisation during primary ciliary formation, cellular proliferation and activation, however, hyper-acetylation of AT has been associated with hepatocyte damage in ethanol fed mice. Fibrocystin is a receptor protein, of unknown function, that also localizes to primary cilia. Mutations of fibrocystin cause a structural dysfunction of primary cilia associated with polycystic kidney and liver disease. Aims. To investigate acAT and fibrocystin expression in human liver tissue during cellular activation associated with inflammatory/malignant disease. Methods. Routine paraffin embedded liver tissue subjected to low temperature epitope retrieval was probed with a commercially available mouse monoclonal antibody to acAT or an antibody raised to the c – terminus of wild type human fibrocystin. Antigen localisation was visualised using a ChemMate Envision detection system (Dako). The cohorts were:- Adult polycystic liver disease (PLD); PBC; PSC; Allograft Rejection (AR) Autoimmune Hepatitis (AIH); HCV; Chronic Alcoholic Liver Disease (ALD); alpha-1-AT deficiency (AAT); Biliary Atresia (BA); Hepatocellular Carcinoma (HCC). Normal liver tissue (NL) surplus to transplantation requirements was used as a control. (n=5 for each group). Results. acAT:- normal donor liver was consistently negative. All other disease groups showed very strong acAT staining of bile ducts particularly in periseptal regions of ductular reactivity. Periseptal/periportal hepatocytes and bile ducts were very strongly +ve in ALD, HCV and HCC. Staining of cystic epithelium in PLD was variable and frequently -ve whereas tumour cells in HCC were frequently +ve. acAT staining was very strong in perivenular hepatocytes and surrounding inflammatory cells in AR. Fibrotic and cirrhotic lesions contained strongly positive inflammatory and stromal cells. By contrast, most bile ducts in normal tissue were strongly +ve for fibrocystin whereas staining was frequently low or absent in all diseased liver tissue. Conclusions. Acetylation of alpha tubulin is a sensitive marker of cellular activation in most common inflammatory liver diseases and the absence of fibrocystin staining reflects ongoing damage to the intrahepatic biliary tree, rather than a phenomenon specific to the so called ciliopathies.

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CRITICAL ROLE OF EARLY GROWTH RESPONSE-1 TO GALACTOSAMINE / LIPOPOLYSACCHARIDE-INDUCED ACUTE LIVER INJURY IN MICE

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Early growth response-1 (Egr-1) is a transcription factor that functions as a master regulator of gene expression in response to proinflammatory signals and cellular stress and plays a critical role in the pathology of ischemia/reperfusion injury, cholestasis, atherosclerosis, pancreatitis and ethanol-induced fatty liver in mouse models. The purpose of this study was to test the hypothesis that Egr-1 is also involved in the development of galactosamine/lipopolysaccharide (GalN/LPS)-induced acute liver injury. GalN/LPS exposure bi-phasically increased hepatic Egr-1 mRNA accumulation at 1h and then at 4-5.5h after treatment. The effects of GalN/LPS were next compared in wild type and *egr-1 -/-* mice. Within 4-5.5h after GalN/LPS exposure, wild type mice exhibited evidence of hepatocyte injury, cell death, and extensive areas of hemorrhage; these parameters were attenuated *egr-1 -/-* mice. Liver to body weight ratios and ALTs were lower in *egr-1-/-* mice 5.5h post GalN/LPS administration compared to wild type mice. The initial proinflammatory response to GalN/LPS administration in *egr-1-/-* mice was similar to wild type, with no differences in TNFα, MIP-2, MCP-1 or ICAM-1 mRNA or protein between genotypes at 1h. However, expression of these genes was lower in the livers of *egr-1-/-* mice at subsequent time points. The observed decrease in the hepatic inflammatory milieu was associated with a decrease in neutrophil extravasation from hepatic sinusoids into the liver parenchyma in *egr-1-/-* mice, compared to wild type mice, 4h after GalN/LPS. In addition, there were fewer TUNEL positive cells in livers from *egr-1-/-* mice 5.5h post GalN/LPS. Finally, the decreases in inflammatory and chemotactic responses in *egr-1-/-* mice were associated with increased survival in *egr-1-/-* mice after GalN/LPS. In conclusion, these data demonstrate, for the first time, that Egr-1 plays a critical role during later phases of GalN/LPS-induced acute liver injury. Supported by NIH grants AA015833 to MTP, AA003624 to GEA and AA0138868 to LEN

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INHIBITION OF THE KUPFFER CELL INCREASED SERUM HMGB1 LEVELS AND THE MORTALITY IN A RAT SEPTIC PERITONITIS MODEL

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High-mobility group protein 1 (HMGB1) is a nonhistone nuclear protein whose function depends on cellular location. Inside the cell, HMGB1 modulates a variety of important cellular processes, including transcription, whereas outside the cell, HMGB1 acts as an inflammatory cytokine and mediate sepsis in animal models. In in vitro studies, proinflammatory molecules can induce HMGB1 release from macrophages. Since the Kupffer cell (KC), the resident hepatic macrophage, is a predominant population of macrophage lineage, the specific purpose of this study was to investigate whether inhibition of the KC affect the expression of HMGB1 in sepsis. Rats were given saline or

gadolinium chloride (GdCl3), a KC toxicant, 24 hours before cecal ligation and puncture (CLP). Survival was assessed for 7 days after CLP. Liver tissues and blood samples were harvested, and the expression of inflammatory mediators was assessed. Inhibition of the KC increased significantly the mortality 7 days after CLP. Plasma endotoxin and serum TNF-α levels were significantly greater in the GdCl3 than in the control group 12 hours after CLP. Furthermore, serum HMGB1 levels were significantly greater in the GdCl3 group than the control group. To assess the expression of HMGB1 in the liver after CLP, immunohistochemical staining for HMGB1 was performed. The expression of HMGB1 was detected on cells in the hepatic sinusoid 12 hours after CLP. These cells recognized morphologically as KCs. Furthermore, the number of the HMGB1-positive KCs was significantly greater in the GdCl3 group than the control group. The mRNA expression of HMGB1 in the liver was assessed by RT-PCR each time after CLP. The expression was significantly greater in the GdCl3 group compared with the control group after CLP. It was reported that GdCl3 eliminates ED1- and ED2-positive macrophages, which were morphologically large KCs, and ED1-positive and ED2-negative KCs were still observed after treatment of GdCl3. Therefore, the mRNA expression of HMGB1 was assessed in each subpopulation of the KC. The Kupffer cells were isolated by a counterflow centrifugal elutriation to separate cells based on cell size and density. The expression of HMGB1 was greatest in the small KCs, which were ED1-positive and ED2-negative cells assessed by flow cytometry, compared with large KCs, which were ED1- and ED2-positive cells. Thus, the large KC plays a beneficial role on the host defense during sepsis. Furthermore, the small KC, which may include infiltrating cells into the liver, is a predominant source of HMGB1 in the liver.

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1041
MRNA EXPRESSION ANALYSIS OF IMMUNOREGULATION, APOPTOSIS AND FIBROSIS IN LIVER DISEASES

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Background: T-regulatory cells (Tregs) are important mediators of immune suppression. Their presence prevents self-immune reactions by inducing regulatory signals to APCs and/or effector T-cells. In previous studies the persistence of viral infection in liver has been associated with the expansion of Tregs in the peripheral blood of the affected patients. Our aim was to study, at the transcriptional level, the presence of Tregs in liver tissues of patients with various diseases. Patients and methods: Liver biopsies from 20 patients (7 with chronic HBV hepatitis and/or cirrhosis, 4 with chronic HCV hepatitis and/or cirrhosis, 6 with NASH, 2 with methotrexate-related toxicity and 1 with autoimmune cirrhosis) and 5 controls (patients with minimal disease or post-mortem normal tissue) were studied. Total RNA was extracted and cDNA was synthesized using standard protocols. Quantitative real-time PCR analysis was performed for the detection of mRNA expression of: Foxp3 (determinant of the presence of natural Tregs), TGF-β1 and IL-10 (cytokine determinants of inducible Tregs – Th3 and Tr1, respectively), TGFβRI, TGFβRII, Smad2, Smad3, Smad4, Smad7 (receptors and mediators of TGF-b signaling) and TRAIL, Fas, FasL (mediators of apoptosis). REST software was used for the statistical analysis. Results: Irrespective of the type of liver damage (viruses, drugs, NASH, autoimmunity) an expansion of natural Tregs was present, evident through the dramatic increase of Foxp3 expression(for HBV OR: 28,025, 95%CI: 3,01 - 255,78,p=0.002, for HCV OR: 16,11, 95%CI: 3,837 - 96,625, p=0.025, for NASH OR: 20,194, 95%CI: 4,603 -165,843, p=0.01, for methotrexate-related toxicity OR: 28,1, 95%CI: 9,391 - 107,627, p=0.001, for autoimmune cirrhosis 25.4 fold) accompanied by a significant increase in mediators of apoptosis (Fas-FasL, TRAIL), compared with normal controls (p<0.01). Furthermore, an increase in the expression of Smad7 (indicator of increased TGF-b signaling) was observed in patients with cirrhosis, compared with normal controls (OR:2,392, 95%CI: 1,604 - 3,575,p=0.012) Conclusions: Our results suggest that acute or chronic tissue damage, irrespective aetiology, results in increased apoptosis, followed by increased phagocytosis and possibly in self-antigen presentation by APCs. Eventually, this could lead in an expansion of natural Tregs that would prevent catastrophic autoimmunity in the damaged tissue. Elucidation of the pathways mediating this potential model could increase our understanding of the role that Tregs play in disease propagation and, for the first time, could have wider implications for the manipulation of Tregs in a therapeutic setting.

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1042
EXPRESSION OF NOTCH SIGNALING AND ANTIGEN PROCESSING MOLECULES IN PROGRESSIVE PATHOGENENIC BIOPSIES OF CHRONIC HEPATITIS B INFECTION

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Background: The Notch signaling plays a key role in cell-fate determination and differentiation, functioning in a cell- and tissue -specific manner. Both upregulated and downregulated Notch1 signalling is seen in carcinogenesis of different tissues. Whereas, in stem cells, notch activity inhibits differentiation and promotes carcinogenesis but in primary epithelial cells high notch activity leads to exit from the cell cycle and commitment to differentiation. Notch1 exerts specific protective effects against HPV-virus and down-modulation of Notch1 was observed in the late stages of HPV-induced carcinogenesis. Aim: To study notch signaling in different stages of HBV induced liver disease and its role in liver disease progression. Patients and Methods: PBMCs of chronic hepatitis B (CHB) (n=10) (age 29.4±13.2 yr., M:F::8:2) patients and healthy controls (n=10) (age 27.9 ± 4.6 yr., M: F ::7:3) and liver biopsies from normal liver – 3 (age 49±3.8 yr.M:F::2:1), CHB patients (age 39±12.8, M:F::3:1) HBV cirrhosis -4 (age-36.2±20.0, M:F::3:1) and HBV related HCC –3(age-55.5±7.2 yr., M:F::3:0) were studied. Total RNA was extracted from PBMCs and biopsies and Notch1, Hes1, Jagged1, and NFKB genes were analyzed by quantitative RT-PCR. Results: Compared to PBMCs of CHB patients, the liver biopsies showed significantly lower expression of Notch1 (p=0.034), Jagged 1 (p=0.021) but not Hes 1 (Table 1). The reduction in expression of these genes was significantly greater in HCC patients compared to CHB and controls. In comparison to control biopsies and PBMCs in CHB, cirrhotic tissue showed significantly lower expression of NFKB (p=0.02). Conclusions: In different stages

of liver disease due to HBV, the PBMCs show higher expression of notch signaling molecules, but in the target liver tissue, the expression of these molecules is significantly inhibited, maximum being in CHB and HCC. This expression pattern of Notch1 signaling suggests that this protein may play a permissive or tumor-promoting function in chronic HBV infection

	CHB PBMCs	CHB Bx	P	Cirrhotic Bx	P	HCC Bx	P
Notch1	0.39	-0.006	0.034	-0.03	NS	-0.06	0.05
Hes1	0.82	-0.26	0.4	-0.29	NS	-0.5	NS
Jagged1	0.63	0.057	0.021	0.02	NS	-0.34	0.034
NFKB	2.2	-0.3	0.027	-0.42	0.021	-0.25	0.034

Disclosures:

The following people have nothing to disclose: Nirupma T. Pati, Sujoy Bose, Sukriti Baveja, Hissar Syed, Shiv K. Sarin

1043
INTRAHEPATIC STATUS OF REGULATORY T CELLS IN AUTOIMMUNE HEPATITIS, PRIMARY BILIARY CIRRHOSIS, CHRONIC HEPATITIS C, AND CHRONIC HEPATITIS B

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Background/Aims: Regulatory T cells (Tregs) maintain immunological tolerance and suppress autoreactive immune responses. We evaluated the intrahepatic status of Tregs in patients with autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), chronic hepatitis C (CH-C), or chronic hepatitis B (CH-B). Methods: We analyzed 85 patients (20 AIH, 22 PBC, 27 CH-C, and 16 CH-B) and 14 controls. Using liver tissue samples obtained by needle biopsy or from marginal parts of resected metastatic liver tumors in controls, immunohistochemical analyses of forkhead box P3+, which is a specific marker for Tregs, CD4+, and CD8+ cells were performed. Results: Intrahepatic Tregs were significantly infiltrated in patients with liver diseases than in controls. There were significantly fewer intrahepatic Tregs in AIH patients than in PBC patients (p=0.037). Patients with a low frequency of intrahepatic Tregs were detected significantly less in the AIH and CH-B groups than in the PBC and CH-C groups. We found significantly less infiltration of CD4+ T cells in AIH than in other diseases (p<0.05). Liver-infiltrating CD8+ T cells were detected more frequently in the CH-B group than in other groups (p<0.003). Conclusions: Intrahepatic Tregs were increased in both patients with autoimmune liver diseases and those with viral hepatitis. In autoimmune liver diseases, intrahepatic Tregs in AIH patients were fewer than in PBC patients.

Frequency of intrahepatic FOXP3+, CD4+, and CD8+ cells in patients with each liver disease.

	AIH	PBC	CH-C	CH-B	Control
%FOXP3+	5.2±2.4*,**	7.2±3.5 *,**	6.2±3.7 **	6.3±1.6 **	1.1±1.1 **
%CD4+	37.8±11.0†	47.4±11.0 †	44.0±9.3 †, ¶	50.6±8.7 †, ¶, Ω	41.0±10.9Ω
%CD8+	32.2±12.2‡	33.3±10.4 ‡	30.5±7.0 ‡	44.2±9.3 ‡	32.0±1.9‡

The values are means ± SD.

*p = 0.037 (AIH vs PBC); **p < 0.001 (Patients vs Control) p = 0.007, 0.045, 0.001 (AIH vs PBC, CH-C, CH-B); ¶p = 0.026 (CH-C vs CH-B); Ω p = 0.013 (CH-B vs Control); p = 0.003, 0.002, 0.001, 0.001 (CH-B vs AIH, PBC, CH-C, Control).

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1044
SPECIFIC CONTRIBUTION OF NADPH-OXIDASE IN DISTINCT CELL TYPES DURING HEPATIC FIBROSIS.

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Background: *NADPH-oxidase* is a multi-protein complex that produces differential amount of reactive oxygen species. In addition to its main activity in host defence, *NADPH* plays a central role in the regulation of hepatic fibrosis via induction by angiotensin II, leptin and PDGF. *NADPH* is functionally expressed in several cell types involved in hepatic fibrogenesis, such as hepatic stellate cells (HSC), Kupffer cells (KC) and hepatocytes. However the contribution of this complex in distinct cell types during hepatic fibrosis is unclear. **Aims:** To determine the role of *NADPH-oxidase* in different cell populations during hepatic fibrogenesis. **Methods:** Hepatic fibrosis was induced by bile duct ligation (BDL) for 21 days or by methionine-choline deficient (MCD) diet for 10 weeks in wild-type (WT) and mice deficient in $p47^{phox}$, a central component of both phagocytic and non-phagocytic *NADPH-oxidase*. Hepatic fibrosis was evaluated by Sirius red staining and mRNA expression of collagen α1(I), αSMA, TGFβ and TIMP-1 by quantitative real time PCR. Hepatic steatosis was determined by oil red-O staining. Hepatocellular injury was assessed by serum ALT and AST levels. $p47^{phox}$ chimeric mice were generated by the combination of liposomal clodronate, irradiation and bone marrow (BM) transplantation of $p47^{phox}$-/- BM into WT recipients and vice versa. **Results:** Upon BDL, the increase of hepatic collagen and hydroxyproline content was lower in $p47^{phox}$-/- mice compared with WT mice. The chimeric mice that contained $p47^{phox}$-/- KCs and WT HSCs showed 25% reduction of fibrosis, as assessed by Sirius red staining. In contrast, the chimeric mice with WT KCs and $p47^{phox}$-/- HSCs displayed about 60% reduction of fibrosis. In order to evaluate the potential contribution of *NADPH* in parenchimal cells, such as hepatocytes, we found no significant differences between WT and $p47^{phox}$-/- mice in MCD-diet induced lipid accumulation and hepatocytes injury as assessed by AST and ALT. In contrast, fribrogenesis was significantly suppressed in $p47^{phox}$-/- mice compared with WT mice treated with the MCD-diet, confirming the importance of $p47^{phox}$ in HSCs during hepatic fibrosis. **Conclusion:** *NADPH-oxidase* and its component $p47^{phox}$ play critical roles in KCs and HSCs during fibrogenesis. Non-phagocytic form of *NADPH-oxidase* in HSCs contributes to hepatic fibrosis more than the phagocytic form of *NADPH-oxidase* in KCs. In addition, $p47^{phox}$ in hepatocytes does not play a role in fibrogenesis induced by the MCD-diet. Our results demonstrate the importance of *NADPH* in HSCs and suggest that potential selective inhibition of *NADPH* in HSCs could have anti-fibrotic activity, without effecting host defences.

Disclosures:

The following people have nothing to disclose: Samuele De Minicis, Ekihiro Seki, Christoph Osterreicher, Robert F. Schwabe, David Brenner

1045
TLR4/MYD88/NF-KB-MEDIATES HEPATIC STELLATE CELLS ACTIVATION BY THE REGULATION OF TGF-B PSEUDORECEPTOR BAMBI IN HEPATIC FIBROSIS

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Background: The gram-negative bacterial cell wall component lipopolysaccharide (LPS) is elevated in the plasma of patients with liver cirrhosis by increased intestinal permeability. Toll-like receptor(TLR) 4 recognizes LPS and results in NF-kB activation via MyD88-dependent pathway. TLR4 signal also activates IRF-3 via TRIF-dependent pathway. It was assumed that increased LPS level in portal may activate TLR4 on Kupffer cells(KCs), subsequently activating hepatic stellate cells(HSCs) by indirect or direct action of KCs, resulting in promoting liver fibrosis. Surprisingly, TLR4 on HSCs, but not KCs, are critical in hepatic fibrosis as we have previously shown using TLR4-chimeric mice. In addition, TLR4 signal activates HSCs by downregulation of Bambi, an inhibitor of TGF-b signal. However, the precise mechanism of Bambi regulation is unknown. Aim: To define the mechanism by which TLR4 signal regulates Bambi expression and HSC activation. Methods: Hepatic fibrosis was induced by bile duct ligation(BDL) on WT, MyD88-/- and TRIF-/-mice. To evaluate liver fibrosis, we measured collagena1(I), aSMA, TGF-b1 and TIMP-1 mRNA expression by real time PCR(qPCR). Collagen promoter activity was assessed in HSCs isolated from collagen-GFP transgenic mice following infection with control or IkB super-repressor(sr) adenovirus after stimulation with TGF-b plus LPS. Bambi, KC, and IP-10 mRNA expressions in HSCs infected with control or IkBsr adenovirus or in HSCs isolated from WT, MyD88-/- and TRIF-/- mice were assessed by qPCR. Results: Upon LPS stimulation, Bambi mRNA expression was downregulated in control virus-infected HSCs, but not in the HSCs infected with IκBsr virus. Control, but not IkBsr virus infected-HSCs have an increase in collagen promoter activity after TGF-b plus LPS stimulation. In addition, Bambi mRNA expression was downregulated in WT and TRIF-/- HSCs, but not in MyD88-/- HSCs. The MyD88-dependent inducible gene KC was upregulated in WT or TRIF-/- HSCs, but not in MyD88-/- or IkBsr-expressing HSCs. In contrast, the MyD88-independent inducible gene, IP-10 was upregulated in WT, MyD88-/- or TRIF-/- HSCs, but not in IkBsr-expressing HSCs. Analysis of profibrogenic gene expression showed upregulation of collagena1(I), aSMA, TGF-b1 and TIMP-1 in WT and TRIF-/- BDL liver. In contrast, profibrogenic gene expressions were less increased in MyD88-/- BDL liver. Conclusion: Bambi mRNA expression is controlled in a TLR4/ MyD88/NF-kB-dependent pathway. Consistent with Bambi regulation, the initial event of hepatic fibrogenesis is regulated by the TLR4/MyD88/NF-kB axis. In addition, our results suggested that TRIF does not play roles in TLR4 signal in HSCs.

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The following people have nothing to disclose: Ekihiro Seki, Samuele De Minicis, David A. Brenner, Robertf F. Schwabe

1046
GLYCATED FIBRONECTIN STIMULATES HEPATIC STELLATE CELL (HSC) ACTIVATION AND TYPE I COLLAGEN PRODUCTION IN PRIMARY CULTURES

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INTRODUCTION: Type II diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Diabetes has been also found to be an independent predictor of fibrosis in nonalcoholic steatohepatitis (NASH). Metabolic milieu of type II diabetes is characterized by hyperglycemia and high insulin levels due to the insulin resistance. We have previously reported that high insulin levels stimulate type I collagen synthesis by HSC. As this effect was more potent in a high glucose media, in this study we examined the role of nonenzymatic glycation of matrix component fibronectin in HSC activation and fibrogenesis. MATERIAL AND METHODS: HSC were isolated from transgenic mice harboring transgene with Green fluorescent protein fused to COL1A1 gene promoter, using a collagenase pronase digestion followed by density gradient centrifugation. HSC were plated on plastic in low and high glucose media and treated with insulin. Additionally, HSC were initially plated on plastic (to ensure purer population of HSC), than replated at day 3 on plastic, native fibronectin and glycated fibronectin. Fibronectin was glycated for 30 days in 0.5M solution of glucose 6-phosphate at 37oC. HSC were imaged at day 6 and 7 for GFP expression and harvested at day 7 for protein analysis. Type I collagen was detected by Western blot of total cell extract at culture day 7. Adhesion and proliferation assays were performed at day 6 of culture. RESULTS: Insulin treatment of primary HSC cultures stimulated type I collagen expression only in high glucose conditions. HSC plated on glycated fibronectin showed increased proliferation and type I collagen synthesis when compared with cells plated on native fibronectin and plastic. In addition, significantly more HSC plated on glycated fibronectin expressed GFP transgene in culture on both day 6 and 7. Adhesion assay showed significantly more HSCs attached on glycated fibronectin than on unaltered fibronectin, indicating possible role of integrins in this effect, a concept supported by our previous studies, demonstrating the importance of fibronectin receptor, alpha5beta1 integrin, in early stages of HSC activation. We detected higher levels of the focal adhesion kinase in HSC plated on glycated fibronectin, suggesting that integrin signaling may be involved in exaggerated HSC activation on glycated matrix. CONCLUSION: Glycated fibronectin stimulates activation and type I collagen synthesis in primary HSC cultures. Glycation of extracellyular matrix in the liver of diabetic patients, may be a factor in stimulating fibrogenesis and contributing to a higher rate of fibrosis in these patients.

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The following people have nothing to disclose: Milan Dodig, Min Li, Srinivasan Dasarathy, Arthur J. McCullough

1047
BENEFICIAL EFFECT OF ANGIOTENSIN-BLOCKING AGENTS ON LIVER FIBROSIS IN PATIENTS WITH HEPATITIS C

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Background: The morbidity and mortality related to chronic Hepatitis C viral (HCV) infection is related to liver fibrosis progression. Current treatments for HCV are limited to interferon and ribavirin, which have unproven effectiveness and limited tolerability as long-term agents in nonresponders. Rational approaches are needed to identify viable antifibrotic agents. Several lines of evidence have implicated angiotensin II as a driver of liver fibrosis. Various animal and clinical studies have

shown that angiotensin-blocking agents attenuate liver fibrosis, but none has evaluated histological data in patients infected with HCV. In this study we evaluated the histological effect of angiotensin-blocking agents in patients with chronic HCV. Methods: This was a retrospective chart review of 234 patients from our institution during the years 2001-2006 with chronic HCV and concomitant hypertension (HTN), and an available liver biopsy. 143 patients (Group I) received angiotensin-blocking agents (angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blocking agents (ARBs) to treat HTN and 91 patients (Group II) received other anti-hypertensive agents (including beta-adrenergic blocking agents, calcium antagonists, alpha-adrenergic blocking agents and diuretics). All available biopsies were reassessed and restaged by a single, blinded pathologist. Results: The two groups were similar with respect to age, sex, genotype, HCV RNA levels and estimated duration of disease, but the two groups varied significantly with regards to the total proportion of diabetic patients (Group I: 43/143 (30%); Group II: 10/91 (11%), p value 0.0007), a result of the recommended management of hypertensive diabetics with ACE inhibitors. There was a significant difference in ISHAK fibrosis score (Group I: 3.20; Group II: 3.73, p value 0.04). Subgroup analysis of non-diabetics demonstrated an even more significant difference between the two groups in ISHAK fibrosis score. (Group I: 3.07; Group II 3.69, p value 0.023). Conclusion: The administration of angiotensin-blocking agents in patients with HCV infection and HTN was associated with histological evidence of decreased fibrosis. In patients without diabetes, this difference was even more pronounced. These data provide support for prospective evaluation of ACE inhibitors or ARBs as possible hepatic fibrosis inhibitors in patients with HCV.

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1048
ROLE OF P75 SIGNALLING IN THE PATHOGENESIS OF LIVER FIBROSIS IS CONTEXT AND LIGAND STRUCTURE DEPENDENT

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Hepatic myofibroblasts (MFB) express p75, the low-affinity nerve growth factor (NGF) receptor, and sortilin, a putative receptor for proNGF. We propose mature NGF is pro-apoptotic whilst proNGF is both protective from serum-deprivation induced apoptosis and proliferative. During resolution of experimental fibrosis or partially reversible cirrhosis levels of proNGF fall relative to mature NGF. We describe functions of p75 signalling in a murine model of liver fibrosis. Wild-type and p75NTR/exonIII-/- mice that do not express the ligand binding domain but express the intracellular domain were injured with carbon tetrachloride and allowed to recover. At peak fibrosis livers from p75-/- animals contained fewer α-smooth muscle actin (α-sma) positive cells, a marker of MFB, compared with wild-type animals. Wild-type livers had 6.9x more non-parenchymal cells staining positively for a proliferation marker (Ki-67) than p75-/- animals. In recovery there was significantly retarded histological resolution in the livers of p75-/- animals, determined by both semi-quantitative scoring and morphometric image analysis of Sirius red stained sections. There was also a significantly delayed loss of α-sma positive cells. The percentage of MFB undergoing apoptosis, determined by caspase 3 activation, was significantly reduced (day 7, 34% v 75%, $p<0.05$) in the livers of p75-/- animals relative to wild-type animals indicating that the persistence of MFB is likely to be due to reduced apoptosis. Our findings are broadly supported by the determination of the expression of procollagen-1, TIMP-1 and α-sma mRNA in injured liver by real-time PCR. Deletion of p75NTR/exonIII-/- does not influence the activation of hepatic MFB or the expression of procollagen-1, TIMP-1 and α-sma in vitro, determined by real-time PCR and immunofluorescence. However, MFB isolated from p75-/- mice and activated by culture on plastic significantly fail to respond to mitogens (fetal calf serum and PDGF), consistent with our in vivo findings in livers of p75-/- animals and suggesting a tonic proliferative role for p75 in hepatic MFB. We have demonstrated a dual role for p75 signalling in liver fibrogenesis. During injury p75 has a profibrotic role via proNGF mediation of MFB proliferation, and protection from apoptosis. In contrast, during recovery from liver fibrosis when proNGF levels fall and mature NGF dominates, p75 has anti-fibrotic effects by mediating MFB apoptosis.

Disclosures:

The following people have nothing to disclose: Timothy J. Kendall, R Christopher Benyon, John Iredale

1049
LIVER FIBROSIS PERSISTS FOLLOWING NEUTROPHIL DEPLETION DURING LIVER REPAIR

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BACKGROUND & AIMS: Cholestatic liver disease from extrahepatic biliary obstruction is characterized by progressive fibrosis. Biliary decompression initiates liver repair and matrix metabolism. We previously demonstrated neutrophil migration to fibrotic portal areas that colocalizes, and is associated with active MMP8. Further, a reduction of neutrophils via Kupffer cell depletion/inactivation resulted in inhibition of matrix metabolism and liver repair. We hypothesize that neutrophils mediate matrix resorption during liver repair and questioned whether specific neutrophil depletion in cholestatic rats would alter the resolution of fibrosis following biliary decompression. METHODS: Male Sprague-Dawley rats underwent biliary ductal suspension (BDS) for 7 days (=60% maximal fibrosis) followed by decompression (repair; 0d). Rats were treated with either saline or the mouse anti-rat neutrophil IgM antibody RP3 beginning 48 hours prior to repair and continuing daily following decompression. Bilirubin levels and collagen morphometry (sirius red) verified decompression. Complete blood counts (CBC) with differential counts were performed. Liver tissue was obtained at 0 and 2 days of repair ($N\geq3$/group) and processed for immunohistochemistry, quantitative PCR and morphometric analysis. RESULTS: Bilirubin and weight data confirmed cholestasis and subsequent repair following decompression. CBC and differential confirmed successful neutrophil depletion following RP3 treatment (1.16X10^3 neutrophils at 0 days of repair versus 5.74X10^3 for saline controls; $p\leq0.05$) without depletion of other circulating leukocytes. Immunohistochemical staining confirmed neutrophil depletion within the liver tissue in RP3 treatment groups when compared to saline controls (27.94 versus 359.67 cells per mm^2; $p\leq0.05$) without altering populations of infiltrating monocytes (ED1) and Kupffer cells (ED2). Sirius

red stained sections, examined by morphometry, indicated that portal tract collagen-1 remained elevated in neutrophil depleted animals (7.72% area versus 3.52% in saline controls; $p \leq 0.05$) despite normal reductions in bilirubin and liver enzymes. Quantitative real time PCR results for neutrophil depleted livers demonstrated dramatic increases and a loss of regulation of expression of the neutrophil chemoattractant CINC1, matrix metalloproteinases and tissue inhibitors of metalloproteinases. CONCLUSIONS: We conclude that functioning neutrophils are critical to liver repair following cholestatic injury. Further, neutrophil migration into repairing liver provides inhibitory feedback for normal chemokine and matrix metalloproteinase expression during cholestatic injury and repair.

Disclosures:

The following people have nothing to disclose: Mark Harty, Elaine F. Papa, Grant A. Ramm, Stephen H. Gregory, Thomas F. Tracy

1050
EVIDENCE OF SNAIL1 TRANSCRIPTION FACTOR INVOLVEMENT IN HEPATIC STELLATE CELLS ACTIVATION PROCESS

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Background: Following liver injury, hepatic stellate cells (HSC) undergo a process of activation from the quiescent fat-storing phenotype to a highly proliferative myofibroblast-like phenotype. Snail1 is a transcription factor best known for its ability to trigger ephitelial-mesenchymal transition (EMT), to influence mesoderm formation during embryonic development and to favour cell survival. Recent studies showed that the aberrant activation of Snail1 is sufficient to trigger renal fibrogenesis and that is expressed in fibroblasts in vivo during the healing process. We therefore hypothesised that Snail1 could be involved in HSC transdifferentiation process. Methods: Primary HSC were isolated from livers of healthy mice and cultured on plastic up to 10 days (in vitro activation model) or from livers of CCl4-treated mice (8 weeks, twice-weekly, i.p.) and cultured 1 day (in vivo activation model). Activation status of HSC was monitored by assessing mRNA level of HSC activation-related genes: α-smooth muscle actin (αSMA), α(1)I collagen (COL1A1), tissue inhibitor of metalloproteinase-1 (TIMP1) and fibronectin (FN). Snail1 expression in HSC and liver was screened by immunostaining and qRT Real Time PCR, whereas Snail1 activity was checked indirectly quantifying mRNA of Snail1-target genes: E-cadherin (E-cad), desmoplakin (DSP) and metalloproteinase-9 (MMP9) by qRT Real Time PCR. Results: We observed a significant increase in Snail1 mRNA level in livers of mice treated with CCl4 for 8 weeks in comparison with livers of healthy mice. These data were confirmed by immunohistochemistry studies which evidenced Snail1 positive cells in fibrotic livers. In vitro studies showed that Snail1 is expressed by HSC and that its transcription is augmented in culture activated (10 days culture) and in vivo activated HSC as compared to quiescent HSC (1 day culture). At protein level we could observe the nuclear translocation of Snail1 after the fourth/fifth day of culture. We next analysed the expression of genes which have been shown to be directly (E-cad, DSP) and indirectly (MMP9, FN) regulated by Snail1. Their expression resulted substantially unchanged until the fourth day of culture, afterwards E-cad and DSP expression significantly decreased while FN and MMP9 expression increased. Summary and conclusion: In activated HSC Snail1 is up-regulated and shows a nuclear localization and the expression of some target genes results significantly modified. Our data support a role for Snail1 transcription factor in liver fibrogenesis and its involvement in HSC transdifferentiation process.

Disclosures:

The following people have nothing to disclose: Melania Scarpa, Alessia Grillo, Paola Brun, Federica Ditadi, Giorgio Palù, Ignazio Castagliuolo, Diego Martines

1051
INCREASED MORTALITY, ENHANCED LIVER FIBROSIS AND DIMISHED ACUTE-PHASE RESPONSE AFTER BILE-DUCT LIGATION IN HEPATOCYTE SPECIFIC C-MET/GP130 DEFICIENT MICE

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Background: C-Met (HGF receptor) and gp130 (IL6 signal transducer) mediate essential signals known to be involved in proliferation and survival of liver cells. However, a detailed knowledge about their role in hepatocytes during the progression of liver fibrosis is lacking. **Material and Methods:** Conditional c-Met (Δc-Met) and gp130 (Δgp130) knockout mice (Cre-loxP system) were subjected to bile duct ligation (BDL). Expression of Cre-recombinase was regulated by a hepatocyte-specific pre- and a postnatally activated albumin promoter. **Results:** While generating conditional hepatocyte specific Δc-Met mice using the prenatal (d10.5) active Cre-recombinase resulted in embryonic lethality. In contrast the postnatally activated promoter produced vital offsprings of mice lacking c-Met in hepatocytes. After BDL we observed reduced survival rates in Δc-Met (75%) and Δc-Met/Δgp130 (25%) mice. Both conditional knockout mice had higher transaminases and a significantly enhanced apoptosis rate compared to controls ($p>0,05$). The knockout mice also displayed more necrotic areas and an enhanced periportal infiltration and proliferation. In contrast, hepatocyte proliferation determined by BrdU uptake was significantly reduced in Δc-Met ($p>0,05$) and Δc-Met/Δgp130 ($p>0,05$) compared to wt. Analysis of the Acute-Phase-Response (APR) in Δc-Met/Δgp130 mice revealed a complete lack in SAA, SOCS3 and STAT3 induction. Surprisingly, Δc-Met mice also displayed a significantly reduced induction of APR-genes, while preserving the activation of STAT3. In regards of the development of liver fibrosis conditional knockout mice showed a significantly stronger activation of hepatic stellate cells 48h after BDL (control<Δc-Met<<Δc-Met/Δgp130). This resulted in enhanced collagen expression and a higher degree of liver fibrosis in long-term BDL surviving mice (most in Δc-Met/Δgp130). **Conclusions:** Our results demonstrate the essential protective role of the HGF/IL6 signalling after BDL. Abolished APR, higher apoptosis and reduced hepatocyte proliferation rates together with a stronger activation of hepatic stellate cells ultimately leads to a higher degree of liver fibrosis in mice lacking c-Met and gp130.

Disclosures:

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1052
AMELIORATION OF BILIARY FIBROSIS BY AN ALPHAV-BETA 6 INTEGRIN ANTAGONIST IS MEDIATED BY INHIBITION OF CHOLANGIOCYTE ADHESION TO FIBRONECTIN AND CHOLANGIOCYTE TGFBETA1 ACTIVATION

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Background and aims: AlphaV beta6 is an epithelial integrin expressed during tissue development, inflammation, wound healing and carcinogenesis. It acts as a receptor for fibronectin and tenascin, and possibly as co-receptor for the activation of latent TGFbeta1. Mice lacking this integrin display resistance to the induction of pulmonary fibrosis and alphaVbeta6 inhibition can ameliorate rat biliary fibrosis (Patsenker et al, AASLD 2006). We therefore examined the molecular mechanisms underlying the antifibrotic effect of alphavbeta6 inhibition. Methods: Adhesion assays were performed on fibronectin using the human cholangiocyte cell line TFK-1. TGFbeta1 activation was measured by human TGFbeta1 ELISA. Rats subject to bile duct ligation (BDL) for 6 weeks and MDR2-/- mice at age 4 weeks age were treated i.p. with the specific alphaVbeta6 antagonist EMD527040 at 20 or 60 mg/kg/day for 4 and 3 weeks, respectively. Beta6 expression was determined by immunohistology, fibrosis was measured as hepatic hydroxyproline content and fibrosis-related transcripts were determined by quantitative RT-PCR. Bile duct proliferation was quantified by double immunohistochemistry for CK19 and Ki-67 using a point counting technique. Results: AlphaVbeta6 was absent in normal livers, but highly expressed on proliferating bile duct epithelia in fibrosis, with 100-fold increased transcript levels. Its inhibition in vitro reduced cholangiocyte adhesion to fibronectin and endogenous activation of TGFbeta1 by 50%. The alphaVbeta6 antagonist prevented peribiliary collagen accumulation in fibrotic animals by 40-50%. This was paralleled by downregulation of profibrogenic and upregulation of fibrolytic genes, improved liver architecture and function. Conclusions: alphaVbeta6 integrin is strongly upregulated in proliferating bile duct epithelia and drives fibrogenesis via adhesion to fibronectin and auto/paracrine cholangiocyte TGFbeta1 activation. Its pharmacological inhibition blocks the progression of primary and secondary biliary fibrosis.

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TOLL-LIKE RECEPTOR-9 ACTIVATION AMELIORATES HEPATIC FIBROSIS ASSOCIATED WITH LYMPHOCYTE INTERACTIONS

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Background: CD8 subsets mediate hepatic fibrosis and NK cells have an anti fibrotic effect. Oligodeoxynucleotides (ODNs) with immunomodulatory motifs (CpG) are acting through TLR9 to stimulate NK cells. We hypothesized that TLR9 would generate an anti fibrotic response. **Methods:** (I) Hepatic fibrosis was induced by CCl4 administration X2/wk for 6 wks in WT and TLR9-/- mice and was compared to naïves. (II) To isolate the effect of TLR9 in lymphocytes, we transferred splenocytes from WT or TLR9-/- donors to irradiated WT recipients. Then fibrosis was induced in recipients by CCl4. (III) CCl4 was induced in WT male mice (groups A to D) for 6 wks. During the last 2 wks of CCl4 administration, mice were also weekly treated by hydrodynamic based transfection with either saline (group A), CpG ODN (gr. B), pGEM7 (gr. C) and were compared to control CpG ODN injected mice (gr. D) and naive controls (Group E). Different routes of ODN administrations were also examined. (IV) As TLR9 activation is mediated by IL-6 and to further evaluate if the ODN effect on fibrosis is TLR9-mediated we used the IL-6-/- mice. Thus, a similar design in (III) was used in IL-6-/- mice and compared to WT response. **Results:** (I) Compared to WT mice, TLR9-/- mice showed a significant increase of fibrosis. While NK cells were unchanged in TLR9-/- groups compared to the WT, both CD4 and CD8 distributions were reduced in TLR9-/- groups. Post fibrosis; CD4 remains unchanged but CD8 increased. (II) Irradiated WT recipients with TLR9-/- lymphocytes also showed increased fibrosis. While NK cells were unchanged, both CD4 and CD8 distributions were increased in recipients with TLR9-/- lymphocytes and keeping CD8 predominance. (III) Control groups A/D displayed fibrosis levels higher than the ODN treated groups (B/C). Serum ALT levels were elevated in all CCl4 groups but were decreased in the CpG and pGEM7 treated groups. CD8 increased in control groups A/D but was alleviated in treated groups B/C. CD4 cells were decreased and T-regs increased in all fibrotic groups, but without remarkable differences. NK cells decreased following fibrosis in control groups A/D, however, both treated groups B/C showed an increase. Different routes of ODN administration showed similar anti fibrotic effects. (IV) Administration of ODNs to fibrotic IL-6-/- mice, did not affect fibrosis or lymphocyte distribution, supporting that ODNs plays a role in fibrosis alleviation via TLR9 activation. **Conclusions:** TLR9 activation is involved in immune modulation of fibrosis. CpG and pDNA displayed significant anti-fibrotic activity, accompanied by CD8 suppression and increased NK cells.

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ANGIOTENSIN 1-7 REDUCES BILE DUCT PROLIFERATION AND HEPATIC FIBROSIS IN THE BILE DUCT LIGATED RAT

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Introduction: Angiotensin 1-7 (Ang-(1-7)) is a biologically active peptide of the renin angiotensin system (RAS) which opposes the deleterious actions of angiotensin II. The effects of Ang-(1-7) infusion on the progression of liver disease have never previously been described. We have used the bile duct ligation (BDL) model to determine the impact of Ang-(1-7) on the development of hepatic fibrosis, generation of growth factors and feedback mechanisms within the RAS. Methods: Male Sprague-Dawley rats underwent sham operation (n=11) or BDL (n=22). Animals were studied in three groups. Sham operated rats and 11 BDL rats were implanted with intraperitoneal osmotic minipumps delivering normal saline. A further 11 BDL rats were implanted with pumps delivering Ang-(1-7) (28μg/kg/hr i.p.). Animals were killed after 2 weeks and plasma and liver tissue collected for analyses. Results: Ang-(1-7) significantly improved plasma

bilirubin and GGT concentrations and reduced hepatic hydroxyproline content and collagen 1A1 gene expression (P<0.05). Alpha smooth muscle actin (alpha-SMA) immunohistochemistry showed significant attenuation in the Ang-(1-7) treatment group (P<0.05). Similarly, quantitative PCR demonstrated a reduction in alpha-SMA gene expression (P<0.05). These findings were supported by morphometric analysis of picrosirius staining and bile duct proliferation assessment in liver sections. Bile duct ligation resulted in significant gene up-regulation of key components of the RAS, including ACE, ACE2, AT1 and the putative Ang-(1-7) receptor, Mas (P<0.01). However, infusion of Ang-(1-7), resulted in a down regulation of ACE and Mas gene expression (P<0.05). The ACE gene data was further supported by ACE autoradiography of liver tissue which showed a significant suppression of ACE protein in the Ang-(1-7) treated group (P<0.001). The gene expression of growth factors CTGF and VEGF were both attenuated by Ang-(1-7) infusion (P<0.05). Data were analyzed by analysis of variance (ANOVA) with Newman-Keuls Multiple Comparison Test. Conclusions: The RAS is upregulated following bile duct ligation. Ang-(1-7) infusion reduces hepatic fibrosis and bile duct proliferation. This is associated with down-regulation of key components of the RAS (ACE and Mas) as well as critical growth factors (CTGF and VEGF). Reduced gene and protein expression of SMA indicate that Ang-(1-7) may attenuate hepatic stellate cell activation. These novel findings indicate that the ACE2/Ang-(1-7)/Mas axis is activated in chronic liver injury and may represent a potential target for antifibrotic therapy.

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1055
ROLE OF C-C CHEMOKINE RECEPTOR 2 (CCR2) DURING CONSTITUTION AND RESOLUTION OF FIBROSIS INDUCED BY CARBONE TETRACHLORIDE IN MICE

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Inflammatory reaction is part of the fibrogenic process and it is accompanied by an infiltration of leukocytes that is regulated by chemokines. The C-C- chemokine receptor, CCR2, has been identified as the primary receptor that mediates monocyte chemoattractant protein-1 (MCP-1) responses. MCP-1 and CCR-2 are up-regulated during the progression of fibrosis in humans. During liver injury, MCP-1 is expressed by hepatocytes, endothelial, Küpffer cells and stellate cells (HSC). In contrast, CCR-2 expression is mainly associated with macrophages and a subpopulation of lymphocytes. These cells have been shown to play a major role in both the constitution and the resolution of liver fibrosis induced by carbone tetrachloride (CCl4) in mice. However, the role of CCR2 in these processes has never been evaluated. We used CCR2 knockout mice (CCR2-/-) to determine whether CCR2 is involved both in the establishment and/or the resolution of fibrosis. CCR2-/- mutant (n=18) and wild type (n=18) male mice of the same Balb/c Ola background were injected twice weekly during 6 weeks with CCl4. Animals were killed either 48 hours (peak of fibrosis) or 7 days (partial resolution of fibrosis) after the end of the injury. We observed a significant lower level of the fibrotic scar at the peak of fibrosis in mutant animals compared to their control littermates as assessed by red sirius quantification. This effect was correlated with a reduction in the proportion of activated macrophages present in the lesion as defined by quantification of CD68 positive macrophages. In contrast, 7 days after the end of the fibrogenic challenge, whereas fibrosis was diminished in CCR2+/+ mice, CCR2-/- mice experienced no regression of their liver injury, and presented marked regions of calcification. This persistence of hepatic injury in mutant animals was correlated with a maintain in TIMP1 mRNA expression. In conclusion, these findings demonstrate that CCR2 stimulates liver fibrogenesis by inducing macrophage infiltration that will subsequently activate HSC and confirm the critical role of macrophages and inflammatory cells for the fibrogenic process. In addition, CCR2 is also involved in the resolution of the fibrotic scar, with an opposite role allowing macrophages to eliminate injured hepatocytes.

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INCREASED SUSCEPTIBILITY TO HEPATIC STEATOSIS, INFLAMMATION AND ADVANCED FIBROSIS IN APOLIPOPROTEIN-E DEFICIENT MICE

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The contribution of hyperlipidemia, one of the most important metabolic risk factors, to the progression of liver disease is not established. In this study, we employed apolipoprotein E-deficient ($ApoE^{-/-}$) mice, a mouse model of hypercholesterolemia, to assess the relationship between the dysregulation of lipid metabolism and the progression of lipoperoxidative-induced liver damage. To this end, $ApoE^{-/-}$ and wild-type mice were exposed to carbon tetrachloride for 4, 6 and 8 weeks. Two additional groups received only olive oil. As compared to wild-type, $ApoE^{-/-}$ mice had increased hepatic steatosis as revealed by oil red-O staining. Moreover, the number of F4/80-positive cells, indicative of macrophage infiltrate, as well as necroinflammation were increased in liver sections from $ApoE^{-/-}$ mice. At a more advanced stage, $ApoE^{-/-}$ mice exhibited exacerbated fibrosis, assessed by Sirius red staining and α-SMA immunostaining. Interestingly, the number of non-parenchymal cells with a TUNEL-positive nucleus was significantly reduced in $ApoE^{-/-}$ mice. Moreover, liver injury (i.e. ALT and AST) was more severe in $ApoE^{-/-}$ than in wild-type mice. Importantly, significant direct correlations were identified between serum cholesterol and hepatic steatosis, fibrosis and ALT levels. Relevant changes in the expression of genes involved in inflammation and oxidative stress, including MCP-1, RANTES receptor, IL-1α, PPARδ and genes of the arachidonate cascade, were detected in the liver of $ApoE^{-/-}$ mice by Low Density Arrays. To explore the mechanisms underlying the increased susceptibility of $ApoE^{-/-}$ mice to liver injury, we exposed cell types relevant to liver physiology to cholesterol oxidized products (oxysterols) and oxidized LDL. These lipid products induced TGF-β_1 release and MCP-1 expression in macrophages, increased oxidative stress in hepatocytes and up-regulated TIMP-1 expression and IL-8 secretion inde-

pendently of NF-κB activity in hepatic stellate cells. Taken together, these findings underscore the concept that metabolic factors such as hyperlipidemia may play an important role in the progression of liver injury.

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DELETION OF THE THROMBIN RECEPTOR GENE, PAR 1, AMELIORATES LIVER FIBROSIS AND DECREASES TGFβ EXPRESSION BY HEPATIC STELLATE CELLS IN A MURINE MODEL OF CIRRHOSIS

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Thrombin, a pleuripotent serine protease, is principally known for modulating hemostasis but can promote extracellular matrix (ECM) production and has been implicated in liver fibrogenesis. Profibrotic effects of thrombin are thought to occur through protease activated receptor 1 (PAR1) signaling. A previous study has shown that PAR 1 antagonism ameliorates liver fibrosis in a rat bile duct ligation model (Fiorucci 2004). The aim of this study was to investigate whether deletion of the PAR 1 gene protected against liver fibrosis in a model of hepatocyte inflammation and fibrosis and to identify potential mechanisms. Methods: Twice weekly intraperitoneal carbon tetrachloride injections for 5 and 8 weeks were used to induce liver fibrosis in 9 week old wild type and PAR 1 knock-out(-/-) C57/BL6 mice. Liver sections were stained with Sirius red and fibrosis was quantified by computer assisted morphometry. Hepatic collagen content was quantified by hydroxyproline assay. α Smooth muscle actin(α SMA) expression was detected by Western blot. Hepatic stellate cells (HSC) were isolated from wild type and PAR 1 -/- mice and cultured on plastic in M199 media supplemented with 10% fetal bovine serum. TGFβ, a profibrogenic cytokine for HSC, was measured by ELISA. Results: PAR 1 knockout mice showed significantly less ECM deposition compared to wildtype. Morphometric analysis of Sirius red stained sections revealed significantly less collagen deposition per total area in PAR 1 -/- vs. wildtype at week 5 (23% reduction, p = 0.04) and at week 8 (33% reduction, p=0.0001). In addition, hepatic hydroxyproline content was significantly lower in PAR 1 -/- vs wildtype at week 5 (18 % reduction, p = 0.04) and at week 8 (28% reduction, p=0.005). In liver sections, there was a trend towards less α SMA activation in the PAR 1 knockout mice compared to wildtype (p=0.09). Cultured HSC from PAR 1 -/- mice expressed significantly less TGFβ (68% reduction, p=0.01) and significantly less α SMA (28% reduction, p<0.05) compared to wild type C57/BL6. Conclusion: Mice with deletion of the thrombin receptor gene, PAR 1, exhibit less fibrosis than wild type mice in a necroinflammatory model of liver fibrosis. The protective effect is magnified with longer duration of liver injury. HSC isolated from PAR1 knockout animals express less TGF β and less α SMA than wildtype animals, suggesting less HSC activation and thus a potential mechanism for the antifibrotic effect. The observation that fibrosis was not completely ameliorated with PAR 1 gene deletion suggests a multifactorial pathway for hepatic fibrogenesis.

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HUMAN DISCOIDIN DOMAIN RECEPTOR 1: INTRAHEPATIC FORMS AND IN VITRO FUNCTIONS

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Discoidin Domain Receptor 1 (DDR1) is a tyrosine kinase receptor that binds and is activated by collagens. Being a receptor of collagen, DDR1 expression needs to be tightly regulated. Transcriptional profiling of human cirrhosis with DNA array and quantitative realtime RT-PCR detected elevated mRNA expression of DDR1 compared to non-diseased liver (Shackel NA, et al Am J Pathol. 2002;160:641). The study aims to characterize DDR1 expression in cirrhotic and non-diseased liver and to examine the cellular effects of DDR1 expression. Immunoblots of 10 cirrhotic and 5 non-diseased livers used the anti-DDR1 (C-20 peptide) antibody (Santa Cruz). In situ hybridisation (ISH) used a Digoxigenin (DIG)-labeled (Roche) human DDR1 566 bp riboprobe. Immunostains included cytokeratin 18 (CK18), DDR1 (C-20), CD45, CK19 and CK7. Green fluorescent protein (GFP)- DDR1 isoform A fusion protein was transiently expressed in the Huh7 cell line for cell adhesion and transwell migration assays on collagen-I, fibronectin and matrigel. The actin cytoskeleton was stained using phalloidin-594. Two molecular forms of DDR1 protein were differentially expressed in cirrhotic versus non-diseased liver: A 132 kDa form of DDR1 in non-diseased liver but not cirrhotic liver and an 85kDa form present only in cirrhotic liver. As DDR1 is subject to protease - mediated cleavage after collagen - induced activation, this differential expression may indicate more intense activation of DDR1 protein in cirrhotic liver. To determine the cellular localization of DDR1, ISH and immunofluorescence were performed. Intense DDR1 mRNA expression was detected in hepatocytes at the portal/parenchymal interface and the reactive bile duct cells in the septum. DDR1 protein was predominantly in leucocytes, the luminal aspect of biliary epithelium and the bile canalicular domain of hepatocytes, indicating the presence of DDR1 on the apical domain of these epithelial cells. DDR1 overexpression increased Huh7 cell adhesion to collagen-I but decreased adhesion to fibronectin and matrigel. DDR1 overexpressing Huh7 cells showed decreased migration on collagen-I, fibronectin and matrigel. As DDR1 is known to be involved in cellular morphogenesis, the actin cytoskeleton was visualised but was unaltered in DDR1 overexpressing cells. Flow cytometry analysis indicated that DDR1 overexpression did not regulate the expression of matrix metalloproteinase (MMP) 1, 2, 3 or 9. These findings suggest that increased DDR1 expression and activation in cirrhotic liver may have an important role in the pathogenesis of cirrhosis by influencing extracellular matrix – hepatoctye interactions including adhesion and migration.

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The following people have nothing to disclose: Sunmi Song, Nicholas A. Shackel, Xin M. Wang, Katerina Ajami, Geoffrey W. McCaughan, Mark D. Gorrell

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BXD RECOMBINANT INBRED MOUSE LINES - A GENETIC REFERENCE POPULATION FOR DISSECTION OF THE COMPLEX GENETICS OF LIVER FIBROSIS

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Background: Previously we have shown that quantitative trait locus (QTL) analysis can detect susceptibility loci for liver fibro-

sis, based on genome-wide linkage analysis in experimental crosses of inbred mouse strains (Hillebrandt et al. Gastroenterology 2002;123:2041-51). In previous studies we identified multiple QTLs and a quantitative trait gene (C5) in a large F2 intercross of fibrosis-susceptible and resistant strains of inbred mice (Hillebrandt et al. Nat Genet 2005;37:835-43). Our aim now was to establish a murine genetic reference population for integrated and replicated analyses of the pathobiology of liver fibrogenesis. Methods: We availed of BXD recombinant inbred lines generated by (i) crossing the two inbred strains C57BL/6J and DBA/2J, and (ii) inbreeding the F2 progeny by brotherXsister matings for at least 20 generations. Each of the 80 lines shows a unique mosaic gene set of the two founder strains and has been genotyped for 13377 single nucleotide polymorphisms. We induced liver fibrosis by challenging the mice twice weekly with CCl4 (12 injections i.p.; 0.7 mg/kg). For phenotypic characterization, we determined histological stages of liver fibrosis after Sirius red staining and measured hepatic collagen contents. For the identification of profibrogenic QTLs, genome-wide scans were performed with dense SNP maps, as implemented in WebQTL. Results [N = 10; Mean Collagen 272 μg/g liver ± SE 10 ; P < 0.01]: In the BXD panel, significant strain differences in susceptibility to fibrosis exist. After 6 weeks of treatment, histological stages of fibrosis vary from F0 to F3, and strain means of hepatic collagen contents range from 138 to 367 μg/g liver. Of note, collagen contents shows a normal distribution among the BXD inbred lines, consistent with polygenic inheritance of liver fibrosis in our model. WebQTL analysis identifies multiple QTLs co-localizing with eQTLs that determine the expression levels of profibrogenic candidate genes. Conclusions: The observed differences in susceptibility to liver fibrosis among the BXD lines demonstrate that this set provides a genetic reference panel for integrated analysis of profibrogenic susceptibility genes. We conclude that the use of such a panel, coupled with transcriptome profiling, will lead to novel insights into the complexity of the genetic control of liver fibrogenesis and allow modelling of gene networks during chronic liver injury. Supported by the German Network for Systems Genetics

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GENETIC ASSOCIATION STUDY OF PROFIBROGENIC GENE VARIANTS USING TRANSIENT ELASTOGRAPHY FOR PHENOTYPIC CHARACTERIZATION OF LIVER FIBROSIS

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Introduction: Transient elastography (TE) has been employed to determine the stage of liver fibrosis in patients with chronic liver diseases with close correlation to liver histology. Recently based on a genome-wide scan, a novel "gene signature" consisting of 7 single nucleotide polymorphisms (SNPs) has been proposed to identify the risk of progressive fibrosis in patients with chronic hepatitis C virus (HCV) infection (Huang et al. *Hepatology* 2007; epub). Our **aim** now was to evaluate this gene signature in a large cohort of patients with chronic liver diseases who underwent TE for assessment of liver fibrosis. **Patients and methods:** We enrolled 516 patients with chronic liver diseases, including a subset of 256 patients with chronic HCV infection. Liver stiffness measurements (LSM) were performed by TE; in addition we included patients with unequivocal signs of liver cirrhosis in whom TE failed (e.g. because of ascites). We stratified patients in a severe fibrosis group (LSM >9.5 kPa, or signs of liver cirrhosis) and a no-fibrosis group (LSM < 7.5 kPa). Patients were genotyped for the 7 SNPs identified by Huang et al. using PCR-based *Taqman* assays. **Results:** The genetic analyses revealed that carriers of the common allele of a SNP in the ornithine decarboxylase antizyme inhibitor (*AZIN1*) gene are at increased risk for severe liver fibrosis (OR = 1.75; 95% CI = 1.01 - 3.04; p < 0.05). This association was even more pronounced in the group of patients with chronic HCV infection (OR = 2.89; CI = 1.28 - 6.52; p < 0.01). Overall, there was also a trend (p = 0.069) for an association with the common allele of a SNP in the 5' region of the adaptor-related protein complex 3 (*AP3S2*) gene (*rs2290351*), which showed a significant association in the HCV sub-group (OR = 6.56; p < 0.05). However, inverse risk alleles and no associations between LSM and the other SNPs included in the gene signature in our cohort or in the HCV sub-group indicate that additional studies are needed to validate the predictive value of the profibrogenic gene signature. **Conclusions:** Our study demonstrates that TE qualifies for non-invasive phenotyping of fibrosis patients in genetic association studies. Furthermore, some of the recently identified cirrhosis risk variants are also associated with progressive liver fibrosis in our cohorts of patients with chronic HCV infection in particular and chronic fibro-proliferative liver diseases in general.

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SYNERGISTIC ANTI-FIBROTIC EFFICACY OF STATIN AND PROTEIN KINASE C INHIBITOR IN HEPATIC FIBROSIS

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Background & Aims: Activated hepatic stellate cells (HSCs) are major participants in hepatic fibrosis, and thus, the suppression of HSC activation or survival has been proposed as anti-fibrotic therapies. Statin has anti-fibrotic efficacy in human fibrosing diseases, such as pulmonary and renal fibrosis, and therefore, is implicated in hepatic fibrosis. However, statin can also activate protein kinase C (PKC), which augments hepatic fibrogenesis, and thereby, is likely to reduce statin's anti-fibrotic efficacy. Therefore, we hypothesized that the simultaneous treatment of statin and PKC inhibitor may synergistically enhance anti-fibrotic efficacy in hepatic fibrosis. Methods: LX-2 cells (an immortalized human HSC line), pravastatin and enzastaurin (PKC inhibitor) were used in this study. Hepatic fibrosis model was established in BALB/c mouse by injecting carbon tetrachloride intraperitoneally twice per week for 6 weeks. Statin and PKC inhibitor were administered by gavages 5 days per week for 5 weeks. Cellular apoptosis was explored using DAPI or TUNEL staining and immunoblot analysis. Hepatic fibrosis and HSC activation were assessed by morphometric analysis of histological findings and immunohistochemistry for α smooth muscle actin. Results: Statin induced LX-2 cell apoptosis through activating mitochondrial apoptotic signals. While PKC inhibitor did not induce LX-2 cell apoptosis, the addition of this inhibitor significantly increased statin-induced apoptosis, and this was due to enhanced caspase 9 activation in these cells. The per-

centages of TUNEL-positive HSCs were significantly increased in mice treated with statin+PKC inhibitor as compared to control or single compound-treated mice. The extent of collagen deposition and the percentage of area occupied by activated HSCs were significantly decreased in mice treated with statin+PKC inhibitor as compared to control or single compound-treated mice. Conclusions: These results demonstrate that the simultaneous treatment of statin and PKC inhibitor synergistically enhanced anti-fibrotic efficacy in in vitro and in vivo model of hepatic fibrosis. Therefore, this combination strategy may have therapeutic implication in reducing hepatic fibrosis.

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THE IMPACT OF BONE MARROW STEM CELLS ON LIVER FIBROSIS IS CRITICALLY DETERMINED BY THEIR ROUTE OF CELL TRAFFICKING

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Introduction Use of stem cells has been advocated in the setting of chronic liver disease, with one group showing that infusion of murine bone marrow during chronic liver injury reduces the amount of liver cirrhosis. Other groups have shown that transplanted bone marrow contributes directly to the production of liver fibrosis, and is a major determinant in the amount of fibrosis generated. These conflicting data may arise due to the different locations/niche from which the stem cells originate. **Hypothesis** We propose that the niche from which stem cells are derived critically determines their biological impact on liver fibrosis. **Methods** Liver fibrosis was induced in the NODscid mouse by twice weekly IP injection of CCl4. Bone marrow derived from a ROSA26 mouse was administered in two differing ways: (1) **Transplantation.** Mice were irradiated (250 Rad) and then received BM infusion via tail vein. Mice received CCl4 liver injury two weeks after this for a 9 week duration. (2) **During injury.** Mice received BM infusion via tail vein after 5 weeks of CCl4 liver injury, but with no concomitant irradiation. CCl4 injury continued for a further 4 weeks. Mice were either culled after 9 weeks CCl4 injury (**peak**) or 4 weeks after cessation of injury (**recovery**). Liver fibrosis was quantified histologically using image analysis and by qPCR for Col-1. Presence of Rosa26 cells was confirmed by beta Galactosidase whole mount staining. **Results** 1) NODscid mice **transplanted** with Rosa26 BM had higher levels of liver fibrosis (21.3%, p=0.03) at peak than NODscid mice which only received CCl4 liver injury. 2) Infusion of Rosa26 BM **during injury** led to no difference in the amount of peak liver fibrosis (compared with an injury only control group). 3) However, infusion of Rosa26 BM **during injury** led to markedly improved resolution of liver fibrosis (24% reduction from peak) at the 4 week **recovery** period (compared with 2.4% and 21% reduction seen in chronically injured NODscid and Rosa-26 mice). The livers of these mice were seen to contain multiple Rosa26+ cells with morphology in keeping with macrophages. **Conclusion** 1. Transplanted BM responds in an evolved fashion to liver injury and contributes to liver fibrosis. 2. BM infused in the midst of liver injury, without prior host bone marrow reconstitution, functions differently and adopts an anti-fibrotic phenotype. Specifically, we demonstrate that it aids resolution of liver fibrosis once the injurious stimulus is removed.

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TGFβ1 STIMULATES THE HUMAN α1(I) COLLAGEN PROMOTER BY COOPERATION BETWEEN SMAD2/4 AND SP1 TRANSACTING FACTORS

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Hepatic fibrosis and cirrhosis result from the excessive deposition of predominantly type I collagen, composed of two α1 chains and one α2 chain. TGFβ1, a principal pro-fibrogenic cytokine, activates the human α2(I) collagen promoter via Smads (2, 4). However, the role of Smads in activation of the human α1(I) collagen promoter remains unknown. Sp1, an ubiquitous zinc-finger family transcription factor, binds to numerous sites along the length of the human α1(1) collagen promoter. The enhancement of expression of distinct genes by cooperation of Sp1 and Smad proteins has been described previously. The aim of this study was to determine the roles of Smads and Sp1 in mediating the effect of TGFβ1 signaling on activation of the human α1(I) collagen promoter. The binding of Smads and Sp1 to the proximal collagen promoter was determined by EMSA and their specific sites of binding were determined by sequential 2 bp mutations of the promoter. Transient transfection experiments were performed with cultured human LX-2 stellate cells. Pre-designed shRNA plasmids were used to knockdown Smads and Sp1. Smads and Sp1 separately bound to adjacent sites of the proximal - 174 α1(I) collagen promoter, and expression vectors of Smad2 and Sp1 separately as well as their combination activated the collagen promoter. Smad2 or Sp1 knockdown decreased activation of the promoter in cells not treated with TGFβ1, but not in TGFβ1-treated cells. However, simultaneous knockdown of Smad2 and Sp1 effectively decreased activation of the promoter with or without the presence of TGFβ1. Smad4 knockdown alone had no effect in either case, but simultaneous knockdown of Smad4 and Sp1 again effectively decreased activation of the promoter in either TGFβ1 treated or untreated cells. The knockdowns were confirmed by Western blot and/or mRNA determined by real time PCR. In conclusion, Smad2, Smad4 and Sp1 cooperate in enhancing human α1(I) collagen promoter activity and type I collagen production in human hepatic stellate cells.

Disclosures:

The following people have nothing to disclose: Polina Sysa, James J. Potter, Xiaopu Liu, Esteban Mezey

1064
A HUMAN RECOMBINANT ANTIBODY FOR TARGETING ANTI-FIBROGENIC THERAPEUTICS TO MYOFIBROBLASTS IN VIVO

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Liver fibrosis is caused by the trans-differentiation of hepatic stellate cells into myofibroblasts, which then secrete many of the factors responsible for fibrogenesis in chronically damaged liver. There is no treatment for fibrosis currently indicated although stimulating the apoptosis of myofibroblasts has been

shown to reduce fibrosis severity in animal models of disease. Since liver macrophages may be essential for effective fibrosis recovery, a means to specifically target myofibroblasts with a therapeutic that promotes apoptosis has been examined. A human recombinant antibody (termed C1-3) was generated to a peptide sequence present on an extracellular domain of synaptophysin, a plasma membrane protein expressed in activated hepatic stellate cells and myofibroblasts. FITC-labelled C1-3 antibody bound to human and mouse liver myofibroblasts in vitro and was taken up into cells by endocytosis. FITC-labelled C1-3 antibody was then injected i.p. into control mice or mice with liver fibrosis (through treatment with CCl4). Antibody appeared in circulating blood within 20 minutes and concentrations peaked at 2 hours. Tissue analysis indicated that the antibody was localised to the fibrotic liver and was not detected in brain, kidney spleen or muscle. Fibrotic liver levels of antibody were maximal after 6 hours and binding was to cells within the scar region as determined by immunohistochemistry or fluorescence microscopy in liver sections. Co-staining for α-smooth muscle actin indicated that the antibody localised specifically only to myofibroblasts. The antibody was not detected in livers from control mice injected with the antibody. In rats, gliotoxin reverses fibrosis through the stimulation of myofibroblast apoptosis, although hepatocyte and Kupffer cell apoptosis also occur. To examine whether targeting gliotoxin to liver myofibroblasts with the C1-3 antibody may be more effective, mice with liver fibrosis were injected with either free gliotoxin; an equivalent dose of gliotoxin conjugated to C1-3 or an equivalent dose of gliotoxin conjugated to an unrelated antibody that does not target any liver cells. Both free gliotoxin and C1-3-gliotoxin stimulated an increase in myofibroblast apoptosis and reduced the numbers of α-smooth muscle actin positive cells in liver sections. However, C1-3-gliotoxin was significantly more effective than free gliotoxin and prevented the increase in hepatocyte and kupffer cell apoptosis seen with free gliotoxin. The C1-3 antibody is therefore able to selectively target myofibroblasts in a mouse model of liver fibrosis. Antibody conjugation did not prevent antibody targeting or therapeutic activity.

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1065
ENHANCED IL-6 PRODUCTION BY HEPATIC STELLATE CELLS IN RESPONSE TO IMATINIB MESYLATE STI571 - (GLEEVEC ®), A NOVEL LINK BETWEEN RECEPTOR TYROSINE KINASE INHIBITION AND HEPATIC REGENERATION

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Background: The hepatic stellate cell (HSC) is a key target of antifibrotic therapies. Gleevec® (imatinib mesylate), is among a new class of small molecule receptor tyrosine kinase (RTK) inhibitors, and is indicated for treatment of CML and GI stromal tumors. Because Gleevec inhibits β-PDGFR signaling, a major pathway of HSC proliferation, we assessed its activity in culture and in vivo, and examined downstream targets of its activity in an immortalized human stellate cell line (LX-2) using cDNA microarray. Methods and Results: Gleevec dose-dependently inhibited LX-2 cell proliferation (0.5 μM – 2.0 μM) with no effect on viability at 12h and 24h. This was associated with progressive inhibition of β-PDGFR phosphorylation as assessed by Western Blot. Mitochondrial activity and superoxide anion production were decreased in response to Gleevec, as assessed by Mito-tracker and FACS. To identify HSC genes altered by Gleevec treatment, cDNA microarray (Affymetrix U133 Chip) was performed on cells with or without Gleevec (2 μM) treated for 12 hours. There was a significant up-regulation of 28 genes involved in cell growth, metabolism or apoptosis, of which 7 were validated by qRT-PCR. This included Interleukin-6 (IL-6), which was elevated 4-fold ($P<0.05$), and correlated with progressive IL-6 secretion in LX-2 supernatants. Gleevec decreased gene expression in LX-2 of collagen α1(I), α-SMA, β-PDGFR, TGFβ1 receptor type 1, MMP2 and tissue inhibitor of metalloproteinase II (TIMP II). In vivo, Gleevec administered to rats (5 d/wk) beginning 4 weeks after 3d per week IP dosing of thioacetamide, led to reduced lobular inflammation, and collagen content as assessed by pathological scoring and/or morphometry, with significant reductions in portal pressure and downregulation of key fibrogenic genes in whole liver. Importantly, hepatic IL-6 mRNA levels were significantly (1.4 to 2-fold) increased in TAA-treated animals receiving Gleevec compared to animals receiving TAA and vehicle. Conclusions: These findings uncover an unexpected link between inhibition of HSC activation by Gleevec and enhanced secretion of IL-6, a key regenerative cytokine. Impaired IL-6 production during liver injury could be related to up-regulation of one or more RTK targets inhibited by Gleevec. Clarification of specific RTK pathways in HSCs that are linked to IL-6 secretion will broaden our understanding of this agent's effects and refine anti-fibrotic and regenerative therapies in humans.

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1066
CD147 REGULATION OF HEPATOCYTE DERIVED MATRIX METALLOPROTEINASES: A NOVEL PATHWAY INVOLVED IN LIVER FIBROGENESIS

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Introduction: We have previously shown CD147 is upregulated in the human cirrhotic liver and expressed predominantly on hepatocytes. CD147 is an abundant glycoprotein and a potent inducer of matrix metalloproteinases (MMPs-1, 2, 3 & 9) in malignant cells and fibroblasts. The progression of liver fibrosis is characterised by the dysregulation of extracellular matrix turnover, primarily mediated by MMPs. Therefore, we hypothesize that CD147 regulates hepatocyte-derived MMP expression which is capable of matrix remodelling. Methods: Cellular localisation of CD147 and MMPs was determined by immunohistochemistry and immunofluorescence in human cirrhotic liver explants. Progressive changes in CD147 expression were analysed in a rat bile duct ligated model of injury (0, 1, 2, 3 & 4 weeks). MMP and tissue inhibitors of metalloproteinase (TIMP) activity was analysed in primary rat hepatocytes (with and without CD147 blocking antibody) and hepatic stellate cells (HSCs) by zymography and reverse zymography respectively. In-vivo functional studies of CD147 were performed in mice treated with carbon tetrachloride for 4 weeks using an anti-CD147 blocking antibody (mAb RL73.2). Injury was assessed by histological analysis and severity of fibrosis quantitated by hydroxyproline estimation. Results: CD147 mRNA

and protein were increased in progressive injury leading to cirrhosis in humans and in the rat bile duct ligated injury model. Localisation studies showed CD147 and MMPs-1, 2 & 9 are expressed predominantly on hepatocytes. In-vitro studies of MMP from rat primary hepatocytes showed significantly increased MMP-2 & 9 comparable to either rat primary quiescent or in-vivo activated HSCs. Further, significant TIMP-1 & 2 activity was found in media from primary HSCs but not in media from hepatocytes. Importantly, MMP-2 & 9 activity was reduced following administration of an anti-CD147 blocking antibody. In-vivo antibody treatment blocking CD147 was associated with a significant reduction in hepatic inflammation, the preservation of hepatic architecture and increased in collagen I deposition ($p<0.01$). Conclusion: Our studies demonstrate that CD147 regulates hepatocyte derived MMP-2 & 9 production, and that this pathway has a distinct role in remodelling of extracellular matrix in liver injury and fibrogenesis.

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1067
DETECTION AND PREVENTION OF HEPATIC FIBROSIS TARGETING TGF-β ACTIVATION REACTION

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Transforming growth factor (TGF)-β, playing a pivotal role in the pathogenesis of liver diseases, is produced as a high molecular weight latent form, and thus must be activated before exerting its biological activities. TGF-β activation is the reaction, by which 25 kD active TGF-β molecule is released from the latent complex. We previously showed that TGF-β is activated by plasmin (PLN) and plasma kallikrein (PLK) during pathogenesis of liver fibrosis and impaired liver regeneration, respectively, and that blockage of these activation reactions with low molecular weight protease inhibitors prevented the development of the diseases in animal models. Now, we determined that PLN and PLK cleaved between K56-L57 and R58-L59 in LAP portion of human latent TGF-β1, respectively, and produced antibodies that specifically recognize the cut ends of each cleavage site. In Western blotting, the antibodies against the PLK-cut ends (anti-R58 and anti-L59 antibodies) recognized the degradation products produced during PLK-mediated activation of latent TGF-β, but did not recognized uncleaved latent TGF-β and only weakly recognized PLN-cleaved latent TGF-β degradates. A similar result was obtained with the antibodies against the PLN-cut ends (anti- K56 and anti-L57antibodies). The anti-R58 antibodies strongly stained liver sections from patients with fulminant hepatitis compared to normal liver sections from patients that died from pulmonary embolism. On the other hand, the anti-L59 antibodies were successively used to establish ELISA to quantitate LAP degradates released into and circulating in the serum in rat fibrosis models. In bile duct ligation models, we found that serum LAP degradates concentration in fibrotic animals was 7-fold higher compared with those in the control animals (no treatment or sham operation), and showing a good correlation to hepatic contents of hydroxyproline and serum GOT/GPT. Furthermore, we produced the peptide containing these cleavage sites as well as its decoy peptide, in which cleavage site amino acids were all mutated to A. These peptides efficiently suppressed the TGF-β activation reaction, prevented the activation of hepatic stellate cells in culture, and prevented impaired liver regeneration observed in LPS-pretreated partially hepatectomized mice. These results suggest a key role for PLN/PLK in the generation of active TGF-β, thereby addressing a potential use for PLN/PLK inhibitors and decoy peptides in hepatitis/fibrosis therapy. These results also suggest a potential usage of a LAP degradate as a biomarker for liver fibrosis.

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C-REACTIVE PROTEIN AMELIORATES HEPATIC FIBROGENESIS THROUGH INHIBITION OF HEPATIC STELLATE CELL TRANSACTIVATION

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Recently, minimal increment in serum C-reactive protein (CRP) levels has been recognized as a sensitive marker of microinflammation in atherosclerotic lesions and hepatic inflammation in non-alcoholic steatohepatitis (NASH). Although it is well-known that CRP participates in regulation of inflammatory responses involving complement activation, the role of CRP in hepatic fibrogenesis has not been well elucidated. In the present study, therefore, we investigated the effect of CRP on transactivation of isolated hepatic stellate cells (HSCs), and experimental hepatic fibrosis in the rat. Methods: HSCs were isolated from male Wistar rats, and cultured in DMEM supplemented with 10% heat-inactivated FBS up to 7 days prior to experiments. Proliferative responses in cultured HSCs were evaluated by BrdU incorporation, and phosphorylation of ERK1/2 and Akt was detected by Western blotting. For experiments in vivo, hepatic fibrosis was induced in male Wistar rats by repeated intraperitoneal injection of thioacetamide (TAA, 200 mg/kg, 3 times/week) for 6 weeks. Some animals were given rat CRP (1 mg/kg, i.p.) simultaneously starting from the third week for 4 weeks. The degree of hepatic fibrosis was assessed by picro-sirius red staining, and expression of smooth muscle α-actin (αSMA) in the liver was detected by immunohistochemistry. Results: In day-3 cultured HSCs, PDGF-BB (5 ng/ml)-induced increases in BrdU uptake were blunted in the presence of human CRP in a dose-dependent manner, reaching 57% inhibition at a concentration of 1 mg/dl ($p<0.05$, vs. PDGF alone). The similar tendency was observed in 7 day-cultured, activated HSCs. More importantly, this anti-proliferative effect of CRP was observed not only in human CRP but also in rat CRP, which lacks complement activation capability. PDGF-induced phosphorylation of ERK1/2 was also inhibited by CRP; however, phosphorylation of Akt was not decreased even in the presence of CRP. On the other hand, TAA-induced hepatic fibrosis was ameliorated markedly when CRP was given simultaneously for 4 weeks. Indeed, induction of αSMA in the liver was prevented remarkably, indicating that CRP also inhibited transactivation of HSCs in vivo. Conclusions: These findings clearly indicated that CRP has an anti-fibrogenic property including direct inhibitory effect on HSC transactivation. The anti-proliferative effect of CRP on HSCs appears to be independent on complement activation, and most likely involves inhibition of the MAP kinase pathway. It is therefore postulated that CRP produced from hepatocytes plays a regulatory role in progression of hepatic fibrosis in NASH.

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1069
THE FUNCTIONAL CONTRIBUTION OF BONE MARROW-DERIVED CELLS TO LIVER FIBROSIS IN A MOUSE MODEL OF CHRONIC LIVER INJURY

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Aims: A significant proportion of the hepatic fibrogenic cell population in chronic liver disease in humans and animal models is of bone marrow (BM) origin. The purpose of this study was to 1) evaluate the relative contribution of the BM-derived (exogeneous) and intra-hepatic (endogeneous) fibrogenic cell compartments to hepatic fibrosis in a carbon tetrachloride (CCl4) model of injury; 2) to determine whether the BM can influence the liver's fibrotic response to injury by manipulation of BM genotype. Methods: r/r collagen transgenic mice (r/r) have an exaggerated fibrotic response to liver injury compared to C57B6 wild-type (Wt) equivalents due to constitutive resistance of collagen I to degradation. Recipient female mice were myelo-ablated and received BM transplantation from male donors according to the following four experimental groups: 1) Wt BM into Wt recipient, 2) r/r BM into Wt recipient, 3) Wt BM into r/r recipient, 4) r/r BM into r/r recipient. Liver fibrosis was induced by 8 weeks of intraperitoneal CCl4. Liver tissue was harvested immediately post injury (day 1) and into recovery (day 9) and fibrosis quantified by digital image analysis of histological sections. (groups of n=6) Results: r/r collagen mice display increased hepatic fibrosis to CCl4 injury over controls, a phenotype enhanced into recovery after liver injury (4.63% vs 3.22% p=0.038, and 4.06% vs 2.40% p=0.004, respectively). Wt mice receiving BM transplantation from r/r donors have an increased hepatic fibrotic response and r/r transgenic mice receiving BM transplantation from Wt donors have a diminished fibrotic response to injury compared to controls, particularly into the recovery phase (2.80% vs 2.40% p=0.013, and 2.78% vs 4.06% p=0.008, respectively at day 9). The degree of liver fibrosis in the two mismatch BM transplanted groups was approximately equivalent (3.70% & 3.63% at day 1, 2.80% & 2.78% at day 9, in the r/r BM into Wt recipient and Wt BM into r/r recipient groups respectively) and levels of fibrosis were intermediate between wholly wild-type or r/r mice indicating that BM-derived cells and endogenous intrahepatic fibrogenic cell populations contribute equally to the pathogenesis of liver fibrosis. (results expressed as mean % surface area collagen) Conclusions: In this in vivo functional assay of liver fibrosis, both circulating BM-derived cells and intrahepatic derived fibrogenic cell populations contribute equally to the development of liver fibrosis. Moreover, manipulation of the fibrogenic propensity of the BM can influence the ensuing fibrotic response of the liver to a chronic injurious stimulus.

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The following people have nothing to disclose: Yiannis N. Kallis, Rob Goldin, John Iredale, Stuart Forbes

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ACCELERATED ORTHOTOPIC HCC GROWTH IS LINKED WITH INCREASED PRO-ANGIOGENIC AND PRO-METASTATIC FACTORS IN MURINE LIVER FIBROSIS

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Background & Aims: Most experimental therapy studies are performed in mice that bear subcutaneous or orthotopic hepatomas but are otherwise healthy and non-fibrotic. However, the majority of hepatocellular carcinoma develops in patients suffering from pre-existing liver fibrosis. Therefore, we wanted to investigate tumor establishment and development after tumor cell implantation into mice with pre-existing liver fibrosis. Methods: Liver fibrosis was induced by thioacetamide and ethanol co-administration in female C3H/He mice. Orthotopic HCC was established by subcapsular intrahepatic injection of 105 HCC cells (Hepa129) into the left liver lobe. Fibrosis induction was verified by measuring hepatic microcirculation (HM) using laser doppler flowmetry one week before implantation and collagen staining. Ten day post implantation tissue samples were harvested for mRNA/cDNA preparation. Transcript levels of VEGF-A, -C, -D and their receptors VEGFR-1/-2 and -3 were determined besides MMP-2 and MMP-9 by semiquantitative real time PCR (LightCycler, Roche Diagnostics, Mannheim, Germany). Results: The combination of TAA/EtOH induced liver damage resulting in F4 (max. score F6) fibrosis as demonstrated by collagen staining. Accordingly, functional relevance was demonstrated by a relative decline (about 50%) in HM in fibrotic mice liver. Tumor growth was considerably accelerated in fibrotic mice compared to the non-treated controls ten days after tumor cell implantation. Mean tumor volume in fibrotic and non-fibrotic mice reached 956 [mm3] and 260 [mm3] (p= 0.0073), respectively. Additionally, metastatic spots were mainly observed in fibrotic but not in non-fibrotic mice (27 vs. 3.1, p<0.0001). Advanced tumor stage was associated with elevated transcription levels VEGFR-1, -2 and high VEGFR-3 values were observed in tumors from fibrotic mice. VEGF-C/-D levels were even higher elevated by pre-existing fibrosis. Furthermore, intratumoral MMP-2 and MMP-9 transcription was also increased in liver fibrotic mice. In contrast to VEGF-C and VEGF-D, VEGF-A transcription itself was not affected by pre-existing fibrosis at the time point of examination. Conclusion: Our data show, that tumor growth and metastasis were considerably accelerated in fibrotic livers in mice and that advanced tumor stage was associated with elevated intratumoral Flk-1 and Flt-1 and increased hepatic MMP-2 and MMP-9 transcription levels. Elevated intratumoral VEGFR-3 receptor status and increased intratumoral VEGF-C/-D transcription levels had been linked with advanced metastasis. This work was supported by a Deutsche Krebshilfe grant.

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CONCORDANCE BETWEEN FIBROTEST® (FT) AND FIBROSCAN® (FS): A NEW NON-INVASIVE METHODOLOGY FOR IMPROVING ACCURACY IN A WORLD WITHOUT A GOLD STANDARD

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Background. Liver stiffness measurements (LSM) using FS, and FT, are widely used as non invasive fibrosis markers. The aim was to identify factors associated with FS accuracy using FT as a non-invasive endpoint and vice versa. Methods. The proof of concept was considered according to the manufacturer's recommendations for including patients (pts)with low risk profiles of false negatives/positives (LR), and excluding those with high risk (HR); for FS: a success rate >60% (SR60), at least 10 valid LSM (V10) and an interquartile range <30% (IQR30); for FT: a security algorithm (SECU) profile excluding extremes compo-

nents values. The hypothesis was that the concordance between FS and FT would be improved by excluding HR pts. Statistical analysis used kappa test (K) for 2 (K2) and 3 (K3) fibrosis stages, and the regression curve fitting F-test (R2). Other factors possibly associated with variability were identified by the same methods: operator effect, steatosis (as diagnosed with SteatoTest), necroinflammatory activity (diagnosed with ActiTest), abdominal and thoracic skin folds, BMI and NAFLD. The database used 2,005 consecutive pts who simultaneously underwent LSM and FT. Results. Among 1,391 pts (69%) with LR for FS, K2=0.41(0.02) m(se) vs 0.31(0.04), K3= 0.33(0.02) vs 0.21(0.03) among the 614 (31%) pts with HR (all P<0.001). K were higher in 1,803 V10 (K2=0.41, K3=0.31) vs 202 non-V10 (K2=0.16, K3=0.08); in 1,743 SR60 (K2=0.41, K3=0.32) vs 262 non-SR60 (K2=0.20, K3=0.11); and in 1,517 IQR30 (K2=0.42, K3=0.32) vs 488 non-IQR30 (K2=0.25, K3=0.19) (All P<0.001 for K2, K3 and F). Patients with 5 to 9 valid measurements (K=0.08) and IQR between 30 to 40% had lower K and F in comparison with V10 (P<0.001) and IQR30 (P=0.04). HR (V10, SR60, IQR30) for FS was significantly associated (all P<0.006) with thoracic skin fold, abdominal skin fold, weight, NAFLD, steatosis and one operator. HIV coinfection was not associated with HR. Among 1,921 pts (96%) with LR for FT, K2=0.39 and K3=0.30 vs 0.12 and 0.12 in 84 excluded by SECU (4%) (all P<0.001 for K2, K3 and F). Among the 1,334 pts with LR for both FS and FT, the concordance was K2=0.43, K2=0.34, and R2=0.32; advanced activity grade (P=0.01) and the same metabolic factors previously associated with HR were associated (all P<0.01) with lower K2, K3 and F. No other factors were identified, including steatosis, age, gender, ALD, HBV, HCV or HIV coinfection. Conclusion: This study has validated the concept of using the strength of concordance between two estimates of liver fibrosis for the identification of factors associated with variability. Metabolic factors were highly associated with LSM variability.

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1072
LACK OF INDUCIBLE NITRIC OXIDE SYNTHASE IN A MOUSE MODEL WITH CARBON TETRACHLORIDE INJURY LEADS TO INCREASED APOPTOSIS AND DECREASED FIBROSIS

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Background / Aim: The role of nitric oxide (NO) on liver injury and fibrosis is unclear. The purpose of this study was to determine the effects of inducible NO synthase deficiency (iNOS-/-) on liver injury and fibrosis produced in mice by chronic CCl4 administration. METHODS: Wild type (wt) or mice with iNOS deficiency were subjected to biweekly CCl4 injections over 8 weeks, while controls were given isovolumetric injections of olive oil. RESULTS: Serum AST and ALT levels were lower after CCl4 in the iNOS-/- than in the wt animals, which correlated with decreased necrosis on liver histology. Also, there were a lower number of stellate cells (α-SMA immunostaining), a lesser degree of fibrosis (morphometric determination of sirius stained slides), and twice as many apoptotic cells (TUNEL assay) after CCl4 in the iNOS-/- as compared to wt animals. In addition, immunostaining for ssDNA, another measure of apoptosis, was significantly higher in the iNOS-/- CCl4 group as compared to wt CCL4 mice. Liver malondialdehyde, an end product of lipid peroxidation by reactive oxygen species, was lower in the iNOS-/- animals. α1(I) collagen mRNA, determined by real time PCR, was markedly increased after CCL4 in the wt and to a significantly lesser extent in the iNOS-/- mice. Tissue inhibitor metalloproteinase 1 (TIMP-1) mRNA, matrix metalloproteinase 2 (MMP-2) mRNA and MMP-9 mRNA were increased after CCl4 in the wt but not in the iNOS-/- mice. Conclusion: NO protects against CCL4 induced apoptosis. In the absence of iNOS, there is decreased necrosis, increased apoptosis, and reduced liver fibrosis.

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1073
EXTRA-CELLULAR FIBRONECTIN INDUCED HEPATIC OVAL CELL PROLIFERATION THROUGH CYCLOOXYGENASE-2-INDUCED PROSTAGLANDIN SIGNALING

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Background: We investigated the activation and proliferation of hepatic oval cells (HOC's) in the liver, using the 2-acetylaminofluorene (2-AAF)/70% partial hepatectomy (PHx) experimental model used in rats. These cells can differentiate into hepatocytes and bile ductular cells for liver regeneration. To understand the molecular mechanism underlying this process, we investigated that extra-cellular matrix protein induced hepatic oval cell proliferation through cyclooxygenase-2-induced prostaglandin signaling. Methods: The reverse transcription polymerase chain reactions (RT-PCR), immunohistochemical staining and Western blot analyses were performed to examine the induction of COX-2 in total liver homogenate and HOC's obtained from 2-AAF/70 % PHx. We investigated whether sorted Thy1+ HOC's would express prostaglandin receptor genes, and extra-cellular matrix proteins, such as connective tissue growth factor (CTGF) and fibronectin, by a Western blot analysis. For the assay of HOC's proliferation on the fibronectin-coated culture dishes, we cultured HOC's with COX-2 inhibitor, 100 uM NS398. Then, HOC's were treated with NS398 after 24 h followed by a change in medium to hepatocyte growth factor (HGF), which determined the activation of COX-2. Results: The COX-2 gene and protein was found to be upregulated in the injured rat liver, using the 70% partial hepatectomy (PHx) with 2-acetylaminofluorene (2-AAF) treatment, and sorted HOC's. Also, the sorted Thy-1+ HOC's induced prostaglandin receptor gene and the extra-cellular matrix protein such as, CTFG and fibronectin. HOC's cultured with 100 uM NS398 significantly decreased COX-2, CTFG and fibronectin. In addition, HOC's were cultured with 100 uM NS398 which decreased BrdU positive cells; however, proliferation of HOC's cultured in the fibronectin pre-treated dishes was not inhibited in spite of 100 uM NS398 treatment. In addition, the COX-2 suppression by 100 uM NS398 was up-regulated by 20 uM HGF. Conclusions: Our results demonstrated that COX-2 expression in activated HOC's occurred through cyclooxygenase-2-induced prostaglandin signaling during liver regeneration, and that extra-cellular fibronectin induced HOCs' proliferation through cyclooxygenase-2-induced prostaglandin signaling.

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1074
TREATMENT OF ESTABLISHED CIRRHOSIS WITH SV40 VECTORS ENCODING INSULIN-LIKE GROWTH FACTOR I

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Liver transplantation is the only curative treatment for advanced liver cirrhosis. Therapies aimed at halting the progression of the disease are urgently needed. Previous studies have shown that the administration of recombinant insulin like growth factor-I (IGF-I) induces hepatoprotective effects in experimental cirrhosis. However, the necessity of using high daily doses of the protein for a long period of time makes this therapy very costly hampering their clinical application. As an alternative therapeutic approach, we have evaluated whether sustained IGF-I expression within the liver from viral vectors based on the Simian Virus 40 (rSVIGF-I) could exert therapeutic effects in liver cirrhosis. We have previously shown that intraportal injection of rSVIGF-I in rats prior to the development of liver cirrhosis induces the expression of hepatocyte growth factor (HGF) in the liver and delays the progression of the disease. In the present study we have tested the effect of the vector in rats that had already developed advanced liver cirrhosis. Liver cirrhosis was induced in Sprague-Dawley male rats by intragastric administration of CCl4 for 12 weeks and then a single dose of rSVIGF-I (1011 vp/rat) was administered by different routes. The following administration routes were tested: intraportal (IP), intrahepatic (IH), intraarterial (IA) and intrabiliar (IB). At the end of the study, cirrhotic rats treated with rSVIGF-I showed reduced serum bilirubin, transaminases and liver fibrosis scores as well as increased expression of serum albumin as compared to mock-infected cirrhotic animals. The effects were more prominent in rats treated by IP or IA injection, suggesting that these routes could allow better access of the vector to hepatocytes in fibrotic livers. IGF-I expression correlated with the increase of mRNA levels of IGF-I binding protein 3 (whose synthesis is induced by IGF-I) and with upregulation of HGF, a factor that seems to mediate hepatoprotective effects of IGF-I. These results indicate that established cirrhosis can be reverted by IGF-I and also, that rSVIGF-I are promising vectors for the treatment of this disease.

Disclosures:

The following people have nothing to disclose: Luciano Sobrevals, Nerea Razquin, Gloria Gonzalez-Aseguinolaza, Carlos Rodriguez, Puri Fortes, Jesus Prieto

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MULTIPLE GENETIC MARKERS IN TLR4 REGION ARE ASSOCIATED WITH CIRRHOSIS RISK IN CHC PATIENTS

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BACKGROUND: We have recently developed a 7 gene Cirrhosis Risk Score (CRS) signature that can predict the risk of developing cirrhosis in patients with chronic hepatitis C (CHC) (Hepatology, 2007, in press, E-pub April 26). Among them T399I is located in the Toll like receptor 4 (TLR4) gene, which has an identified role in hepatic fibrosis. **AIM:** To determine the role of other genetic markers in or near TLR4 in cirrhosis risk. **METHODS:** We developed assays for an additional 157 single nucleotide polymorphisms (SNPs), covering the region from 98 kb upstream to 161 kb downstream from the original T399I SNP. These 157 SNPs were genotyped in 1,020 CHC patients (708 Caucasians) enrolled from 2 US centers. All patients had liver biopsy prior to treatment and clinical data. In all statistical analyses, Cases were defined as subjects with Bridging fibrosis/Cirrhosis (F3-4) while Controls were No-fibrosis (F0) on liver biopsy. We first performed univariate analysis to identify individual markers significantly associated with cirrhosis risk. We then used the Haplo.Score program to determine significant haplotypes in this region. Last, logistic regression was used to determine the relative importance of each SNP conditional on the effect of other SNPs for all possible pairs in the region. **RESULTS:** Of 157 SNPs in the TLR4 region, nine were significantly associated with cirrhosis risk with $p<0.005$. Their odds ratios (OR=1.68-2.96) were comparable to that of the original T399I SNP. The frequency of the risk alleles is 23.8-94.3%. Among the nine SNPs, one missense (D299G), three intronic and four intergenic SNPs were located in or near the TLR gene; one intronic SNP was in an uncharacterized gene (47kb from TLR4). The two missense TLR4 SNPs T399I and D299G are in nearly complete linkage disequilibrium ($R^2=0.934$). In addition, a 2-SNP haplotype containing the risk allele of the original T399I SNP showed a 24-fold stronger association with cirrhosis risk than T399I by itself (p value 1.37E-05 vs. 3.34E-04). Analysis of the relative importance of the SNPs in the entire region identified one intronic SNP in TLR4 and one intronic SNP in the nearby uncharacterized gene that were associated with cirrhosis risk independent of T399I, suggesting these 3 SNPs may be responsible for the cirrhosis risk in this region. **CONCLUSIONS:** We found additional SNPs and haplotypes associated with cirrhosis risk at an equivalent or more significant level than the original T399I. Identification of additional markers in the remaining six gene regions of CRS is ongoing. These findings could lead to a more predictive signature for cirrhosis risk and improve the management of CHC patients.

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BMP-7: THE KEY ANTAGONIST OF TGF-β IN LIVER FIBROSIS?

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Background: Transforming growth factor-β1 (TGF-β1) has been accepted as the master cytokine not only in the fibrotic response in the liver. Bone morphogenetic protein-7 (BMP-7), a member of the same superfamily of cytokines, antagonizes profibrogenic functions of TGF-β1 in diverse cellular systems. In the liver a regenerative function of BMP-7 was described but the expression of BMP-7 in isolated liver cells as well as its antifibrotic potential is controversially discussed. Results: First of all we could detect BMP-7 in isolated liver cells. Although hepatic stellate cells (HSC) and hepatocytes express all necessary BMP-7 receptors, only hepatocytes express BMP-7 while HSC do not. This was shown by RT-PCR and immunohistochemstry. In vitro we could show that HSC are responsive to BMP-7 by western blot analysis of phosphorylated Smad1/5 and an increased luciferase activity when using the artificial BMP-reporter (BRE)2-

luciferase. When applied in parallel we could clearly demonstrate that BMP-7 antagonizes the profibrogenic actions of TGF-β1, in that the TGF-β1-induced collagen I and PAI-1 synthesis was completely blocked. Furthermore, we could show by using the (CAGA)12-MLP luc reporter that the basis for this antagonism is most likely a reduced activation of Smad3. Although hepatocytes show a similar sensitivity towards BMP-7 compared to HSC, TGF-β1 induced (CAGA)12-MLP luc activity was not influenced by BMP-7. Nevertheless BMP-7 was able to reduce the LDH concentration in TGF-β1 treated heaptocytes, implying an anti-apoptotic effect. Finally we could demonstrate, in a model of liver fibrosis induced by bile duct ligation (BDL), an increase in BMP-7 expression confirmed by an adenoviral (BRE)2-luc reporter activated in vivo and RT-PCR. Conclusion: We conclude that BMP-7 is a cytokine which is involved in the maintenance of normal liver function. We figured out two different aspects of BMP-7 function: 1) The upregulation of BMP-7 during liver injury protects hepatocytes from TGF-β-mediated apoptosis. 2) BMP-7 directly antagonizes TGF-β-induced extracellular matrix synthesis regulated by Smad3.

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KUPFFER CELL-DERIVED CONNECTIVE TISSUE GROWTH FACTOR (CTGF) AND TRANSFORMING GROWTH FACTOR BETA (TGFB) MEDIATE THE PROFIBROGENIC EFFECTS OF LEPTIN IN VITRO

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Introduction The mechanism(s) by which leptin exerts its profibrogenic effects are currently unclear. Some data suggest that leptin acts directly on hepatic stellate cells (HSCs) to trigger fibrogenic gene expression. We hypothesized that in vitro cell culture models will help elucidate the mechanisms whereby leptin activates profibrogenic gene expression in HSCs. Methods Non-parenchymal liver cells (HSCs, KCs and SECs) were isolated from wt or leptin-resistant fatty (fa/fa) Zucker rat and cultured for various times from 2 to 6 days. Recombinant rat leptin (10 nM, 100 nM) was subsequently added. Gene expression of Type I collagen, TIMP1, TGFb1 and CTGF were assessed by real time PCR. HSC a-smooth muscle actin (a -SMA) and KC JAK/STAT3, MAPK and PI-3K signalling pathways were assessed by immunoblot. The WST-1 assay was used for HSC proliferation. Immunocytochemistry was performed to assess CTGF protein expression. Electrophoretic mobility shift assays (EMSA) were performed for AP-1 and NF-kB DNA binding ability. Results Leptin treatment of HSCs in culture failed to potentiate cellular activation. In contrast, when conditioned medium from KCs treated with leptin was transferred to HSCs, profibrogenic gene expression, a -SMA protein and cellular proliferation were augmented. These effects were leptin receptor dependent since Zucker rat KC conditioned medium treated with leptin did not activate stellate cells. We next sought to determine whether the fibrogenic cytokines TGFb1 and CTGF were important in mediating these effects. In KCs treated with leptin, the expression of mRNAs for TGFb1 and CTGF were significantly increased. Likewise, by immuno-histochemistry, we were able to demonstrate increased levels of CTGF protein in KCs treated with leptin compared to controls. We were unable to demonstrate any increase in TGFb1 protein in KC lysates or in KC-conditioned medium by ELISA or immunoblotting. Since CTGF expression is stimulated by TGFb, we next inhibited TGFb production in KCs treated with or without leptin using a soluble TGFb type II receptor. Kupffer cells treated with the soluble receptor but not a control immunoglobulin demonstrated down regulation of CTGF protein. Finally, leptin potentiated pSTAT3, pAKT and pERK1/2 in KCs as well as enhanced AP-1 and NF-kB DNA binding activities in KCs. Conclusion: Leptin and its functional receptors play a crucial role in HSC activation and liver fibrosis principally through indirect effects via soluble mediators released from KC. Increased expression of CTGF and TGFb are in part responsible for mediating these effects.

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LOSARTAN REDUCES THE EXPRESSION OF PROFIBROGENIC GENES AND INFLAMMATION IN PATIENTS WITH CHRONIC HEPATITIS C

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Background & Aims: There are no antifibrotic therapies for patients with chronic hepatitis C in whom viral infection cannot be cured. Angiotensin II type 1 receptor (AT1) blockers have been shown to reduce liver fibrogenesis in experimental models of liver disease, but their effects on liver fibrogenesis in humans is unknown. We investigated the effect of losartan, an AT1 blocker, on liver fibrogenesis in patients with chronic hepatitis C who did not respond to antiviral therapy. Methods: Fourteen patients with chronic hepatitis C and significant liver fibrosis (F2-F4) received oral losartan (50 mg/day) for 18 months. Two paired liver biopsies were performed before and after treatment. The degree of fibrosis and inflammation were evaluated by histological analysis (METAVIR). Collagen content, the amount of myofibroblasts and inflammatory cells were estimated by measuring the percentage of the whole biopsy area stained with Sirius Red, anti-α-SMA and anti-CD43, respectively. Liver fibrogenesis was assessed by quantifying mRNA expression of key genes encoding extracellular matrix proteins, fibrogenic mediators, inflammatory cytokines, pro-oxidant proteins and components of the renin-angiotensin system through real-time PCR. Results: Oral losartan was well tolerated in all patients. Treatment with losartan was associated with a decrease in inflammatory activity in 8 patients (57%) and a decrease in the fibrosis stage decreased in 7 patients (50%). Treatment with losartan was associated with a significant reduction in the expression of key genes involved in liver fibrogenesis. The mean reduction in the expression of procollagen α1(I), procollagen α4(I), ut-PA, MMP2 and Rac1 was 30%, 23%, 40%, 27% and 21%, respectively. Patients with reduced inflammatory activity showed a pronounced down-regulation of procollagen α1(I) (44%) and additional down-regulation of TIMP-1 and inflammatory cytokines (MCP-1 and Gro-α). Patients with improved fibrosis showed a reduction in collagen content and in the amount of myofibroblasts compared to patients without fibrosis improvement. Patients with improved inflammation showed a significant decrease in the CD43-positive area compared to patients in whom inflammation did not improve. Losartan treatment did not affect renal function, serum liver tests or viral load. Conclusions: Oral losartan for 18 months decreases the expression of genes involved in liver fibrogenesis and

reduces the inflammatory activity in patients with chronic hepatitis C. The effects of AT1 blockers in chronic hepatitis C should be further investigated in randomized controlled studies.

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ATORVASTATIN ATTENUATES ANGIOTENSIN-II INDUCED INFLAMMATORY AND FIBROGENIC ACTIONS IN THE LIVER

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Background & Aims: Statins, besides their lipid lowering properties, exert beneficial effects in chronically damaged tissues. Angiotensin II (Ang II) plays an important role in liver fibrogenesis by inducing oxidative stress, promoting hepatic inflammation and stimulating TGFβ1 and collagen gene expression. We investigate whether atorvastatin modulates Ang II-induced pathogenic effects in the liver. Methods: Male Wistar rats were infused with saline or Ang II (50 ng/Kg/min) for 4 weeks through a subcutaneous osmotic pump. Rats received either vehicle or atorvastatin (40 mg/Kg.day) by gavage. H&E liver specimens were blindly scored by an expert pathologist. The degree of hepatic inflammation, oxidative stress as well as the expression of genes involved in the hepatic wound healing response to injury were assessed by CD43 immunostaining, 4-HNE immunostaining and quantitative PCR, respectively. The effect of atorvastatine on Ang II-pathogenic effects were also assessed in cultured hepatic stellate cells (HSC), a major fibrogenic cell type. Cell proliferation (3H-thymidine incorporation), NFκB activation (reporter gene assay and p65 nuclear translocation), secretion of interleukin-8 (ELISA) and gene expression (quantitative PCR) were studied. Results: Infusion of Ang II infusion in to normal rats resulted in resulted in hepatic inflammation, as indicated by an increased necroinflammatory score and infiltration of CD43-positive cells. Ang II also caused thickening of hepatic vessels. These effects were markedly blunted by the concomitant administration of atorvastatine. Ang II infusion also caused hepatic oxidative stress (4-HNE immunostaning), which was prevented by atorvastatine. Finally, Ang II infusion increased the hepatic expression of TNFα and interleukin-6, which was attenuated in the presence of atorvastatine. In addition, atorvastatin reduced Ang II–induced procollagen a1(I) and TGFβ1 gene expression in the rat livers. We next explored whether the beneficial effects of atorvastatin on Ang II-induced actions are also reproduced at the cellular level. We studied cultured primary HSC. Ang II (10-8M) stimulated cell proliferation, pro-inflammatory actions (NFkB activation, ICAM-1 expression and interleukin-8 secretion) as well as expression of procollagen a1(I) and TGFb1. All these effects were reduced in the presence of atorvastatin (10-7M). Conclusions: Atorvastatine attenuates the inflammatory and fibrogenic effects of Ang II in the liver. Therefore, statins could have beneficial effects in conditions characterized by hepatic inflammation and fibrogenesis.

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T CELL DERIVED MEMBRANE MICROPARTICLES CONTAINING EMMPRIN INDUCE PROFIBROLYTIC ACTIVATION IN HEPATIC STELLATE CELLS

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BACKGROUND: T cell interactions with hepatic stellate cells (HSC) may be a determinant of liver fibrosis progression. Microparticles (MP) are small membrane vesicles shed from various cell types during activation and apoptosis. In most chronic liver diseases, such as viral and autoimmune hepatitis, activated T cells are driving force of disease progression and their turnover is greatly increased at the sites of active tissue remodeling. Here we studied and analyzed the effect of T-cell derived MP on the profibrogenic activation of HSC. METHODS: MP were generated from Jurkat T cells subjected to apoptosis induction with staurosporine, and purified from cell-free supernatant by differential ultracentrifugation. Characterization and quantification of MP was done by electron microscopy FACS. MP proteins were analyzed by MALDI-TOF mass spectrometry. Human LX-2 HSC (gift of Dr. S.L. Friedman) were incubated with increasing numbers of MP for up to 36h. Profibrogenic and fibrolytic transcripts were quantified by quantitiative RT-PCR. To study membrane interaction of MP with HSC, tracking experiments were performed using PKH26 fluorescent-labeled MP. RESULTS: Apoptosis induction in Jurkat cells lead to 7-9 fold increase of MP production compared to untreated cells. Electron microscopy revealed that preparations of MP contained round empty vesicles with a lipid bilayer and sizes between 60 and 700 nm. Incubation of freshly isolated MP with HSC strongly induced fibrolytic MMP-1 and -3 mRNA transcripts (3- and 30-fold, respectively), while profibrogenic TIMP-1 mRNA, was unchaged and procollagen α1(I) was moderately downregulated. Tracking experiments revealed rapid transfer of fluorescent label from MP to HSC membranes already after 30 min, being retained for at least 12h. MALDI-TOF mass spectrometry of purified MP revealed high levels of CD147/Emmprin/Basigin, an MMP inducer that is putatively responsible for the observed effects. CONCLUSIONS: 1. T cell derived MP potently induce fybrolytic activation of HSC; 2. This suggest a novel cytokine-independent mechanism of fibrogenesis modulation by activated/apoptotic T celsl in chronic liver diseases. 3. EMMPRIN is a prime candidate to mediate the fibrolytic switch.

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CELECOXIB SHOWS ANTI-FIBROTIC EFFECT IN HEPATIC FIBROSIS MODELS IN RAT AND INDUCES APOPTOSIS OF HEPATIC STELLATE CELLS THROUGH INHIBITION OF AKT ACTIVATION

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Background/Aim: We previously reported that celecoxib, a COX2 selective inhibitor, shows anti-proliferative and pro-apoptotic effect on human hepatic stellate cells (HSC). It has been reported that selective COX2 inhibitors such as JTE-522 or SC-236 attenuates hepatic fibrosis in rat. However, Hui et al. reported that celecoxib potentiates hepatic fibrosis in CCl_4-treated rat. We investigated the mechanism of pro-apoptotic effect of celecoxib in human HSC and evaluated anti-fibrotic effect of celecoxib in bile duct ligation (BDL)- and thioacetamide (TAA)-induced hepatic fibrosis models in rat. Methods: We used immortal human HSC established by functional expression of the telomerase catalytic subunit. We treated HSC with celecoxib (50μg/ml) after infection of adenovirus expressing constitutively active Akt (Ad5myrAkt) or adenovirus expressing GFP (Ad5GFP). Hepatic fibrosis was induced in Wistar rats by BDL for 4 weeks or by peritoneal TAA injection for 5 weeks. Celecoxib (20mg/kg/day) was orally administered from the beginning of liver injuries. Untreated BDL- and TAA -rats served as controls. Extracellular matrix (ECM) was Trichrome stained and quantified by morphometry. Hydroxyproline content in liver tissue was measured. Expression of α-SMA was assessed by immunohistochemistry. Hepatic mRNA levels of COX2, α-SMA, TGF-β1, collagen α1(I) was quantified by realtime RT-PCR. Results: Celecoxib induces HSC apoptosis and inhibits Akt phosphorylation in HSC. Celecoxib-induced HSC apoptosis is significantly attenuated in HSC infected with Ad5myrAkt but not in those infected with Ad5GFP. AST, ALT, and alkaline phosphatase levels are decreased in celecoxib treated group in TAA-rats ($p<0.01$) but not in BDL-rats. Liver tissue morphometry after Trichrome staining shows that celecoxib decreases hepatic ECM deposition in both BDL-rats (BDL only vs. BDL+celecoxib 52.8% vs. 37.9%, $p=0.035$) and TAA-rats (TAA only vs. TAA+celecoxib 17.9% vs. 10.8%, $p<0.001$). Hepatic hydroxyproline content and α-SMA expression are significantly decreased by celecoxib in both BDL- and TAA-rats. In both BDL-rats and TAA-rats, COX2, α-SMA, TGF-β1, and collagen α1(I) mRNA is upregulated. Celecoxib treated rats shows significantly decreased mRNA expression of COX2, α-SMA, TGF-β1, and collagen α1(I) compared with untreated rats in both BDL- and TAA-induced hepatic fibrosis models. Conclusions: Celecoxib shows anti-fibrogenic effects in both BDL- and TAA- rats suggesting celecoxib as a potential anti-fibrotic agent for hepatic fibrosis. Celecoxib-induced inhibition of Akt activation in hepatic stellate cells at least partially contributes to the anti-fibrotic effect of celecoxib.

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TELMISARTAN PREVENTS HEPATIC FIBROSIS AND ENZYME-ALTERED PRENEOPLASTIC LESIONS IN RAT LIVER CIRRHOSIS INDUCED BY A CHOLINE-DEFICIENT L-AMINO ACID-DEFINED DIET

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Objective: Nonalcoholic steatohepatitis(NASH)may develop into fibrosis, cirrhosis and hepatocellular carcinoma. The renin-angiotensin system is related to liver fibrosis and hepatic stellate cells(HSCs)play an important role in the liver fibrogensis.Telmisartan, an angiotensin II type 1 receptor(AT1)antagonist,can function as an agonist of peroxisome proliferator-activated receptor γ. So we hypothesized that telmisartan could improve NASH related fibrosis. Materials and Methods: The effect of telmisartan was studied by in vitro and in vivo. In vitro, HSCs were isolated from rat liver, and the cells were treated with telmisartan at the dose of 0 to 25μM. Using Western blotting, we checked HSC activation marker of α-SMA and how HSCs express AT1 and ANG II. Using real-time PCR, we determined fibrotic gene expression. In vivo, we used choline-deficient L-amino acid–defined diet induced rat liver fibrotic model, which is an established model of NASH. The total study periods were 10 weeks. The rats were treated with telmisartan at the dose of 0, 0.5, 1.0 and 2.5mg/kg body weight/day. The liver fibrosis index was measured by Azan and Sirius red stain and immunohistochemistry of α-SMA. The liver tissue expression of fibrotic gene and the serum biochemical marker were also determined. Furthermore, the preneoplatic lesion of placental glutathione S-transferase(GST-P)positive lesions was analyzed. Results: In vitro, telmisartan inhibited HSCs expression of α-SMA and decreased the mRNA expression of type 1 procollagen and TIMP-1, 2 in a dose-dependent manner. HSCs expressed AT1 and ANG II and the expression increased as the cells undergoing activation. In BrdU incorporation assay, we found telmisartan blocked ANG II induced HSCs proliferation. In vivo, telmisartan(0.5, 1.0 and 2.5mg/kg)treated rats showed decreased extracellular matrix accumulation by 25%, 41% and 65% of control ($p<0.01$ respectively); simultaneously, telmisartan reduced the area of α-SMA positive cells by 27%, 35% and 49% of the control($p<0.01$ respectively). The development of GST-P-positive preneoplastic lesions in each group revealed in area as (125.1±42.9;87.0±21.3,58.8±20.4,45.9±17.4 μm2/section) and the number as(4.8±3.0,2.5±1.8,2.1±1.4,0.8±0.6/section), which were showed significant reduced by telmisartan ($p<0.01$ respectively). The mRNA expression of type 1 procollagen and the serum hyaluronic acid, total bile acid were significantly decreased by telmisartan. Conclusions: Telmisartan prevented liver fibrosis and attenuated enzyme-altered preneoplastic lesions in NASH related liver fibrosis via inhibition of HSCs activation. Telmisartan will be a candidate drug for NASH patients.

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The following people have nothing to disclose: Haiyan Jin, Naoki Yamamoto, Shuji Terai, Fujio Murakami, Isao Sakaida

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NATRIURETIC PEPTIDE IMPROVED LIVER FIBROSIS IN RAT MODEL USING NEW TREATMENT SYSTEM

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[Aim/Backgroud]: The natriuretic peptide (NP) are used as the acute heart failure treatment in clinical and it is reported that the suppression of fibrosis in the heart and the lung recently. In this study, we made the in vivo model that can estimate the effect of new drugs on liver fibrosis by new injection system using 24 hour continuous infusion pump system. Using the in vivo model, we checked the effect of atrial natriuretic peptide (ANP) or C-type natriuretic peptide (CNP) on liver fibrosis.[Methods]:Wister rats were injected with dimethylnitrosamine(DMN) twice a week via interperitoneal (i.p.)for 4 weeks to make the liver fibrosis. Fibrotic rats were assigned to three groups(each group n=6): DMN only, DMN+ANP(1.0μg/kg/min), DMN+ANP(0.1μg/kg/min), DMN+CNP(1.0μg/kg/min), DMN+CNP(0.1μg/kg/min). Each group was given by continuous intravenous dosage system used 24 hour infusion pump for 3 weeks after 1 week of DMN administration. The liver fibrosis index was measured by H-E, Azan, Sirius-Red staining. The state of hepatic stellate cell was analyzed by α-SMA staining. Laboratory data was also analyzed. Collagen type I, TIMP-1, TIMP-2 and MMP2 mRNA expression was analyzed by real time RT-PCR system. [Results]: The NP treated groups showed decrease of serum AST (mean value : ANP 81.8 vs CNP 95.4 vs Control 2000 IU/l P<0.01) ALT (mean value : ANP 63.8 vs CNP 44.8 vs Control 2000 IU/l P<0.01). Liver fibrosis was suppressed by ANP group or CNP group more than control group in both Azan and Sirius Red Stain (P<0.01). In Western blot analysis, ANP or CNP group inhibited α-SMA expression(P<0.01). ANP or CNP also significantly inhibited Type 1 procollagen, TIMP-1, TIMP-2, MMP9 and MMP2 mRNA expression (all of P<0.01). [Conclusion]: The natriuretic peptide (NP) inhibited liver fibrosis in our in vivo model. The NP will be a candidate drug for liver cirrhosis patients.

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TRANSIENT ELASTOGRAPHY (FIBROSCAN®) IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION AND HAEMOPHILIA A

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Introduction: In patients with bleeding disorders and chronic hepatitis C virus (HCV) infection liver biopsy for assessment of fibrosis stage is generally avoided because of potential hazardous complications, high costs, and low patients' acceptance. Thus, non-invasive methods for fibrosis staging are warranted. **Methods and patients:** We recruited 83 patients with chronic HCV infection and haemophilia A (HCV-A) and 297 HCV patients without haemophilia A (HCV-wo); a significant proportion of patients was co-infected with HIV (40% and 12%, respectively). In addition, we included 29 HCV patients and sustained virological response to interferon therapy(HCV-SVR). We determined serum surrogate markers for fibrosis (AST/platelets ratio index [APRI], Forns index) and performed transient elastography (FibroScan®) in all patients, and recorded histological stages of liver fibrosis if available. Correlation of serum markers and liver stiffness was tested using Pearson's correlation coefficient and linear regression analysis. **Results:** In the HCV-wo group, histological stages of liver fibrosis show a highly significant linear correlation with all non-invasive fibrosis tests (transient elastography, APRI and Forns index ($n = 88$; $p < 0.001$). HCV-A patients display more advanced liver fibrosis, as indicated by differences in liver stiffness (15.4 vs. 11.4 kPa; $p = 0.011$) and Forns index (8.98 vs. 8.27; $p < 0.05$), albeit there are younger than HCV-wo patients (42 vs. 55 yrs; $p < 0.001$). In HCV-A patients, elastography results correlate with serum markers tests ($p < 0.001$), but this correlation is not present in HCV-A patients with pronounced fibrosis in elastography (> 12.5 kPa). Of note, responders to interferon therapy display lower liver stiffness than non-responders or naive patients (7.2 vs. 14.0 kPa; $p < 0.001$; 7.2 vs. 10.9 kPa; $p < 0.01$), whereas Forns index and APRI show no differences. **Conclusions:** Despite younger age, haemophilic patients present with more advanced fibrosis, most likely due to co-infection with HIV. Responders to interferon therapy show lower liver stiffness than patients with ongoing HCV infection. We conclude that transient elastography and serum surrogate markers help to identify haemophilic patients at risk for rapid progression of fibrosis. Moreover, transient elastography might be useful for monitoring regression of fibrosis after resolution of HCV infection.

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INHIBITION OF ANGIOGENESIS BY A VITRONECTIN RECEPTOR ANTAGONIST WORSENS LIVER FIBROSIS

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Background and aims: Hepatic fibrogenesis is dependent on neovascularization. However, the impact and role of angiogenesis in liver fibrosis progression are ill defined. The vitronectin receptor (integrin alphaVbeta3) is central to angiogenesis by mediating migration, proliferation and tubulogenesis of endothelial cells, but also drives fibrogenic activation of hepatic stellate cells (Xu et al, JBC 2004; Patsenker et al, J Hepatol 2007). We therefore studied the effect of alphaVbeta3 inhibition in models of liver fibrosis in vivo. Methods: Liver fibrosis in rats was induced by bile duct ligation (BDL) for 6 weeks or by i.p. thioacetamide (TAA) for 12 weeks. The alphaVbeta3 inhibitor EMD121974 (cilengitide) was given i.p. twice daily at 15 mg/kg from week 2-6 after BDL and for 8 weeks after discontinuation of TAA. Liver collagen was determined as hydroxyproline content, and fibrosis related gene expression was measured by quantitative RT-PCR. Angiogenesis was assessed by immunostaining with CD31 antibody and vessel counting using a point counting technique. Results: Treatment of both BDL and TAA fibrotic rats with cilengitide resulted in a further significant increase of hepatic collagen accumulation by 30%, upregulation of profibrogenic gene expression, and was accompanied by an increase of GGT and bilirubin (4.7-, and 1.3-fold vs. untreated fibrotic controls, respectively) in rats with BDL, while liver enzymes and bilirubin remained unchanged in TAA-treated rats that received cilengitide. In both fibrosis mod-

els alphaVbeta3 inhibition decreased vessel formation in portal areas significantly by 20-25% compared to untreated fibrotic rats, while vessel density in sinusoidal areas resembled that of normal livers. Conclusions: 1. Inhibition of angiogenesis by a specific alphaVbeta3 integrin antagonist worsens the progression of liver fibrosis in vivo, despite antifibrotic effects on hepatic stellate cells in vitro, suggesting a beneficial role of neoangiogenesis in portal fibrosis. 2. Antiangiogenic therapy may worsen liver fibrosis in tumor patients with liver fibrosis.

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1086
HYPERDYNAMIC CIRCULATION IN INFANTS WITH BILIARY ATRESIA AND PORTAL HYPERTENSION

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The hyperdynamic circulation (HC) is a well described phenomenon in adults with cirrhosis, yet it has not been carefully examined in children. Infants with biliary atresia (BA) often develop cirrhosis and prominent portal hypertension (PH) with its complications, ascites and variceal bleeding. Goal: To evaluate the presence of HC in infants with BA and PH. Methods: Chart review of patients < 1 year with BA and PH that underwent pretransplant cardiac evaluation between 1/1994 to 12/2006. PH was defined by: 1. presence of endoscopic evidence of esophageal/ gastric varices, or 2. Splenomegaly or ascites by clinical examination and/or imaging, and radiologic evidence of esophageal /gastric varices or hypersplenism. Evaluation included vital signs and echocardiographic assessment including measurement of left ventricular systolic and diastolic dimensions, septal and posterior wall thickness,quantification of shortening fraction, detailed diastolic function assessment (mitral valve inflow Doppler) and assessment of longitudinal function (Doppler Tissue, DTI, derived indices of both systolic and diastolic function). Patients with congenital heart disease or receiving medications that can affect HR or BP were excluded. Twice as many healthy aged matched infants that underwent cardiac evaluation served as controls. Results: 9 patients (5M) mean age 7±1.8 months and 18 controls (12M) mean age 6.52±2.2 months (p=0.76) were included. 8/9 patients underwent prior portoenterostomy; 7/9 had one or more episodes of variceal bleeding. Mean Hb level at echocardiography was 10 ±1.5 gr/dl. There was no statistical difference in the HR, Pulse Pressure (Table 1) and DTI parameters between the groups. Shortening fraction, systolic and diastolic BP were significantly higher in infants with BA. Conclusion: Circulatory differences were observed in infants with BA and PH. The findings are distinct from the classical syndrome described in adults. Resting heart rate is relatively unchanged and blood pressure is increased. The hyperdynamic circulation is instead manifest by evidence of increased contractility. These novel findings have important implications for the management of portal hypertension in young children.

Table 1

	Patients n=9 Mean ±SD	Controls n=18 Mean ± SD	P value
Mean resting HR	139 ± 27	126 ± 13	0.11
Mean Systolic BP (mmHg)	95 ± 14	84 ± 9	0.02
Mean Diastolic BP (mmHg)	55 ± 8	44 ± 7	0.002
Pulse pressure (mmHg)	39 ± 7	40 ± 9	0.9
Mean Left Ventricle End Systolic Septal Thickness (cm)	0.89± 0.12	0.62±0.06	<0.0001
Mean left Ventricle End Diastolic Septal Thickness (cm)	0.60±0.09	0.43±0.06	<0.001
Mean Shortening Fraction (%)	42.5±4.5	37.6± 3.2	0.02

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ADAPTIVE CHANGES OF HEPATOCYTE TRANSPORTERS AND NUCLEAR RECEPTORS IN PEDIATRIC PATIENTS WITH EARLY AND LATE-STAGE CHOLESTASIS

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The adaptive changes of hepatocyte transport proteins and nuclear receptors during obstructive cholestasis in humans are important yet remain unclear. To investigate the early-stage and late-stage adaptive responses, we investigated liver samples from children with chronic obstructive liver disease, including patients with biliary atresia during Kasai operation(n=8, 64+/-17 days), and patients who progressed to late-stage cholestasis underwent orthotopic liver transplantation (n=8, 10.50+/-3.12 months). Expression levels of the sinusoidal and canalicular transporters, nuclear receptors, and CYP enzymes were analyzed by real-time RT-PCR and immunofluorescent staining in comparison to age-matched pediatric controls and neonatal intrahepatic cholestasis. At early-stage cholestasis, most canalicular transporters and sinusoidal uptake transporters were down-regulated, including BSEP, MDR3, MRP2, NTCP, and OATPs; except that OSTα/β were up-regulated. At late-stage obstructive cholestasis, BSEP returned to normal, MDR3 and MDR1 were up-regulated, and sinusoidal exporter OSTα/β and MRP4 were up-regulated. FXR was down-regulated at early-stage cholestasis, returned toward normal levels at late-stage cholestasis, and the expression level of PXR was decreased profoundly in late-stage obstructive cholestasis, while SHP and CAR were unchanged. Immunofluorescent staining showed unchanged BSEP, increased MDR3, MDR1, and decreased MRP2 signals well-targeted to the canalicular membrane at late-stage obstructive cholestasis, with higher expressions in zone 1 and 2 than zone 3. The expression levels of multiple genes were directly related to FXR levels, confirming FXR as the main regulator or bile acid transport. In conclusion, different adaptive changes occurred in early-stage and late-stage cholestasis in human. FXR-BSEP remained unchanged at late-stage cholestasis to maintain bile acid homeostasis. PXR was markedly down-regulated at the progress of late-stage obstructive cholestasis, and could be a potential target for therapy.

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THE ROLE OF ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY IN DIAGNOSIS OF BILIARY ATRESIA IN INFANTS

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Aim: To investigate role and safety of endoscopic retrograde cholangiopancreatography (ERCP) in diagnosing biliary atresia (BA) in infants with prolonged conjugated jaundice, where standard clinical work up including laboratory tests, ultrasound and liver biopsy have failed to produce a definite diagnosis. Patients and Methods: We retrospectively analysed our database for infants younger than 100 days who underwent ERCP for diagnosis of BA from June 1997 to May 2007. Results: Amongst 2079 infants investigated for prolonged jaundice, 224(10.7%) was diagnosed with BA over the observation period. ERCP was performed in 48(2.3%) infants (24 male; median age: 58.5 days, range 19 – 98 days; median weight: 4 kg, range, 2.07- 5.3 kg). All infants had acholic stools, including 3 with associated alpha-1-antitrypsin PiZZ deficiency and 2 with bile salt export pump deficiency (PFIC-2). Forty-seven infants had liver biopsy, while the remaining one had haemophilia C. Liver histology showed non-specific cholestasis (n=18, 38%), giant cell hepatitis (n=12, 26%), large bile duct obstruction (n=10, 21%) and mixed cholestatic and hepatitic features (n=7, 15%). Cannulation of papilla was unsuccessful in 3 (6.25%) infants. These three infants were eventually diagnosed with BA on laparotomy. Of the remaining 45 children, ERCP demonstrated intrahepatic biliary tree in 20 (42%), while 25 (52%) proceeded to laparotomy. Of those, BA was confirmed macroscopically and histologically in 22 (46%). Three remaining infants who underwent explorative laparotomy, but not Kasai portoenterostomy had CMV hepatitis, neonatal cholestasis and right and left hepatic duct hypoplasia. Amongst the 20 children in whom ERCP ruled out BA, 10 (50%) had neonatal sclerosing cholangitis, 1 (5%) Caroli disease and 9 (45%) normal biliary tree. After ERCP 3 infants (6.25%) had slight intraabdominal extravasation of the contrast, but none had clinical pancreatitis or peritonitis. Conclusion: ERCP is a safe procedure for diagnosing BA even in small infants with the positive predictive value of 88% and negative predictive value of 100%. In specialized centres this procedure could prevent unnecessary laparotomy in majority of infants with ambiguous clinical findings.

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PREDOMINANCE OF T HELPER 1 VIRUS SPECIFIC IMMUNE RESPONSE FAVOURS VIRUS CONTROL IN CHILDHOOD HEPATITIS B VIRUS INFECTION

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Background: The outcome of hepatitis B virus (HBV) infection results from the interaction between virus and host immune response. In adults, a vigorous and broad HBV-specific T helper1 (Th1) skewed immune response is associated with successful control of viral replication. The role of chemokine interferon (IFN)-γ-inducible protein-10 (IP-10), that predicts outcome of hepatitis C, has not been investigated in chronic HBV infection. Aims: To investigate Th1 and Th2 HBV-specific immune reactivity and plasma concentrations of IP-10 in paediatric patients in five different stages of HBV infection. Patients: One hundred paediatric patients (median age 13.1 years, 54 males) were divided into 5 groups according to presence/absence of HBeAg, HBsAg and aminotransferases activity (Table 1). Six children infected with hepatitis C infection served as controls (median age 13.7 years, 3 males). Methods: HBV-specific Th1/Th2 immune responses were determined by IFN-γ, interleukin-2 (IL-2), IL-4 and IL-10 Elispots after stimulation of peripheral blood mononuclear cells (PBMC) with HBV antigens (HBcAg, HBeAg and HBsAg). The non-specific stimuli phytohemagglutinin, bacterial lipopolysacharide and tetanus toxoid antigen served as positive controls. Plasma IP-10 concentrations were measured by ELISA. HBV DNA viral load was quantified by real-time PCR (log_{10} copies/ml). Results: Higher HBV-specific production of IFN-γ and IL-2 (Th1) was detected in group E than groups A and D [IFN-gamma HBV core: 198±106 vs. 13.4±2.7 and 8.4±5.2 specific spot forming cells per million of PBMC (spSFC/10^6 PBMC) respectively, p=0.006]. Group C and B patients showed higher HBV-specific release of IL-4 and IL-10 (Th2) than children in group E (IL-10 HBV-core: 144±89 and 50±12.5 vs. 15±5 spSFC/10^6 PBMC respectively, p=0.028). An inverse correlation between HBV core-specific IFN-γ production and HBV DNA viral load was detected (r=-0.28, p=0.016). Although levels of IP-10 did not differ significantly among the five groups, they tended to be higher in groups B and C than in group E (174±33 and 144±21 vs. 72±16 pg/ml, p=0.11). Conclusions: Efficient control of HBV replication is associated with strong Th1 and weak Th2 HBV-specific immune response and possibly with low IP-10 production.

Group	HBeAg	HBsAg	ALT	N	Stage
A	Positive	Positive	Normal	46	Immunotolerant
B	Positive	Positive	Elevated	24	Immunoactive
C	Negative	Positive	Normal	22	Low replication
D	Negative	Positive	Elevated	2	HBeAg negative hepatitis
E	Negative	Negative	Normal	6	Self-limited

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UNIQUE FULL LENGTH HEPATITIS B VIRUS GENOMES ISOLATED FROM CHILDREN WITH HEPATOCELLULAR CARCINOMA

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[Background & Aims]: The cause of early oncogenesis in hepatitis B virus (HBV) -related childhood hepatocellular carcinoma (HCC) remains unclear. This study is aimed at investigating whether the existence of possible unique HBV strains contributes to the early oncogenesis of childhood HCC. [Methods]: We investigated the full-length genomic sequences of HBV in the serum of 7 children with HCC and another 21 children with chronic HBV infection. HBV full-length sequences from 48 Asian adults with HCC and 16 Asian adults with chronic HBV infection, which were retrieved from GenBank, were used for com-

parison. Using phylogenetic tree construction and direct sequence comparison, we analyzed the distinct features of HBV genomes from children with HCC. [Results]: Phylogenetic analysis indicated that the majority (71.4%) of HBV from children with HCC contained recombinant sequences of both genotypes B and C and clustered in two very closely related clans that were clearly distinguishable from HBV genomes of patients (children and adults) with chronic HBV infection and adults with HCC. Sequence analysis identified a consensus 12-bp pre-S2 deletion in 57.1% of HBV genomes of children with HCC. Other characteristic mutations were also found throughout HBV genome. [Conclusion]: The majority of full-length sequences of HBV isolated from children with HCC belonged to recombinant strains of genotype B and C, which are clearly distinguishable from HBV genomes of patients with chronic HBV infection and adults with HCC. Those HBV strains from HCC chidlren contained unique mutations, particularly a short pre-S2 deletion, which may be associated with early HCC development.

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INTRAFAMILIAL TRANSMISSION OF HEPATITIS C VIRUS IN CHILDREN: CORRELATION WITH VIROLOGICAL AND IMMUNOLOGICAL PARAMETERS

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Background: Intrafamilial transmission of hepatitis C virus (HCV) infection has been reported, however the extent of its role and the factors associated with it are poorly understood. Objectives: To determine risk factors of transmission in children potentially exposed to HCV when one or both parents are infected. Patients and Methods: We examined 20 families (total of 93 subjects: 40 adults, 53 children) where one or both parents were HCV infected. Children living with infected parent/s were enrolled, a questionnaire inquiring about HCV risk factors was completed,and serum was screened for HCV antibodies (ELISA). Positive samples were confirmed by polymerase chain reaction and tested for genotype, HCV RNA viral load, HVR1 sequence analysis (nucleotide positions 1156 to 1234), HCV-specific CD4+ and cytotoxic CD8+T cell responses (ELISpot), natural killer cells, and cytokines. Generalized linear mixed effects models were used to analyze the data to account for correlation between children from the same family. Results: Fifteen children (28%) were HCV infected and none had risk factors other than contact with the infected parent/s. Boys were more likely to be infected than girls (Odds Ratio (OR)=2.3; 95% confidence interval (CI): 0.6, 8.3; p = 0.23). Genotype and nucleotide sequencing of the HVR1 region of HCV isolates infecting parents and children had > 95% homology. When both parents were HCV infected, 43% of children were infected compared to 9% when one parent was infected (OR = 8.0; 95% CI: 1.6, 40.6; p = 0.012). Among families with only one infected parent, children of infected mothers had 9.5 times greater odds of being infected than children of infected fathers (95% CI: 0.4, 217, p = 0.16). Although older parental age and higher maternal HCV viral load were associated with higher odds of child infection, only higher paternal viral load was significant (OR=1.02 per 10,000 copies, 95%CI:1.001, 1.04, p = 0.039). Those delivered vaginally had 2.5 higher odds of infection than those delivered via Cesarean section (95% CI: 0.3, 23.1, p = 0.41). Interestingly, 74% of children with no detectable viremia had HCV-specific CD4 + or CD8+ T cell responses of > 30 SFC x 106 (95% CI: 49%, 90%). Conclusion: Our data show that biparental HCV infection and high paternal HCV RNA levels were significantly associated with intrafamilial pediatric HCV infection. The observation that HCV-specific CD4+ and CD8+T-cell responses exist in HCV negative children may imply a protective potential for these responses and might have important implications for vaccine development and epidemiologic studies.

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HCV AUTOIMMUNITY IN A U.S. MULTI-CENTER COHORT OF TREATMENT NAïVE CHILDREN

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Background: Auto-antibodies are described frequently in adults with chronic HCV, with thyroid antibodies present in14% and autoimmune hepatitis antibodies in up to 90%. These antibodies have prevented some adults from undergoing or successfully completing treatment with interferon. Although children with HCV generally tolerate treatment better than adults and have a higher response rate, little is known about the frequency with which autoantibodies are present. Understanding this baseline feature may be important in developing recommendations for treatment of pediatric patients with chronic HCV. Aim: Our aim was to assess the incidence and describe the correlates of positive antibodies, thyroid peroxidase (TPO), anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA) and anti-Liver Kidney Microsomal antibody (LKM) in treatment naïve children (ages 5 to 18 years) with chronic HCV . Methods: From 12/2004 to 6/2006, 128 children were screened at entry to the multi-center, NIH funded Peds-C Network clinical trial. Labs were determined centrally and a single pathologist scored all biopsy slides. Assays for ANA, ASMA, ALKM, and TPO were measured at baseline. Results: Subjects had a mean age of 11.2 years (56% male), 77% had vertically acquired HCV, 15% unknown, 7% transfusion, less than 1% drug or sex. 82% were Caucasian, 6% African American, 9% Hispanic,

4% Asian. Genotype 1a was present in 60%, 1a/1b in 1%, 1b in 22%, Genotype 2a in 5%, 2bin 1%, Genotype 3b in 10%, Genotype 6a in1%. Mean ALT was 60.2 U/L. Liver histology demonstrated moderate inflammation in 38%/severe in 3%. Moderate fibrosis 5% / cirrhosis 2%. Of the 128 children screened, 18 (14%) had measurable auto-antibodies - 44% were ASMA positive (range 1:80-1:160), 33% ANA positive (range 1:80-1:320), 17% LKM positive (range 33.9-65.2 units, ULN20.1), and 6% TPO positive (108.8 IU/ml ULN2.1 IU/ml). Using Chi square and Fisher T, no significant correlates of antibody positivity were found with age, gender, race/ethnicity, ALT, mode of acquisition, genotype, degree of liver fibrosis or inflammation scores. A trend toward older age was found for ANA positivity, with the three oldest patients having the highest titers (ages 16, 16 and 17yr. ANA 1:320) Conclusion: This study demonstrates less frequent TPO, LKM, ASMA and ANA positivity in children with Chronic HCV compared to adults. We speculate that these autoantibodies may be of importance in the side-effect profile and treatment response in children treated with antivirals.

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TREATMENT OF HEPATITIS B USING INTRON A VS PEGINTERFERON IN COMBINATION WITH LAMIVUDINE IN CHILDREN

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Aim: To assess the safety, tolerability and efficacy of 2 combination therapy regimens using interferon (IFN) and lamivudine (LAM) to treat E antigen (Ag) positive pediatric hepatitis B (HBV) infected patients. Methods: 34 patients (age 2.5-6 years) at a single center were assessed over a 5 years. Baseline labs included liver profile (LFT), complete blood count (CBC), thyroid function test (TFT), HBV E Ag, HBV E antibody (Ab) and HBV DNA quantitative PCR. All patients had a liver biopsy with the intent to treat to grade and stage disease. Exclusion criteria included HIV co-infection, stage 4 disease, severe thrombocytopenia, thyroid disease, uncontrolled depression and history of non-compliance. Two treatment regimens were used. Group A: 16/34 patients received Intron A, 5 million unit/m2 SQ daily (max dose 10 million units) for 5 months with LAM (staggered by one month) 4mg/kg/day orally (max dose 100mg) until E Ag seroconversion or resistance. Group B: 6/34 patients received of Peginterferon Alfa 2b (Peg-IFN), 1.5mcg/kg/week for 24 weeks with LAM as above. Monthly assessments included vital signs, weights, LFT, CBC, TFT, HBV DNA, HBV E Ag, and HBV E Ab. Results: Twenty-four males and 10 females were assessed. Liver biopsies demonstrated Stage 0-3, Grade 0-3 disease. Group A demographics: 4 Asian, 2 Hispanic, 6 African American, 4 Caucasian with vertically transmitted disease in 13/16. At screening all had HBV DNA > 200 million copies, 6/16 had normal LFT. On therapy15/16 had undetectable HBV DNA with 8/16 achieving E Ag seroconversion at mean of 44 weeks (range 22-69). Safety data: none developed thyroid dysfunction, 2/16 patients had neutropenia (1 treated with IFN dose reduction, 1 treated with Neupogen), 1 patient required an antidepressant, 1 patient stopped IFN due to seizure, and 2/16 required nutritional supplements for weight loss. One subject developed LAM resistance. Group B demographics: 3 Asian, 2 Hispanic, 1 African American all with vertical transmission. At screening all had HBV DNA > 200 million copies and normal LFT. No patient cleared DNA on therapy and no patient achieved E Ag seroconversion. Two of 6 required nutritional supplements for weight loss and no other adverse effects occurred. Conclusions: Combination therapy with IFN and LAM in a staggered manner is safe and effective in pediatric patients with chronic HBV infection. Combination therapy with Peg-IFN and LAM at the dosing assessed is not equally efficacious and should not be used in pediatric patients until further clarification of dosage and frequency.

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HBEAG SEROCONVERSION CORRELATES WITH A HIGH NUMBER OF CORE GENE MUTATIONS AND WITH GENOTYPES B AND D IN PAEDIATRIC PATIENTS

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Background: The outcome of hepatitis B virus (HBV) infection results from the interaction between virus and host immune response. While effective immune recognition usually controls viral replication, escape mutations may arise from the immune system selective pressure, with consequent persistent infection. Aim: To investigate whether there is a relationship between presence of mutations within HBV core gene, HBV genotypes, HBV DNA viral load and HBeAg seroconversion in five different HBV infection settings. Patients: One hundred paediatric patients (median age 13.1 years, 54 males) were divided into 5 groups according to their HBeAg/HBsAg status and aminotransferases activity (Table 1). Methods: HBV DNA was isolated from serum samples. The presence of point mutations within HBV pre-core and core promoter regions and amino acid substitutions within immunodominant epitopes of HBV core gene was analysed by direct sequencing (mutations/patient). HBV genotypes were determined by direct sequencing. HBV DNA viral load was quantified by real-time PCR ($\log_{10}$ copies/ml). Results: A higher number of mutations within the HBV pre-core region and HBV core gene was detected in HBeAg negative patients than in those HBeAg positive (pre-core: 4.5±0.2 vs.2.6±0.2, p=0.001 and core: 5.5±0.4 vs. 4.1±0.3, p=0.04). Genotypes B and D were more prevalent in HBeAg negative children than in those HBeAg positive (80% vs. 58%, p<0.001). HBV-DNA viral load was significantly higher in groups A (mean ± SEM, 7.9±0.2), B (6.8±0.3), and D (7.5±1.2) compared to C (3.4±0.3) and E (1.0±0.4) (p=0.001). Among HBeAg negative children, those with genotypes B and D had a higher number of pre-core mutations and tended to have a lower number of core mutations than those with genotypes A, C and E (pre-core: 5.5±0.4 vs. 4.1±0.2, p=0.04; 4.7±0.3 vs. 5.7±0.3, p=0.1, respectively). Conclusions: Irrespective of genotype, emergence of HBV core gene mutations is associated to HBeAg seroconversion. In patients with genotypes B and D, that more frequently lose HBeAg, seroconversion is associated also to a high number of pre-core mutations. To what extent these mutations result from the selective pressure of the immune system or are virally determined remains to be clarified.

Group	HBeAg	HBsAg	ALT	N	Stage
A	Positive	Positive	Normal	46	Immunotolerant
B	Positive	Positive	Elevated	24	Immunoactive
C	Negative	Positive	Normal	22	Low replication
D	Negative	Positive	Elevated	2	HBeAg negative hepatitis
E	Negative	Negative	Normal	6	Self-limited

Disclosures:

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GRAFT HISTOLOGY UP TO 10 YEARS AFTER PEDIATRIC LIVER TRANSPLANTATION: HIGH INCIDENCE OF SEVERE FIBROSIS, CORRELATED WITH TRANSPLANT RELATED FACTORS

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Introduction: Previously we showed that 31% of liver grafts have portal fibrosis at 1 year after pediatric liver transplantation (LTx; Transplantation 2000; 6:236). The presence of fibrosis correlated positively with transplant related factors, including cold ischemia time (CIT) and biliary complications. Aim of the study: To assess progress and development of fibrosis in liver grafts in the long term after pediatric LTx and to identify possible risk factors. Methods: We reviewed protocol biopsies obtained at 5 and 10 yrs after LTx from the originally included 77 patients/grafts. We analyzed the relationships between histology and parameters of transplantation and follow up. Severity of fibrosis was evaluated using a three point scale: 0 = no fibrosis, 1 = portal fibrosis without bridging (mild fibrosis), 2 = portal fibrosis with occasional bridging (moderate fibrosis), 3 = diffuse portal bridging (severe fibrosis/cirrhosis). Results: At 5 and 10 yrs after LTx, 66/77 (85%) and 55/77 (71%) biopsies were available, resp. Missing biopsies at 10 yrs after LTx included 7 deaths and 6 retransplants. Between 1 yr and 5 yrs after LTx, the prevalence of fibrosis increased from 31% to 67%, but remained stable thereafter (10 yrs: 65%). However, the percentage of patients with severe fibrosis continued to increase, from 10% (5 yrs) to 27% (10 yrs). The presence of fibrosis at 1 yr tended to correlate with re-transplantation (p=0.054). The incidence of severe fibrosis after 10 yrs was significantly higher in patients who had fibrosis at 1 yr after LTx, compared with those without fibrosis at 1 yr (p= 0.004). At 5 years, ASAT and γGT values were significantly higher in patients with fibrosis than in those without fibrosis (ALAT 23 ± 13 vs. 36 ± 28 U/l; GGT 30 ± 52 vs. 112 ± 149 U/l; resp., each p <0.05). At 10 yrs after LTx, corresponding values were not significantly different. Sixty four percent (64%) of patients without fibrosis at 1 yr after LTx had developed some degree of fibrosis at 10 yrs (25/39). The development of fibrosis starting after 1 yr was strongly related to CIT, donor/recipient ratio and type of preservation fluid (these factors were also strongly interrelated). The CIT of the grafts that developed late fibrosis was longer than that of grafts without fibrosis at 10 yr (11.4 ± 3.9 h vs. 7.2 ± 3.8 h; p = 0.004). Conclusion: At 5 and 10 yrs after pediatric LTx, the majority of grafts have fibrosis in protocol biopsies. At least in 25% of the grafts, fibrosis progresses to cirrhosis in 10 yrs after pediatric LTx. Even fibrosis that only develops after the first year post LTx is strongly correlated with transplant related factors.

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IMPROVED PREDICTIVE VALUE OF PELD WITH THE ADDITION OF SERUM SODIUM AND PLATELET COUNT

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Aim: To re-evaluate the prognostic value of the Paediatric End-stage Liver Disease (PELD) score with the inclusion of parameters that indicate portal hypertension reflecting on platelet count (Plt), aspartate aminotransferase (AST) to Platelet Ratio Index (APRI), spleen size and haemodynamic decompensation [serum sodium (Na)] at listing and at time of transplantation (LT). Methods: Medical records of 85 patients (38 male) with biliary atresia waiting for liver transplantation from 2001-2006, were reviewed retrospectively. 3 patients died and 5 patients were admitted in PICU requiring ventilation prior to transplantation. Median age at transplantation was 1 year [range, 0.39-13]. PELD score and above mentioned parameters were recorded at the time of listing and at the time immediately before liver transplantation. A multivariate and a univariate Cox regression analysis were performed for the endpoints death/PICU admission whilst on the list. Three models were evaluated for their predictive power for both endpoints. The area under the receiver operating characteristic curve (AUC ROC) was used to compare models. Results: The median time between listing and LT was 84 [range, 13-682] days. The median PELD score at the time of listing was 15 [range, –10 to 36], which significantly increased to 17 [range, –5 to 42] just before LT (p<0.001). At the time of listing the median values were for sodium 136mmol/L [range, 128-144], for platelet count 160x10\S\9/l [range, 29-577], and for APRI 2.7 [range, 0-27.84]. Paired statistics between existing PELD and our variables did not improve its prediction of endpoints. In a multivariate Cox regression models PELD+Plt, PELD+Na and PELD+Plt+Na (King's PELD) all variables had a significant association (P<0.7). The regression coefficients of predictors were used as multiplicative factors to calculate the new severity scores. A univariate logistic regression analysis for death was performed (King's PELD: odds ratio 2.72, 95% CI 1.01-7.32; p 0.05).The ROC analysis that was done to the King's PELD showed that the best cut-off point to dichotomise was 48.84 to predict death (odds ratio 6.73, 95%CI 1.63-27.84; p 0.01). The new scoring system is: -0.42*Age(<1yr)-0.55*Bili+2.34*INR-1.78*Alb+0.46*Growth+0.47*Plt+11.09*Na The median King's PELD at the time of listing was 48 [range, 46-50] and prior to transplantation was 46 [range, 44-48]. Conclusions: The PELD does not reflect the severity of disease in our cohort. The new King's PELD is a better predictor of endpoints on the waiting list. Further prospective analysis is in progress.

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