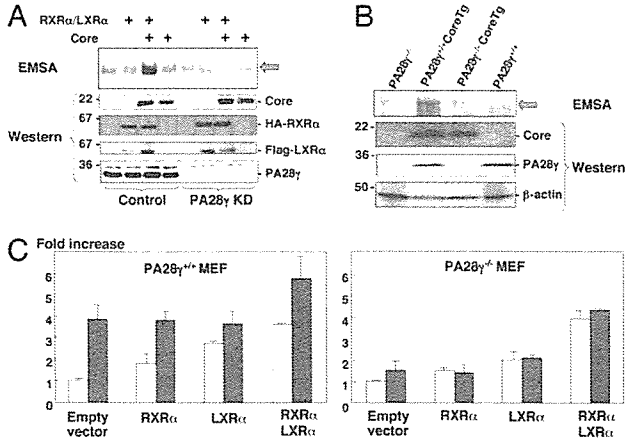

Fig. 5. PA28γ is required for HCV core-dependent activation of the *srebp-1c* promoter. (*A*) Effect of PA28γ knockdown on the LXRα/RXRα–DNA complex. FLAG-LXRα and HA-RXRα were expressed in FLC4 (control) or PA28γ-knockdown (PA28γ KD) cells together with or without HCV core protein. Cells were harvested at 48 h posttransfection, and nuclear extracts were mixed with the reaction buffer for EMSA. (*Upper*) The resulting mixtures were subjected to PAGE and blotted with horseradish peroxidase-streptavidin. The mobility shift of the LXRE probe is indicated by an arrow. (*Lower*) Expression of HCV core, HA-RXRα, FLAG-LXRα, and PA28γ in cells was detected by immunoblotting. (*B*) Effect of PA28γ knockout on the LXRα/RXRα–DNA complex in the mouse liver. (*Upper*) Nuclear extracts were prepared from the livers of 2-month-old PA28γ$^{-/-}$, PA28γ$^{+/+}$CoreTg, PA28γ$^{-/-}$CoreTg, and PA28γ$^{+/+}$ mice and subjected to EMSA. The mobility shift of the LXRE probe is indicated by an arrow. (*Lower*) The expression of HCV core, PA28γ, and β-actin in the livers of the mice was detected by immunoblotting. (*C*) Effect of HCV core protein on *srebp-1* promoter activity in PA28γ-knockout fibroblasts. A plasmid encoding firefly luciferase under the control of the *srebp-1c* promoter was transfected into MEFs prepared from PA28γ$^{+/+}$ (*Left*) or PA28γ$^{-/-}$ (*Right*) mice together with a plasmid encoding a *Renilla* luciferase. An empty plasmid or plasmids encoding mouse RXRα or LXRα were also cotransfected into the cells together with (gray bars) or without (white bars) a plasmid encoding HCV core protein. Luciferase activity under the control of the *srebp-1c* promoter was determined, and it is expressed as the fold increase in relative luciferase activity after standardization with the activity of *Renilla* luciferase.

tiates *srebp-1c* promoter activity in an LXRα/RXRα-dependent manner.

HCV Core Protein Activates the *srepb-1c* Promoter in an LXRα/RXRα- and PA28γ-Dependent Manner. To examine whether PA28γ is required for HCV core-induced enhancement of *srebp-1c* promoter activity in human liver cells, a PA28γ-knockdown human hepatoma cell line (FLC4 KD) was prepared. Enhancement of binding of the LXRE probe to LXRα/RXRα by coexpression of HCV core protein and LXRα/RXRα in FLC4 cells was diminished by knockdown of the PA28γ gene (Fig. 5*A*). Furthermore, formation of the LXRα/RXRα–LXRE complex was enhanced in the livers of PA28γ$^{+/+}$CoreTg mice but not in those of PA28γ$^{-/-}$, PA28γ$^{+/+}$, or PA28γ$^{-/-}$CoreTg mice (Fig. 5*B*). The expression of the HCV core protein in the mouse embryonic fibroblasts (MEFs) of PA28γ$^{+/+}$ mice induced the activation of the mouse *srebp-1c* promoter through the endogenous expression of LXRα and RXRα (Fig. 5*C Left*). Further enhancement of the activation of the *srebp-1c* promoter by HCV core protein in PA28γ$^{+/+}$ MEFs was achieved by the exogenous expression of both LXRα and RXRα. However, no enhancing effect of HCV core protein on *srebp-1c* promoter activity was observed in PA28γ$^{-/-}$ MEFs (Fig. 5*C Right*). These results support the notion that HCV core protein enhances the activity of the *srepb-1c* promoter in an LXRα/RXRα- and PA28γ-dependent manner.

Table 1. HCC in mice at 16–18 months of age

Mouse and sex	Total no. of mice	No. of mice developing HCC	Incidence, %
PA28γ$^{+/+}$CoreTg			
Male	17	5	29.4
Female	28	3	10.7
PA28γ$^{+/-}$			
Male	16	0	0
Female	4	0	0
PA28γ$^{-/-}$			
Male	23	0	0
Female	13	0	0
PA28γ$^{-/-}$CoreTg			
Male	15	0	0
Female	21	0	0

PA28γ Plays a Crucial Role in the Development of HCC in PA28γ$^{+/+}$ CoreTg Mice. The incidence of hepatic tumors in male PA28γ$^{+/+}$ CoreTg mice older than 16 months was significantly higher than that in age-matched female PA28γ$^{+/+}$CoreTg mice (6). We reconfirmed here that the incidence of HCC in male and female PA28γ$^{+/+}$CoreTg mice at 16–18 months of age was 29.4% (5 of 17 mice) and 10.7% (3 of 28 mice), respectively. To our surprise, however, no HCC developed in PA28γ$^{-/-}$CoreTg mice (males, 15; females, 21), although, as expected, no HCC was observed in PA28γ$^{+/-}$ (males, 16; females, 4) and PA28γ$^{-/-}$ mice (males, 23; females, 13) (Table 1). These results clearly indicate that PA28γ plays an indispensable role in the development of HCC induced by HCV core protein.

Discussion

HCV core protein is detected in the cytoplasm and partially in the nucleus and mitochondria of culture cells and hepatocytes of transgenic mice and hepatitis C patients (6, 23, 24, 26). Degradation of HCV core protein was enhanced by deletion of the C-terminal transmembrane region through a ubiquitin/proteasome-dependent pathway (27). We previously reported (18) that PA28γ binds directly to HCV core protein and then enhances degradation of HCV core protein in the nucleus through a proteasome-dependent pathway because HCV core protein was accumulated in nucleus of human cell line by treatment with proteasome inhibitor MG132. In this work, accumulation of HCV core protein was observed in nucleus of hepatocytes of PA28γ$^{-/-}$CoreTg mice (Fig. 1*D*). This result directly demonstrates that HCV core protein migrates into the nucleus and is degraded through a PA28γ-dependent pathway. However, HCV core protein accumulated in the nucleus because knockout of PA28γ gene abrogated the ability to cause liver pathology, suggesting that interaction of HCV core protein with PA28γ in the nucleus is prerequisite for the liver pathology induced by HCV core protein. We have previously shown (18) that HCV core protein is degraded through a PA28γ-dependent pathway, and Minami *et al.* (28) reported that PA28γ has a cochaperone activity with Hsp90. Therefore, degradation products of HCV core protein by means of PA28γ-dependent processing or correct folding of HCV core protein through cochaperone activity of PA28γ might be involved in the development of liver pathology. We do not know the reason why knockout of the PA28γ gene does not affect the total amount of HCV core protein in the liver of the transgenic mice. PA28γ-dependent degradation of HCV core protein may be independent of ubiquitination, as shown in SRC-3 (21), whereas knockdown of PA28γ in a human hepatoma cell line enhanced the ubiquitination of HCV core protein [supporting information (SI) Fig. 6], suggesting that lack of PA28γ suppresses a ubiquitin-independent degradation but enhances a ubiquitin-dependent degradation of HCV core protein. Therefore, the total amount of HCV

core protein in the liver of the mice may be unaffected by the knockout of the PA28γ gene.

Our results suggest that the interaction of HCV core protein with PA28γ leads to the activation of the *srebp-1c* promoter along an LXRα/RXRα-dependent pathway and the development of liver steatosis and HCC. HCV core protein was not included in the LXRα/RXRα–LXRE complex (Fig. 3*A*), suggesting that HCV core protein indirectly activates the *srebp-1c* promoter. Cytoplasmic HCV core protein was shown to interact with Sp110b, which is a transcriptional corepressor of RARα-dependent transcription, and this interaction leads to the sequestering of Sp110b in the cytoplasm, resulting in the activation of RARα-dependent transcription (29). The sequestration of an unidentified corepressor of the LXRα/RXRα heterodimer in the cytoplasm by HCV core protein may also contribute to the activation of the *srebp-1c* promoter. Although the precise physiological function of PA28γ-proteasome activity in the nucleus is not known, PA28γ has previously been shown (21) to regulate nuclear hormone receptors by means of the degradation of its coactivator SRC-3 and to participate in the fully Hsp90-dependent protein refolding (28). It appears reasonable to speculate that degradation or refolding of HCV core protein in a PA28γ-dependent pathway might be involved in the modulation of transcriptional regulators of various promoters, including the *srebp-1c* promoter. Saturated or monounsaturated fatty acids have been shown to enhance HCV RNA replication in Huh7 cells containing the full-length HCV replicon (7). The up-regulation of fatty acid biosynthesis by HCV core protein may also contribute to the efficient replication of HCV and to the progression of HCV pathogenesis.

Expression of HCV core protein was reported to enhance production of reactive oxygen species (ROS) (30), which leads to carbonylation of intracellular proteins (31). Enhancement of ROS production may trigger double-stranded DNA breaks and result in the development of HCC (30, 32, 33). HCV core protein could enhance the protein carbonylation in the liver of the transgenic mice in the presence but not in the absence of PA28γ (SI Fig. 7), suggesting that PA28γ is required for ROS production induced by HCV core protein. Development of HCC was observed in PA28γ$^{+/+}$CoreTg mice but not in PA28γ$^{-/-}$CoreTg mice (Table 1). Enhancement of ROS production by HCV core protein in the presence of PA28γ might be involved in the development of HCC in PA28γ$^{+/+}$CoreTg mice.

It is well known that resistant viruses readily emerge during the treatment with antiviral drugs targeting the viral protease or replicase, especially in the case of infection with RNA viruses. Therefore, antivirals targeting the host factors that are indispensable for the propagation of viruses might be an ideal target for the development of antiviral agents because of a lower rate of mutation than that of viral genome, if they have no side effects to patients. Importantly, the amino acid sequence of PA28γ of mice is identical to that of human, and mouse PA28γ is dispensable because PA28γ knockout mice exhibit no abnormal phenotype except for mild growth retardation. Therefore, PA28γ might be a promising target for an antiviral treatment of chronic hepatitis C with negligible side effects.

In summary, we observed that a knockout of the PA28γ gene from PA28γ$^{+/+}$CoreTg mice induced the accumulation of HCV core protein in the nucleus and disrupted the development of both steatosis and HCC. Activation of the *srebp-1c* promoter was up-regulated by HCV core protein both *in vitro* and *in vivo* through a PA28γ-dependent pathway, suggesting that PA28γ plays a crucial role in the development of liver pathology induced by HCV infection.

Materials and Methods

Histology and immunohistochemistry, real-time PCR, and detection of proteins modified by ROS are discussed in *SI Materials and Methods*.

Plasmids and Reagents. Human PA28γ cDNA was isolated from a human fetal brain library (18). The gene encoding HCV core protein was amplified from HCV strain J1 (genotype 1b) (34) and cloned into pCAG-GS (35). Mouse cDNAs of RXRα and LXRα were amplified by PCR from the total cDNAs of the mouse liver. The RXRα and LXRα genes were introduced into pEF-FLAGGspGBK (36) and pcDNA3.1 (Invitrogen, Carlsbad, CA), respectively. The targeting fragment for human PA28γ knockdown (GGATCCGGTGGATCAGGAAGTGAAGTTCAAGAGA-CTTCACTTCCTGATCCACCTTTTTTGGAAAAGCTT) was introduced into the BamHI and HindIII sites of pSilencer 4.1 U6 hygro vector (Ambion, Austin, TX). Mouse anti-FLAG (M2) and mouse anti-β-actin antibodies were purchased from Sigma (St. Louis, MO). Rabbit polyclonal antibody against synthetic peptides corresponding to amino acids 70–85 of PA28γ was obtained from AFFINITI (Exeter, U.K.). Horseradish peroxidase-conjugated goat anti-mouse and anti-rabbit IgGs were purchased from ICN Pharmaceuticals (Aurora, OH). Rabbit anti-HCV core protein was prepared by immunization with recombinant HCV core protein (amino acids 1-71), as described in ref. 24. Mouse monoclonal antibody to HCV core protein was kindly provided by S. Yagi (37). The plasmid for expression of HA-tagged ubiquitin was described in ref. 27.

Preparation of PA28γ-Knockout HCV CoreTg Mice. The generation of C57BL/6 mice carrying the gene encoding HCV core protein genotype 1b line C49 and that of PA28γ$^{-/-}$ mice have been reported previously (22, 25). Both strains were crossbred with each other to create PA28γ$^{-/-}$CoreTg mice. PA28γ$^{-/-}$CoreTg mice were identified by PCR targeted at the PA28γ or HCV core gene (22, 25). Using 1 μg of genomic DNA obtained from the mouse tail, the PA28γ gene was amplified by PCR with the following primers: sense, PA28-3 (AGGTGGATCAGGAAGTGAAGCTCAA); and antisense, PA28γ-5cr (CACCTCACTTGTGATCCGCTCTCT-GAAAGAATCAACC). The targeted sequence for the PA28γ-knockout mouse was detected by PCR using the PA28-3 primer and the PAKO-4 primer (TGCAGTTCATTCAGGGCACCGGA-CAG). The transgene encoding HCV core protein was detected by PCR as described in ref. 25. The expression of PA28γ and HCV core protein in the livers of 6-month-old mice was confirmed by Western blotting with mouse monoclonal antibody to HCV core protein, clone 11-10, and rabbit antibody to PA28γ. Mice were cared for according to the institutional guidelines. The mice were given ordinary feed, CRF-1 (Charles River Laboratories, Yokohama, Japan), and they were maintained under specific pathogen-free conditions.

All animal experiments conformed to the Guidelines for the Care and Use of Laboratory Animals, and they were approved by the Institutional Committee of Laboratory Animal Experimentation (Research Institute for Microbial Diseases, Osaka University).

Preparation of Mouse Embryonic Fibroblasts. MEFs were prepared as described in ref. 22. MEFs were cultured at 37°C under an atmosphere of 5% CO_2 in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% FBS, penicillin, streptomycin, sodium pyruvate, and nonessential amino acids.

Transfection and Immunoblotting. Plasmid vectors were transfected into the MEFs and 293T cells by liposome-mediated transfection by using Lipofectamine 2000 (Invitrogen). The amount of HCV core protein in the liver tissues was determined by an ELISA as described in ref. 37. The cell lysates were subjected to SDS/PAGE (12.5% gel), and they were then transferred onto PVDF membranes. Proteins on the membranes were treated with specific antibody and Super Signal Femto (Pierce, Rockford, IL). The results were then visualized by using an LAS3000 imaging system (Fuji Photo Film, Tokyo, Japan). The method of immunoprecipitation test is described in ref. 18.

Reporter Assay for *srebp-1c* Promoter Activity. The genomic DNA fragment encoding the *srebp-1c* promoter region (located from residues −410 to +24) was amplified from a mouse genome. The fragment was introduced into the KpnI and HindIII sites of pGL3-Basic (Promega, Madison, WI), and it was designated as pGL3-srebp-1cPro. The plasmids encoding RXRα and LXRα were transfected into MEFs together with pGL3-srebp-1cPro and a control plasmid encoding *Renilla* luciferase (Promega). The total DNA for transfection was normalized by the addition of empty plasmids. Cells were harvested at 24 h posttransfection. The ligand of RXRα, 9-*cis*-retinoic acid (Sigma), and that of LXRα, 22(*R*)-hydroxylcholesterol (Sigma) were added at a final concentration of 5 μM each to the culture medium of 293T cells transfected with pGL3-srebp-1cPro together with expression plasmids encoding RXRα, LXRα, and HCV core protein at 24 h posttransfection. Cells were harvested 24 h after treatment. Luciferase activity was measured by using the dual-luciferase reporter assay system (Promega). Firefly luciferase activity was standardized with that of *Renilla* luciferase, and the results are expressed as the fold increase in relative luciferase units.

Electrophoresis Mobility Shift Assay (EMSA). EMSA was carried out by using a LightShift Chemiluminescent EMSA kit (Pierce) according to the manufacturer's protocol. Nuclear extract of the cell lines and liver tissue was prepared with an NE-PER nuclear and cytoplasmic extraction reagent kit (Pierce). Briefly, double-stranded oligonucleotides for EMSA were prepared by annealing both strands of each LXRE of the *srebp-1c* promoter (5′-GGACGCCCGCTAGTAACCCCGGC-3′) (16). Both strands were labeled at the 5′ ends with biotin. The annealed probe was incubated for 20 min on ice with nuclear extract (3 μg of protein) in a reaction buffer containing 10 mM Tris·HCl (pH 7.5), 50 mM KCl, 1 mM DTT, 0.05 μg/μl poly(dI-dC), 2.5% glycerol, 0.05% Nonidet P-40, and 0.1 nM labeled probe, with or without 1 mM nonlabeled probe. The resulting mixture was subjected to PAGE (5% gel) at 120 V for 30 min in 0.5× TBE. The DNA–protein complex was transferred to a Hybond N+ membrane (Amersham, Piscataway, NJ), incubated with horseradish peroxidase-conjugated streptavidin, and visualized by using an LAS3000 imaging system.

Statistical Analysis. The results are expressed as the mean ± SD. The significance of differences in the means was determined by Student's *t* test.

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LETTERS TO THE EDITOR

Anti-HCV agent, ribavirin, elevates the activity of clotting factor VII in patients with hemophilia: a possible mechanism of decreased events of bleeding in patients with hemophilia by ribavirin

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The combination therapy with ribavirin and interferon-α (IFN-α) has been reported to be more effective than IFN-α monotherapy for eradicating hepatitis C virus (HCV) [1,2], including patients with concomitant hemophilia [3]. We observed significant decreases in doses of clotting factors used for hemostatic therapy in hemophiliacs during ribavirin administration (e.g. 3780 units per month before ribavirin treatment and 1600 units per month during ribavirin on the average) [4]. In our hospital, 47 hemophilic patients who had been treated for chronic hepatitis C with IFN-α alone demonstrated no significant reduction in the use of clotting factor. This observation strongly suggests that the addition of ribavirin leads to the reduction of clotting factors used for bleeding in hemophiliacs. One suggestion comes from a case report that described an increase in warfarin dose requirement in a patient with heart valve prosthesis after starting this anti-HCV combination therapy [5].

These observations led us to investigate the ribavirin-induced change in vitamin K-dependent coagulation factors. To this purpose, we have measured the clotting activity of factor (F)VII, X, and prothrombin in hemophilic patients who were receiving the anti-HCV combination therapy. The protocol of therapy and analysis was approved by the Nagoya University institutional review board and written informed consent was obtained from each patient before treatment. Nine hemophilic patients, including seven hemophilia A and two hemophilia B (mean age ± SD: 42.5 ± 10.4 years old), whose characteristics were previously described [4], were entered in this study. The liver biopsy performed before starting the combination therapy did not show cirrhosis but chronic hepatitis in all patients analyzed. During this study, all patients were treated with the same 24-week regimen of IFN-α 2b (Intron A®, Schering Plough, K.K., Osaka, Japan) and oral ribavirin (600–800 mg day^{-1} of Rebetol; Schering-Plough, Kenilworth, NJ, USA). All statistical analyses were performed with STATA ver.7 software (STATA Corp., College Station, TX, USA) and the P-value < 0.05 was considered statistically significant.

The procoagulant activity of FVII in plasma has been elevated in all of nine ribavirin-treated hemophilic patients in comparison with that before ribavirin administration (Fig. 1A). The average and standard deviation for the elevation of FVII activity was 15.7% ± 8.8% ($P < 0.04$ in before vs. during ribavirin treatment; max. 28%; min. 5%). This elevation of FVII activity was independent of improvement of liver function (i.e. albumin, total billirubin, cholinesterase) in the patients (not shown). Only two patients, one has HIV infection and the other has hepatitis B virus concomitant with HCV, did not show a substantial elevation of FVII activity (i.e. 5% and 8%, respectively). We then measured activated FVII (FVIIa) levels in patients' plasma before and during ribavirin treatment using STACLOT® VIIa-rTF (Diagnostica Stago, Asnieres, France) [6] and observed substantial increases in FVIIa (e.g. 25.3 ± 14.8 mU mL^{-1}), which were almost compatible with elevation of FVII clotting activity. The plasma levels of FX and prothrombin were unchanged by ribavirin treatment in all of nine hemophilic patients (not shown). The elevation of FVII clotting activity by ribavirin is consistent with the previous observation of warfarin resistance in a ribavirin-treated patient [5].

To investigate the mechanism of ribavirin-induced elevation of FVII activity, we analyzed the gene expression of FVII in cultured normal human hepatocytes (Cambrex Bio Science Walkersville, Inc., Walkersville, MD, USA) or human hepatoma cell line, HepG2 cells (ATCC, Manassas, VA, USA),

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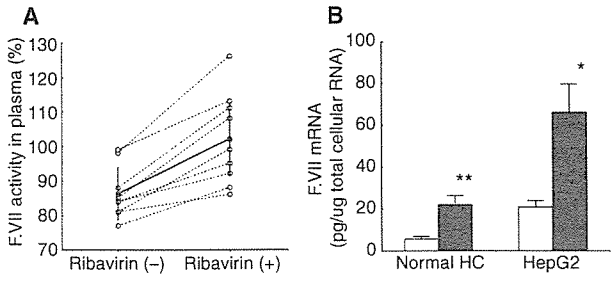

Fig. 1. Clotting activity of FVII in plasma of hemophilic patients and the mRNA expression of FVII in cultured human hepatocytes with or without ribavirin. (A) Each clotting activity of FVII in plasma of nine hemophilic patients before and at 4 weeks after starting ribavirin therapy was shown as open circle and dashed line, respectively. The average and SD of all patients was expressed as closed circle and error bar (without ribavirin: 86.3% ± 7.6%; with ribavirin: 102.0% ± 10.3%; $P < 0.04$). (B) Normal human hepatocytes or HepG2 cells had been cultured with (■) or without (□) ribavirin for 48 h. The mRNA expression of FVII was quantitated by real-time RT-PCR assay. Each value is expressed as the mean and SD from three sets of experiments. All of real-time RT-PCR assays were performed in duplicate. $*P < 0.02$; $**P < 0.01$.

which were cultured in medium with ribavirin at clinically therapeutic concentration (150 μg mL^{-1}) in the presence of IFN-α 2b (0.75 μg mL^{-1}; kindly provided by Schering Plough, K.K.). The expression level of mRNA for FVII, FX, and prothrombin, was determined by real-time quantitative RT-PCR with the ABI Prisms 7700 Sequence Detection (Perkin-Elmer Biosystems, Foster City, CA, USA) and SYBR Green PCR Kit (Perkin-Elmer Biosystems), according to the manufacturer's recommendations. The sequences of primer pairs used to quantify mRNA of the above genes were described in the NCBI Sequence Viewer. Variations in sample loading were assessed by measuring β-actin mRNA. Comparison of quantitative RT-PCR results between two groups was performed with the two-sample *t*-test. Welch's method was applied when variance between two groups was unequal (statistical significance: $P < 0.05$).

Significant induction of FVII mRNA was demonstrated in cultured normal hepatocytes (fourfold; $P < 0.01$) or HepG2 cells (threefold; $P < 0.02$) at 48 h after ribavirin treatment (Fig. 1B). No significant induction of mRNAs for FX and prothrombin was detected in ribavirin-treated cultured hepatocytes or HepG2 cells (not shown). In hepatocytes, ribavirin may stimulate to synthesize FVII by binding specifically to the promoter region of FVII gene (under current investigation).

It is possible that not only the induction of FVII but also changes in other coagulation factors during ribavirin therapy may be responsible for the decreased events of bleeding in hemophiliacs. However, the elevation of FVII activity in plasma could contribute most to the increased hemostatic potential in hemophilic patients because the cell-based tissue factor-activated FVII would play a central role in initiating coagulation and in activating platelets followed by large scale thrombin generation [7]. Clinically, recombinant activated FVII has been widely used as an antidote to control and prevent excessive hemorrhage in hemophilic patients with inhibitors [8]. Meanwhile, it was reported that even 10–20% of increase in plasma FVII/FVIIa would be an independent risk factor for coronary heart disease in healthy individuals [9,10], suggesting that a substantial elevation of endogenous FVII levels could result in an increased thrombotic potential. In general, the occurrence of spontaneous bleeding events in hemophiliacs is dependent on the critical hemostatic balance. In these conditions, 15–20% elevation of intrinsic FVII activity in plasma (Fig. 1A), because of the continuous induction of endogenous FVII by ribavirin (Fig. 1B), would contribute to the prevention from spontaneous bleeding in hemophiliacs. As a half-life of FVII in plasma is the shortest in all of coagulation factors, the continuous induction of FVII can maintain or increase the hemostatic value *in vivo*. If the prophylaxis to bleeding in hemophilic patients by ribavirin treatment were executable, it would result in much improvement of quality of their life and in large reduction of medical expenses in the country.

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Urinary β_2-Microglobulin as a Possible Sensitive Marker for Renal Injury Caused by Tenofovir Disoproxil Fumarate

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ABSTRACT

Tenofovir disoproxil fumarate (TDF) is renally excreted by a combination of glomerular filtration and active tubular secretion, and its renal safety profiles have been reported based on a limited increase of serum creatinine (sCr) levels. However, renal tubular function has not previously been well monitored. We measured sCr and urinary β_2-microglobulin (U-β_2MG) levels cross-sectionally in 70 patients treated with TDF [TDF(+)] and 90 patients on other antiretroviral therapy who had never been exposed to TDF [TDF(−)]. The mean U-β_2MG was significantly higher in TDF(+) patients than that in TDF(−) patients ($p < 0.0001$), though no statistical difference was detected in their creatinine clearance estimated by using the Cockcroft–Gault equation. Multivariate analysis showed that coadministration of boosted lopinavir (LPV) and patients' body weight were associated with U-β_2MG levels in TDF(+) patients. U-β_2MG levels were significantly higher in those who also received boosted LPV [TDF(+)LPV(+)] ($p = 0.0007$), and abnormally high levels were noted in 67.7% of them. Furthermore, in the TDF(+)LPV(+) group, U-β_2MG levels showed significant negative correlation with patients' body weight ($p = 0.0029$) and abnormal U-β_2MG was observed in all six patients with body weight less than 55 kg. In four patients, a rapid fall in U-β_2MG occurred after cessation of TDF. Relative to sCr, U-β_2MG could be a more sensitive marker of renal tubular injury caused by TDF. Boosted LPV co-administration and low body weight may be risk factors for TDF-induced renal tubular dysfunction, probably because these factors are associated with an increase in TDF concentration.

INTRODUCTION

TENOFOVIR DISOPROXIL FUMARATE (TDF) is an orally bioavailable ester prodrug of tenofovir, an acyclic nucleotide analogue with activity against HIV-1, HIV-2, and hepatitis B virus. TDF is administered once daily in HIV treatment and a combination formula with emtricitabine is currently available. TDF does not have high mitochondrial toxicity compared with stavudine[1,2] and it does not induce a systemic hypersensitivity reaction like abacavir.[3] TDF is currently being prescribed for a growing number of HIV-infected patients. One concern regarding use of TDF is its renal toxicity. Several studies showed a limited incidence of renal dysfunction based on the monitoring of serum creatinine (sCr).[4–7] Some cases of TDF-related renal impairment have occurred in patients with underlying systemic or renal diseases.[8–11] However, the majority of the cases of TDF-related renal dysfunction have occurred in patients without any identified risk factor.[12–15] Therefore, careful monitoring of renal function is necessary for the follow-up of TDF-treated patients.

TDF is excreted unchanged in the urine via a combination of glomerular filtration and active tubular secretion.[16] Proximal renal tubular dysfunction and Fanconi's syndrome have been reported to be associated with TDF usage.[10–15,17–19] β_2-Microglobulin is commonly measured in urine by enzyme-linked immunosorbent assay (ELISA) or latex agglutination assay. It is freely filtered at the glomerulus and is avidly taken up and catabolized by the proximal renal tubules. Therefore, high levels of urinary β_2-microglobulin (U-β_2MG) are associated with various pathological conditions involving the proximal renal tubule.[20] In the present study, we compared U-β_2MG in TDF-treated patients and those on other antiretroviral treatment who had never been exposed to TDF, and assessed the suitability of U-β_2MG as a sensitive marker of TDF-induced proximal renal

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tubular injury compared with creatinine clearance (CrCl) estimated by using the Cockcroft–Gault equation.

MATERIALS AND METHODS

Patients

Between February 2004 and June 2005, U-β_2MG was measured cross-sectionally in 70 TDF-treated patients and 90 patients on other antiretroviral treatments who had never been exposed to TDF in the outpatient clinic of the AIDS Clinical Center, International Medical Center of Japan. The U-β_2MG levels were determined by latex agglutination assay. The normal range of U-β_2MG was <500 μg/liter, determined by the analysis of more than 100 healthy volunteers. A signed consent form was obtained from each participant of this study.

Statistical analysis

The sCr concentrations, body weight, CD4 count, HIV-1 viral load measured on the same day with U-β_2MG, and duration of antiretroviral treatment were also compared. U-β_2MG was analyzed logarithmically because U-β_2MG levels change logarithmically in the cases of renal tubular dysfunction and logarithmic analysis compensated the skewed distribution of U-β_2MG levels in the patients who had never been exposed to TDF [distribution skewness: U-β_2MG, 2.971; log(U-β_2MG), 0.412]. CrCl was calculated using the Cockcroft–Gault equation, which estimates CrCl on the basis of sCr level, body weight, and sex of the patient.[21] All data were expressed as mean ± SD. Differences between groups were examined for statistical significance using the Student's *t*-test. Correlations between values were examined using the Pearson's correlation coefficient and the Fisher's *z* transformation. Multivariate least-squares linear regression was used to assess the associations of multiple factors with high U-β_2MG level. A *p* value less than 0.05 denoted the presence of statistical significance. Statistical analysis was performed using StatView software (SAS Institute).

RESULTS

Patients

Between February 2004 and June 2005, U-β_2MG was measured in 70 TDF-treated patients [TDF(+) group] and 90 patients on other antiretroviral therapy who had never been exposed to TDF [TDF(−) group]. No enrolled patient was taking renal toxic drugs such as ganciclovir or adefovir. In both groups, the HIV-1 RNA loads were less than 400 copies/ml in more than 90% of the patients, suggesting that most patients maintained excellent adherence (Table 1). Most of the analyzed patients were Asian, and around 90% were males. There were no significant differences in age, body weight, CD4 cell count, sCr level, and duration of antiretroviral treatment between TDF(−) and TDF(+) groups.

High U-β_2MG in TDF-treated patients

U-β_2MG was measured at least 1 month after the introduction of TDF treatment in TDF(+) patients and the introduction of antiretroviral therapy in TDF(−) patients. TDF(+) patients had significantly higher logarithmic values of U-β_2MG than TDF(−) patients [2.79 ± 0.85 vs. 2.09 ± 0.43 (log (μg/liter)), $p < 0.0001$], though there was no significant difference in sCr (0.76 ± 0.18 vs. 0.76 ± 0.15 mg/dl) and in estimated CrCl (114.2 ± 34.3 vs. 120.0 ± 29.8 ml/min) between the two groups (Fig. 1A–C). Thirty of 70 (42.9%) TDF(+) patients had abnormally high U-β_2MG levels (>500 μg/liter), although abnormal sCr (>1.1 mg/dl) and abnormal CrCl (<90 ml/min) was observed in only 3 and 11 of them, respectively. There was no significant relation between the duration of TDF treatment and U-β_2MG values in the TDF(+) group. Six patients had abnormally high U-β_2MG values within 3 months after the initiation of TDF treatment. In the TDF(−) group, logarithmic U-β_2MG values showed a normal distribution and abnormally high U-β_2MG values were observed in only 7 of 90 (7.8%). Eleven of the TDF(−) patients had abnormal CrCl and only one TDF(−) patient with compromised CrCl showed abnormal U-β_2MG values. There was a significant negative correlation between CrCl

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF ENROLLED PATIENTS

Characteristics	*TDF (−) (n = 90)*	*TDF (+) (n = 70)*
Sex, no. (%) male	83 (92.2)	61 (87.1)
Ethnicity, no. (%)		
Asian	84 (93.3)	69 (98.6)
White	1 (1.1)	1 (1.4)
African	3 (3.3)	0 (0)
Half Hispanic half Asian	2 (2.2)	0 (0)
Age, mean ± SD (years)	40.4 ± 9.9	42.1 ± 12.3
Body weight, mean ± SD (kg)	64.3 ± 10.2	61.6 ± 9.1
CD4 cell count, mean ± SD (cells/mm^3)	461.9 ± 199.8	437.4 ± 224.0
HIV-1 RNA load, no. (%) <400 copies/ml	82 (91.1)	64 (91.4)
Serum creatinine level, mean ± SD (mg/dl)	0.76 ± 0.15	0.76 ± 0.18
Duration of ART,[a] mean ± SD (months)	40.2 ± 30.7	56.6 ± 30.8
Duration of TDF, mean ± SD (months)	Not applicable	13.0 ± 9.2

[a]ART, antiretroviral therapy.

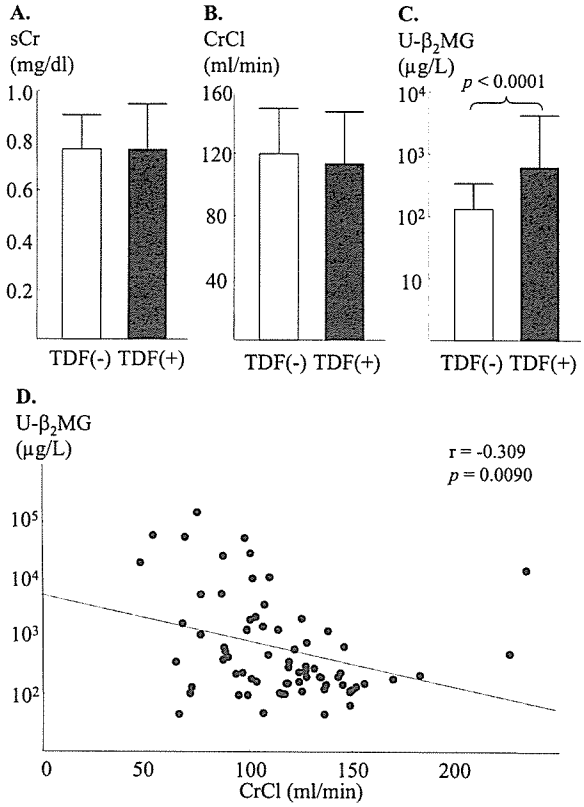

FIG. 1. Renal function of 90 TDF(−) and 70 TDF(+) patients. sCr (**A**), CrCl estimated by using Cockcroft–Gault formula (**B**), and U-β_2MG values (**C**) were compared between 90 TDF(−) (open bar) and 70 TDF(+) (closed bar) patients. Means and SDs are indicated with bars and horizontal lines, respectively. Logarithmic U-β_2MG values of 90 TDF(+) patients are plotted against CrCl estimated by using the Cockcroft–Gault formula (**D**). The regression line is also shown.

and U-β_2MG values in the TDF(+) group ($r = -0.309$, $p = 0.0090$) (Fig. 1D) though it was not significant in the TDF(−) group, which suggests renal insufficiency observed in TDF(+) patients was specifically associated with renal tubular damage.

Co-administration of boosted LPV as a risk factor

According to the results of a pharmacokinetic study,[22] TDF exposure was increased by 32% when administered with lopinavir (LPV)/ritonavir (RTV) therapy, compared with TDF monotherapy, which can be considered to accelerate TDF-induced renal injury. In order to analyze the effect of coadministration of RTV-boosted LPV on TDF-induced renal tubular damage, we classified TDF(+) patients into three subgroups by the usage of LPV and RTV, and compared their U-β_2MG values; those who did not receive LPV or RTV at the time of measurement of U-β_2MG [TDF(+)LPV(−)RTV(−) group, $n = 29$], those on RTV-boosted protease inhibitor-containing treatment other than LPV [TDF(+)LPV(−)RTV(+) group, $n = 10$], and those on the coadministration of RTV-boosted LPV [TDF(+)LPV(+)RTV(+), $n = 31$] (Fig. 2). There was no significant difference in U-β_2MG values between the TDF(+)LPV(−)RTV(−) group and the TDF(+)LPV(−)RTV(+) group [2.50 ± 0.78 vs. 2.49 ± 0.37 (log (μg/liter)), $p = 0.966$], suggesting RTV coadministration had little effect on TDF-induced renal injury. However, the U-β_2MG values of TDF(+)LPV(+)RTV(+) patients [3.17 ± 0.89 (log (μg/liter))] were significantly higher than those in the TDF(+)LPV(−)RTV(−) group ($p = 0.0032$) and those in the TDF(+)LPV(−)RTV(+) group ($p = 0.0257$), and abnormally high levels were noted in 67.7% of TDF(+)LPV(+)RTV(+) patients, indicating that boosted LPV cousage accelerated renal damage by TDF. There were no significant differences in CrCl among the three groups, suggesting that estimated CrCl is less sensitive in detecting TDF-induced renal injury than U-β_2MG.

Low body weight as a risk factor

When multivariate least-squares linear regression was used to assess the multiple factors including age, body weight, CD4 cell count, and TDF usage, TDF usage was a most significant factor associated with a high U-β_2MG value (partial regression coefficient = 0.477, $F = 46.7$). In order to identify other risk factors associated with TDF-induced renal injury, the multiple factors including age, body weight, CD4 cell count, duration of TDF treatment, and boosted LPV usage were assessed in TDF(+) patients. Boosted LPV usage had the greatest positive impact on the U-β_2MG value (partial regression coefficient = 0.394, $F = 12.5$) and patients' body weight had the second greatest impact though it was negative (partial regression coefficient = −0.305, $F = 6.97$) (Table 2). In order to confirm the effect of patients' body weight, U-β_2MG values were plotted against patients' body weight. In the TDF(+)LPV(+)RTV(+)

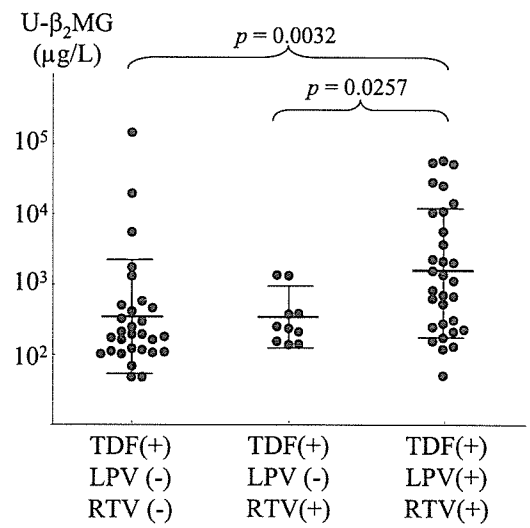

FIG. 2. U-β_2MG levels of TDF-treated patients and LPV and RTV usage. U-β_2MG values were compared among three subgroups of TDF(+) patients divided by usage of LPV and RTV; 29 TDF(+)LPV(−)RTV(−) patients, 10 TDF(+)LPV(−)RTV(+) patients, and 31 TDF(+)LPV(+)RTV(+) patients. Logarithmic means and SDs are indicated with bold and thin horizontal lines, respectively.

TABLE 2. FACTORS ASSOCIATED WITH U-β_2MG LEVEL IN TDF-TREATED PATIENTS

Factor	*Partial regression coefficient*	F *value*
Age (years)	0.131	1.19
Body weight (kg)	−0.305	6.97
CD4 cell count (cells/mm^3)	−0.237	4.03
Duration of TDF (months)	0.208	3.06
LPV usage	0.394	12.5

group, U-β_2MG levels showed significant negative correlation with patients' body weight ($r = -0.511$, $p = 0.0029$) and abnormal U-β_2MG was observed in all six patients with body weight less than 55 kg (Fig. 3). In the TDF(+)LPV(−) group including TDF(+)LPV(−)RTV(−) patients and TDF(+)LPV(−)RTV(+) patients, patients with lower body weight also tended to have higher U-β_2MG values, albeit statistically insignificant ($r = -0.151$, $p = 0.361$) (data not shown). When estimated CrCl was plotted against patients' body weight, CrCl showed significant positive correlation with patients' body weight in the TDF(+)LPV(−) group ($r = 0.467$, $p = 0.0024$), and patients with lower body weight also tended to have lower CrCl in the TDF(+)LPV(+) group, albeit statistically insignificant ($r = 0.181$, $p = 0.334$) (data not shown).

Rapid fall in U-β_2MG after cessation of TDF

Renal injury induced by TDF has been reported to be reversible after cessation of TDF.[10–15,17,18] To confirm this, we examined changes in U-β_2MG over time after switching from TDF to other antiretroviral agents. During this study period, TDF was switched to other agents in four cases that had abnormally high U-β_2MG levels before switching the treatment regimen. The U-β_2MG levels were substantially decreased by 0.86–2.15 log at 5–8 months after switching treatment, and in one case the level was normalized (<500 μg/liter). Thus, as reported by other investigators,[10–15,17,18] TDF-induced renal injury seems reversible, as evident by changes in U-β_2MG values.

DISCUSSION

Renal safety of TDF has been reported based on minimal change in sCr within the normal range during TDF treatment.[4–7] However, we found that 30 of 70 TDF-treated patients had abnormally high U-β_2MG while only 3 of them had abnormal sCr and only 11 had abnormal CrCl, suggesting that the incidence of renal tubular injury in TDF-treated patients is larger than previously estimated, and that U-β_2MG can be used as a more sensitive marker of TDF-induced renal injury than sCr.

U-β_2MG levels have been reported to be reproducible in urine samples collected from the same subjects on multiple occasions,[23] and its measurement is considered a useful tool for noninvasive monitoring of the renal allograft after kidney transplantation.[24] Measurement of urinary protein may be also helpful for monitoring TDF-induced renal injury, though its low specificity might be problematic because not only renal tubular injury but also glomerular damage such as diabetic nephropathy could increase protein excretion in the urine.[20] Furthermore, it was reported that proteinuria is often observed in HIV-infected patients on initial evaluation.[25,26] Therefore, monitoring markers specific to renal tubular injury, such as U-β_2MG, may be useful for HIV-infected patients treated with TDF.

Our results also showed that boosted LPV-containing treatment and low body weight were associated with high U-β_2MG in TDF-treated patients. Surprisingly, all TDF- and boosted LPV-treated patients with body weight <55 kg had abnormally high U-β_2MG. It is possible that boosted LPV usage and low body weight accelerate TDF-induced renal injury by increasing TDF plasma concentrations. In fact, many of the reported cases of TDF-induced renal injury have been in those treated with boosted LPV-containing regimens,[10,12–15,17] and Peyriere *et al.*[10] reported seven cases of TDF-induced renal tubular dysfunction and 6 of them had a low body weight (<60 kg). Patients with low body weight have unusually low sCr levels because creatinine is mainly derived from muscle and lean patients have disproportionately less muscle mass.[27] Therefore, body weight should be taken into consideration when evaluating renal function with sCr values, but this may not be sufficient for full assessment. A simple renal tubular injury is often not associated with an increase in sCr,[20] and mild TDF tubular toxic effects may be missed by the simple and convenient approach of the formula-based estimation of ClCr based on sCr and patient demographics. Thus, sensitive markers, such as U-β_2MG values, may be of use in monitoring HIV-infected patients during TDF treatment, especially when boosted LPV is coadministered or the patient has low body weight.

Abnormally high U-β_2MG levels indicate renal tubular injury, though its clinical significance in TDF-treated patients is still unknown. Recently, Gallant *et al.*[5] reported that TDF treat-

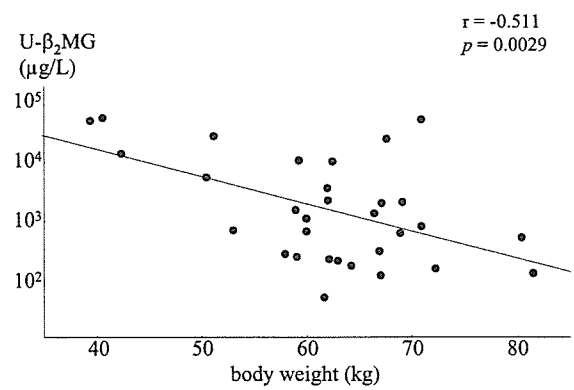

FIG. 3. U-β_2MG values and estimated CrCl in TDF-treated patients. U-β_2MG values of 31 TDF(+)LPV(+) patients are plotted against patients' body weight. The regression line is also shown.

ment causes a mild decrease of bone density but this was not associated with increased frequency of bone fractures. However, persistence of renal tubular injury in TDF-treated patients may result in significant mineral loss and osteoporosis. It was reported that bone deformities and/or fractures were found in 28% of the pediatric patients with hereditary renal tubular disorders during 10 years of observation.[28] Only a longer trial looking at outcomes of TDF-treated patients with high U-β_2MG levels would confirm its clinical significance. It is also possible that since most of our patients were Asians, TDF excretion might be different from that reported in other races. Our study is limited by its cross-sectional design and lack of TDF concentrations in the plasma. Prospective and longitudinal analysis of U-β_2MG value and TDF plasma concentration in a larger cohort is warranted.

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REVIEW

Living donor liver transplantation to patients with hepatitis C virus cirrhosis

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Abstract

Living donor liver transplantation (LDLT) is an alternative therapeutic option for patients with end-stage hepatitis C virus (HCV) cirrhosis because of the cadaveric organ shortage. HCV infection is now a leading indication for LDLT among adults worldwide, and there is a worse prognosis with HCV recurrence. The antivirus strategy after transplantation, however, is currently under debate. Recent updates on the clinical and therapeutic aspects of living donor liver transplantation for HCV are discussed in the present review.

Key words: Hepatitis C virus; Living donor liver transplantation; Interferon; Rivabirin

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INTRODUCTION

The use of live donors for liver transplantation was initiated more than a decade ago as a solution to the cadaveric donor shortage for pediatric recipients[1]. After the first successful case in an adult patient in 1994[2], this procedure is now widely applied to adult recipients, especially in countries where the availability of brain-dead donors is severely restricted[3] and also in the United States and European countries, due to a critical shortage of cadaveric organs. Improved surgical techniques and the introduction of new immunosuppressive agents have enhanced the long-term results of living donor liver transplantation (LDLT), leading to an increased demand for liver transplantation that exceeds the number of potential donor organs. In initial experiences with adult LDLT in Japan, the most common indication was cholestatic liver disease, including primary biliary cirrhosis and primary sclerosing cholangitis in Japan. The number of LDLT patients indicated for hepatitis C virus (HCV) has recently increased rapidly.

A recent study[4] of deceased donor liver transplantation (DDLT) reported that HCV infection was associated with a 23% increase in mortality and a 30% increase in the rate of graft failure. The poor results might be due to the recurrence of HCV disease in the graft[5]. HCV-induced graft hepatitis and fibrosis/cirrhosis occur in 75% to 80% and 10 % to 30% of recipients, respectively, at 5 years[6,7]. Once liver cirrhosis is established, the cumulative probability of developing clinical decompensation is close to 50% after 1 year and survival after decompensation is extremely short[8]. Cholestatic hepatitis occurs in approximately 10% of patients infected with HCV and leads to accelerated graft failure and death[9]. One of the hottest debates is the possibility of increased severity of recurrent HCV in LDLT patients. The benefit of LDLT might be offset if the outcome of LDLT for HCV patients is worse than that of DDLT. In this review, we describe current trends and controversies in LDLT for patients with HCV. Our results for LDLT and HCV are also reported.

CURRENT STATUS OF LDLT

According to the Japan Liver Transplantation Society[10], the number of adult patients (≥18 years old) is increasing annually, and has reached 300 in 2003. The most common indication for adults has been hepatocellular carcinoma (n = 311), followed by primary biliary cirrhosis (n = 255), and HCV-related cirrhosis without carcinoma (n = 113). The 1, 3, and 5 year survival rates of all the adult patients were 76%, 72%, and 69%, respectively. Those of HCV-positive patients were 76%, 73%, and 65%, respectively.

In the United States in 2000, there was a high level of enthusiasm for adult LDLT, with 49 centers performing at least one LDLT. Overall, in experienced centers, about a third of adults on the waiting list had a potential living donor and half of them had undergone LDLT; thus, LDLT might be applicable for up to 15% of individuals on the list[11]. The enthusiasm was, however, quickly tempered

Table 1 Comparison between LDLT and DDLT for hepatitis C virus cirrhosis

Study			N		Dif[1]	Protocol biopsy	Findings
Author	Year	Institution	LDLT	DDLT			
Gaglio[23]	2003	Colombia U.	23	45	Yes	No	Cholestatic hepatitis in 17% of LDLT and 0% of DDLT (P = 0.001). No significant difference in incidence of Rec.
Shiffman[28]	2003	Virginia Commonwealth U.	22	53	No	Yes	79% patient survival in LDLT and 91% in DDLT during 3 year (NS). No significant difference in inflammation score in liver specimen after 3 years
Russo[29]	2004	UNOS data	279	3955	No	No	87% 1-year patient survival in both.
Thuluvath[30]	2004	UNOS data	207	408	No	No	No significant difference in patient survival (P = 0.6).
Van Vlierberghe[32]	2004	Ghent U.	17	26	No	No	Rec in 35% of LDLT and 38% of DDLT during 1 year (P = 0.1)
Bozorgzadeh[34]	2004	Rochester U.	35	65	No	No	Rec in 77% of LDLT and 72% of DDLT during 1 year (NS), 89% patient survival in LDLT and 75% in DDLT during 39 mo (NS)

[1] Difference in short-term outcomes or severity of virus recurrence between living and deceased donor liver transplantation. Abbreviations: Rec, Virus recurrence; U, University; NS, not significant; UNOS, United Network for Organ Sharing.

by the death of a donor in 2002 in the United States[12]. Since 2001, the number of patients who have undergone LDLT has declined[13]. Currently less than 5% of all adult liver recipients use living donors. By July 2005, 2734 LDLT cases had been performed. There were 1761 adult patients and HCV was the most common indication. HCV is the most common indication for LDLT[14] and the number of HCV-positive patients is stable, approximately 100 per year between 2000 and 2002.

By the end of 2003, 1743 LDLT cases were recorded in the European Liver Transplantation Registry[15]. According to the Transplant Procurement Management[16], the number of LDLT peaked in 2003 and has gradually decreased over recent years. LDLT accounts for approximately 5% of the total liver transplants performed in Europe. Among the 806 LDLT cases from October 1991 to December 2001[17], the overall 5-year graft survival rate was 75%, better for children than for adults (80% *vs* 66% at 3 years). Cirrhosis secondary to HCV infection is a leading indication for LDLT among adults in Europe[18]. The number of LDLT patients is shown in the Table 1.

INDICATIONS

In areas with low deceased donor organ availability, the indications for LDLT are similar to those for DDLT. In contrast, in Western countries, LDLT is conducted in an attempt to alleviate the shortage of donor organs and to decrease the mortality among the patients awaiting transplants. That is, a balance needs to be achieved between the candidate's liver disease severity and the adequacy of a partial graft for transplantation. The candidate's liver disease should be advanced to the extent that transplantation is justified, but the liver disease cannot be so advanced that a partial graft will not provide adequate hepatic mass.

According to Russo's report[19] a substantial proportion of patients were United Network for Organ Sharing (UNOS) status 3 at the time of LDLT (43%). The policy at their centers prior to the implementation of a model for end stage liver disease (MELD)-based allocation was not to proceed with LDLT in patients meeting UNOS status 2A criteria. Their patient survival rate was 57% with an average stay of 23 d in the intensive care unit. In comparison, 1-year patient survival was 82% in DDLT recipients who were UNOS status 2A at the time of transplant[20].

The waiting list mortality increases in patients with advanced liver disease and patients with a MELD score of 25 have a 20% 3-mo mortality[21]. In general, it is uncommon to proceed with LDLT in patients with MELD scores above 25. Thus, depending on the region of the country and the average MELD score at the time of the transplant within the area served by the organ procurement organization, LDLT might offer patients transplantation before they die waiting for a deceased donor liver. The lower MELD score limit with LDLT is more controversial and varies from center to center. Russo[19] commented that they do not proceed with LDLT in candidates with MELD scores under 11.

LDLT AS A RISK FACTOR FOR RECURRENCE OF HCV

One study from Barcelona[22] reported that LDLT patients (n = 22) had younger donors, less graft steatosis, more frequent biliary complications, and earlier and more severe acute hepatitis compared with DDLT (n = 95) patients. A report from Colombia University[23] indicates that cholestatic hepatitis or severe HCV recurrence occurs more frequently in LDLT. These reports indicate that more intensive antiviral therapy might be necessary for recipients of living donor grafts.

The possible causes of HCV recurrence include HLA matching between donor and recipient. Because cellular immune reactions restricted by both HLA class I and II antigens are involved in the recognition of HCV peptides[24], HLA matching between donor and recipient could potentially increase damage to the graft from recurrent viral infections by facilitating host recognition of viral antigens[6]. Recently, a beneficial effect of a complete HLA-DQ mismatch was reported in 14 patients after transplantation for HCV cirrhosis[25]. Another possible cause might be related to liver regeneration[26], although recent data[27] did not support this hypothesis. *In vitro*, HCV internal ribosome entry site activity and replication are higher in actively dividing cells, and it is possible that

viral translation is enhanced by factors that stimulate the regeneration of hepatocytes. Moreover, there are experimental data suggesting that liver regeneration induces low density lipoprotein receptor expression, which might facilitate HCV entrance into the hepatocytes.

In contrast, comparable data between LDLT and DDLT for HCV was recently reported[28]. Russo and colleagues[29] compared patient and graft survival in recipients transplanted for chronic HCV who received a living donor organ (n = 279) and deceased donor organ (n = 3955) using the UNOS liver transplant database. One-year patient survival was 87% in both groups and 2-year patient survival was 83% and 81% in the living donor group and deceased donor group (P = 0.68), respectively. Similar results (DDLT, n = 480 *vs* LDLT, n = 207) were obtained from another analysis using the UNOS data base[30]. Analyses from the Mayo Clinic[31] and Gent University[32] also demonstrated no negative impacts of LDLT on the results of liver transplantation for HCV-related cirrhosis.

These data should be interpreted with caution, however, because of the important clinical distinction between LDLT and DDLT recipients. At the time of transplantation, the LDLT group recipients are far less sick than their DDLT group counterparts[33]. The LDLT (n = 35) and DDLT (n = 65) data from a single institution, Rochester University, were examined[34]. Patient survival, graft survival, rate of HCV recurrence, severity of HCV recurrence, graft loss from HCV, and interval for HCV recurrence in DDLT and LDLT were similar. It remains unclear, however, whether LDLT is truly disadvantageous compared to DDLT for HCV-positive patients because the number of cases or follow-up duration is not yet sufficient.

According to the data from Russo[29], from 1999 to 2000, the 1-year patient survival in the LDLT group increased from 69% to 90% (P = 0.04), and 1-year graft survival increased from 63% to 79% (P = 0.16). In contrast, in the DDLT group, 1-year patient and graft survival did not substantially change from 1999 to 2000. As a result, 1-year survival rates became similar between the LDLT and DDLT groups in 2000. The results indicated an experience effect and learning curve on outcomes after LDLT for HCV. Therefore, the initial reports indicating poorer results of LDLT might be due to technical problems from a lack of experience. Recent data indicating similar results between LDLT and DDLT might be due to the increased experience with LDLT. The multicenter adult to adult LDLT cohort study (A2ALL) might soon provide some answers to the questions about recurrent HCV after LDLT and DDLT[35].

MANAGEMENT OF HCV

Therapy for reccurrence in DDLT

If HCV recurs earlier and more severely after LDLT, a specific strategy for preventing the detrimental effects of HCV on living donor grafts must be developed. One strategy might be aggressive treatment for HCV. Treatment of recurrent HCV disease with interferon and ribavirin after DDLT is used in some centers[36-38]. One standard regimen includes interferon-alpha2b (3 MU × 3 per week) and ribavirin (1000 mg/d) for 6 mo. In a recent trial, polyethylene glycol-conjugated interferon therapy was used[35,39-44], with a sustained viral response rate ranging from 13% to 47%.

Preemptive therapy for HCV after DDLT

Preemptive therapy in the early post-transplantation period with interferon either alone or in combination with ribavirin has been attempted in DDLT, although its effectiveness is controversial. In one study, HCV-positive recipients were randomized within 2 wk of transplantation to receive either interferon alone (3 MU × 3 per wk, n = 30) or placebo (n = 41) for 1 year[39]. Only 17 patients could complete 1 year of interferon therapy. Eight patients (27%) in the interferon group and 22 (54%) of the untreated patients had recurrent hepatitis (P = 0.02). Patient and graft survival at 2 years did not differ between the groups, however, and the rate of viral persistence was not affected by treatment.

In another controlled trial[45], 24 recipients were randomized at 2 weeks post-transplantation to receive interferon (3 MU × 3 per wk) or placebo for 6 mo. There were no differences in graft or patient survival. There were no differences between groups in the incidence of histological recurrence or its severity differed between groups. Recurrent HCV was delayed 408 d in treated patients versus 193 d in the control cohort.

In a case series by Mazzaferro[46], 36 recipients were treated with interferon-alpha 2b (3 MU × 3 per wk) and ribavirin (10 mg/kg per d). They started treatment at a median of 18 d after the operation and treatment continued for 11 mo. After a median follow-up of 52 mo, the 5-year patient survival was 88%. Serum HCV RNA clearance was obtained in 12 patients (33%). They did not require further antiviral treatment because of negative HCV RNA in serum and normal liver histology for a median of an additional 36 mo. The former two randomized trials on preemptive interferon monotherapy demonstrated minimal benefits of the drug. In contrast, Mazzaferro reported more encouraging results, although their protocol brings into question how long therapy is needed once embarking on a preemptive strategy.

Re-transplantation

The approach to retransplantation for recurrent HCV varies widely among the transplant centers of DDLT[11]. The results after retransplantation for HCV (45% at 5 years) are poorer than that for other causes[47] (56%, P < 0.001). The patients with recurrent HCV in the early timing and graft failure within the first year have poor outcomes after retransplantation. These individuals should be considered contraindicated for retransplantation. The experience of retransplantation for HCV in LDLT has not been well accumulated.

OUR EXPERIENCE

We performed preemptive therapy for LDLT patients with HCV infection[40]. From 1996 to 2004, 67 patients underwent LDLT for HCV cirrhosis at the Tokyo University Hospital. The patients were 51 men and 16

women and their ages ranged from 23 to 63 years (median 55). The HCV genotype was 1b in 53 patients (79%). Forty-one patients (61%) had hepatocellular carcinoma. All the patients received the same immunosuppressive regimens with tacrolimus and methylprednisolone.

All the patients preemptively received antiviral therapy consisting of interferon α-2b and ribavirin, which was started approximately 1 mo after the operation. The therapy was continued for 12 mo after the first negative HCV RNA test. The standard regimen included interferon α-2b (3 MU × 3 per wk) and ribavirin (800 mg/d) for 6 mo. The patients were then observed without the therapy for 6 mo. The therapy was continued for at least 12 mo even if the HCV RNA test remained positive.

Therapy was discontinued when there was significant leukopenia (< 1500 /mL), thrombocytopenia (< 50000 / mL) despite application of granulocyte colony stimulating factor (Gran®, Sankyo, Co. Ltd., Tokyo, Japan), hemolytic anemia (hemoglobin < 8 g/L), renal dysfunction (serum creatinine > 20 mg/L), depressive psychological status, or general fatigue. The subjects were removed from the protocol if they did not continue the therapy for 12 mo due to adverse effects or could not start the therapy due to early death.

Blood counts and liver function tests were checked every 2 wk for the first month, and at 4 wk intervals thereafter. Serum samples were collected once a month for quantitative HCV RNA detection. Protocol liver biopsy was not performed. The log-rank test was used to compare the survival rate of the HCV-positive patients with the HCV-negative patients who underwent transplantation during the same period (n = 168).

A total of 28 patients were excluded from the analysis; 12 patients were removed from the protocol because of early death (n = 9) or because of drug cessation (n = 3). Another 16 patients are currently on the protocol and were therefore excluded from the analysis. Of the remaining 39 patients, 16 (16/39; 41%) obtained a sustained virologic response. The cumulative 5-year survival of the HCV-positive patients was 84%, comparable with that of patients negative for HCV (n = 168, 86%).

CONCLUSIONS

LDLT will remain an indispensable therapeutic tool for HCV related end stage liver disease and an alternative to DDLT. The association between LDLT and early HCV recurrence remains to be determined, although most of the recent papers suggest that live donor graft has no effect on short-term outcome or severity of virus recurrence. If living donor graft is associated with early HCV recurrence and consequently poorer graft survival, an aggressive antiviral protocol might improve the outcome of LDLT for HCV.

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Cyclosporin A for Treatment of Hepatitis C Virus After Liver Transplantation

Cirrhosis secondary to hepatitis C virus (HCV) infection is a leading indication for liver transplantation. HCV recurrence, however, is nearly certain and might worsen patient/graft outcome. A recent paper (*1*) reported that HCV infection is associated with a 23% increase in mortality and a 30% increase in the graft failure rate. The goal of HCV management in the transplantation setting is to prevent graft loss due to recurrent HCV infection, raising questions about a possible role for immunosuppression regimens and antiviral therapy.

A previous report (*2*) indicated that cyclosporin A (CyA) and interferon-alpha2b might effectively inhibit HCV replication in vitro. The antiviral effects of CyA for patients with chronic HCV (*3*) and those for HCV recurrence after transplantation (*4*), however, are controversial. We conducted a pilot study of the use of CyA, interferon, and ribavirin for preemptive therapy of HCV after liver transplantation.

Until October 2003, 41 HCV-positive patients underwent liver transplantation from living donors at the University of Tokyo Hospital. The immunosuppression regimens consisted of steroids and tacrolimus (*5*). The targeted whole-blood tacrolimus level was 15 to 20 ng/ml during the seven days after living donor liver transplantation (LDLT), which was gradually tapered to 5 ng/ml six months after LDLT. Predonisolone was tapered to 0.05 mg/day/kg six months after LDLT but was not stopped. All of the HCV-positive patients preemptively received interferon-alpha2b and ribavirin therapy (*6*), which was started approximately one month after transplantation. HCV RNA level was measured by real-time-polymerase chain reaction (*7*) and Amplicor HCV (Roche Molecular *Systems, Pleasanton*, USA). Of the 41 patients, six died within two years; 14 obtained a viral response (<50 IU/ml by Amplicor HCV) at the end of the treatment period (one year) and the response was sustained for another six months without the antiviral therapy; 21 did not respond to the antiviral therapy. Of the 21 nonresponders, eight patients continued with the protocol, which called for a change from tacrolimus to CyA without changing the antiviral therapy. The targeted CyA trough level was 100 ng/ml.

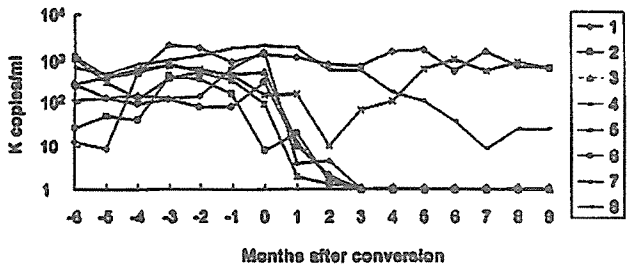

FIGURE 1. Change of the viral titer in the eight patients.

All of the patients were genotype 1b. The serum HCV titer was measured once a month by reverse transcriptase–polymerase chain reaction. The Institution Review Boards at University of Tokyo Hospital approved the protocol.

In five of the eight subjects (63%), the HCV titer was negative by Amplicor HCV within three months after the conversion and remained negative for another six months on CyA, interferon, and ribavirin (Figure 1). Liver and renal functions remained stable in all of the patients, and none of them had complications of acute cellular rejection after the conversion.

Our findings support the use of CyA in combination with interfron and rivabirin for the eradication of HCV in previous nonresponders. These findings suggest that a controlled study to confirm the benefit of CyA for preemptive treatment of HCV after liver transplantation is warranted.

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Influence of Genotypes and Precore Mutations on Fulminant or Chronic Outcome of Acute Hepatitis B Virus Infection

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The outcome of acute hepatitis B virus (HBV) infection is variable, influenced by host and viral factors. From 1982 through 2004, 301 patients with acute HBV infection entered a multi-center cross-sectional study in Japan. Patients with fulminant hepatitis (n = 40) were older (44.7 ± 16.3 vs. 36.0 ± 14.3 years, $P < .0017$), less predominantly male (43% vs. 71%, $P = .0005$), less positive for hepatitis B e antigen (HBeAg) (23% vs. 60%, $P < .0001$), less infected with subgenotype Ae (0% vs. 13%, $P < .05$), and more frequently with Bj (30% vs. 4%, $P < .0001$) than those with acute self-limited hepatitis (n = 261). Precore (G1896A) and core-promoter (A1762T/G1764A) mutations were more frequent in patients with fulminant than acute self-limited hepatitis (53% vs. 9% and 50% vs. 17%, $P < .0001$ for both). HBV infection persisted in only three (1%) patients, and they represented 2 of the 23 infected with Ae and 1 of the 187 with the other subgenotypes (9% vs. 0.5%, $P = .032$); none of them received antiviral therapy. In multivariate analysis, age 34 years or older, Bj, HBeAg-negative, total bilirubin 10.0 mg/dL or greater, and G1896A mutation were independently associated with the fulminant outcome. In *in vitro* transfection experiments, the replication of Bj clone was markedly enhanced by introducing either G1896A or A1762T/G1764A mutation. In conclusion, persistence of HBV was rare (1%) and associated with Ae, whereas fulminant hepatitis was frequent (13%) and associated with Bj and lack of HBeAg as well as high replication due to precore mutation in patients with acute HBV infection. *Supplementary material for this article can be found on the HEPATOLOGY website (http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html).* (HEPATOLOGY 2006; 44:326-334.)

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBc, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; EIA, enzyme immunoassay; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; ALT, alanine aminotransferase.

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The nucleotide sequences of HBV DNA isolates used in this study have been deposited in the international DNA database under accession numbers AB249373-AB249636.

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Approximately 3 billion people, one half of the world population, have been exposed to hepatitis B virus (HBV), of whom approximately 350 million are persistently infected with it.[1] Acute infection with HBV resolves in the great majority but can induce fulminant hepatitis or go on to become chronic. Host and viral factors may influence fulminant or chronic outcome of acute HBV infection, but they are not fully defined.

Eight genotypes have been detected by a sequence divergence greater than 8% in the entire HBV genome of approximately 3,200 nucleotides (nt), and designated by capital alphabet letters from A (HBV/A) to H in the order of documentation.[2-5] They have distinct geographical distributions associated with severity of liver disease as well as response to antiviral therapies.[6-8] Furthermore, subgenotypes have been reported for HBV/A, B, and C and named Aa/A1 (Asian/African type) and Ae/A2 (European type),[9] Bj/B1 (Japanese type) and Ba/B2 (Asian type),[10] as well as Cs/C1 (Southeast Asian type) and Ce/C2 (East Asian type).[11-13] Increasing lines of evidence indicate that subgenotypes of HBV/A and B influence the replication of HBV and bear clinical relevance.[14-16] Furthermore, genotypes affect mutations in precore region and core promoter, thereby influencing the expression of hepatitis B e antigen (HBeAg).[8,17]

During the 23 years from 1982 to 2004, a multi-center cross-sectional study was conducted throughout Japan on 301 patients with acute hepatitis B. We examined the influence of genotypes/subgenotypes on their fulminant or chronic outcome. Furthermore, the influence of G1896A or A1762T/G1764A on replication of HBV was evaluated in an *in vitro* replication model.

Patients and Methods

Patients With Acute Hepatitis B. During 1982 through 2004, 336 consecutive cases of acute hepatitis B were registered in 16 hospitals throughout Japan. These hospitals were from the following eight areas: Hokkaido (represented by J.-H. K. and S.H.), Tohoku (T.K. and K.S.), Kanto (H.T., Y.A. and K.I.), Koshin (E.T. and S.O), Tokai (A.O., Y.T., E.O., M.S., R.U., M.M., and S.K.), Kinki (T.O.), Honshu/Shikoku (Y.M., K.H., and M.O.), and Kyushu (H.Y. and H.S.). The diagnosis of acute hepatitis B was contingent on a sudden onset of clinical symptoms of hepatitis and detection of high-titered antibody to hepatitis B core antigen (anti-HBc) of IgM class in serum. Patients with initial high-titered anti-HBc (≥90% inhibition by a 1:200 diluted serum) were excluded; they were diagnosed as exacerbation of chronic hepatitis B. Patients with acute hepatitis A, hepatitis C, or human immunodeficiency virus co-infection, and drug- or alcohol-induced acute hepatitis also were excluded; hepatitis D virus infection was not examined because of its extreme rarity in Japan.[18] Most of them were followed for clinical outcomes until the disappearance of hepatitis B surface antigen (HBsAg) during 24 weeks or longer after the presentation. The criteria of fulminant hepatitis are based on the report by Trey et al.,[19] with a slight modification in 1981 (Inuyama symposium, Aichi, Japan): coma of grade II or higher and prothrombin time less than 40% developing within 8 weeks after the onset. Serum samples were collected at the presentation and had been stored at −80°C. HBV genotypes, HBV DNA, and HBeAg were determined, and clinical outcomes of acute hepatitis were analyzed. The study protocol conformed to the 1975 Declaration of Helsinki, and was approved by the Ethics Committees of the institutions. Every patient gave an informed consent for this study.

Serological Markers of HBV Infection. HBsAg was determined by hemagglutination (MyCell; Institute of Immunology Co., Ltd., Tokyo, Japan) or enzyme immunoassay (EIA) (Axsym; Abbott Japan, Tokyo, Japan), and HBeAg by enzyme-linked immunosorbent assay (F-HBe; Kokusai Diagnostic, Kobe, Japan) or chemiluminescent EIA (Fujirebio Inc., Tokyo, Japan). Anti-HBc of IgM and IgG classes were determined by radioimmunoassay (Abbott Japan).

Genotypes and Subgenotypes of HBV. The six major HBV genotypes (A-F) were determined serologically by EIA using commercial kits (HBV GENOTYPE EIA; Institute of Immunology). The method depends on the combination of epitopes on preS2-region products detected by monoclonal antibodies, which is specific for each of them.[20] HBV/G was determined by a slight modification of the polymerase chain reaction (PCR) with specific primers.[21]

Subgenotypes of HBV/A designated Ae prevalent in Europe and Aa frequent in Africa as well as Asia,9 which corresponds to subgroup A′ originally reported by Bowyer et al.,[22] were determined by PCR restriction fragment length polymorphism (RFLP) involving nucleotide conversions in an immediate upstream of the precore region that are specific for each of them.[16,23] HBV/Bj (Japanese type) lacking the recombination with C over the precore region and the core gene and Ba (Asian type) with the recombination were determined by its absence or presence on HBV DNA sequences, as well as RFLP based on specific nucleotide substitutions, after the methods described previously.[15,24]

Subgenotypes of HBV/C, Cs (Southeast Asian type) found only in Southeast Asia, including Vietnam, Myanmar, Thailand, Laos, Bangladesh, Hong Kong, and Southern China, and Ce (East Asian type), found in Far

East Asia, including Japan, Korea, and Northern China, were determined by the PCR-RFLP method described previously.[12]

Quantification of HBV DNA and Sequencing. HBV DNA sequences spanning the S gene were determined by real-time detection PCR according to the method of Abe et al.,[25] with the detection limit of 100 copies/mL. HBV DNA sequences bearing core promoter, precore region, and the core gene were amplified by PCR with hemi-nested primers by the method described previously.[10] Negative samples were tested by another more sensitive second-round PCR with HB7F and HBV1917R (5′-CTC CAC AGT AGC TCC AAA TTC TTT A-3′). Thereafter, PCR products were directly sequenced with Prism Big Dye (Applied Biosystems, Foster City, CA) in the ABI 3100 DNA automated sequencer.

Construction of Plasmid and Site-Directed Mutagenesis of HBV DNA. Serum samples were obtained from two patients infected with HBV/Bj and a patient with Ce. HBV DNA was extracted from 100 μL serum using QIAamp DNA blood kit (QIAGEN, GmbH, Hilden, Germany). Four primer sets were designed to amplify two fragments covering the entire HBV genome. Amplified fragments were inserted into pGEM-T Easy Vector (Promega, Madison, WI) and cloned in DH5a competent cells (TOYOBO, Osaka, Japan). At least five clones of each fragment were sequenced and the consensus sequence determined. Among them, those containing the consensus sequence were identified and adopted as templates for further construction. Finally, 1.24-fold the HBV genome (nt 1413-3215/1-2185), just enough to transcribe oversized pregenome and precore mRNA, was constructed into pUC19 vector (Invitrogen Corp., Carlsbad, CA). For site-directed mutagenesis, the wild-type HBV was digested by *HindIII* and *EcoO65I* and ligated with the fragment carrying T1762/A1764 to produce 1.24-fold the genome carrying the core-promoter double mutation. Similarly, 1.24-fold the HBV genome with the precore stop-codon mutation (1896A) was generated. Further details are available online at: http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html.

Cell Culture and DNA Transfection. For the standard replication assay, 10-cm-diameter dishes were seeded with 1×10^6 Huh7 cells each. After 16 hours of culture, cells were transfected with 5 μg DNA construct using the FuGENE 6 transfection reagent (Roche Diagnostics, Indianapolis, IN) and harvested 3 days later. Transfection efficiency was measured by cotransfection with 1 μg reporter plasmid expressing secreted alkaline phosphatase and estimating its enzymatic activity in the culture supernatant.

Southern Blot Hybridization. HBV DNA samples from cells at day 3 in culture were separated on 1.2% (wt/vol) agarose gel, transferred to a positive-charged nylon membrane (Roche Diagnostics), and hybridized with full-length HBV DNA labeled with alkaline phosphatase. Detection was performed with CDP-star (Amersham Biosciences, Piscataway, NJ), and signals were analyzed in the LAS-1000 image analyzer (Fuji Photo Film, Tokyo, Japan).

Statistical Analysis. Categorical variables were compared between groups by the chi-squared test and noncategorical variables by the Mann-Whitney *U*-test. A *P* value less than .05 was considered significant. Multivariate analyses with logistic regression were used to determine independent factors for fulminant hepatitis. STATA Software (StataCorp LP, College Station, TX) version 8.0 was employed for analyses.

Results

Demographic and Clinical Differences in Patients Infected With Various HBV Genotypes/Subgenotypes. Genotypes of HBV were not classifiable in 28 (8%), and sufficient clinical data were not available in 7 (2%) of the 336 patients with acute hepatitis B. Exclusive of these 35 patients, 301 (90%) were left for evaluation of HBV genotypes in reference to clinical outcome.

HBV genotypes/subgenotypes were Aa in 10 (3%), Ae in 33 (11%), Ba in 22 (7%), Bj in 22 (7%), Cs in 11 (4%), Ce in 192 (64%), D in 5 (2%), and G in 6 (2%); none of them were infected with F or H (Table 1). All six patients with HBV/G were co-infected with another genotype; Ae in two, Ba in two, and Ce in the remaining two. The mean age was lower in the patients with HBV/Ae than Ba ($P = .0001$), Aa ($P < .01$), Bj or Cs ($P < .05$ for each) and Ce than Ba ($P < .05$). Men predominated in HBV infections with foreign (Ae and Ba) compared with domestic genotypes (Bj and Ce) ($P < .05$).

HBeAg was detected in 79% of patients with HBV/Ae at a frequency much higher than that with Bj ($P < .005$), Ce ($P < .001$) or Ba ($P < .05$). HBeAg in four of the six (67%) patients with HBV/G was coded for by HBV of the other genotypes co-infecting them, because it has two stop codons and an insertion in the core gene that prohibit encoding HBeAg.[21] HBV DNA levels as well as HBeAg-positive rates at the presentation were higher in HBV/Ae than Ce ($P < .005$) or Bj ($P < .05$) infection.

The peak alanine aminotransferase (ALT) level was higher in HBV/Bj than Ae infection ($P < .05$). Fulminant hepatitis was significantly more frequent in patients infected with HBV/Bj (55%) than the other genotypes ($P < .05$); it occurred in two of the five (40%) patients with HBV/D, also. In reflection of severe clinical course,

Table 1. Clinical Characteristics of Patients Acutely Infected With HBV of Distinct Genotypes/Subgenotypes

Features	Genotypes/Subgenotypes							
	Aa (n = 10)	Ae (n = 33)	Ba (n = 22)	Bj (n = 22)	Cs (n = 11)	Ce (n = 192)	D[a] (n = 5)	G[a,b] (n = 6)
Age (years)	42.2 ± 13.1	31.2 ± 10.3[d]	41.5 ± 10.7[e]	43.5 ± 19.1	38.5 ± 11.1	36.3 ± 15.0	38.6 ± 20.8	42.7 ± 17.5
Men	8 (80%)	30 (91%)[f]	19 (86%)[g]	9 (41%)	7 (64%)	122 (64%)	2 (40%)	6 (100%)
HBeAg positive	7 (70%)	26 (79%)[h]	11 (50%)	8 (36%)	8 (73%)	101 (53%)	1 (20%)	4 (67%)
ALT (IU/L)	1875 ± 759	2070 ± 1113[i]	2523 ± 1185	3472 ± 2720	2269 ± 995	2610 ± 1719	2559 ± 1672	2142 ± 722
Duration of elevated ALT (weeks)[c]	7.9 ± 5.8	9.5 ± 6.2	8.8 ± 3.7[j]	6.0 ± 2.5	10.1 ± 7.5	7.7 ± 5.1	5.7 ± 2.1	9.8 ± 1.5
Total bilirubin (mg/dL)	14.1 ± 10.3	9.0 ± 7.2	9.3 ± 5.9	10.9 ± 9.0	11.0 ± 13.8	9.8 ± 10.7	8.2 ± 2.2	13.0 ± 7.8
HBV DNA (log copies/mL)								
Median	4.76	6.08[k]	5.15	4.93	5.61	4.94	5.91	5.97
(range)	(2.90-8.08)	(2.00-8.46)	(2.00-8.19)	(2.00-8.44)	(2.00-8.50)	(2.00-9.06)	(2.00-8.37)	(3.35-7.11)
<2.00 (undetectable)	0 (0%)	1 (3%)	2 (9%)	3 (14%)	2 (18%)	28 (15%)	1 (20%)	0 (0%)
Medication with								
Lamivudine	1 (10%)	9 (27%)	2 (9%)	5 (23%)	2 (18%)	28 (15%)	4 (80%)	2 (33%)
Steroid	0	3 (9%)	0	5 (23%)	1 (9%)	16 (8%)	0	0

[a]Patients with HBV genotype D or G were not included in the analysis.
[b]All patients with HBV genotype G were co-infected with HBV of another genotype; Ae in two, Ba in two, and Ce in two.
[c]Exclusive of the 16 patients who died of fulminant hepatitis, 3 receiving liver transplantation and 10 without clinical data available.
[d]$P = .0001$, Ae vs. Ba. $P < .01$, Ae vs. Aa. $P < .05$, Ae vs. Bj or Cs.
[e]$P < .05$, Ba vs. Ce.
[f]$P = .0001$, Ae vs. Bj. $P < .005$, Ae vs. Ce.
[g]$P < .005$, Ba vs. Bj. $P < .05$, Ba vs. Ce.
[h]$P < .005$, Ae vs. Bj. $P < .01$, Ae vs. Ce. $P < .05$, Ae vs. Ba.
[i]$P < .05$, Ae vs. Bj.
[j]$P < .01$, Ba vs. Bj. $P < .05$, Ba vs. Ce.
[k]$P < .005$, Ae vs. Ce. $P < .05$, Ae vs. Bj.

the peak ALT level tended to be high in patients with HBV/Bj.

Presumed infection routes of 301 patients were sexual transmission in 172 (57%), blood transfusion in 4 (1%), medical accidents in 17 (6%), and unknown in the remaining 108 (36%).

Clinical Outcome of Patients With Acute Hepatitis B. Fulminant hepatitis developed in 40 (13%) patients. To cope with severe acute liver disease, lamivudine and steroid were administered to 53 (18%) and 25 (8%) patients, respectively. Fulminant hepatitis led to death in 16 (5%) patients, and three (1%) received liver transplantation. Exclusive of the 40 patients with fulminant hepatitis who received various treatments and five without clinical data, 256 (85%) were followed for the chronic outcome (Fig. 1). Serum ALT levels stayed elevated for longer than 24 weeks for the diagnosis of chronic hepatitis in eight (3%) of them. Among them, five had cleared HBsAg from serum until then, and therefore, their liver function abnormality was not attributed to persistent HBV infection. Table 2 summarizes persistence of HBV infection in the 256 patients with acute hepatitis; 253 (99%) lost serum HBsAg by 6 months. Hence, HBV infection evolved into chronicity in only 3 of the 256 (1%) patients, representing 2 of the 32 (6%) infected with HBV/Ae and 1 of the 21 (5%) with Ba. All of the three with chronic outcome had low-titered IgG anti-HBc at the presentation, and two of them had been negative for HBsAg before the presentation. None of them had received lamivudine or steroid treatment during their acute phase of illness. Of the patients without antiviral therapy, chronic outcome was significantly more frequent in those infected with HBV/Ae than non-Ae genotypes (9% 2/23 vs. 0.5% 1/187, $P = .032$).

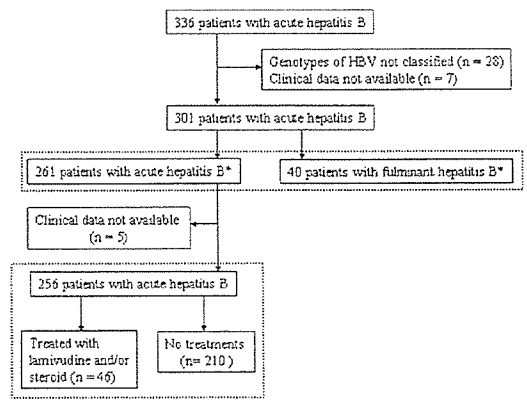

Fig. 1. A flow diagram of 336 patients studied. Comparison was made between patients with fulminant and acute self-limited hepatitis (upper dotted area), and the chronicity was compared between patients with and without treatments (lower dotted area). *Of 301 patients, 37 were negative for HBV DNA, including 27 with acute and 10 with fulminant hepatitis.