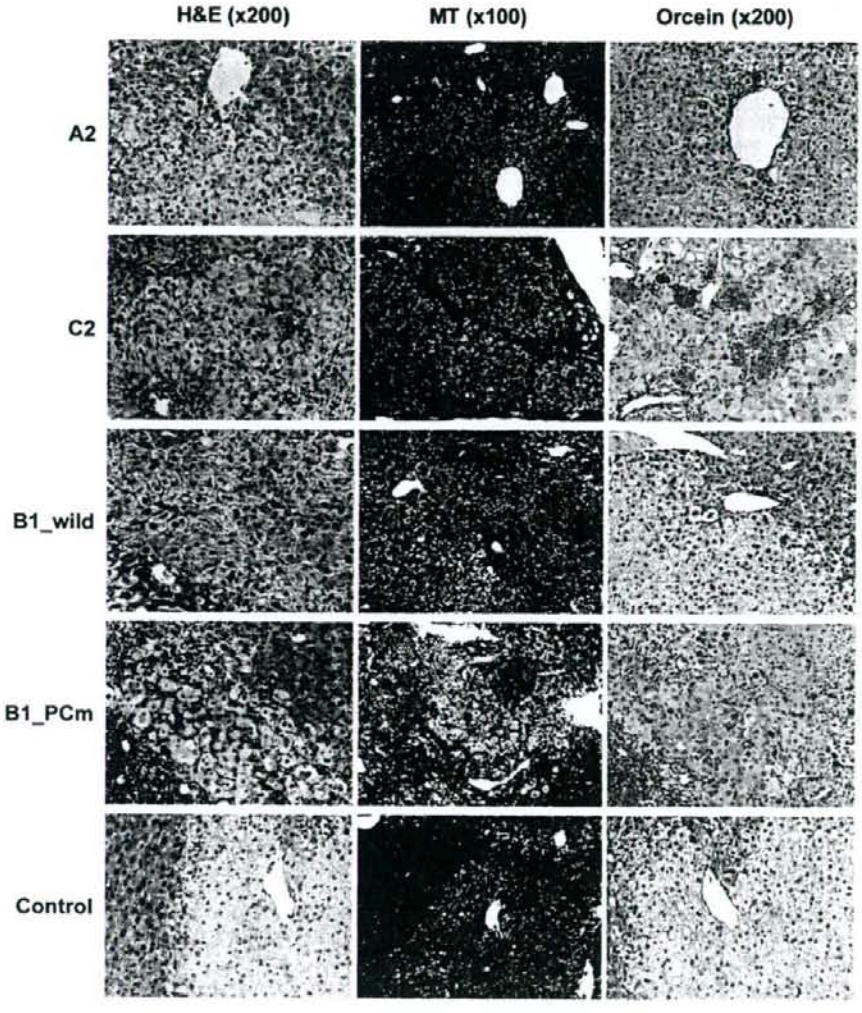

Figure 3. Immunohistochemical analysis of liver tissue. Comparison of liver histology in mice long term (25 weeks) infected with HBV/A2, C2, B1_wild, B1_PCm, and noninfected control. Liver sections stained with H&E, MT, or orcein are shown. After deparaffinization, tissue slides were stained according to each method. Representative staining of C2 and B1_PCm showed a ground-glass appearance, fibrosis, and cytoplasmic positivity of human hepatocytes by orcein staining (brown), whereas these were absent in A2, B1_wild, and control mice. Original magnifications: H&E and orcein, 200×; MT, 100×.

expression of HBV DNA and antigens. This has allowed for an assessment of the direct cytopathic potential of different HBV genotypes (ie, particular subgenotypes) to be investigated without the host-related bias, under conditions of the absence of immune pressure. In addition, this may represent a novel mouse model for human liver fibrosis associated with ROS production leading to the activity of TGF-β by viral infection but not chemical trigger. The study thereby has shown that infection with HBV/C2 in contrast to HBV/A2 or B1_wild has induced an abundant ground-glass appearance of the human hepatocytes along with an increased fibrosis in the humanized liver of the chimeric mice in an immunosuppressive condition. A strong staining of α-SMA observed in broad areas of fibrosis indicated activation of HSCs in cases of HBV/C2 and B1_PCm, but not in A2 and B1_wild. In the chronic state, ground-glass hepatocytes could play a critical role in HSC activation. In connection with this study, we have evaluated the liver damages in chimeric mice killed at 3 months postinfection (early phase dynamics). The viral dynamics and ROS production of HBV/C2 or B1_PCm evaluated in the early phase indicated levels of alterations similar to those observed after long-term infection (Supplementary Figure 2; see Supplementary material online at www.gastrojournal.org). Fibrosis stage and orcein staining levels (ground-glass appearance), however, were expressed in lesser levels than in the long-term infected mice, suggesting that the liver damage can be detected even in the early stage of the infection, but its level correlates with the duration of exposure to oxidative stress.

Our previous report showed that the intracellular virion retention and endoplasmic reticulum stress were the highest for HBV/C2.[21] Our data obtained in vitro and

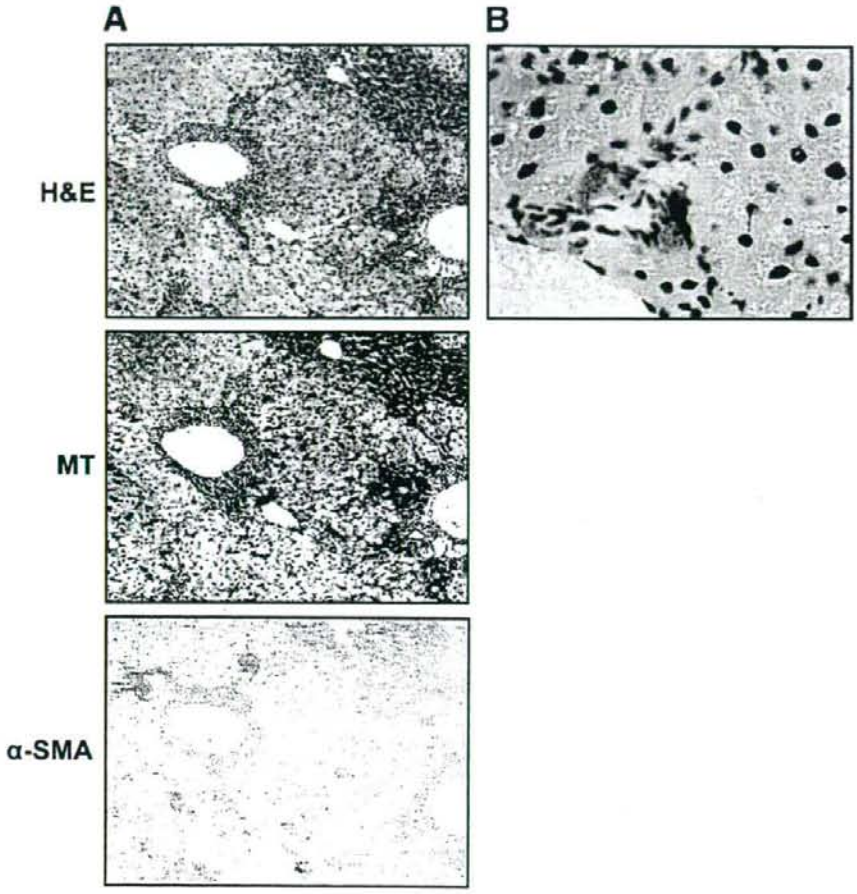

Figure 4. Confirmation of liver fibrosis by immunostaining using anti-α-SMA antibody. (*A*) Liver sections stained with H&E, MT, or immunostaining using anti-α-SMA antibody (as described in the Materials and Methods section). (*B*) Nuclei stained *brown* with the antibodies indicate human origin, and α-SMA is stained in *red*, located in the cytoplasm without a stained nucleus. Shown are representative staining of images expressing fibrosis. Original magnification, 200×.

in vivo may explain in part previous results accumulated from clinical studies indicating that HCC more often was associated with HBV/C and the mean age of patients with HCC is younger in the HBV/C-infected group compared with the HBV/B1-infected group.[28,29] On the other hand, the low replicative capacity and hepatic injury of HBV/A2 may contribute to the ability of the subgenotype to evade the immune response and chronically persist in up to 10% of acutely infected adults (which is exceptionally rarely observed with HBV/C or HBV/B).[11,30–32] High levels of HBsAg secretion for HBV/A2 are in contrast with its low replicative activity, and this may be an important mechanism for the immune escape. However, some cautions must be exercised when extrapolating the results of in vivo models to patients because immune responses are not taken into account.

The hepatic injury during acute and chronic HBV infection genuinely is considered to be caused by the host's immune response against the infected hepatocytes. However, in some immunosuppressed chronic HBV patients, high viremia and liver fibrosis may occur.[11,33] Previous reports have shown that HBV genotypes E or G cause intracellular changes and hepatocellular damage in human hepatocytes in severe combined immunodeficient mice transgenic for urokinase-type plasminogen activator.[2,3] We showed here that activation of oxidative stress led to TGF-β1 production in chimeric mice as reported in previous studies.[26] Accumulation of oxidative damage, 8-OHdG, might enhance the possibility of carcinogenesis as observed in HCC patients. These findings suggest that hepatic injuries could arise in the absence of a mature immune system and the difference of genotype would affect the cytopathic potential of the virus.

Chimeric mice were infected with HBV recovered from serum or culture medium containing virion from Huh7 cells transfected with HBV construct.[2,20,21,36,37] In our previous study, by using a single clone corresponding to HBV/A or C, we showed 2 logs difference during weeks 4–7 in the serum levels of HBV DNA between the cohort of mice inoculated with HBV/C and HBV/A.[21] In the present study, we extended the examination of the geno-

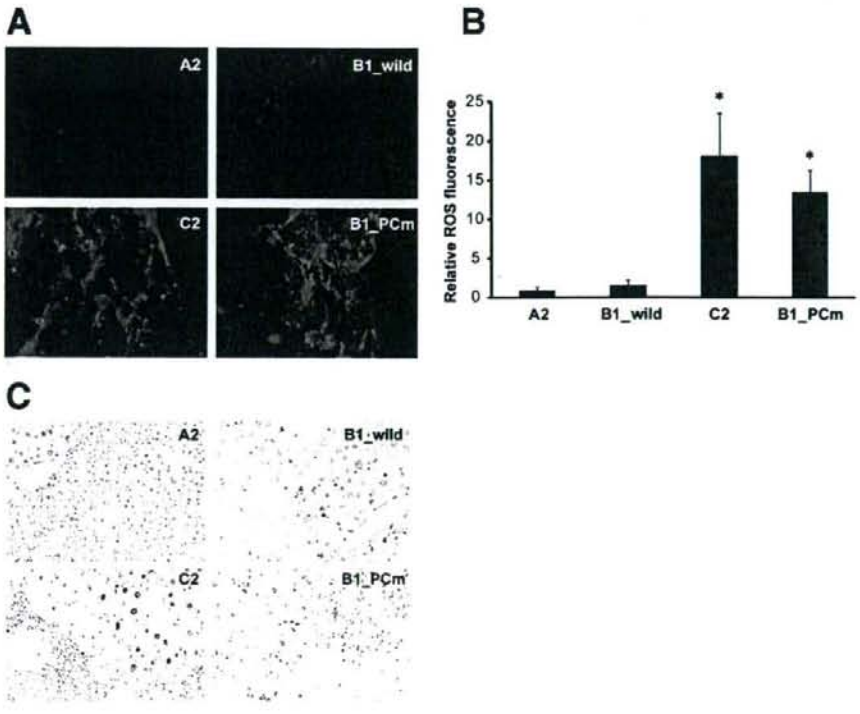

Figure 5. Differences in production of oxidative damage among HBV genotypes. (*A*) Frozen liver sections of mice inoculated with different HBV genotypes were stained by dihydroethidium. Fluorescence was detected with a laser scanning microscope. (*B*) Fluorescence intensities in randomly selected areas of digital images were quantified by National Institutes of Health image analysis software. *$P < .01$: A2 or B1_wild vs C2 or B1_PCm. (*C*) Oxidative damages in liver tissue were evaluated by staining of 8-OHdG–positive nuclei. Original magnifications, 200×.

type differences by using 3 clones, representative of each genotype. The results of the present study in concordance with our previous study showed that the replication efficiency of HBV/C is significantly higher than that of HBV/A, as was indicated by 2 logs difference during weeks 6–8 in the levels of HBV DNA detected in murine sera ($P < .05$). The ability of HBV/A to express more HBsAg, and that of HBV/C to produce more HBcrAg revealed in our previous in vitro study,[21] were both thereby confirmed by the present in vivo replication model using the chimeric mice.

Previous clinical observations on HBV/B1[11,28] prompted a deeper investigation on the impact of the PC mutation on the virologic characteristics of the genotype. The unique characteristic of HBV/B1_wild stood out among genotypes harboring no major mutations. The HBV/B1_wild group revealed low replication efficiency with window periods and low antigen expression. The lower replicative activity and hepatic injuries of HBV/A2 and B1_wild may partially explain why carriers with either HBV/A2 or HBV/B1 often are asymptomatic in contrast to those with HBV/C infection.[26,28,30] In our study, the PC mutation was the only difference between HBV/B1_PCm and HBV/B1_wild clone, and the former showed higher replication efficiency and severe damage in liver tissue. The antigen levels of the HBV/B1_PCm increased rapidly and decreased earlier than those of the HBV/A2 or C2 clone, whereas HBV/B1_wild showed that the concentrations of HBV antigens remained low for several months postinfection. These particular characters were observed for the HBV/B1_PCm group inoculated with sera from both preinfected mice and patients with fulminant hepatitis. The majority of patients with fulminant hepatitis and fatal acute exacerbation have been found to have the G1896A mutation.[11,40,41] A greater incidence of fulminant hepatitis might be associated with the high replication and protein production in the early phase, as was shown on the HBV/B1_PCm clone in this study. The defect of immunologic tolerance as a result of the absence of HBeAg may play an important role in the fulminant course of precore mutation in HBV infection.[42] This would concur with a previous report by Bocharov et al which proposed that enhanced HBV replication would efficiently stimulate immune responses, represented by the cytotoxic T-lymphocyte response,[43] suggesting that enhanced replication by HBV/B1 with G1896A mutation might lead to an extremely high cytotoxic T-lymphocyte response, resulting in fulminant hepatitis. But in this study, HBV/B1_PCm showed similar responses to HBV/C2 infection because chimeric mice did not have an immune system that was strong enough to invite strong cytotoxic T-lymphocyte response against viral infection. To uncover these unique characteristics of PC mutant, further study would be needed by using the infection model but not gene transfer.

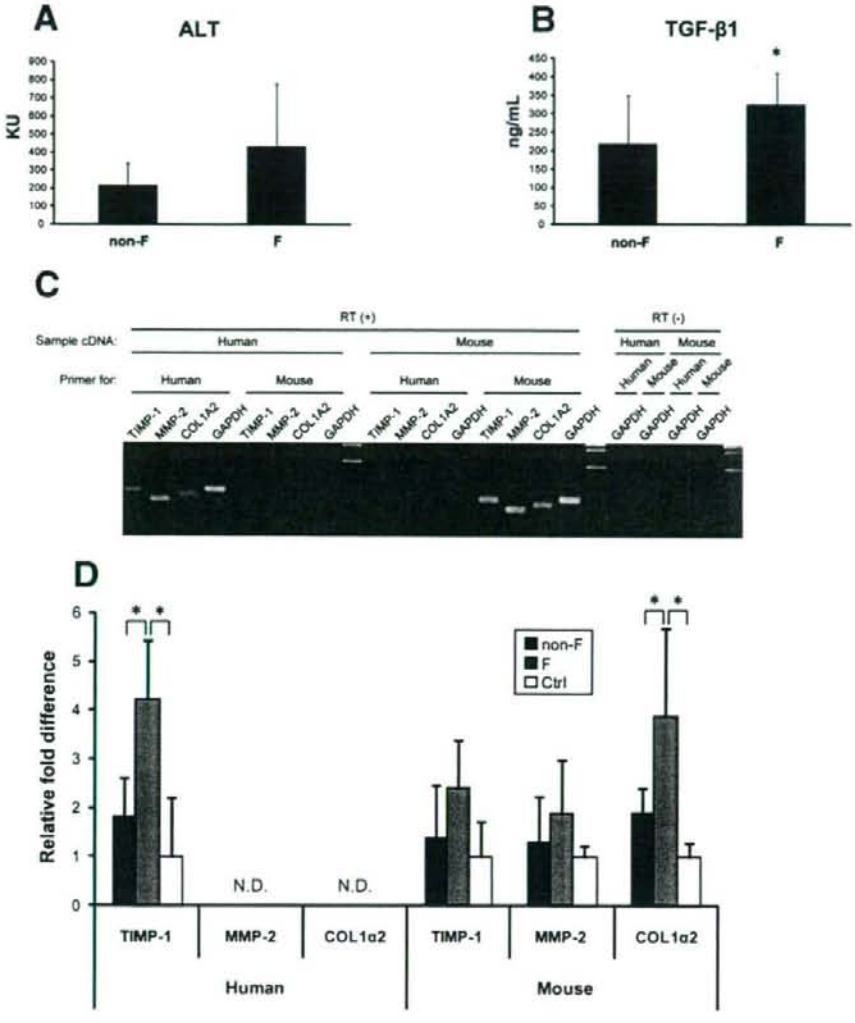

Figure 6. Differences in the expression levels of fibrosis-related genes among HBV genotypes. Quantification of (*A*) ALT and (*B*) TGF-β1 levels in mouse sera with enzyme-linked immunosorbent assay (see Supplementary Materials and Methods section. non-F, no fibrosis group (A2 and B1_wild); F, fibrosis group (C2 and B1_PCm). *$P < .01$: non-F vs F. (*C*) The specificity of each PCR using species-specific primer sets. The species-specific primer sets were established to determine whether mRNA of fibrosis-related genes were of human or mouse origin. Liver tissue of a HCC patient or a mouse without transplantation of human hepatocytes was used to check the primer sets for real-time detection PCR. The PCR products were run on 2% agarose gels to confirm the molecular sizes as well as species-specific amplifications. (*D*) Quantification of mRNA expression on fibrosis-related genes in each group by real-time reverse transcription PCR. non-F group, n = 15; F group, n = 22; control, n = 8; ND, not detected; *$P < .001$.

Finally, the discrepancy between in vitro[21] and in vivo (present study) observations on HBV/B1_wild might have been caused by differences in the cells used for transfection (Huh7 cells) and infection (human hepatocytes from Caucasoid donors), respectively. Nonrecombinant type HBV/B strains (B1 and B6) have been detected in limited areas including Japan[11] and Alaska,[15] which were settled mainly by Mongoloid people. The existence of a window period on HBV/B1 might indicate a possibility that a receptor or co-receptor used by HBV/B1 is not equal to one adopted by other genotypes as shown in the human herpes virus.[16] Further studies using human hepatocytes from Mongoloid people would be required.

In conclusion, using an in vivo experimental system, we show that different HBV genotypes and even particular mutations are associated with different virologic and histopathologic characteristics. Infection with HBV/C2 as well as PC mutant of the HBV/B1 in immunosuppressive conditions can induce a direct cytopathic effect in the humanized part of the murine liver. This mouse model appears to be useful in the evaluation and prediction of pathogenic effects of various genotypes of HBV and certain HBV mutations.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2008.10.048.

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Received April 20, 2008. Accepted October 23, 2008.

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The authors disclose the following: Supported by a grant-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology, and a grant-in-aid from the Ministry of Health, Labour, and Welfare of Japan, the Toyoaki Foundation.

The authors thank Drs C. Tateno, H. Yokomichi, K. Kuramoto, and T. Nakamura of PhoenixBio Co, Ltd for providing chimeric mice with a high replacement for hepatocytes; Dr T. Wakita of the National Institute of Infectious Diseases, Tokyo, Japan for quantifying the alanine aminotransferase level; Dr Ikehara of the National Institute of Advanced Industrial Science and Technology for the differential diagnosis of neutrophil/monocyte in liver tissue; Dr S. Nishina of Yamaguchi University Graduate School of Medicine for assistance with histological reactive oxygen species evaluation; Ms K. Tatematsu of Nagoya City University Graduate School of Medical Sciences for performing sequencing; and Mr S. Sato and Ms Y. Tanizaki of Nagoya City University Hospital for slicing liver tissues of chimeric mice.

The nucleotide sequences of HBV-DNA isolates used in this study have been deposited in the international DNA database under the following accession numbers: AB246337, AB246338, AB246341, AB246342, AB246344, AB246345, and AB362931–362933.

Supplementary Data

Materials and Methods

Plasmid Constructs of HBV DNA and Sequencing

The 1.24-fold HBV genomic constructs used in the present study were prepared as described previously.[1] The constructs were designed to transcribe oversized pregenome and precore mRNA. Table 1 shows the list of 12 plasmids used in this study. Nine wild-type clones were used including 3 HBV/A (Ae/A2), 3 HBV/B (Bj/B1), and 3 HBV/C (Ce/C2). An additional 3 HBV/B plasmids identical to the earlier-mentioned HBV/B clone were constructed with precore stop-codon (PC) mutation (G1896A), which abolishes HBeAg expression. Briefly, for site-directed mutagenesis, the wild-type clone was digested by *Hind*III and *Eco*O65I and ligated with the fragment carrying the PC mutation (G1896A). Cloned HBV-DNA sequences were confirmed with Prism Big Dye (Applied Biosystems, Foster City, CA) in the ABI 3100 automated sequencer. Furthermore, the HBV DNA spanning the complete genome were amplified from murine sera and cloned into the pGEM-T Easy Vector (Applied Biosystems) with followed sequencing.

Cell Culture and Transfection

Huh7 cells were transfected with plasmids equivalent to 5 μg of HBV-DNA constructs with use of the Fugene 6 transfection reagent (Roche Diagnostics, Indianapolis, IN), and harvested after 3 days in culture. Transfection efficiency was monitored by cotransfecting 0.5 μg of reporter plasmids expressing secreted alkaline phosphatase in the culture media.

Determination of HBV Markers

HBsAg and HBeAg were determined by chemiluminescent enzyme immunoassay using commercial kits (Fujirebio Inc, Tokyo, Japan). HBcrAgs, which included both HBeAg and HBcAg, were measured in serum using the chemiluminescent enzyme immunoassay as described previously.[2,3] HBcAg was measured by enzyme-linked immunosorbent assay as previously reported.[2]

Detection and Quantification of Serum HBV DNA

HBV-DNA sequences spanning the S gene were amplified by real-time detection PCR by the method of Abe et al.[1] The detection threshold of the method is 100 copies/mL (equivalent to 20 IU/mL). However, because of the small volume of the serum available from each mouse for the HBV-DNA quantification, 10-fold template dilution was used, which resulted in a higher detection threshold of the method in this study: 1000 copies/mL (200 IU/mL). Quantification standards used in the assay were prepared based on World Health Organization standard serum containing HBV genotype A (kindly provided by Dr Hiroshi Yoshizawa of Hiroshima University). The amplification and detection were performed in the ABI Prism 7700 Sequence Detection System (Applied Biosystems) according to the protocol.

Detection of 8-OHdG in Liver Tissue

The slides obtained from frozen tissues for 8-OHdG determination were placed in Bouin's fixative overnight at room temperature, and washed in water for 20 minutes. Tissues were incubated with 0.3% H_2O_2 in methanol for 30 minutes and rinsed in phosphate-buffered saline (PBS) buffer. The slides were placed in 0.05 N NaOH in 40% ethanol for 12 minutes, rinsed in PBS, and incubated with 250 μg/mL ribonuclease for 1 hour. An avidin/biotin block (Vector Laboratories) was applied for 20 minutes, and super block and mouse-to-mouse blocking reagent (ScyTek Laboratories, Logan, UT) were used to eliminate background staining caused by endogenous mouse immunoglobulin (Ig)G. The primary 8-OHdG antibody (Japan Institute for the Control of Aging, Shizuoka, Japan) then was applied to the slides overnight at 4°C (20 μg/mL, 1:100). To detect positive cells binding primary antibody, these slides were treated with Vectastain Elite ABC kit (Vector Laboratories).

Quantification of TGF-β1 and ALT Levels in Sera

Serum TGF-β1 and ALT levels were determined by using commercially available enzyme-linked immunoassay kits (Bender MedSystems GmbH, Vienna, Austria; and Nissui Pharmaceutical Co, LTD, Tokyo, Japan) according to the manufacturer's instructions, respectively.

Quantification of Gene Expression Levels of Fibrosis Markers

Fresh liver tissues (n = 45) from killed mice were used for quantification of fibrosis markers. Total RNAs were isolated using the RNeasy Mini Kit, and DNA contamination of samples was eliminated using the RNase-free DNase Set (Qiagen, Hilden, Germany), according to the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized in reaction mixtures with SuperScript II RNase H^- Reverse Transcriptase kit (Invitrogen), adding 0.5 μg oligo$(dT)_{12\text{-}18}$ primer at 70°C for 10 minutes. Reaction mixtures were incubated sequentially at 42°C for 60 minutes, at 95°C for 5 minutes, and at 60°C for 5 minutes. To check DNA contamination of samples, PCR was performed using isolated samples without reverse transcriptase. Primer sets to detect species-specific cDNA were designed using Primer Express software (Applied Biosystems) and are shown in Supplementary Table 1. Equal aliquots (1 μL) of cDNA were amplified by real-time detection PCR according to the manufacturer's Power SYBR Green PCR Master Mix instructions (Applied Biosystems) using the ABI Prism 7700 Sequence Detection System (Applied

Biosystems) in triplicate. The PCR conditions were as follows: (1) stage 1, 50°C for 2 minutes; (2) stage 2, 95°C for 10 minutes; and (3) stage 3, 95°C for 15 seconds followed by amplification at 60°C for 1 minute. Stage 3 was repeated for 40 cycles. Specificity of the amplification products was confirmed by examination of dissociation reaction plots, and a distinct single peak indicated a single DNA sequence amplified by the real-time detection PCR. The PCR products were run on 2% agarose gels to confirm the molecular sizes as well as species-specific amplifications (Figure 6C). Data were analyzed by the 2 [-Delta Delta C(t)] method using Sequence Detector version 1.7 software (Applied Biosystems),[5] and were normalized using human or mouse-specific glyceraldehyde-3-phosphate dehydrogenase. A standard curve was prepared by serial 10-fold dilutions of human or mouse cDNA. The curve was linear over 7 logs with a 0.998 correlation coefficient.

Immunofluorescence Immunofluorescence was performed as previously reported.[1] Briefly, fresh-frozen specimens were cut at 5–6 μm by cryostat, and fixed in acetone at room temperature for 10 minutes. Liver sections were blocked with Antibody Diluent (Dako, Glostrup, Denmark), incubated with rabbit anti-HBc antibody (Dako) at room temperature for 1 hour, and then incubated with goat anti-rabbit IgG antibody conjugated with Cy3 (Chemicon) or goat anti-human albumin antibody labeled with FITC (Bethyl Laboratories Inc, Montgomery, TX). Sections were observed in a fluorescent microscopy (Eclipse E800M; Nikon, Tokyo, Japan).

Statistical Analysis

Group means were compared by an independent Student *t* test or 1-way analysis of variance.

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Supplementary Table 1. Sequence of Species-Specific Primers on Fibrosis-Related Genes

Primer	Sequence
hTIMP1/F1	5'-ATGGCCCCCTTTGAGCCC-3'
hTIMP1/R1	5'-GTCTGGTTGACTTCTGGTGTC-3'
mTIMP1/F1	5'-ATGGCCCCCTTTGCATCT-3'
mTIMP1/R1	5'-GTCTCGTTGATTTCTGGGGAA-3'
hMMP2/F1	5'-CCTTCTTGTTCAATGGCAA-3'
hMMP2/R1	5'-GGACAGAAGCCGTACTTGC-3'
mMMP2/F1	5'-CCTTCCTGTTCAACGGTCG-3'
mMMP2/R1	5'-GGGCAGAAGCCATACTTGC-3'
hCOL1α2/F1	5'-AGGAAATGGCTACCCAACTT-3'
hCOL1α2/R1	5'-TTAGAGCCCTGTAGAATG-3'
mCOL1α2/F1	5'-AGGAAATGGCAACTCAGCTC-3'
mCOL1α2/R1	5'-TTGGAACCCTGCAGAAGC-3'
hGAPDH/F2	5'-CACCAGGGCTGCTTTTAACTC-3'
hGAPDH/R2	5'-AGATGGTGATGGGATTTCCA-3'
mGAPDH/F2	5'-CACCAGGGCTGCCATTTGCAG-3'
mGAPDH/R2	5'-AGATGGTGATGGGCTTCCCG-3'

COL1α2, collagen type 1α2; F, sense primer; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; h, human specific; m, mouse specific; MMP2, matrix metalloproteinase 2; R, antisense primer; TIMP1, tissue inhibitor of metalloproteinase 1.

Journal of Medical Virology 80:1880–1884 (2008)

Change of Hepatitis B Virus Genotypes in Acute and Chronic Infections in Japan

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During 35 years from 1971 to 2005, 153 patients with acute and 4,277 with chronic HBV infection visited the Toranomon Hospital in Tokyo, Japan. They were grouped into seven 5-year periods, and HBV genotypes/subgenotypes were determined. Patients with acute HBV infection were younger ($P = 0.046$), predominantly male ($P = 0.004$), possessed higher alanine aminotransferase levels ($P < 0.001$), positive more frequently for HBeAg ($P < 0.001$), and had lower HBV DNA loads ($P = 0.014$) than those with chronic infection. Sexual transmission was more frequent in patients with acute than chronic HBV infection (67% vs. 3%, $P < 0.001$). The number of patients with acute infection increased throughout 1971–2005. Patients with chronic infection increased since 1971, peaked in 1986–1990 and then decreased. The number of patients increased since 1990–2000 again, however, reflecting recent boost of acute HBV infection. The distribution of HBV genotypes was considerably different between patients with acute and chronic infections (A, B, and C: 28.6%, 10.3%, and 59.5% vs. 3.0%, 12.3%, and 84.5%, respectively, $P < 0.001$). Since 1991, genotype A foreign to Japan started to increase sharply in patients with acute infection, and gradually in those with chronic infection. There was a trend for the foreign subgenotype B2/Ba to increase recently ($P < 0.05$). Despite immunoprophylaxis of high-risk babies born to carrier mothers with hepatitis B e antigen, implemented nationally since 1986, acute and chronic infections with HBV have been increasing in Japan. Based on genotypes/subgenotypes changing with time, the resurgence of hepatitis B could be attributed to infections, with foreign HBV genotypes/subgenotypes, spreading swiftly by sexual contact. ***J. Med. Virol. 80: 1880–1884, 2008.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: acute hepatitis; chronic hepatitis; genotypes; hepatitis B virus: subgenotypes

INTRODUCTION

Worldwide, an estimated 350 million people are infected persistently with hepatitis B virus (HBV), and approximately a third develop serious liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC) during the lifetime [Lee, 1997]. Universal vaccination of newborns has been implemented successfully in Taiwan [Ni et al., 2001], the United States [MMWR, 2002] and elsewhere. Catch-up vaccination is extended to children and adults for preventing HBV infection further.

Japan is unique in that, since 1986, passive and active immunopropylaxis has been performed annually on some 4,000 babies born to mothers who are infected with HBV and have hepatitis B e antigen (HBeAg) in the serum [Koyama et al., 2003; Noto et al., 2003]. This policy is based on a high risk of such babies to develop persistent HBV infection, in contrast to a low risk of babies born to carrier mothers with antibody to HBeAg (anti-HBe) [Okada et al., 1976]. As a result, the prevalence of HBV infection has been decreasing in Japan during past decades, which is reflected in the age-specific frequency of hepatitis B surface antigen (HBsAg) among first-time blood donors. The prevalence of HBsAg is low in blood donors born after 1981 at 0.23%, in remarkable contrast to 1.5% in those born between 1941 and 1950 [Tanaka et al., 2004].

The high incidence of HBV infection in men aged 20 years or older has been noted in the United States since 1999 [MMWR, 2004], forecasting continued new

Grant sponsor: Ministry of Health, Labour and Welfare of Japan.

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Accepted 17 July 2008
DOI 10.1002/jmv.21309
Published online in Wiley InterScience (www.interscience.wiley.com)

infections in the next two decades. There is a possibility that Japan, as well as any country in the world, will suffer from resurgent HBV infection that might be inapparent in the general population. During 35 years from 1971 to 2005, a city hospital in the Metropolitan Tokyo was visited by 4,430 patients infected with HBV. Patients with acute and chronic infections increased since 1996, thereby indicating that HBV infection has not been controlled efficiently in Japan.

MATERIALS AND METHODS

Patients

During 35 years from 1971 through 2005, 4,430 patients with HBV infection visited the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo, including 153 with acute and 4,277 with chronic HBV infection. Genotypes were A in 158 (3.6%) patients, B in 521 (11.8%), C in 3,564 (80.5%), D in 7 (0.2%), F in 3 (0.06%), H in 2 (0.04%) and not typeable in the remaining 175 (3.9%) patients. The median age of the patients was 37 years (range: 0.1–83) at the presentation, and included 3,210 (72.1%) men. Acute infection was diagnosed by high-titered antibody to hepatitis B core antigen of the IgM class and/or the development of HBsAg in previously seronegative individuals. Chronic hepatitis was diagnosed by liver biopsy carried out by laparoscopy and/or ultrasonic images, and liver cirrhosis by liver biopsy and/or ultrasonographic images plus laparoscopic findings. The number of patients with acute and chronic hepatitis B changed through 35 years, and the genotypes/subgenotypes were surveyed for predicting future trends of HBV infection in Japan. The study design conformed to the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of the institution. Every patient gave an informed consent for this study.

Markers of HBV Infection

HBsAg and the corresponding antibody (anti-HBs) were determined by hemagglutination (MyCell, Institute of Immunology Co., Ltd., Tokyo, Japan), and HBeAg by enzyme-linked immunosorbent assay (F-HBe, Sysmex, Kobe, Japan). HBV DNA was determined by the polymerase chain reaction (PCR) followed by hybridization (Amplicor HBV Monitor, Roche Molecular Systems, Inc., Branchburg, NJ) and the results were expressed in log copies/ml over a range from 2.6 to 7.6. HBV genotypes (A–H) were determined by enzyme-linked immunosorbent assay (HBV GENOTYPE EIA, Institute of Immunology) [Usuda et al., 1999, 2000] and PCR-Invader assay with genotype-specific probes [Tadokoro et al., 2006]. Subgenotypes of A, B and C were determined by sequence analysis, restriction fragment length polymorphism [Sugauchi et al., 2004a, 2004b; Tanaka et al., 2005] and PCR-Invader assay [Tadokoro et al., 2006].

Statistical Analysis

Frequencies were compared between groups by the Chi-squared test and Fisher's exact test, and medians by the Mann–Whitney's *U*-test. Analysis of data was conducted with the computer program SPSS ver.11.0 (SPSS Inc., Chicago, IL). The trend of subgenotypes B1/Bj and B2/Ba was analyzed by the Cochran-Armitage trend test with SAS version 9.1.3 software (SAS Institute, Inc., Cary, NC). A P value less than 0.05 was considered significant.

RESULTS

Patients With HBV Infection During 35 Years (1971–2005)

During 35 years from 1971 through 2005, the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo was visited by 4,430 patients infected with HBV, including 153 with acute and 4,277 with chronic infection. Table I compares the demographic, clinical and virological characteristics between the patients with acute and chronic HBV infection at the baseline. Patients with acute HBV infection were younger ($P = 0.046$), predominantly male ($P = 0.004$), had higher alanine aminotransferase levels ($P < 0.001$),

TABLE I. Baseline Characteristics of Patients Infected With HBV Who Visited Toranomon Hospital During 35 Years (1971–2005)

Features[a]	Acute infection (n = 153)	Chronic infection (n = 4,277)	Differences (P value)
Age in years	34 (19–69)	38 (0.1–83)	0.046
≤39	99 (65%)	2,358 (55%)	
40–59	49 (32%)	1,642 (38%)	
≥60	5 (3%)	277 (7%)	
Men	125 (82%)	3,067 (72%)	0.004
ALT (IU/L)	1,460 (19–6,876)	58 (12–3,520)	<0.001
Sexual transmission	102 (67%)	129 (3%)	<0.001
Liver disease			
Symptom-free	0	1,035 (24%)	
Chronic hepatitis	0	2,617 (61%)	
Cirrhosis	0	405 (10%)	
Hepatocellular carcinoma	0	220 (5%)	
HBeAg	100 (65%)	2,131 (50%)	<0.001
HBV DNA (log copies/ml)	5.9 (<2.6 to >7.6)	6.4 (<2.6 to >7.6)	0.014

[a]Data are expressed in number of patients with percentage in parentheses or the median value with a range in parentheses.

were positive more frequently for HBeAg ($P < 0.001$), and had lower HBV DNA loads ($P = 0.014$) than those with chronic infection. Sexual transmission was more frequent in patients with acute than chronic HBV infection (67% vs. 3%, $P < 0.001$).

The number of new patients presenting with acute and chronic HBV infections during a 5-year period was compared during 1971 through 2005 (Fig. 1). In the initial four 5-year periods (1971–1990), both patients with acute and chronic HBV infections increased linearly. In the fifth 5-year period (1991–1995), however, patients with acute or chronic HBV infection decreased to less than those in the previous 5-year period (1986–1990). In the next 5-year period (1996–2000), nevertheless, patients with acute HBV infection began to increase while a decrease in chronic HBV infection was observed. In the seventh 5-year period (2001–2005), patients with acute HBV infection kept increasing. In addition, there was a small but appreciable increase of patients with chronic HBV infection in comparison with the previous 5-year period (1996–2000). Taken altogether, acute HBV infection resurged since 1991 accompanied by an increase in chronic HBV infection since 2001.

HBV Genotypes in Patients Infected With HBV

HBV was typeable in 126 of the 153 (82.4%) patients with acute and 4,121 of the 4,277 (96.4%) with chronic HBV infection (Table II). Genotype A, foreign to Japan, was more frequent in acute than chronic HBV infection (28.6% vs. 3.0%, $P < 0.001$). There were no differences in the distribution of endemic genotypes B and C; combined, they accounted for 69.8% and 96.8%, respectively, in patients with acute and chronic HBV infections. Foreign genotypes other than A (D–H) were detected in 2 (1.6%) and 10 (0.24%) patients with acute and chronic HBV infections, respectively. One each genotype D and H were found in patients with acute HBV infection; and 6 with genotype D, 3 genotype F and 1 genotype H in those chronic infection. Among patients with chronic HBV infection, genotype B was more frequent (566/3,481 [16.3%] vs. 28/508 [5.5%], $P < 0.001$), while genotype C was less common (2,915/3,481 [83.7%] vs. 480/508 [94.5%], $P < 0.001$), in those with chronic hepatitis than cirrhosis and/or HCC.

TABLE II. Distribution of Genotypes in Patients With Acute and Chronic HBV Infections

Genotypes[a]	Acute (n = 126)	Chronic (n = 4,129)	Differences (P value)
A	36 (28.6%)	122 (3.0%)	<0.001
B	13 (10.3%)	508 (12.3%)	NS
C	75 (59.5%)	3,489 (84.5%)	NS
D	1 (0.8%)	6 (0.1%)	NS
E	0	0	NS
F	0	1 (0.02%)	NS
G	0	0	NS
H	1 (0.8%)	3 (0.07)	NS

[a]Data are expressed in number of patients with percentage in parentheses.

Subgenotypes of HBV

Subgenotypes of A, B, and C were determined in patients with HBV infection. Of the 158 patients infected with genotype A, 15 (9.5%) were classified into subgenotype A1/Aa and 121 (76.6%) into A2/Ae; the remaining 22 (13.9%) were not typeable. Likewise, of the 521 patients with genotype B, 388 (74.5%) were infected with the domestic subgenotype B1/Bj and 102 (19.6%) with foreign subgenotype B2/Ba; subgenotypes in the remaining 31 (6%) patients could not be determined. Figure 2 compares the proportion of these subgenotypes among the seven 5-year periods. By the trend analysis, subgenotype B2/Ba was increasing recently ($P < 0.05$). Subgenotypes of C were domestic C2/Cs in all the 1,610 HBV isolates tested. The foreign subgenotype C1/Ce was not detected in any patient infected with HBV genotype C.

Change in the Distribution of Genotypes in Patients Infected With HBV

Figure 3 illustrates distributions of genotypes A–C in patients with acute and chronic HBV infection during

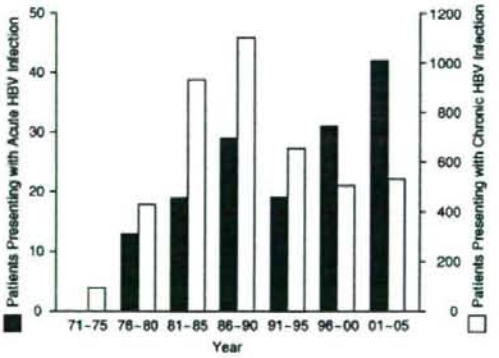

Fig. 1. Patients with acute and chronic HBV infection who visited Toranomon Hospital during 35 years from 1971 to 2005. Numbers are indicated in different scales for patients with acute and chronic HBV infections for seven 5-year periods.

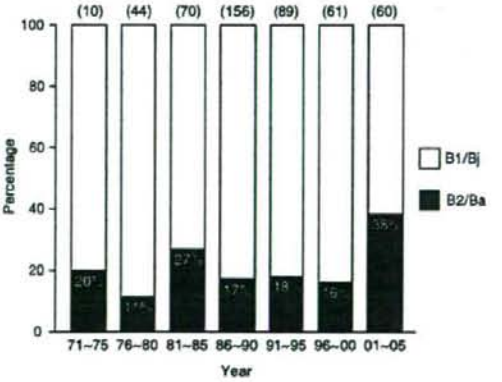

Fig. 2. Distribution of subgenotypes of genotype B shifting during 1971 through 2005. The number of patients is shown in parentheses for each seven 5-year period.

seven 5-year periods. The genotype distribution in patients with acute HBV infection changed through seven 5-year periods. The proportion of genotype A started to increase sharply in the fifth 5-year period (1996–1995) and accounted for 43% and 40%, respectively, in the sixth (1996–2000) and seventh (2001–2005) 5-year periods. The proportion of genotype A during the fifth and seventh 5-year periods (1991–2005) was significantly higher than that during the second through fourth 5-year periods (1976–1990) (39% [33/84] vs. 8% [3/40], $P < 0.001$). Before 1995, genotype A was detected in men <35 years but not found in those >36 years (7/21 [33%] vs. 0/25 [0%]). However, genotype A became comparably frequent since 1996 (15/29 [52%] vs. 14/32 [44%]).

By remarkable contrast, the distribution of genotypes in patients with chronic HBV infection remained fairly constant, although the proportion of genotypes A kept increasing constantly. Thus the proportion of genotype A during the fifth through seventh 5-year periods (1991–2005) was greater than that during the first to fourth 5-year periods (1971–1990) (4.3% [70/1,638] vs. 2.4% [51/2,128], $P < 0.001$).

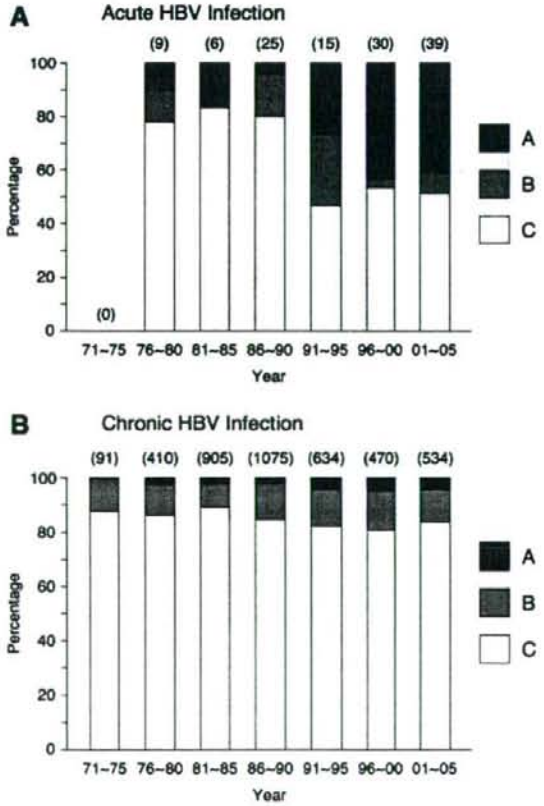

Fig. 3. Time-dependent distribution of HBV genotypes in patients with acute and chronic HBV infections during 1971 through 2005. Distribution of genotypes A–C in patients with acute HBV infection (A) and those with chronic HBV infection (B) are shown for seven 5-year periods. The number of patients is shown in parentheses for each seven 5-year period.

DISCUSSION

The Department of Hepatology at the Toranomon Hospital was visited by 153 with acute and 4,277 patients with chronic HBV infection during 35 years from 1971 through 2005. Patients with acute HBV infection were younger, more commonly male and had been infected by sexual contact more frequently than those with chronic infection. Patients were grouped by the year when they visited the department, and they were compared among seven 5-year periods spanning 1971–2005, for the purpose of estimating time-dependent trends of acute and chronic HBV infections in Japan.

Remarkably, patients presenting with acute HBV infection increased during the past 35 years (Fig. 1). Patients with chronic HBV infection peaked in 1986–1990 and then decreased until 1996–2000. They did not decrease further, but instead, increased slightly in the 21st century. Such a recent increase in chronic HBV infection would reflect resurgence of acute infection, which is supported by the analysis of genotypes.

The distribution of HBV genotypes was much different between patients with acute and chronic infections. Of note, infection with genotype A was much more frequent in acute than chronic infection (28.6% vs. 3%, $P < 0.001$). HBV genotypes have distinct geographic distribution [Miyakawa and Mizokami, 2003; Fung and Lok, 2004; Norder et al., 2004]. The Japanese have been infected with genotypes B and C since the prehistoric era [Yamashita et al., 1975], and foreign genotypes represented by A (both subgenotypes A1/Aa and A2/Ae) were introduced by travelers and immigrants after the end of World War II. Since 1991, foreign genotypes have been increasing in acute HBV infection in Japan [Sugauchi et al., 2006]. As for chronic HBV infection, genotype C was more prevalent in patients with cirrhosis and/or HCC than in those with chronic hepatitis (480/508 [94.5%], vs. 2,915/3,481 [83.7%], $P < 0.001$), standing in corroboration with previous studies [Kao et al., 2000; Orito et al., 2001].

There was a dramatic change in the distribution of HBV genotypes in patients with acute HBV infection during the past 35 years. This change is attributed to ever increasing infection with genotype A in them. It accounted for only 8.1% before 1990, in marked contrast to 39.3% after 1991 ($P < 0.001$). The recent resurgence of acute infection in Japan could be due to increase in the transmission with HBV of foreign genotypes. The gradual increase of genotype A, in patients with chronic HBV infection since 2001, would be accounted for by an increase of acute infection with this genotype in Japan. In support of this view, infection with genotype A tends to persist, infection even in adulthood, and becomes chronic in 10% of infected adults [Suzuki et al., 2005; Kobayashi et al., 2006]. In an outbreak transmitted by a surgeon, 5 of the 16 (31%) patients infected with genotype A became HBV carriers [Harpaz et al., 1996].

There are two types of risk for exposure to HBV. One is avoidable and mediated by promiscuous sexual contacts and the use of illicit intravenous drugs. The other is not preventable and can involve citizens without high-risk behaviors. For instance, HBV can be transmitted from patient to patient in dental care [Redd et al., 2007]. HBV can spread from carrier surgeons who are negative for serum HBeAg [Perry et al., 2006]. In 2002, the largest outbreak of HBV involving 38 patients occurred in a physician's office in New York City by multidose vials contaminated with HBV [Samandari et al., 2005]. There is a pressing need to investigate and determine the risk of HBV transmission in the health care setting [Allos and Schaffner, 2007]. Fortunately, risks of HBV infection can be avoided by vaccination. Mass vaccination of newborns and catch-up vaccination, such as those conducted in the United States [MMWR, 2002], Taiwan [Ni et al., 2001] and elsewhere, would need to be considered in Japan. The ultimate national protection would be universal vaccination of all age groups.

In conclusion, acute HBV infection is increasing in Japan in spite of immunoprophylaxis of high-risk babies implemented nationally since 1986. Based on genotypes/subgenotypes changing with time, the increase may be attributed to infections with HBV of foreign genotypes/subgenotypes predominantly by sexual contact. Since HBV genotype A, with a high propensity to persist, prevailed in acute infection, chronic infection would increase in the foreseeable future. Effective measures have to be taken for preventing HBV transmission among young men at high risk in Japan.

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Hepatology Research 2008; **38**: 877–885 doi: 10.1111/j.1872-034X.2008.00343.x

Original Article

Potential of laparoscopy in chronic liver disease with hepatitis B and C viruses

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Aim: The definitive diagnosis of chronic liver disease is made either by a histological examination of a biopsy specimen or upon visualization of the liver surface at laparoscopy. The aim of this retrospective cohort study is to assess whether histological or laparoscopic findings are associated with hepatocellular carcinoma (HCC) development.

Methods: A retrospective review of paired laparoscopy and histology reports was performed on 4124 hepatitis virus-positive patients who underwent laparoscopy: 2804 patients had hepatitis C virus (HCV group) and 1320 patients had hepatitis B virus (HBV group). Based on the irregularities of the liver surface, the laparoscopic findings were classified into three groups in progression order: smooth, irregular, or nodular. The histological findings were classified according to the extent of fibrosis into four stages (stages 1–4) in progression order.

Results: The number of patients with HCC development was 565 in the HCV group and 115 in the HBV group. The Coxregression hazard model showed that HCC appearance in the HCV group was independently associated with laparoscopic findings (relative risk based on every progression of one rank [RR], RR = 4.31, $P < 0.0001$) and histological findings (RR = 2.56, $P < 0.0001$). In the HBV group, however, HCC appearance in was mainly associated with laparoscopic findings (RR = 2.12, $P < 0.0001$) compared to histological findings (RR = 1.13, $P = 0.403$).

Conclusion: Our data indicate that laparoscopic findings of the liver are dominant predictors for HCC development compared with histological findings in patients with HBV.

Key words: hepatitis B virus, hepatitis C virus, hepatocellular carcinoma, laparoscopy, liver biopsy

INTRODUCTION

HEPATITIS C VIRUS (HCV) or hepatitis B virus (HBV) is one of the common causes of chronic liver disease in the world. Chronic hepatitis C or B infection can be associated with progressive liver disease that may evolve insidiously to cirrhosis.[1–4] In addition, HCV or HBV is a major risk for hepatocellular carcinoma (HCC).[5–9] HCC is one of the major causes of death, especially in Asian countries. It is necessary for physicians to make an accurate diagnosis for the management for chronic hepatitis. A definitive diagnosis of chronic liver disease in patients with hepatitis B or C is important in the prognosis and management of patients. The definitive diagnosis of chronic liver disease is made either by a histological examination of a biopsy specimen or upon visualization of the liver surface at laparoscopy.

Keeling revolutionised investigations of liver disease using the original description of laparoscopy in 1923.[10] Laparoscopy-guided liver biopsy, however, is considered by many to be the most accurate method of diagnosing liver disease, especially liver cirrhosis.[11–16] However, the use of laparoscopy as a diagnostic tool in liver disease has decreased over the past decade.[17,18] The reason for decreasing laparoscopic examinations is that there are misconceptions about the overall safety and complication rate. The use of laparoscopy is generally more complex than that of ultrasonography (US)-guided biopsy.

With this in mind, the present cohort study aimed to compare the accuracy of liver descriptions made during laparoscopy with histological reports from biopsies in patients with chronic viral hepatitis. At the same time,

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Received 8 January 2008; revision 16 January 2008; accepted 16 January 2008.

we assessed whether laparoscopic or histological findings are associated with HCC appearance in patients with HCV or HBV.

METHODS

Patient population

THE NUMBER OF patients who were diagnosed by using both laparoscopy and histology between April 1985 and April 2000 in the Department of Hepatology, Toranomon Hospital (Tokyo, Japan) was 6640. Of these, 4124 patients met the following criteria: (i) positive for HCV-RNA or hepatitis B surface antigens (HBsAg); (ii) negative for antinuclear antibodies or antimitochondrial antibodies in the serum, as determined by radioimmunoassay or spot hybridization; (iii) no history of treatment with corticosteroids, immunosuppressive agents, or antiviral agents; (iv) no evidence of HCC nodules as shown by US and/or computed tomography (CT); and (v) macroscopic examination and classification by three laparoscopy experts (YA, KI, or HK) who have performed laparoscopy-guided biopsies of >1000 episodes. Patients with either of the following criteria were excluded from the study: (i) α-fetoprotein of 400 ng/mL or higher; (ii) positive for both HCV-RNA and HBsAg; and (iii) advanced and decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites. The 2806 patients with HCV-RNA and without HBsAg were regarded as HCV group. The 1302 patients with HBsAg and without HCV-RNA were regarded as the HB group. The physicians in charge explained the purpose and method of the laparoscopic-guided liver biopsy to each patient and/or patients' family, who gave their informed consent for participation. This study was approved by the Institutional Review Board of our hospital. Written informed consent was obtained from all patients before the procedure commenced.

Laparoscopy

Abdominal US, electrocardiogram, and a chest X-ray were performed before laparoscopy. The patients were deprived of food and water at the day of examination. Thirty minutes before exploration, 50 mg pethidin and 0.5 mg atropine were injected intramuscularly. If necessary, patients received sedativa or analgetics during the laparoscopic intervention. During laparoscopy, each patient was given continuously an isotonic electrolyte solution intravenously. Patients were monitored by pulsoxymetry and blood pressure manometer. After local anesthesia, the pneumoperitoneum was installed by puncturing at Kalk's point with the Verres needle followed by insufflation of 2–3 L nitrous oxide. After insertion of the laparoscope in a trocar with a safety shield at Kalk's point, macroscopic exploration of liver followed. Liver biopsies were taken generally from an area on the anterior surface of the right lobe of the liver using a Silbermann needle at least 3–4 cm from the liver edge, containing at least five portal areas. After laparoscopy-guided liver biopsies, hemostasis was achieved through gelatin sponge (Gelform, Nipponkayaku, Tokyo, Japan) placement to the biopsy site.

Based on the irregularities of the liver surface, the laparoscopic findings were classified into three groups in progression order: smooth (an essentially smooth liver surface or with limited areas of depression), irregular (a liver surface showing increased numbers of interconnected depressions, possibly resembling ripples or speck), and nodular (a liver surface with nodular formations) as shown Figure 1.

Histopathological evaluation

Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin–eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of the specimens for the examination was more than six portal areas. Histopathological interpretation of specimens was made by experienced liver pathologists who had no clinical information. Baseline liver histology of chronic hepatitis was classified according to the extent of fibrosis into four stages in progression order: stage 1, periportal expansion; stage 2, portoportal septa; stage 3, portocentral linkage or bridging fibrosis; and stage 4, liver cirrhosis.[19]

Viral markers of HCV and HBV

The diagnosis of HCV infection was based on the detection of the serum HCV antibody and positive RNA. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, IL, USA). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV monitor test version 2.0, Roche, Tokyo, Japan). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI, USA). The used serum samples were stored −80°C at the time of the laparoscopic examination.

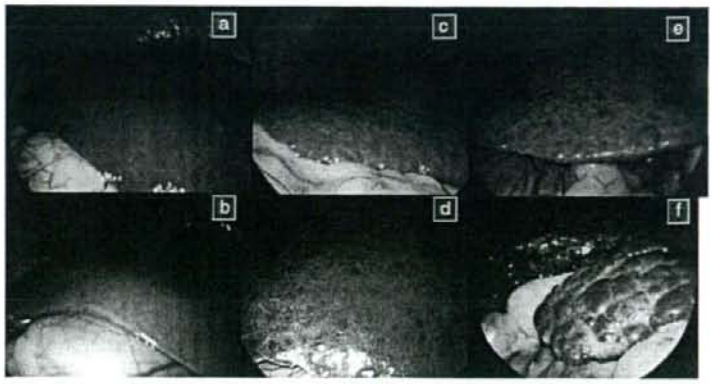

Figure 1 Based on the irregularities of the liver surface, laparoscopic findings were classified into three groups in progression order: smooth, an essentially smooth liver surface or with limited areas of depression (a,b); irregular, a liver surface showing increased numbers of interconnected depressions, possibly resembling ripples or specks (c,d); and nodular, a liver surface showing nodular formations with or without specks (e,f).

Follow up

Patients were followed up monthly to tri-monthly after the first medical examination at our hospital. The physical examinations and biochemical tests were conducted at each examination together with regular check-ups using abdominal CT or US imaging in each patient. Three hundred and nineteen patients were lost to follow up. Because the appearance of HCC and death was not identified in these 319 patients, they wee considered as censored data in the statistical analysis.[20] Moreover, the patients treated with antivirus drugs were regarded as withdrawals at the time of starting the antivirus drugs. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings on CT and US. A microscopic examination of fine-needle biopsy material was carried out in patients whose angiograms did not demonstrate a typical HCC image.

Statistical analysis

Non-parametric procedures were employed for the analysis of background features of the patients, including the Mann–Whitney *U*-test and χ^2-test. The cumulative appearance rate of HCC was calculated from the time of the laparoscopy examination to the appearance of HCC, using the Kaplan–Meier method. Differences in the development of HCC were tested using the log-rank test. We analyzed whether laparoscopic or histological findings were associated with the incidence rate of HCC by the Cox proportional hazard model. A *P*-value of less than 0.05 in the two-tailed test was considered significant. Data analyses were performed using the SPSS computer program version 11.0 (SPSS, Chicago, IL, USA).

RESULTS

Patients' characteristics

THE CHARACTERISTICS OF the 4124 patients with HCV or HBV are shown in Table 1. These patients comprised of 2804 with HCV infection (HCV group) and 1320 with HBV infection (HBV group). There were significant differences in many backgrounds between the two groups as shown in Table 1. The number of patients with HCC development was 565 in the HCV group and 115 in the HBV group.

Relationship between laparoscopic findings and histological stage

The relationship between the laparoscopic findings and histological stage in the HCV group are shown in Table 2. Almost all the patients with a smooth liver surface had stage 1 or 2; 89.9% (879/981) of patients with an irregular liver surface had stage 2 or 3; and although patients with a nodular liver surface had mainly stage 4, 21.8% (71/326) of these patients had stage 1, 2, or 3.

The relationship between the laparoscopic findings and histological stage in the HBV group are shown in Table 3. Almost all the patients with a smooth liver surface had stage 1 or 2; 88.7% (361/407) of patients with an irregular liver surface had stage 2 or 3; and although 58.5% (120/206) of patients with a nodular liver surface had mainly stage 4, approximately 40% of these patients had stage 1, 2, or 3. The incidence of stage 4 in HBV patients with a nodular surface was smaller than that in HCV patients ($P < 0.0001$).

Table 1 Clinical characteristics at laparoscopy and liver biopsy†

	HBV group	HCV group	*P*-value
n	1320	2804	
Age	36.6 ± 10.5	51.1 ± 11.6	<0.001
Sex (male percentage)	76.6% (1011)	57% (1597)	<0.001
AST (IU/L)	113.5 ± 154.9	89.5 ± 102.2	<0.001
ALT (IU/L)	194.7 ± 255.7	130.1 ± 145.7	<0.001
Total bilirubin (mg/dL)	0.82 ± 0.45	0.84 ± 0.43	0.553
γ-GTP (IU/L)	64.8 ± 82.9	98.2 ± 83.1	<0.001
Platelet count ($\times 10^4/mm^3$)	15.6 ± 12.6	20.3 ± 17.4	<0.001

†Data are number of patients or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidases; HBV, hepatitis B virus; HCV, hepatitis C virus.

Cumulative appearance rates of HCC

The cumulative development rates of HCC based on the histological findings in the HCV group are shown in Figure 2. In the patients with histological findings of stage 1 or 2, the HCC development rates differed due to differences in the laparoscopic findings. The cumulative development rates of HCC based on the laparoscopic findings in the HCV group are shown in Figure 3. In the patients with laparoscopic findings of an irregular liver surface, the HCC development rates differed due to differences in the histological findings. However, in the patients with laparoscopic findings of a smooth or nodular liver surface, the HCC development rates were not significantly different due to difference in the histological findings.

The cumulative development rates of HCC based on the histological findings in HBV group are shown in Figure 4. In the patients with histological findings of stage 1 or 2, the HCC development rates differed due to difference in laparoscopic findings. However, in patients with stage 3 or 4, HCC development rates were not statistically different in spite of the differences in the laparoscopic findings. The cumulative development rates of HCC based on the laparoscopic findings in the HBV patients are shown in Figure 5. In the patients with the same laparoscopic findings, the HCC development rates did not differ due to differences in the histological findings.

HCC appearance rates based on laparoscopic and histological findings were evaluated by the Cox propor-

Table 2 Relationship between laparoscopic findings and histological stage in patients with chronic type C hepatitis

Laparoscopic finding	Histological stage				
	Stage 1	Stage 2	Stage 3	Stage 4	Total
Smooth	1390 (92.7%)	106 (7.1%)	4 (0.3%)	0	1500
Irregular	65 (6.6%)	631 (64.5%)	248 (25.4%)	37 (3.8%)	981
Nodular	3 (0.9%)	16 (4.9%)	52 (16.0%)	255 (78.2%)	326
Total	1458	753	304	292	2807

Table 3 Relationship between laparoscopic findings and histological stage in patients with chronic type B hepatitis

Laparoscopic findings	Histological stage				
	Stage 1	Stage 2	Stage 3	Stage 4	Total
Smooth	645 (91.2%)	61 (8.6%)	1 (0.1%)	0	707
Irregular	40 (9.8%)	275 (67.6%)	86 (21.1%)	6 (1.5%)	407
Nodular	4 (1.9%)	20 (9.7%)	62 (30.1%)	120 (58.5%)	206
Total	693	352	159	126	1320

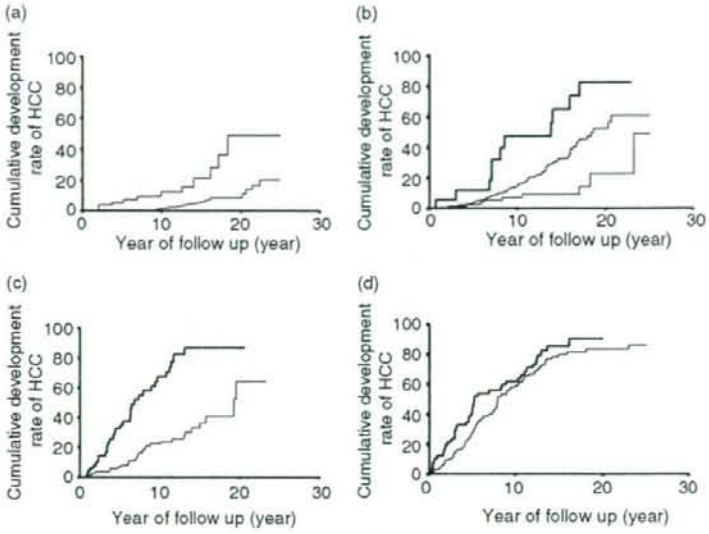

Figure 2 Cumulative development rates of hepatocellular carcinoma (HCC) based on the histological findings of hepatitis C virus patients. (a) Patients with stage 1 ($P < 0.0001$); (b) patients with stage 2 ($P < 0.0001$); (c) patients with stage 3 ($P < 0.0001$, $P = 0.055$); (d) patients with stage 4 ($P = 0.081$). (——) Nodular, (——) Irregular, (——) Smooth.

tional hazard model as shown in Tables 4 and 5. The multivariate Cox regression hazard model using two factors of laparoscopic and histological findings showed that HCC development in the HCV group were independently associated with laparoscopic findings (relative risk based on every progression of one rank [RR], RR = 4.31, $P < 0.0001$) and histological findings (RR = 2.56, $P < 0.0001$). In the HBV group, however, HCC development in was mainly associated with laparoscopic findings (RR = 2.12, $P < 0.0001$) compared to histological finding (RR = 1.13, $P = 0.403$).

The multivariate analysis showed that both laparoscopic and histological findings were associated with HCC appearance in the HCV group. However, in the HBV group, laparoscopic findings were important predictors compared to histological findings.

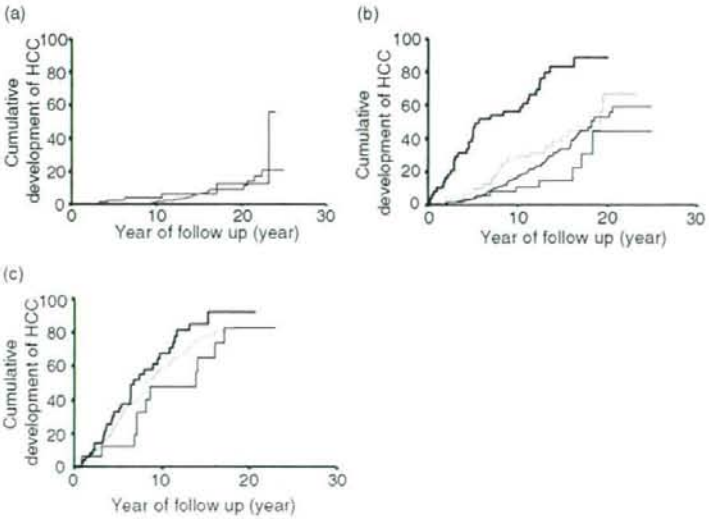

Figure 3 Cumulative development rates of hepatocellular carcinoma (HCC) based on the laparoscopic findings in hepatitis C virus patients. (a) Patients with smooth liver surface ($P < 0.391$); (b) patients with irregular liver surface ($P < 0.0001$); (c) patients with nodular liver surface ($P < 0.055$) (——) Stage 4, ( ) Stage 3, (——) Stage 2, (——) Stage 1.

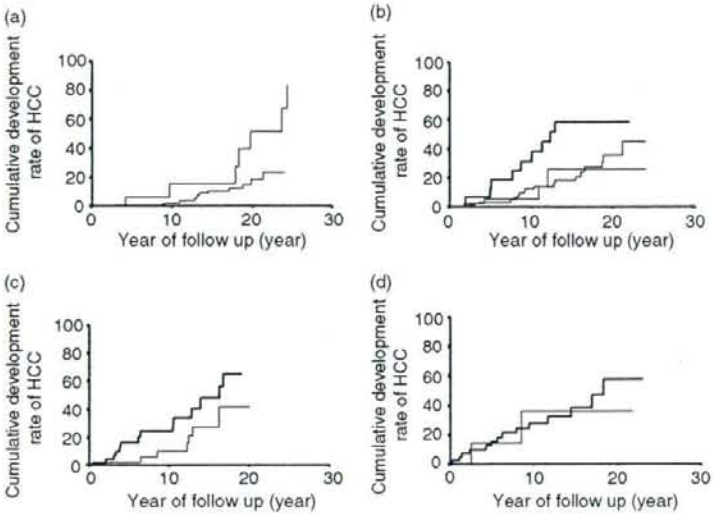

Figure 4 Cumulative development rates of hepatocellular carcinoma (HCC) based on the histological findings in hepatitis B virus patients. (a) Patients with stage 1 ($P < 0.013$); (b) patients with stage 2 ($P < 0.016$); (c) patients with stage 3 ($P < 0.116$); (d) patients with stage 4 ($P < 0.858$). (—) Nodular, (—) Irregular, (—) Smooth.

Adverse event

Seven patients had the following major operative complications: pneumothorax ($n = 6$) and shock due to vasovagal reflux ($n = 1$) at the time of liver biopsy. There were no complications of bleeding at the site of the liver biopsy.

DISCUSSION

WE HAVE DESCRIBED the difference of laparoscopic and histological findings in patients with HCV or HBV. The present study was limited by a retrospective cohort trial. Other limitations of the study were

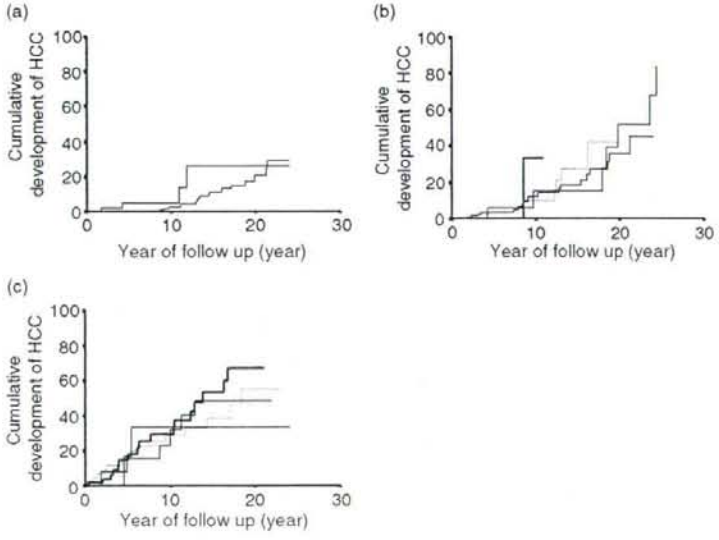

Figure 5 Cumulative development rates of hepatocellular carcinoma (HCC) based on the laparoscopic findings in hepatitis B virus patients. (a) Patients with smooth liver surface ($P < 0.252$); (b) patients with irregular liver surface ($P < 0.736$); (c) patients with nodular liver surface ($P < 0.785$). (—) Stage 4, ( ) Stage 3, (—) Stage 2, (—) Stage 1.