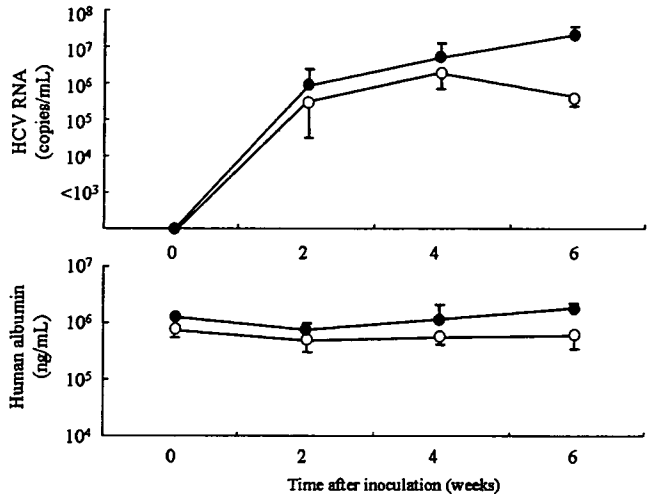


Fig. 2. Changes in HCV RNA and human albumin concentrations in serum of mice infected with clonal HCV. Each of three mice were inoculated intrahepatically with in vitro transcribed genotype 1a HCV RNA (closed circles) or intravenously with a culture medium collected from Huh7 cells transfected with JFH-1 genome intravenously (open circles). Data are mean ± S.D.

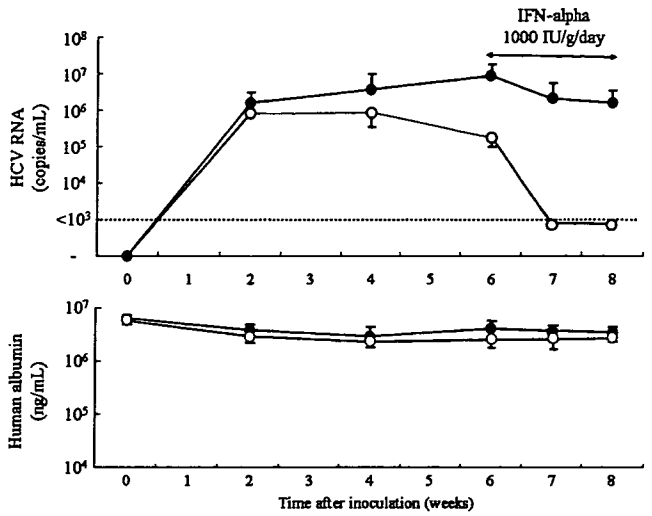


Fig. 3. Passage experiment and response to IFN-alpha therapy in mice infected with HCV genotypes 1a and 2a clones. Serum samples (10 μl) obtained from genotype 1a and 2a clonal HCV-infected mice sera (see Fig. 2) were inoculated intravenously into each of three naïve chimeric mice. Six weeks after infection, all six mice were injected intramuscularly with 1000 IU/g/day of IFN-alpha daily for 2 weeks. Closed circles: genotype 1a HCV-infected mice, open circles: genotype 2a HCV-infected mice. Data are mean ± S.D.

### 3.4. Variable susceptibility of HCV clones to IFN therapy

We treated each of the three mice infected with genotype 1a and 2a clones by passage experiments with 1000 IU/g of IFN-alpha daily for 2 weeks. Such treatment induced only a slight decrease in HCV in genotype 1a-infected mice; the viral load decreased only 0.6 and 0.7 log after 1 and 2 weeks of treatment, respectively (Fig. 3). In contrast, the same treatment reduced HCV genotype 2a RNA to undetectable levels after 1 and 2 weeks of IFN therapy. During IFN-treatment, serum HSA levels did not decrease in mice infected with genotype 1a or 2a HCV. Histopathological examination showed no morphological changes or apoptotic hepatocytes in replaced

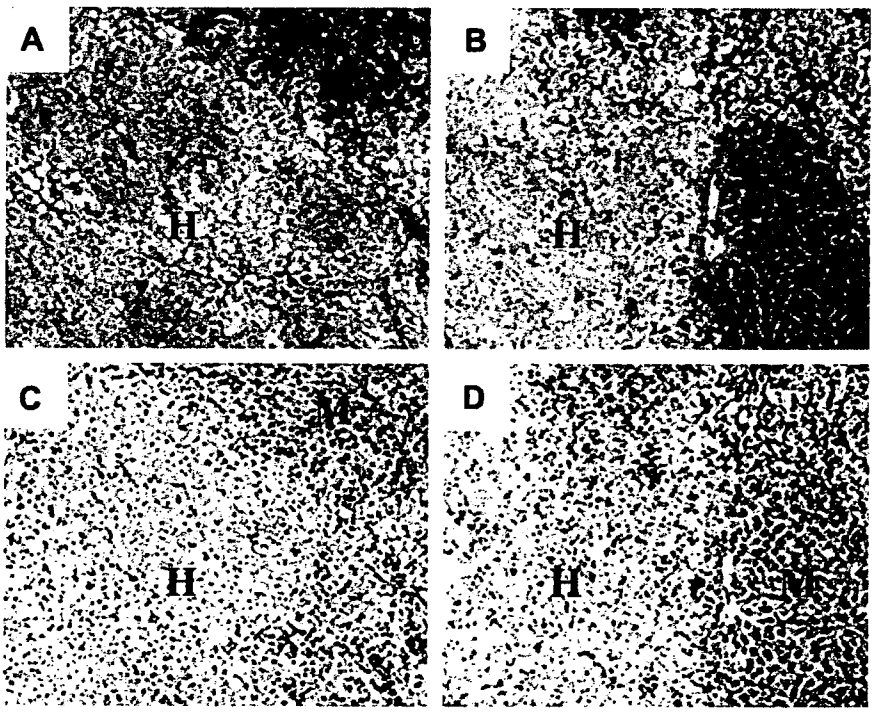


Fig. 4. Histochemical analysis of the tissues of infected chimeric mice. Liver samples obtained from mice infected with genotype 1a (A, C) and genotype 2a (B, D) stained with hematoxylin–eosin staining (A, B) or by immunohistochemical staining with anti-human serum albumin antibody (C, D). Regions are shown as human (H) and mouse (M) hepatocytes, respectively. (Original magnification, ×100.)

human hepatocytes in mice infected with each genotype after 2-week IFN-treatment (Fig. 4). These results suggest that the decrease in HCV is due to the direct anti-viral effect of IFN and not induced by liver cell damage. The difference in the virus titer and susceptibility to IFN are considered to be due to the characteristics of the genotypes.

## 4. Discussion

In this study, we established a reverse genetics system of HCV genotype 1a and 2a clones using human hepatocyte chimeric mice. The HCV genotype 2a clone, JFH-1, has remarkable features, i.e., infects cultured Huh7 cell line as well as establish infection in chimeric mouse [7]. It has been reported that HCV genotype 1a clone, H77-S, also infects Huh7 cell line and produces infectious virion [14]. In the present study, we intrahepatically inoculated genotype 1a infectious clone, CV-H77C. As reported in chimpanzee [13,15–17], we were able to establish genotype 1a infection using human hepatocyte chimeric mice. Using this technique, it is hoped that we can conduct further experiments in the future using genetically engineered HCV clones. Experiments using chimeric clone described by Lindenbach et al. [7] should also provide further information regarding the variable replication property of HCV genomes. Modifying genomes with nucleotide substitutions allowed examination of the functions of HCV peptides as we showed with HBV [12].

As reported recently by Kneteman et al. [10], the mouse model system is useful for evaluating the effect of anti-HCV drugs such as IFN, protease inhibitors and polymerase inhibitors. As we showed in this study, the response to IFN therapy varied according to HCV genotype. Further experiments are necessary to determine whether differences in response to IFN are due to the different replication ability (replication level of genotype 2a clone was slightly lower than that of genotype 1b, see Figs. 2 and 3) or differences in genotypes, as has been reported in clinical studies [18]. As we showed in this study (Fig. 4), there is no hepatocyte damage or inflammation in the liver of the infected chimeric mouse. Thus, this model is suitable for the study of mechanisms involved in HCV replication and IFN resistance.

The intrahepatic injection method used in this study simplified our experiments using genetically engineered virus. This is particularly important in studies of protease inhibitors and polymerase inhibitors because HCV will easily develop resistance against these small molecule agents.

Previous studies identified amino acid sequences that correlate with different susceptibilities of genotype 1b HCV against IFN therapy, namely, interferon sensitivity determining region [19] and the PKR-eIF2 phosphorylation homology domain [20,21]. To elucidate such issues, we are currently trying to establish genotype 1b infection system using the method described in this paper.

In summary, we showed in the present study the successful application of a genetically engineered HCV in human hepatocyte chimeric mice. Using this mouse model, we showed that genotypes 1a and 2a HCV clones exhibit different susceptibilities to IFN-alpha therapy. Our mouse model seems useful for the study of HCV virology and resistance of HCV against IFN and for the development of new anti-HCV therapy.

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# Serum HBV RNA is a Predictor of Early Emergence of the YMDD Mutant in Patients Treated with Lamivudine

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**Lamivudine (LAM) is a nucleoside analogue widely used for the treatment of chronic hepatitis B virus (HBV) infection. Emergence of resistant strains with amino acid substitutions in the tyrosine-methionine-aspartate-aspartate (YMDD) motif of reverse transcriptase is a serious problem in patients on LAM therapy. The amount of covalently closed circular DNA in the serum is reported to be higher in patients who develop YMDD mutants than in those without mutants. However, there is no useful serum marker that can predict early emergence of mutants during LAM therapy. Analysis of patients who were treated with entecavir (n = 7) and LAM (n = 36) showed some patients had high serum levels of HBV RNA. Median serum levels of HBV RNA were significantly higher in patients in whom the YMDD mutant had emerged within 1 year (n = 6, 1.688 log copies/ml) than in those in whom the YMDD mutant emerged more than 1 year after treatment (n = 12, 0.456 log copies/ml, $P = 0.0125$) or in whom the YMDD mutant never emerged (n = 18, 0.688 log copies/ml, $P = 0.039$). Our results suggest that HBV RNA is a valuable predictor of early occurrence of viral mutation during LAM therapy.** (HEPATOLOGY 2007;45:1179-1186.)

The hepatitis B virus (HBV) is a member of the hepadnaviridae family. Worldwide, approximately 350 million people are estimated to be chronically infected with HBV.[1] Patients with chronic HBV infection develop chronic hepatitis, cirrhosis, and hepatocellular carcinoma, accounting for approximately 1 million deaths per year.[2] Recently, inhibitors of reverse transcriptase have been developed and widely used for patients with chronic HBV infection. Lamivudine (LAM), a cytosine nucleoside analogue, was first developed as an antiviral agent against HIV and later was used effectively against HBV because HBV also uses reverse transcriptase for replication.[3,4] Because LAM suppresses HBV replication, patients who are treated with LAM show a decreased level or disappearance of HBV DNA in serum and hepatitis B e antigen, normalization of serum alanine aminotransferase (ALT) level, and histological improvement.[5-12] However, discontinuation of therapy often leads to reactivation of HBV.[6,8,13,14] Therefore, long-term therapy is necessary for many patients with chronic HBV infection. During long-term LAM therapy, drug-resistant mutants with amino acid substitutions in the tyrosine-methionine-aspartate-aspartate (YMDD) motif emerge, resulting in expression of HBV DNA increasing again and in worsening of hepatitis.[6,10,15-18] Moreover, some patients develop a severe flare-up of hepatitis that could lead to fatal hepatic failure. Therefore, prediction of the emergence of YMDD mutants is an important issue.

In our hunt for useful serum markers to detect the early emergence of YMDD mutants, we noticed some patients who showed a discrepancy in the expression of HBV DNA measured by the transcription-mediated amplifica-

*Abbreviations: cccDNA, covalently closed circular DNA; ETV, entecavir; HBV, hepatitis B virus; LAM, lamivudine; PCR, polymerase chain reaction; RT, reverse transcription; TMA-HPA, transcription-mediated amplification and hybridization protection assay; YMDD, tyrosine-methionine-aspartate-aspartate.*

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**Table 1. Clinical Characteristics of the 3 Groups**

| | Group A | Group B | Group C |
|---|---|---|---|
| Number | 6 | 12 | 18 |
| Age, median (range) | 50 (37-67) | 49 (31-66) | 49 (27-68) |
| Sex (M:F) | 3:3 | 9:3 | 13:5 |
| Observation period (months) | 34.5 (13-58) | 38 (16-64) | 34 (13-58) |
| Time before emergence of mutants (months) | 8.5 (4-11) | 19 (13-36) | |
| HBV DNA (LGE/ml) | 7.8 ± 0.95 | 6.13 ± 0.84 | 6.64 ± 1.63 |
| Hbe-antigen-positive | 4 (66.7%) | 6 (50%) | 10 (55.6%) |
| Hbe-antibody-positive | 1 (16.7%) | 6 (50%) | 9 (50%) |
| ALT (U/l) | 136.1 ± 122.8 | 114.5 ± 104.1 | 129.8 ± 206.4 |

Group A: patients who showed early emergence of the mutants (within 1 year).
Group B: patients who developed resistance after 1 year of LAM therapy.
Group C: patients in whom mutants did not develop.

tion and hybridization protection assay (TMA-HPA) and that measured by the Amplicor HBV Monitor test. Because the former method detects both HBV DNA and HBV RNA, we thought that the difference in measurement by the 2 methods was a result of the presence of a large amount of HBV RNA.[19-21] We thus studied patients with chronic HBV infection who were being treated with LAM or entecavir (ETV) for the presence of HBV RNA. We also assumed that the presence of a large amount of HBV RNA would indicate that transcription and virus particle formation were still active in such patients. We thus assessed the value of this indicator in the prediction of the emergence of YMDD mutants during LAM therapy.

## Patients and Methods

***Patients.*** We studied 36 patients with chronic hepatitis B who were being treated with LAM from 2001 to 2006 at Hiroshima University Hospital, Kawakami Clinic, and Hiroshima Red Cross Hospital and Atomic Bomb Survivors Hospital. We also analyzed 7 patients who were being treated with ETV from 2004 to 2006 at Hiroshima University Hospital. No patients showed clinical signs of cirrhosis or hepatocellular carcinoma. They were not treated with other antiviral agents, corticosteroids, or immunosuppressant drugs during LAM/ETV therapy. The LAM-treated patients were 25 men and 11 women whose median age was 52 years (range 27-68 years; Table 1). They were divided into 3 groups (groups A, B, and C) according to how long it took for YMDD mutants to appear. Group A (n = 6) was composed of patients who showed early emergence of the mutants (within 1 year); group B (n = 12) had patients who developed resistance after 1 year of LAM therapy; and group C (n = 18) was composed of patients who did not show resistance to LAM therapy. Each of the 36 patients received 100 mg of LAM daily for 4-58 months (median, 21.5 months). All patients continued LAM therapy throughout the course of the study. Patients in the ETV group were 6 men and 1 woman whose median age was 37 years (32-50 years). They received 0.01-0.5 mg of ETV daily for 21-28 months (median, 25 months), and all patients continued ETV therapy throughout the course of the study. Blood samples were obtained from patients of both groups just before commencement of antiviral therapy and every 4 weeks during therapy. Informed consent was obtained from each patient.

***Quantification of HBV DNA.*** HBV DNA serum level was determined by using the TMA-HPA (Fujirebio Inc., Tokyo, Japan) and the Amplicor HBV monitor test (Roche Diagnostics, Tokyo, Japan). The measurement range of the former assay is $10^{3.7}$-$10^{8.7}$ genome equivalents (GE)/ml (3.7-8.7 LGE/ml),[22] whereas the range of the latter test was $10^{2.6}$-$10^{7.6}$ copies/ml (2.6-7.6 log copies/ml).[23] These quantitative assays of HBV DNA were performed at the Special Reference Laboratory (Tokyo, Japan).

***Extraction of Nucleic Acid of HBV and Reverse Transcription.*** Nucleic acid was extracted from 100 μL of serum by the SMITEST (Genome Science Laboratories, Tokyo, Japan) and dissolved in 20 μL of $H_2O$ for DNA analysis or 8.8 μL of ribonuclease-free $H_2O$ for RNA analysis. The latter solution was reverse-transcribed by using random primer (Takara Bio Inc., Shiga, Japan) and M-MLV reverse transcriptase (ReverTra Ace, TOYOBO Co., Osaka, Japan). In the next step, 25 pM of random primer was added to 8.8 μL of nucleic acid extract and heated at 65°C for 5 minutes. The samples were set on ice for 5 minutes. Then 4 μL of 5× reverse transcription (RT) buffer, 2 μL of 10 mM dNTPs, 2 μL of 0.1 M dithiothreitol (DTT), 8 units of ribonuclease inhibitor, and 100 units of M-MLV reverse transcriptase were added to each sample. The reaction mixture was incubated at 30°C for 10 minutes and 42°C for 60 minutes, followed by inactivation at 99°C for 5 minutes.

***Quantitative Analysis of HBV DNA by Real-Time Polymerase Chain Reaction.*** One microliter of DNA solution or cDNA solution was amplified by real-time polymerase chain reaction (PCR) with an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA) according to the instructions provided by the manufacturer. Amplification was performed in a 25-μL reaction mixture containing SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 200 nM of forward primer (5′-TTTGGGGCATGGACATTGAC-3′, nucleotides 1893-1912), 200 nM of reverse primer (5′-GGTGAACAATGGTCCGGAGAC-3′, nucleotides 2029-2049), and 1 μL of DNA or cDNA solution. After incubation for 2 minutes at 50°C, the sample was heated for 10 minutes at 95°C for denaturing, followed by a PCR cycling program consisting of 40 2-step cycles of 15 seconds at 95°C and 60 seconds at 60°C. The lower detection limit of this assay was $10^3$ copies/ml.

***Confirmation of Presence of HBV RNA in Serum by RNase Digestion.*** To confirm the presence of HBV RNA, nucleic acid extracted from the serum samples by SMITEST (Genome Science Laboratories, Tokyo) was digested with 1 μg/μL of RNase A (Wako Pure Chemical Industries, Osaka, Japan) at 37°C for 60 minutes, digested with proteinase K (New England Biolabs Inc., Ipswich, MA) at 37°C for 60 minutes, extracted with phenol/chloroform, precipitated with ethanol, and dissolved in water. Treated nucleic acid with or without RNase was analyzed by real-time PCR after reverse transcription with a random primer and reverse transcriptase, as already described.

***Detection of YMDD Mutant.*** Mutations in the YMDD motif of reverse transcriptase of HBV were examined by PCR with peptide nucleic acid clamping, as described previously.[24]

***Statistical Analysis.*** Differences between groups were examined for statistical significance using the Student t test, and correlations of parameters were examined by the Spearman's rank correlation. A difference with a $P$ value less than 0.05 was considered statistically significant. All statistical analyses were performed with StatView version 5.0 (SAS Institute, Cary, NC).

## Results

***HBV DNA Levels Determined by TMA-HPA and Amplicor HBV Monitor Test During ETV Therapy.*** High expression of HBV RNA was initially observed by measuring HBV nucleic acid with the TMA-HPA and HBV DNA with the Amplicor HBV monitor test. As shown in Fig. 1, expression of HBV nucleic acid was higher than HBV DNA during the initial 6 months of ETV therapy. We assumed that the discrepancy in the measurements by these 2 methods was a result of the large amount of HBV RNA in the serum because the TMA-HPA measures both HBV DNA and HBV RNA, whereas the Amplicor HBV monitor test detects only HBV DNA. We measured the HBV nucleic acid levels in the 7 patients who received ETV therapy 3 and 6 months after the start of therapy. The HBV nucleic acid levels of all 7 patients determined by the TMA-HPA were 10-100 times higher than those determined by the Amplicor HBV Monitor test except for 2 patients who received a small amount (0.01 mg) of ETV (Fig. 2). The small dif-

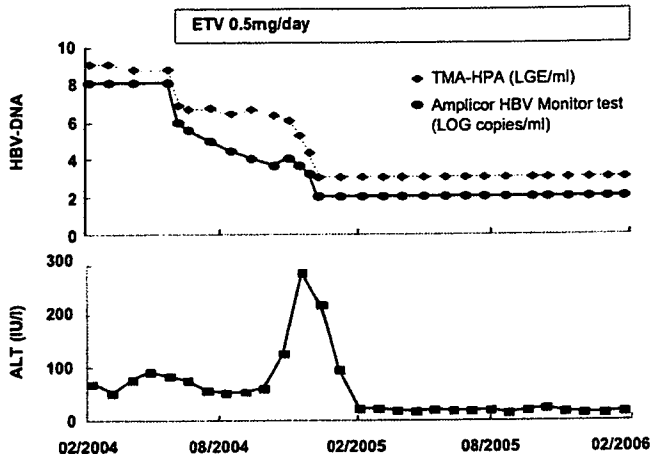


Fig. 1. Time courses of serum HBV DNA and ALT levels of patients treated with ETV. Expression of HBV nucleic acids determined by the TMA-HPA was higher than that determined by the Amplicor HBV Monitor test soon after beginning administration of ETV. The discrepancy was less marked when both measurements were low and when both were negative.

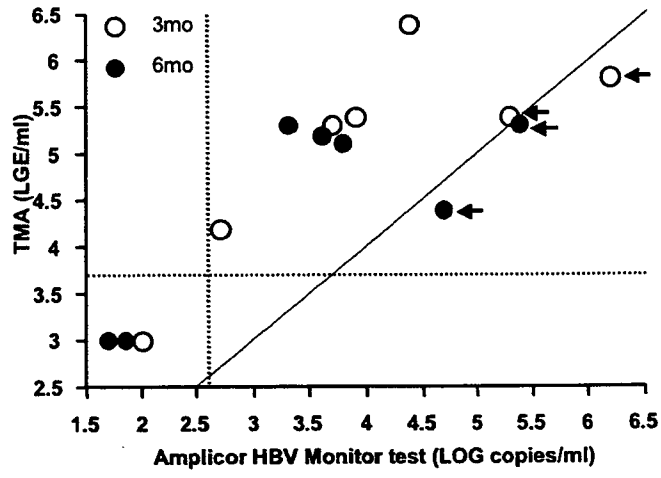


Fig. 2. Correlation of HBV nucleic acid levels determined by the TMA-HPA with HBV DNA levels determined by the Amplicor HBV Monitor test during ETV therapy. Serum samples obtained from the 2 patients who received low-dose ETV (0.01 mg) are indicated by arrows. The vertical and horizontal dotted lines indicate the lower detection limits of the Amplicor HBV Monitor test and the TMA-HPA, respectively.

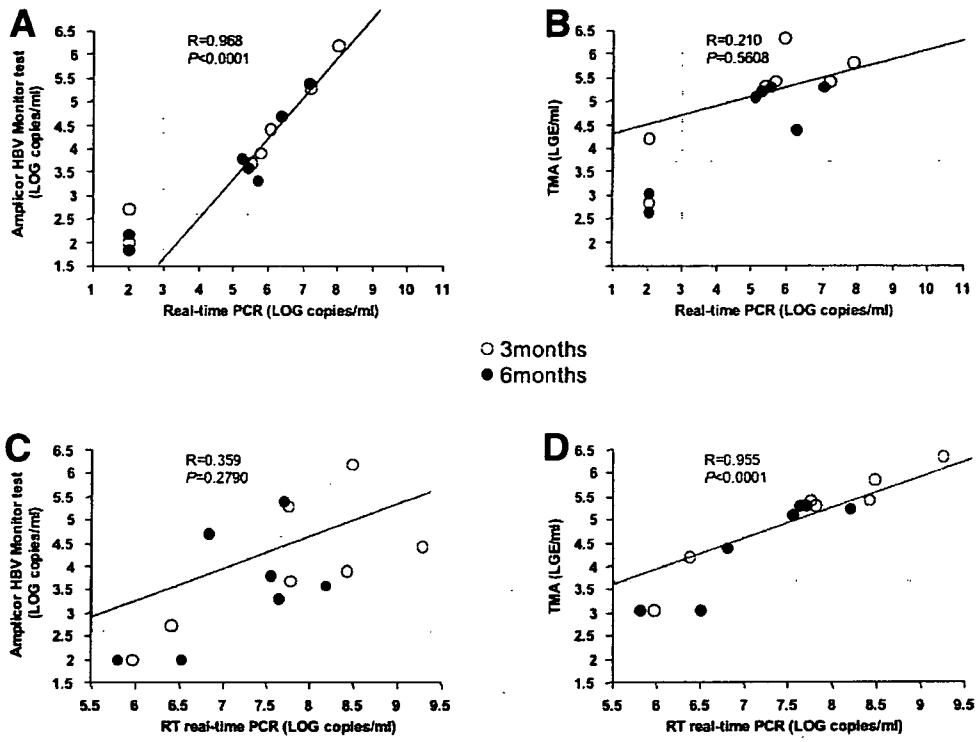


Fig. 3. Correlation between HBV nucleic acid and HBV DNA measurements after 3 and 6 months of ETV therapy. (A) Correlation between HBV DNA level determined by Amplicor HBV Monitor test and that determined by in-house real-time PCR. (B) Correlation of HBV nucleic acid level determined by the TMA-HPA and of HBV DNA determined by real-time PCR. (C) Correlation of HBV DNA level determined by the Amplicor HBV Monitor test with HBV nucleic acid level determined by real-time RT-PCR. (D) Correlation of HBV nucleic acid level determined by the TMA-HPA with that determined by real-time RT-PCR. The vertical and horizontal dotted lines represent the lower detection limits of the Amplicor HBV Monitor test or TMA-HPA and in-house real-time PCR, respectively.

ference in nucleic acid level of these patients is probably a result of the small effect of the small amount of ETV.

***Comparisons of HBV Nucleic Acid and DNA Values Determined by 4 Measurement Methods—TMA-HPA, Amplicor Monitor Test, In-House Real-Time PCR Assay, and Real-Time RT-PCR—in Patients Treated with ETV.*** We measured HBV DNA by in-house real-time PCR and HBV nucleic acid by real-time RT-PCR using serum samples obtained from the patients after 3 and 6 months of ETV therapy and compared these values with those obtained by the TMA-HPA and the Amplicor monitor test. HBV DNA determined by real-time PCR correlated well with that obtained by the Amplicor HBV Monitor test ($r = 0.968$, $P < 0.0001$; Fig. 3A), but not with HBV nucleic acid determined by the TMA-HPA ($r = 0.210$, $P = 0.5608$; Fig. 3B). Expression of HBV DNA determined by the in-house real-time PCR assay was $10^{1.5}$-$10^{2}$ higher than that determined by the Amplicor HBV Monitor test. We confirmed the accuracy of our assay using limiting dilution and detection with nested PCR assay. When we diluted the standard samples used in our in-house assay to 1 copy/$\mu$L, we detected them by nested PCR using 1 $\mu$L of such samples. Three of the 10 (30%) samples tested positive by nested PCR. We thus conclude that our assay accurately measure the amount of HBV DNA in serum.

To examine if measurement by the TMA-HPA reflected the total amount of HBV RNA and HBV DNA in serum samples, we performed real-time RT-PCR using serum samples obtained from patients after 3 and 6 months of ETV therapy. In contrast to the values determined by real-time PCR without RT, the measurement of HBV nucleic acid determined by RT-PCR did not correlate well with that obtained by the Amplicor HBV Monitor test ($r = 0.359$, $P = 0.2790$; Fig. 3C), but did correlate well with that obtained with the TMA-HPA ($r = 0.955$, $P < 0.0001$; Fig. 3D). These results show that the TMA-HPA measures both HBV DNA and HBV RNA in serum. To further confirm the presence of HBV RNA, we digested 3 nucleic acid samples arbitrarily picked from serum samples obtained from patients treated by lamivudine for 3 months, by RNase A. As shown in Fig. 4, RNase treatment reduced the amount of HBV DNA detected by real-time RT-PCR to about 1% of that originally detected.

***HBV DNA Levels Determined by TMA-HPA and Amplicor HBV Monitor Test during LAM Therapy.*** We then investigated the levels of HBV DNA in serum samples obtained from 36 patients after 3 and 6 months of LAM therapy. In some patients, HBV DNA was already negative after 3 and 6 months of therapy (Fig. 5). Similar to the results obtained from patients treated with ETV, comparisons of values obtained from patients who showed measurable HBV DNA levels revealed that HBV nucleic acid levels determined by the TMA-HPA tended to be higher than those determined by the Amplicor HBV Monitor test (Fig. 4).

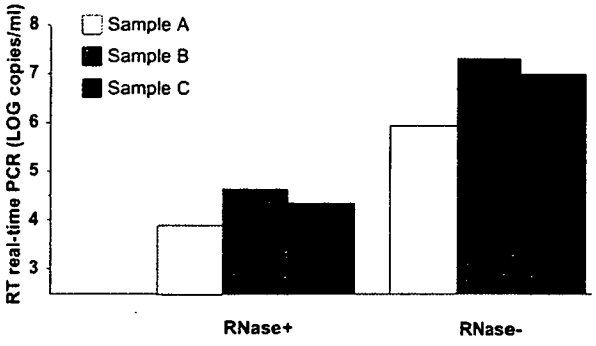


Fig. 4. Presence of HBV RNA confirmed by RNA treatment of 3 nucleic acid samples (samples A-C) obtained from patients after 3 months of LAM therapy. Extracted nucleic acid samples with or without RNase digestion were further digested by proteinase K and ethanol-precipitated after phenol/chloroform extraction. The amount of HBV DNA in each sample was then measured by real-time RT-PCR.

***Comparisons of HBV Nucleic Acid Values and HBV DNA Determined by 4 Measurement Methods—TMA-HPA, Amplicor Monitor Test, In-House Real-Time PCR Assay, and Real-Time RT-PCR—in Patients Treated with LAM.*** We measured HBV nucleic acid and DNA levels by the same 4 methods and investigated the correlations between them after 3 and 6 months of LAM therapy (Fig. 6). HBV DNA levels determined by real-time PCR correlated better with those determined by the Amplicor HBV Monitor test ($r$ = 0.653, $P$ = 0.0083; Fig. 6A) than with those determined by the TMA-HPA ($r$ = 0.456, $P$ = 0.1173; Fig. 6B). Similarly, measurement of HBV nucleic acid by RT-PCR did not correlate well with that obtained by the Amplicor HBV Monitor test (Fig. 6C), but showed better correlation with that obtained by the TMA-HPA ($r$ = 0.452, $P$ = 0.0907, and $r$ = 0.675, $P$ = 0.0114, respectively; Fig. 6D). These results also showed that the TMA-HPA detects both HBV RNA and HBV DNA.

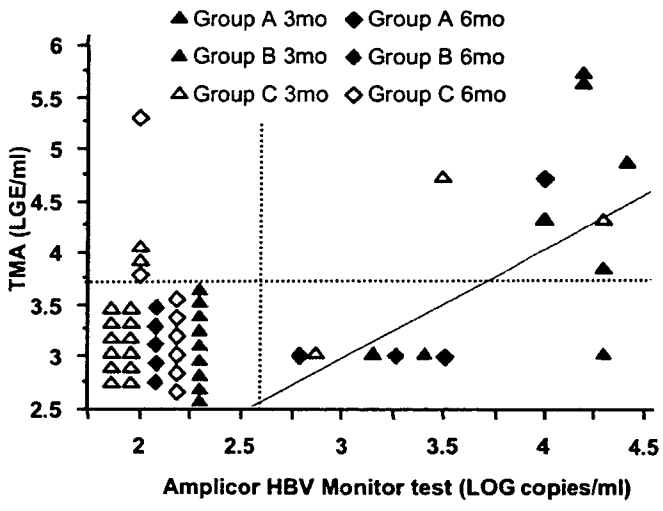


Fig. 5. Correlation of HBV nucleic acid levels determined by the TMA-HPA with HBV DNA levels determined by the Amplicor HBV Monitor test during LAM therapy. During ETV therapy the TMA-HPA showed higher expression of HBV DNA in patients regardless of the presence of the mutation than did the Amplicor HBV Monitor test. The vertical and horizontal dotted lines indicate the lower detection limits of the Amplicor HBV Monitor test and the TMA-HPA, respectively.

***HBV RNA in Serum after 3 Months of LAM Therapy Is Higher in Patients Who Showed Early Emergence of YMDD Mutants.*** In LAM-treated patients, it was assumed that a high serum level of HBV RNA was a marker of the active transcription form of covalently closed circular DNA (cccDNA) and packaging of HBV RNA in the liver. We assumed that YMDD mutants easily emerged under such condition. We compared HBV RNA values (HBV nucleic acid determined by real-time RT-PCR minus HBV DNA determined by real-time PCR) in patients who showed early emergence of mutants (within 12 months) with those who showed late emergence of mutants (more than 12 months) and those who did not show emergence of mutants (Table 1). As shown in Fig. 7, HBV RNA levels were significantly higher in patients who showed early emergence of mutants than the other 2 groups after 3 months of LAM therapy. There was no significant difference in the amount of HBV RNA between group A (patients who showed emergence of mutants within 12 months) and the other 2 groups at the beginning of LAM therapy (data not shown).

## Discussion

In this study, we addressed the discrepant measurements of HBV nucleic acid by the TMA-HPA and the Amplicor Monitor test. The presence of HBV RNA in serum samples of patients with HBV infection has been previously reported.[19-21] Because the TMA-HPA uses RNA transcription and amplification of transcripts by T7 RNA polymerase,[22] we assumed that the discrepancy was a result of the presence of HBV RNA in the serum of LAM- and ETV-treated patients. The presence of HBV RNA in a patient treated with LAM was reported previously.[21] In that report, the authors mainly analyzed truncated HBV RNA, which they assumed was transcribed from the integrated genome.[20, 21] They showed a large difference between HBV DNA and truncated HBV RNA, which did not decrease during LAM therapy. We also detected HBV DNA and HBV nucleic acid by real-time PCR and real-time RT-PCR. The values determined by these 2 methods showed less than a 1 log difference (data not shown); we assume that the effect of truncated HBV RNA in serum was only minimal in our study. As we demonstrated in this study, HBV nucleic acid measured by real-time RT-PCR correlated with that determined by the TMA-HPA. This finding suggests that the

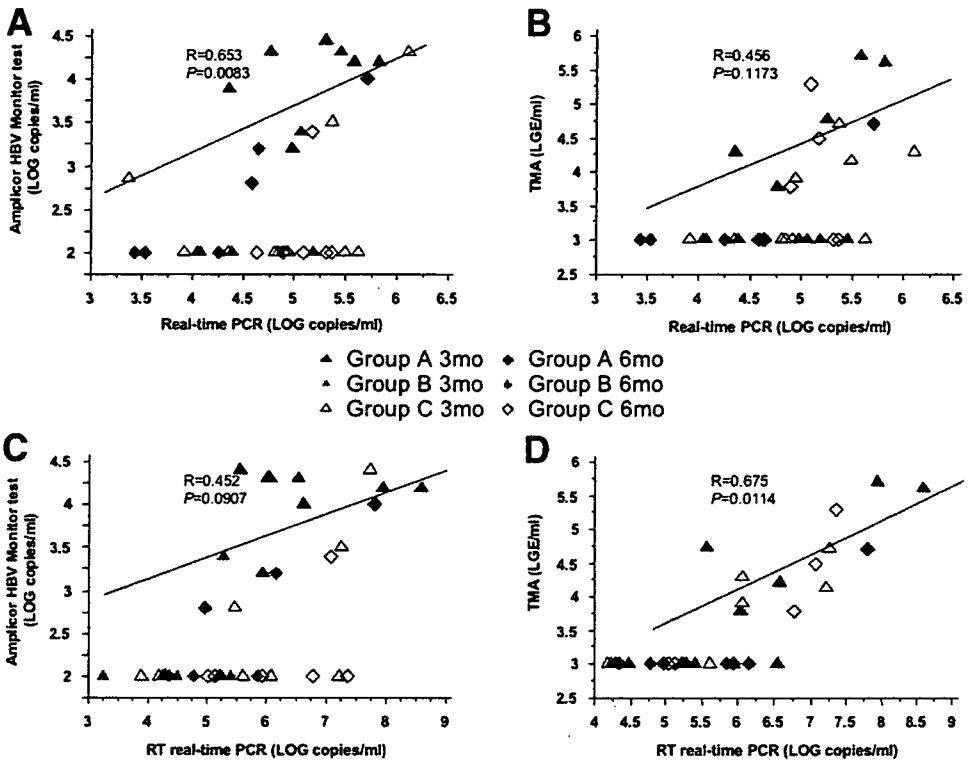


Fig. 6. Correlations between HBV nucleic acid and HBV DNA measurements after 3 and 6 months of LAM therapy. (A) Correlation of HBV DNA level determined by the Amplicor HBV Monitor test with that determined by in-house real-time PCR. (B) Correlation of HBV nucleic acid level determined by the TMA-HPA with HBV DNA by real-time PCR. (C) Correlation of HBV DNA level determined by the Amplicor HBV Monitor test with HBV nucleic acid level determined real-time RT-PCR. (D) Correlations of HBV nucleic acid level determined by the TMA-HPA with that determined by real-time RT-PCR. The vertical and horizontal dotted lines represent the lower detection limits of the Amplicor HBV Monitor test or TMA-HPA and in-house real-time PCR, respectively.

discrepancy in the values measured by the TMA-HPA and the Amplicor Monitor test is a result of the presence of HBV RNA in the serum.

We showed that a large amount of HBV RNA in the serum was produced during the early stage of ETV (Fig. 1) and LAM treatments (within 6 months). Because ETV and LAM work only on reverse transcription, it is difficult to conceive that the level of transcription from the cccDNA was altered by these drugs. Thus, the slow decrease in HBV RNA seems to reflect that a certain amount of cccDNA still existed in the liver and that the virus replication machinery was still actively operational. This is consistent with previous reports that showed that the amount of cccDNA in the liver tissues[25, 26] and in serum,[26] which correlated well with intrahepatic cccDNA,[27] reflected the effect of LAM and is a marker for cessation of therapy without viral level increasing again after stopping the therapy.

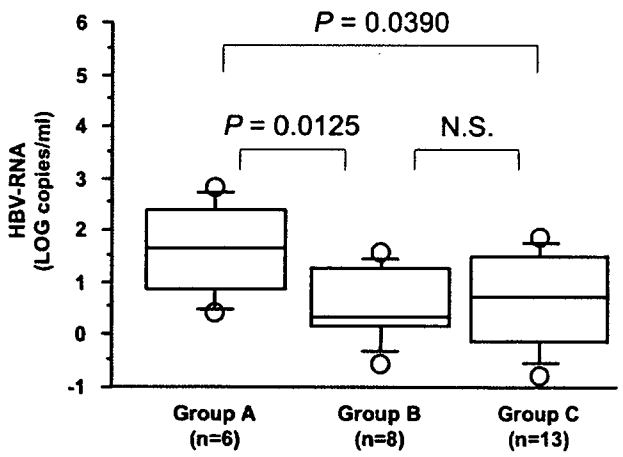


Fig. 7. Box plots of HBV RNA levels of patients in group A (patients who showed emergence of the mutants within 1 year, group B (those who developed resistance after 1 year of LAM therapy), and group C (patients who did not show resistance to LAM therapy). HBV RNA level represents the difference between HBV nucleic acid level determined by real-time RT-PCR minus HBV DNA level determined by in-house real-time PCR. Nine samples that tested negative for in-house real-time PCR were omitted from the analysis (4 samples of group B and 5 samples of group C).

Whether a large amount of HBV RNA originates from a large amount of cccDNA template in hepatocytes or from active transcription (or both) is actually unknown. However, it is assumed that the probability of developing mutants is high in patients who have large amounts of HBV RNA. We thus analyzed the amount of HBV RNA in patients treated with LAM and compared it in patients who showed early emergence of mutants and those who did not. As expected, the amount of HBV RNA in the serum was significantly higher in patients who showed early emergence of mutants than in those who showed late emergence and those who did not show emergence of mutants.

Using complex analysis, previous studies identified several factors predictive of emergence of YMDD mutants such as HBV genotype,[28] ALT level,[29, 30] HBV DNA level

before therapy,[28,30-32] degree of decline of HBV DNA level during therapy,[33,34] presence of hepatitis B e antigen,[17,29,31,32,35] presence of core promoter mutations,[36] deletion of pre-S region,[37] and HBV core–related antigen.[38] We also showed that a slow decrease in HBV nucleic acid measured by the TMA-HPA is a marker of early emergence of mutants. Our finding is important because this assay is routinely used in daily clinical practice. However, the results did not reach statistical significance, probably because of the small number of patients analyzed in our study and the low sensitivity of the assay (detection limit 3.7 log copies/ml). We assume that a sensitive measurement of HBV RNA is useful for predicting the emergence of mutants. Development of such an assay is needed for the proper treatment of patients using different nucleotide and nucleoside analogues. Mechanisms that control transcription of HBV from cccDNA deserve further investigation in order to develop more effective therapies for HBV infection.

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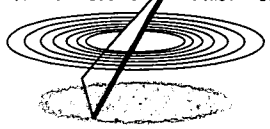
# C型肝炎治療の進歩

*Progress of treatment for chronic hepatitis C*

診断の指針 / 治療の指針



高橋 祥一 茶山 一彰*
*TAKAHASHI Shoichi CHAYAMA Kazuaki*

日本では約150万人，全世界では約1億5千万人が罹患していると推測されているC型肝炎であるが，C型肝炎ウイルス(HCV)の存在が確認されたのは1989年のことであった．わが国では1992年にインターフェロン(IFN)療法が認可されたが，当初の著効率は約3割程度であった(著効：IFN投与終了6ヵ月後のHCV RNA陰性[SVR：sustained viral response])．後にこの理由はgenotypeによるIFNへの反応性の違いと，HCVウイルス量の違いによることが判明し，Genotype 1B，HCV RNA 100KIU/ml以上のいわゆる難治性のHCVに対しては，IFN単独療法の著効率は10%以下で成績は非常に悪かった．しかし2001年IFNα-2b+リバビリン(RBV)の併用療法の開始，2003年のpegIFNα-2a単独療法の開始を経て，2004年の12月にpegIFNα-2b+RBVの併用療法がわが国で認可された．わが国の全国治験のデータでは，Genotype 1b，高ウイルス量の患者に対し，47.6%の著効例が得られた．これは従来のIFN+RBV併用療法における著効率：約20%やpegIFNα-2a単独投与における著効率：約28%と比較して大きな進歩といえる．またウイルス陰性化時期別に著効率をみると，治療開始4週目までにウイルスが陰性化した症例については著効率100%，治療開始4週から12週目までに初めてウイルスが陰性化した症例は著効率71.1%，治療開始12週から24週目までに初めてウイルスが陰性化した症例は著効率36.4%であった．すなわち，ウイルスがいったん血液検査上陰転化してから，さらにいかに長く治療継続するかが，著効が得られるためのポイントになると思われる．また薬剤の減量中止と著効率の関係は，pegIFNとRBVの両方を減量なく完遂した症例は62.5%と高値であったが，どちらか一方を減量した症例で52～53%，両剤減量例でも45.7%と，投与中止例の著効率19.2%と比して著明に高値であり，減量してでも48週間継続することがまず肝要であることが示されている．またpegIFNは週1回の投与であり，従来型のIFNに比して発熱等の自覚症状としての副作用の出現頻度が低く，患者のQOLも大きく改善されている．

このC型慢性肝炎に対する標準的な治療法について「B型およびC型肝炎ウイルスの感染者に対する治療の標準化に関する臨床的研究」班(熊田博光班長：厚生労働科学研究補助金 肝炎等克服緊急対策研究事業)よりC型慢性肝炎治療の新ガイドライン2007が発表されている(表1)．このガイドラインによるとgenotype1の高ウイルス量の患者に対しては上述のごとくpegIFNα-2bまたはα-2a+RBVの併用療法の48週間投与，genotype 2の高ウイルス量症例に対してはpegIFNα-2b+RBVの併用療法の24週間投与が推奨されており，低ウイルス量症例に対してはgenotypeに関係なく，従来型のIFN単独療法24週間またはpegIFNα-2a単独療法の24～48週間投与が推奨されている．

IFNの治療効果判定は，投与終了6ヵ月後のHCVRNAの消失をもって著効(SVR)としており，2004年12月に投与開始となった症例の効果判定が2006年6月であるため，市販後の著効率はようやく最近になって判明し始めてきている．全国規模でのpegIFNα-2b+RBVの併用療法の著効率については今後の報告を待たねばならないが，おそらく1B高ウイルス症例に対する著効率は40～50%とほぼ治験と同等のデータが示されるものと思われる．

当科および広島大学消化器内科関連病院で構成された広島肝臓スタディグループにおけるpegIFNα-2b+RBVの併用療法の途中経過を紹介すると，2004年12月から2007年6月までにpegIFNα-2b+RBVで治療を行ったHCV陽性慢性肝疾患患者は1,185例で，このうちgenotype 1bかつ高ウイルス量(>100KIU/ml)(1b/高)は926例(78%)，1b高ウイルス量以外(others)は259例(22%)であった．pegIFNα-2b+ RBV療法長期投与を除く48週間治療例での現段階でのSVR率は全体で50.1%，1b/高で42.5%，othersで79.3%であった．1b/高での男女別SVR率は男性46.9%，女性34.2%

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Key words C型肝炎 ペグインターフェロン リバビリン

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表1 平成18年度C型慢性肝炎の治療ガイドライン初回投与

| 初回投与 | Genotype 1 | Genotype 2 |
|---|---|---|
| 高ウイルス量<br>1 Meq/mL<br>100KIU/mL<br>300fmol/L<br>以上 | Peg-IFNα2b：Peg-Intron＋Ribavirin(48週間)<br>Peg-IFNα2a：Pegasys＋Ribavirin(48週間) | Peg-IFNα2b：Peg-Intron＋Ribavirin (24週間) |
| 低ウイルス量<br>1 Meq/mL<br>100KIU/mL<br>300fmol/L<br>未満 | IFN(24週間)<br>Peg-IFNα2a：Pegasys(24～48週間) | IFN (8～24週間)<br>Peg-IFNα2a：Pegasys (24～48週間) |

で有意に男性で高く，年齢別，男女別で見ると65歳以下男性，65歳以上男性，65歳以下女性，65歳以上女性の順にSVR率は低下し，65歳以上の女性のSVR率は25.4%であった．線維化の程度とSVR率は既報通り逆相関を認め，F1で54.1%に対し，F4で25%であった．薬剤の投与状況とSVR率の関係はpegIFN，RBVとも減量しなかった群ではSVR率は66.1%，pegIFN減量，RBV減量群ではそれぞれ52.2%，55.7%であったが，48週完遂できず中止した症例では8.1%と非常に低いSVR率であった．薬剤のadherenceとSVR率の関係を見るとpegIFN，RBVともに総投与量の80%以上投与し得た症例はSVR率が64.9%と良好であったが，pegIFNのみ80%以上では52.3%，RBVのみ80%以上では41.7%，pegIFN，RBVともに総投与量の80%未満であった症例はSVR率は20.3%であり，仮に48週間完遂できたとしても不十分な薬剤投与量ではSVRは見込めないことが示された．またALT 40IU/L以下の肝機能正常例に対するIFN治療であるが，SVR率はALT 40IU/L以上で45.3%であるのに対し，ALT 40IU/L以下では40.2%で若干低いが有意差は認めなかった．またIFN投与中にALTのflare upを認める症例はほとんどなく，肝機能正常例でも問題なく治療できると考えられた．一方，othersでは男女別SVR率は男性73.3%，女性83.5%で女性の方が高いが有意差はなく，年齢別男女別のSVR率もいずれも群も比較的高いSVR率を示し，やはりothersでは高い確率でSVRが期待できることが判明した．

欧米の報告では投与開始後12週目でのウイルス陰性化が得られない症例は著効が得られる可能性が低いため，投与中止するよう勧告されているが，結局はウイルス陰性化が得られてから，いかに長く薬剤投与するかが著効率に大きく関係すると思われるので，保険上の問題はあるが，可能ならば48週を超えて72週あるいは96週の治療を行えば，著効率はさらに上がるものと思われる．この点についても今後の報告が待たれる．

今後のC型肝炎治療であるが，現在HIVに対する治療薬開発の研究を応用してHCVに対するプロテアーゼ阻害剤やRNAポリメラーゼ阻害剤が開発中で，米国ではすでに治験中でかなり高い効果が期待されており，近日中に本邦でも治験開始予定である．単剤ではHIVと同様に薬剤耐性株の出現が懸念されているが，IFNを含めた多剤併用療法を確立することにより，難治性の1b抗ウイルス群に対しても，現在の40～50%のSVR率が70%，80%と上昇し，完全根治が得られる日が近づいているのかもしれない．

# B型肝炎

高橋 祥一・茶山 一彰

**ポイント**

- **B型急性肝炎に対しては，重症，劇症化例でなければ，抗ウイルス薬，肝庇護薬を投与せず，B型肝炎ウイルスの自然排除を待つ．**
- **B型慢性肝炎は若年，非進行例ではインターフェロン治療，中高年，肝炎進行例ではエンテカビル投与が第一選択となる．**

B型肝炎ウイルス(hepatitis B virus：HBV)感染によって起きるB型肝炎は，一過性感染である急性肝炎と持続性感染である慢性肝炎に大別され，その臨床像，治療法，予後は大きく異なる．

## B型急性肝炎の病態と治療

B型急性肝炎は，そのほとんどが成人間の性感染であり，HBV持続感染者(HBVキャリア)から非感染者へ血液(体液)を介して感染する．数カ月の潜伏期の後に，全身倦怠感，食欲不振，嘔気，褐色尿，顕性黄疸として発症し，肝トランスアミナーゼ(AST，ALT)が異常高値(1,000 IU/$l$以上)を呈する．その約2週間後に黄疸のピークが出現するが，ほとんどの症例で増殖したHBVは自己の免疫機能で自然排除され，数週間後には肝内のHBVはほぼ消失し，AST，ALTは正常化する．この間，抗HBV薬は特に投与の必要はなく，HBVが自然排除されるまで，食欲低下が強ければ補液する程度である．強力ネオミノファーゲンC®などの肝庇護薬はウイルスの排除を遷延させるため，投与してはならない．一方で稀に劇症化する場合があるが，これに対しては後述の慢性肝炎急性増悪例と同様に，核酸アナログ製剤を中心とした集学的治療が必要である．

## B型慢性肝炎の病態と治療目標

B型慢性肝炎は，HBVキャリアの母からの産道感染，あるいは免疫能が十分発達していない乳幼児期での感染によって起こり，思春期になり肝炎を発症する．約8割の症例では一過性のトランスアミナーゼの上昇の後，HBe抗体が出現して肝炎は鎮静化し，臨床的治癒の状態となって安定化するが，残りの2割の症例では持続性，あるいは間欠的にAST，ALTは上昇し，慢性肝炎から肝硬変，肝不全，あるいは肝細胞癌を発症する．これらの慢性肝炎症例に対しては積極的に治療介入する必要があるが，

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0025-7699/07/¥500/論文/JCLS

C型慢性肝炎治療と比較して根本的に異なるのは，HBVは宿主(患者)からのウイルス完全排除が非常に困難な点である．このためB型慢性肝炎の治療目標は，①HBe抗原陽性からHBe抗体陽性へのセロコンバージョン，②HBV DNAの陰性化，③トランスアミナーゼの正常化であり，HBV DNAの増殖を持続的に抑制し，肝炎を沈静化させて，肝硬変や肝癌への進展を防いでいくことが重要になる．

## B型慢性肝炎の治療

B型慢性肝炎治療については「B型およびC型肝炎ウイルスの感染者に対する治療の標準化に関する臨床的研究」班(厚生労働科学研究補助金 肝炎等克服緊急対策研究事業)より「B型慢性肝炎治療の新ガイドライン2007」が発表されている[1]．35歳未満の若年者でHBe抗原陽性例に対しては，自己の免疫力によりHBe抗原の陰性化や肝炎の収束が期待されるため，インターフェロン(IFN)長期間欠投与を行う．HBe抗原陰性例に対しては原則的に経過観察を行うが，すでに肝硬変に至った症例，ALT高値で数年で肝硬変へ進行すると思われる線維化の進んだ肝炎例ではエンテカビル投与も考慮する．一方，35歳以上の中高年者に対しては，エンテカビル投与が原則となる．

IFN長期間欠投与は，HBe抗原陽性例に対するIFNα製剤の週3回・24週間の投与が保険適応となっている．IFN 24週投与によるセロコンバージョン率は20～40％であり，有効率は高くないが，いったん臨床的治癒に落ち着けば投薬の必要がなくなり，経過観察のみでよいため，肝炎の進行していない若年者に対しては第一選択となる．本邦ではHBe抗原陰性例には保険適応がないが，海外のデータでは有効性が証明されており，欧州ではpeg-IFNの48週投与が行われ，さらに高い効果が報告されている[2]．本邦でも2007年からpeg-IFN投与の治験が始まり，今後の認可が待たれる．

IFNの副作用としてはインフルエンザ様症状，汎血球減少(以上必発)，ときに間質性肺炎，精神神経症状，甲状腺異常，糖尿病の増悪，蛋白尿，眼底出血，脱毛などがあり，特に間質性肺炎，精神疾患は死亡例があるため，注意が必要である．

35歳以上の中高年者あるいは肝病変の進行した症例に対しては，核酸アナログ製剤であるエンテカビルを第一選択とする．核酸アナログとは，細胞内で宿主のデオキシリボ核酸と競合し，ウイルス由来の逆転写酵素によるウイルスDNAの合成を阻害し，ウイルスの複製を抑える働きをする逆転写酵素阻害薬のことである．エンテカビルは，ラミブジン，アデホビルに続く本邦で3番目に認可された核酸アナログ製剤で，ラミブジンと同等の強い抗ウイルス効果をもつ点と，ラミブジンの問題点である薬剤耐性株の出現率が初回治療例では圧倒的に低い点から，現段階では第一選択薬とされている[3]．なお，ラミブジン耐性であるYMDD変異株に対しては，エンテカビル耐性株の出現頻度が高いため，ラミブジン＋アデホビルの併用が推奨されている．

エンテカビル投与は十分な抗ウイルス効果が得られる反面，中止すると肝炎の再燃はほぼ必発であり，一度内服を開始すると中止するのが難しい．自己中止すると，その後のリバウンドにより肝炎の重症化が起きるため，厳重な内服指導を必要とする．エンテカビル耐性株の出現にも注意が必要である．核酸アナログ製剤未治療症例では2年で変異株の出現は0％であるが，長期投与に伴う変異株の出現のリスクは未知である．また，妊娠，授乳に関する安全性は確認されていない．

## B 型劇症肝炎，慢性肝炎急性増悪の治療

急性肝炎の劇症化，また慢性肝炎の急性増悪時は，一度に多数の肝細胞が破壊されるため，急速に肝不全に進行する危険性が高い．この場合は抗ウイルス療法としてエンテカビル，感染肝細胞への免疫反応を抑えるため，ステロイドなどの免疫抑制薬，血漿交換，持続血液濾過透析などの人工肝補助療法による集学的治療を行うが，それでも救命困難な場合もあり，肝移植が行われることもしばしばある．


文　献

1) 熊田博光：B 型及び C 型肝炎ウイルスの感染者に対する治療の標準化に関する臨床的研究，熊田博光(主任研究者)：B 型及び C 型肝炎ウイルスの感染者に対する治療の標準化に関する臨床的研究 平成 16 年度～平成 18 年度 総括・分担研究報告書，pp 3-18，厚生労働省科学研究費補助金 肝炎等克服緊急対策研究事業(肝炎分野)，2007

2) Janssen HLA, et al：Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chornic hepatitis B；A randomized trial. Lancet 365：123-129, 2005

3) Chang TT, et al：A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 354：1001-1010, 2006


トピックス

### 感染症サマーセミナー 2007 開催される

日本感染症教育研究会

さる 7 月 27～29 日の 3 日間にわたり札幌にある手稲渓仁会病院で，日本感染症教育研究会(Infectious Diseases Association of Teaching and Education of Nippon：通称 IDATEN)主催による「感染症サマーセミナー 2007」が開催された．

IDATEN とは，日本における感染症診療の発展と教育の推進を目的に，2005 年に発足した団体．感染症診療のバイブル的存在である『レジデントのための感染症診療マニュアル』(医学書院)の著者である青木眞氏(感染症コンサルタント)をはじめ，大曲貴夫氏(静岡がんセンター感染症科)や五味晴美氏(自治医科大学感染制御部)，大野博司氏(洛和会音羽病院 ICU/CCU)など新しい感染症診療の流れをつくるリーダーを中心に運営されている．

特に夏・冬に，研修医などの若手医師を対象に開催される「感染症セミナー」は，優れた講師陣が揃い，集中的に最新の感染症診療を学べることから，高い人気があり，毎年多数の参加希望者を集めている．北海道での開催となった今回は，道内きっての人気研修病院として知られる手稲渓仁会病院を会場に，応募者のなかから地域や施設などを考慮して選ばれた 91 人(医師 81 人，医学生 10 人)が参加．熱気あふれたセミナーとなった．

今回のテーマとして選ばれたのは，「市中感染症の入院マネジメント」．研修医をはじめとする病棟勤務医が遭遇する感染症患者のマネジメントについて，グループディスカッション，フロアとの対話などを交えた，充実した講義が行われた．感染症診療の第一線にいる気鋭の IDATEN 講師陣や，症例をベースにして考えるなかで感染症診療の鉄則を学ぶという練り上げられたプログラムが，最大の魅力であるのはもちろんだが，講師・参加者間の交流にも力点が置かれており，参加者たちは，感染症診療に携わっていくうえで貴重な基盤を築くことができた模様だ．

なお，来夏のセミナーは，長野県の諏訪中央病院を会場に開催される予定である．

## トピックス

# IV. 性感染症
# 2. B型肝炎

高橋　祥一　　茶山　一彰

要　旨

我が国でのB型慢性肝炎の感染経路のほとんどが母児感染である一方，成人のB型急性肝炎の大部分は性感染によるものであり，特に元来本邦に存在しなかったgenotype Aの感染例が近年多く見られる．

B型急性肝炎の重症化例に対しては，積極的に核酸アナログを投与し，肝炎の進展を抑えるべきであるが，性感染による急性B型肝炎ではHIV（ヒト免疫不全ウイルス）との重感染の可能性があり，投薬時には薬剤耐性ウイルスの出現に対する配慮が必要である．

〔日内会誌　96：2450～2455，2007〕

**Key words**：B型感染ウイルス（HBV），性感染，核酸アナログ，ヒト免疫不全ウイルス（HIV）

我が国でのB型慢性肝炎の原因は，そのほとんどがHBVキャリア，特にHBe抗原陽性キャリアである母親からその子供への産道感染を契機とした持続感染が主なものである．1986年から始まったHBV母児感染予防事業の普及により，母児感染はほとんど成立しなくなり，将来的にHBV感染はなくなると考えられていた．しかしながら，近年急性B型肝炎症例が増加傾向にあり，さらに急性肝炎から慢性化する症例が以前より増加してきていることが分かった．

成人でのB型急性肝炎の大部分は性感染によるものであり，さらに急性肝炎症例のgenotypeの多くは我が国に存在しなかったgenotype Aであることが明らかになってきた．平成17年度の熊田らによる厚労省肝炎等克服緊急対策研究事業報告によると，全国13施設のB型急性肝炎321例のgenotypeはA：26%，B：12%，C：58%であった（図1）[1]．これは本邦のHBVキャリアのgenotype分布，genotype Cが約70%，Bが20数%，genotype A，Dがごくわずかという結果とは異なり，genotype Aの急性感染例が著明に増加してきていることがわかる．genotype

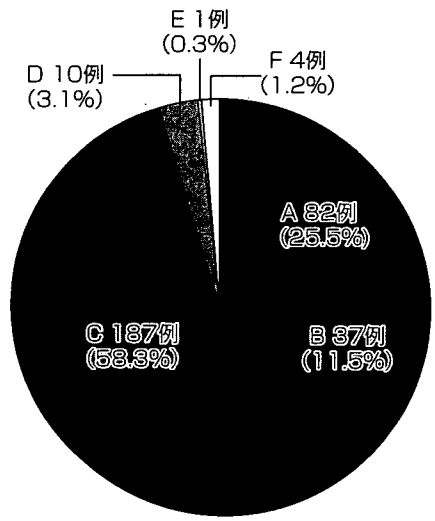


**図1．HBV genotypeを測定し得た321例のHBV genotypeの頻度**（文献1）

たかはし　しょういち，ちゃやま　かずあき：広島大学大学院分子病態制御内科学

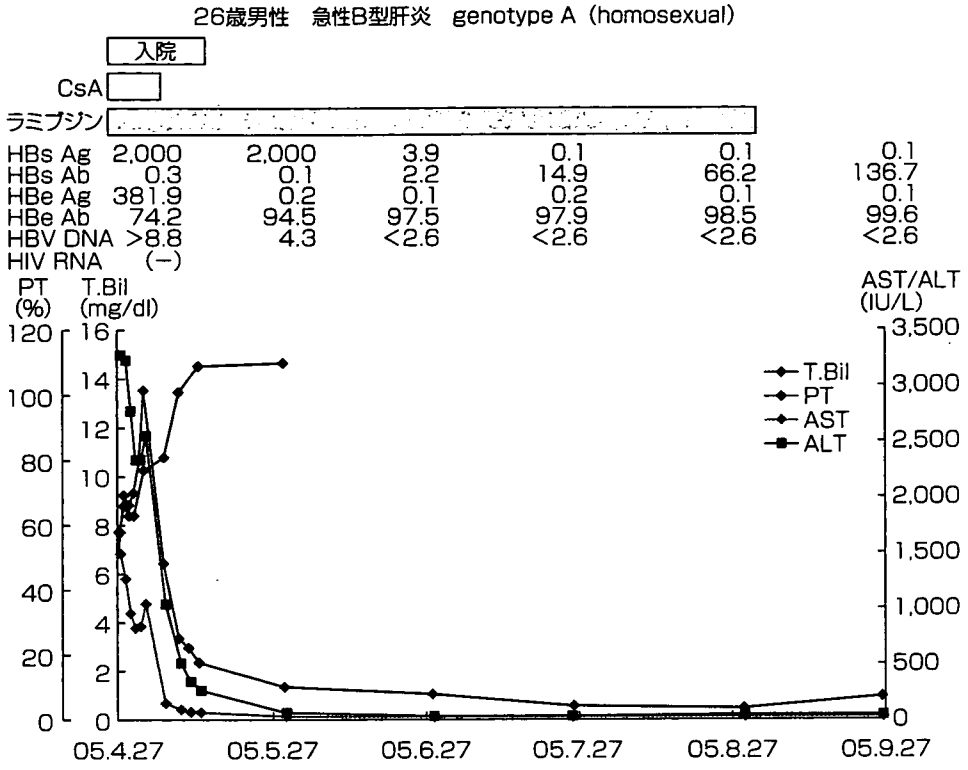


**図 2.　経過表**

Aは本来我が国には存在しなかった，欧米，西アフリカ，フィリピンに多く認められているgenotypeであり，我が国の国際化を反映した現象と考えられる．またgenotype Aはgenotype B，Cに比して慢性化しやすいことが知られている．熊田らの報告によると，genotype Aの慢性化率は14.6% であり，genotype Bの5.4%，genotype Cの4.8% に比して高率であった．従って今後は垂直感染によるHBVの慢性化に変わり，genotype Aの水平感染による慢性化，HBVの蔓延が危惧される[1]．

B型急性肝炎の治療の基本は，自己のリンパ球（免疫力）によるHBV感染肝細胞の排除を促すことにあり，劇症化のおそれがなければ無投薬で経過観察をすることである．食欲低下があれば補液をすることは必要であるが，基本的には肝庇護剤等は慢性化の助長につながるため，使用を控えるのが原則である．なお肝炎の重症化，劇症化のおそれがある場合は積極的に逆転写酵素阻害剤の使用，人工肝補助療法を行い，それでも救命できない場合は生体肝移植を選択することもある．当科の症例を呈示する．本症例はgenotype Aの急性肝炎で入院時PT 58% と劇症化のおそれがあったためラミブジンとサイクロスポリン（CsA）を投与し，回復した（図2）．

一方B型慢性肝炎では，ウイルスの完全な排除が難しいため，HBVDNAの増殖を持続的に抑制していくこと，肝炎を沈静化させて肝病変を改善し，肝硬変や肝癌への進展を防いでいくことが重要になる．実際のB型慢性肝炎治療については「B型およびC型肝炎ウイルスの感染者に対する治療の標準化に関する臨床的研究」班（厚生労働科学研究補助金　肝炎等克服緊急対策研究事業）より平成18年度B型慢性肝炎治療の新ガイドラインが発表されている（表1，2）[2]．HBeのナチュラルセロコンバージョンが期待できる

表1. 平成18年度B型慢性肝炎の治療ガイドライン

35歳未満

| HBV-DNA | ≧7 log copies/ml | <7 log copies/ml |
|---|---|---|
| e抗原陽性 | IFN長期間歇 | IFN長期間歇 |
| e抗原陰性 | 経過観察 | 経過観察 |

(進行例はEntecavir)

表2. 平成18年度B型慢性肝炎の治療ガイドライン

35歳以上

| HBV-DNA | ≧7 log copies/ml | <7 log copies/ml |
|---|---|---|
| e抗原陽性 | ①Entecavir<br>②IFN長期間歇 | Entecavir |
| e抗原陰性 | Entecavir | Entecavir |

年齢が一般に35才前後までであることから，この年齢を目安としてガイドラインが設定されている．35歳未満の若年症例に対してはHBe抗原陽性例に対しては，自己の免疫力によりHBe抗原の陰性化や肝炎の収束が期待されるため，IFN長期間歇投与を基本的に行い，HBe抗原陰性例に対しては原則的に経過観察を行うが，肝病変進行例ではエンテカビル投与も考慮する．なお，B型肝炎では，肝線維化と血小板数などの臨床検査値の増悪は平行しないことも多く，肝病変の進行度は肝生検で診断すべきであり，その結果をふまえて治療すべき症例と，経過観察する症例を選択することが重要である．一方35歳以上の中高年者に対しては，エンテカビル投与が原則となる．エンテカビル(バラクルード®)は2006年9月に発売になったB型慢性肝炎，B型肝硬変に対する逆転写酵素阻害剤であり，B型慢性肝疾患に対する同種の薬剤としては，ラミブジン(ゼフィックス®)，アデフォビル(ヘプセラ®)に次いで本邦で3剤目となる．逆転写酵素阻害剤は，細胞内で宿主のデオキシリボ核酸と競合し，ウイルス由来の逆転写酵素によるウイルスDNAの合成を阻害し，ウイルスの複製を抑える働きをする．もともとHIVの治療薬として開発された経緯があるが，HBVでもその増殖を抑えることがわかり，臨床応用されるようになった．

これらの逆転写酵素阻害剤の作用機序について簡略に解説すると，不完全2本鎖DNAウイルスであるB型肝炎ウイルス(HBV)のウイルスゲノムは，肝細胞の核内でいったん完全2本鎖DNAとなり，宿主のRNA polymeraseでプレゲノムRNAとなった後，ウイルス由来の逆転写酵素を用いてHBVゲノムDNAを複製する．エンテカビルなどの逆転写酵素阻害剤は宿主のゲノムDNAを構成するデオキシアデノシン，デオキシグアノシンなどのヌクレオシドに構造的に非常に類似した核酸アナログとよばれる物質であるが，この核酸アナログがHBVプレゲノムRNAの逆転写反応中に伸長されるHBVゲノムDNA中に取り込まれ，そのとなりのヌクレオシドとは共有結合が行われないことよりDNA伸長反応が止まる，いわゆる「chain termination」という現象によりウイルスゲノムの複製を抑制している(図3)．ちなみにエンテカビルはグアノシンの核酸アナログ，ラミブジンはシチジン，アデフォビルはアデノシンの核酸アナログである．

これらの核酸アナログは，投与開始後数週間で速やかにHBV DNA，ALTの低下が見込める

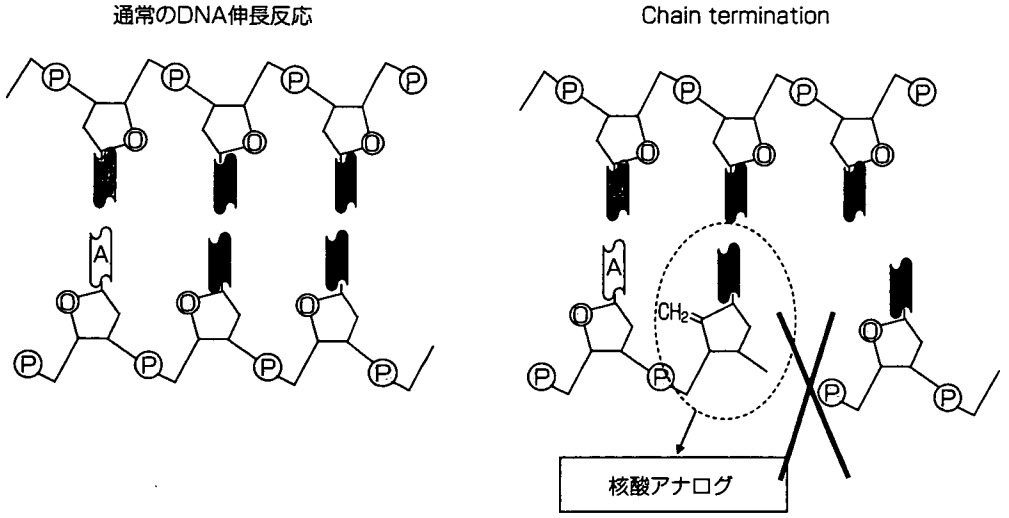


**図3. 核酸アナログの作用機序**

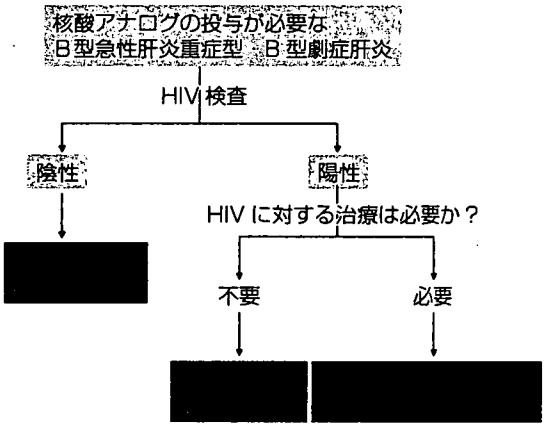


**図4. HBV/HIV重感染者に対して，抗HIV作用を併せ持つ抗HBV核酸アナログは単剤投与してはならない**

一方で，内服中止すると大部分の症例で再燃が認められる点，またHBVゲノムDNAの変異による耐性株の出現する点が大きな問題である．特にラミブジンにおける耐性株の出現率の高さは深刻で，当科のデータでも投与開始4～5年でほぼ半数の患者に耐性株が出現している．ラミブジン耐性株はHBV polymeraseの逆転写酵素領域のYMDDモチーフと呼ばれるアミノ酸配列がYIDDもしくはYVDDに変異することにより起こる（M204I，M204Vと表記）[3]．アデフォビル長期投与による耐性株の出現頻度はラミブジンに比して低いが，2年で3%，4年で18%の変異株の出現を認めている（A181TまたはN236T）．エンテカビルは国外でも発売されて間もないため，長期投与のデータはまだないが，核酸アナログ未使用症例では，投与2年後で現在までのところ，変異株の出現をほとんど認めていない．しかしながら，すでにM204IまたはM204Vの変異を持つラミブジン耐性株に対するエンテカビルの投与においては，1年で7%，2年で9%のエンテカビル耐性株の出現を認めているため，すでにラミブジンに耐性を有する場合には，エンテカビルへの変更ではなく，従来通りラミブジン＋アデフォビルの併用療法を行うべきである．

Genotype AのHBVの感染は，男性間のhomosexual transmissionでしばしば認められ，時としてHBVとHIVの重複感染がみられる．従ってB型急性肝炎患者に対し，核酸アナログを使用しなければならない重症例を診たときは以下の点に注意が必要である（図4）．

HBVとHIVはウイルス複製の際に逆転写され，このステップを阻害するのが，核酸アナログ＝逆転写酵素阻害剤である．従って一部の核酸ア