Table 4 Univariate analysis of association between sustained virological response (SVR) and influential factors (mean ± SE)

Factor	SVR patients (n = 20)	Non-SVR patients (n = 37)	P
Parameters before interferon treatment			
Age (yr)	64.0 ± 0.71	63.8 ± 0.73	0.6637
Sex (male:female)	16:4	18:19	0.0262
Body mass index	23.3 ± 0.56	23.6 ± 0.53	0.3973
Viral load (kirocopies/mL)	1500	1800	0.3616
ALT (IU/L)	94.5 ± 31.1	76.6 ± 11.0	0.3038
WBC (/μL)	5119 ± 313	4832 ± 223	0.3798
Hemoglobin (g/dL)	14.1 ± 0.23	14.1 ± 0.19	0.8473
Platelet (× 10^3/μL)	173 ± 12.4	151 ± 7.9	0.1434
Parameters associated with treatment			
EVR	13/18 (72.2%)	7/32 (21.9%)	0.0008
Cumulative exposure to peg-IFN			
12 wk (≥ 80%/< 80%)	16/17 (94.1%)	20/33 (60.6%)	0.0183
Overall (≥ 80%/< 80%)	15/17 (88.2%)	14/33 (42.4%)	0.0023
Cumulative exposure to RBV			
12 wk (≥ 80%/< 80%)	14/20 (70%)	20/34 (58.8%)	0.5612
Overall (≥ 80%/< 80%)	8/20 (40%)	13/34 (38.2%)	> 0.9999

ALT: Alanine aminotransferase; WBC: White blood cell; EVR: Early virological response; Peg-IFN: Pegylated interferon; RBV: Ribavirin.

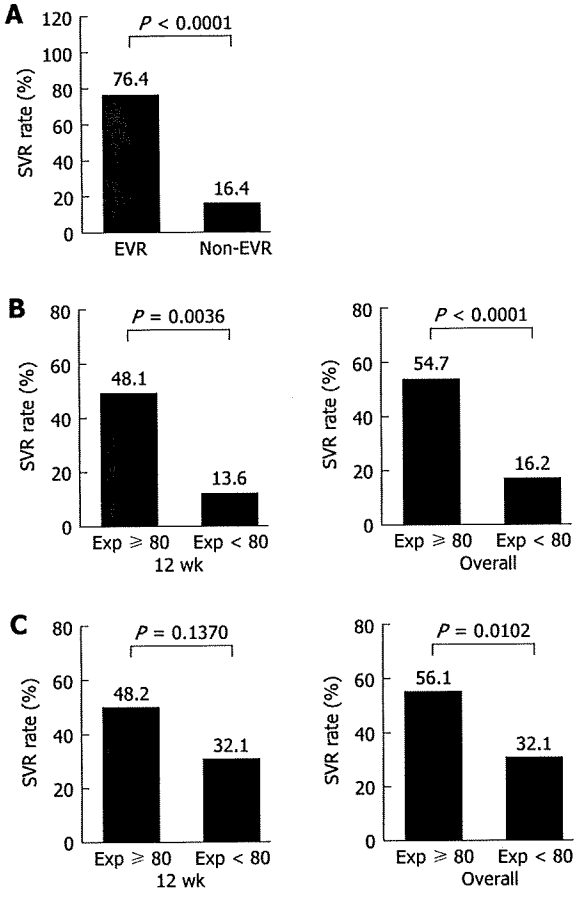

Figure 2 The clinical parameters associated with SVR rate using univariate analysis. A: The relationship between EVR and SVR rate; B: The relationship between cumulative exposure to peg-IFN and SVR rate; C: The relationship between cumulative exposure to RBV and SVR rate.

to be a parameter which may be associated with SVR (Table 4). The SVR rate in females was 17%, which was significantly lower than the SVR rate of 50% in males

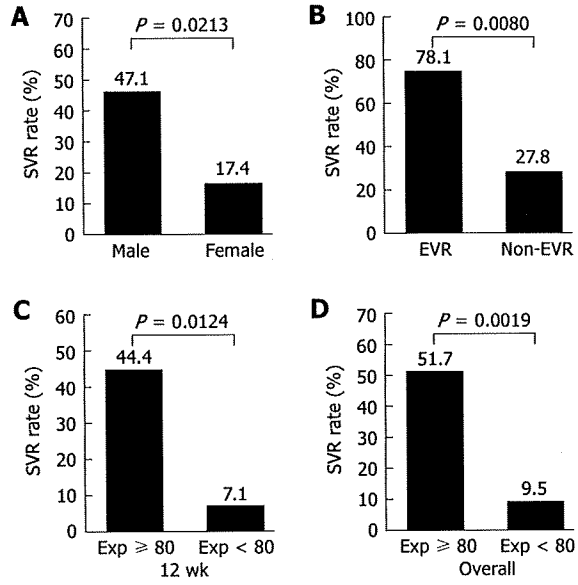

Figure 3 Clinical parameters in the older patient group (> 56 yr) associated with SVR rate using univariate analysis. A: The relationship between sex and SVR rate; B: The relationship between EVR and SVR. C and D: The relationship between cumulative exposure to peg-IFN and SVR.

(P = 0.0262) (Figure 3A). From the factors associated with treatment outcome, EVR and adherence to peg-IFN were demonstrated to be significant (Figure 3B). In particular, the SVR rate in the group with poor adherence to peg-IFN was 7.1% (1 of 14) at the 12th wk and was 9.5% (2 of 21) at the end of treatment. These rates were extremely low compared with the SVR rate of 44.4% (12th wk) and 51.7% (overall) in the group with good adherence to peg-IFN (Figure 3C and D).

DISCUSSION

One hundred of 130 patients completed peg-IFNα-2b plus RBV combination therapy in our hospital and related institutions. Treatment was discontinued in 13 patients (10.8%) due to adverse effects. The treatment showed good tolerability in Japanese patients. A study of peg-IFNα-2b plus RBV combination therapy in Caucasian and African American CHC patients reported a discontinuation rate of 21%[10]. Another study on Japanese CHC patients reported a 21% discontinuation rate[9]. Although we cannot compare these studies directly, it seems that tolerability in this study was satisfactory. At least in a clinical setting, peg-IFNα-2b plus RBV was well tolerated in our study of Japanese patients.

In this study, age, ALT level, EVR achievement, and adherence to Peg-IFN and RBV were associated with a high SVR rate using univariate analysis. After multivariate analysis, EVR and adherence to peg-IFN were demonstrated to be associated with SVR. Of the baseline factors assessed before treatment, age, sex, WBC, α-feto protein level, γ-glutamyl transpeptidase, and LDL-cholesterol have been reported to be associated with a high SVR rate following peg-IFNα-2b plus RBV therapy in CHC Japanese patients[8,11,12]. The results of this study were very similar to those of our

study. Davis *et al*[13] reported that EVR was considered to be associated with SVR in patients with CHC treated with IFN. As a result of this study, EVR was found to be one of the factors which most influenced SVR rate in Japanese patients treated with peg-IFNα-2b plus RBV combination therapy. In our study of older patients (older than the median), sex, EVR, and adherence to peg-IFN were associated with SVR rate. The SVR rate in older females was remarkably low at 17.4% compared to the SVR rate in all females included in the study which was 36.0% (data not shown).

Adherence to peg-IFN was found to influence the SVR rate as a treatment-related factor in this study. SVR rates were low in patients who did not receive 80% or more of the intended dose of peg-IFN. The effect of adherence to IFN on SVR has been reported previously[13-15]. In a study on peg-IFNα-2a/RBV therapy in patients with HCV genotype I, it was reported that the SVR rate in cases who had a reduction in RBV dosage before the 20th wk was remarkably low[15]. Furthermore, a reduction in RBV dosage and/or peg-IFNα-2a dosage after the 24th wk did not influence the SVR rate[15]. On the other hand, a study on African American patients with HCV genotype I reported that a reduction in peg-IFNα-2b dosage influenced the SVR rate more than a reduction in RBV dosage[14]. In the current study, adherence to RBV up to the 12th wk did not significantly influence the SVR rate, but overall adherence to RBV significantly influenced the SVR rate. Unlike the reports on Caucasian and African American patients, it may be that overall adherence to RBV is important in Japanese patients.

It was notable that adherence to peg-IFNα-2b significantly influenced SVR in this study. In the patients who did not receive 80% or more of the intended dose by the 12th wk, the SVR rate decreased markedly. Adherence to peg-IFNα-2b at the 12th wk may be critical in determining whether the treatment should be continued. It is often difficult to maintain adherence to peg-IFNα-2b simply to improve the SVR rate, because IFN dosage and the hematologic adverse effects of this drug are problematic[16,17]. Recently, a 72-wk treatment protocol for late virological responders was reported[18,19]. Further examination of the impact of prolonged administration in patients with poor adherence to peg-IFNα-2b is needed.

In conclusion, peg-IFNα-2b plus RBV combination therapy demonstrated good tolerability in Japanese patients with CHC, and resulted in a SVR rate of 44.3%. Treatment of older female patients and maintenance of adherence to peg-IFNα-2b are important factors in improving SVR rate.

COMMENTS

Background

Pegylated interferon α-2b (peg-IFNα-2b) plus ribavirin (RBV) is a standard treatment of chronic hepatitis C globally. However, the impact of this treatment in an ordinary clinical setting in Asian patients is still unclear.

Research frontiers

It is well documented that data from clinical practice is not comparable to those of clinical trials. This is believed to be derived from differences in recruited patients in phase II and III clinical trials and usual clinical settings (e.g. young *vs* elderly, highly motivated *vs* reluctant, *etc*).

Innovations and breakthroughs

The current study demonstrated that outcome is dependent on therapeutic adherence (> 80% of expected peg-IFN dosage). The overall treatment success [sustained virological response (SVR)] was 44.3%, almost equivalent to those in phase III clinical trials.

Applications

The total SVR rate was equivalent to clinical trials. The elderly, especially female patients showed a lower response to treatment. The reason for this is still unclear and future investigations are feasible in order to understand this observation.

Terminology

SVR indicates sustained virological response, which means sustained (more than 24 wk after treatment) viral clearance from the infected host.

Peer review

It is very important to describe the true clinical impact of global standard treatment in Asian races. Fortunately, the results were almost equivalent to those of other global regions. Although female patients seem to have a disadvantage with this treatment, these patients could have comparable results if adherance to both drugs is maintained.

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Case report

Analysis of the full-length genome of hepatitis B virus in the serum and cerebrospinal fluid of a patient with acute hepatitis B and transverse myelitis☆

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Abstract

Although many extrahepatic manifestations have been described in patients with acute or chronic hepatitis B, there are few reports about neurological disorders. We describe a 55-year-old man who contracted acute hepatitis B virus (HBV) infection and transverse myelitis. His neurological findings were gradually reduced along with the recovery from hepatitis. The cerebrospinal fluid (CSF) was revealed to be positive for HBsAg and HBV DNA. Full-length sequences of HBV in his serum and CSF were determined, and it was revealed that these two isolates had mutations at nucleotide (nt) 1762/1764 in the core promoter region and nt 1896 in the precore region. They were identical to each other except for two ambiguous codes at nt 2020 and 2631 in the CSF isolate. After cloning of the amplicons, substitutions at nt 2020 and 2631 were found in 6 (38%) of the 16 CSF clones. One clone of the 6 CSF clones had an additional substitution at nt 2119. These substitutions were not found in 16 serum clones. The presence of HBV clones unique to CSF suggests that HBV was a possible causative agent of the myelitis.

Keywords: Hepatitis B virus; Acute hepatitis B; Transverse myelitis; Cerebrospinal fluid; Sequence analysis; Full-length genome

1. Introduction

Although hepatocytes are the primary locus of infection of hepatitis B virus (HBV), it was reported that 16% of patients with chronic HBV infection have extrahepatic clinical manifestations (Cacoub et al., 2005). Many of these are thought not due to extrahepatic infection of HBV, but to autoimmune and related phenomena. However, increasing evidence suggests the possibility of HBV infection of non-hepatic cells (Mason et al., 1993; Neurath et al., 1990; Seifer et al., 1990; Umeda et al., 2005). Although HBV DNA has been found in the cerebrospinal fluid (CSF) of infected individuals (Pao et al., 1987), there is little detailed information concerning HBV infection in the central nervous system (CNS). The relation between HBV and neurological disorders is poorly understood. Recently, a case of acute hepatitis B that occurring soon after the acute onset of transverse myelitis was seen in our hospital, suggesting a causal relationship. Because such a case has never been reported previously, evaluation of HBV markers in CSF and sequence analysis of HBV DNA in the serum and CSF were undertaken.

2. Case report

A 55-year-old Japanese male was admitted to our hospital with complaints of numbness in the perianal area, weakness

☆ The nucleotide sequence data reported in this study have been assigned GenBank/EMBL/DDBJ accession numbers AB298720 and AB298721.

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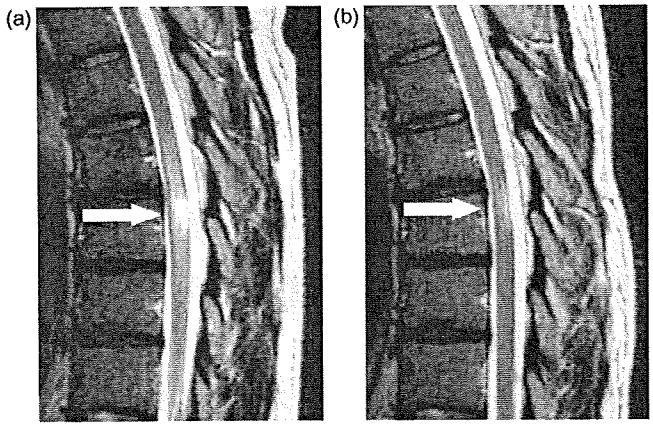

Fig. 1. (a) T2-weighted MRI of the thoracic spinal cord before the onset of acute hepatitis. A high intensity lesion at the Th7-8 level with spinal swelling is observed. (b) T2-weighted MRI 2 months after discharge. The lesion of the spinal cord is diminished.

and numbness of both lower extremities, and difficulty in urination. These symptoms began 2 months previously; peaked in severity after 1 month; then began slowly improving for the next month before admission. T2-weighted magnetic resonance imaging (MRI) of the thoracic (Th) spinal cord showed a high intensity area at the Th7-8 level (Fig. 1a) consistent with transverse myelitis. CSF obtained on hospital day 3 contained 10 monocytes/μL and 69 mg/dL of protein. At this time, liver function tests revealed elevation of alanine aminotransferase (ALT) (491 IU/L). His serum contained hepatitis B surface antigen (HBsAg) and was weakly positive for immunoglobulin M (IgM) antibody to hepatitis B core (HBc) (anti-HBc IgM). Both hepatitis B e antigen (HBeAg) and antibodies to HBeAg (HBeAb) were negative, and the HBV DNA concentration in plasma determined by transcription-mediated amplification (TMA) assay was over 8.6 log genome-equivalent. Ten days later, anti-HBc IgM increased and he was diagnosed with acute hepatitis B. The infection was presumed to have been acquired during sexual contact with a prostitute in Korea 3 months previously. He had no other risk factors for HBV infection. Without antiviral therapy, his liver function recovered from a peak ALT of 2632 IU/L and peak total bilirubin of 14.0 mg/dL. Hepatic encephalopathy, coagulopathy or ascites did not occur. His neurological findings continued to gradually improve along with liver function tests. After discharge on hospital day 30, HBsAg and HBV DNA became to be undetectable and HBsAb has turned to be positive. Two months after discharge, MRI showed that the spinal cord lesion had diminished (Fig. 1b).

To clarify the relationship between HBV infection and myelitis the CSF was evaluated for evidence of HBV infection. The CSF sample obtained in our hospital was positive for HBsAg and contained 1.6×10^6 copies/mL of HBV DNA measured by real-time PCR in the LightCycler system (Roche Diagnostics, Mannheim, Germany) as reported previously (Jardi et al., 2001). The concentration of HBV DNA in the serum at the time of sampling of CSF was 7.7×10^{10} copies/mL and had been 1.1×10^{11} copies/mL the previous day. Anti-HBc IgM in the CSF was not detectable by in-house ELISA (Tsatsralt-Od et al., 2006).

Because red blood cells in the CSF was not determined during the routine examination, the extent of blood contamination could not be evaluated. To determine the origin of the HBV in the CSF, we compared full-length sequences of HBV in serum and CSF. Using the serum and CSF samples obtained on the same day, amplification of the entire HBV genome was performed by methods similar to those described previously (Shibayama et al., 2005). Briefly, two overlapping regions (nt 190–1775 and nt 1673–3215/1–228) of HBV DNA were amplified by nested PCR with PrimeSTAR HS DNA Polymerase (TaKaRa Bio Inc., Shiga, Japan) and primers designed within conserved areas of the HBV genomes of the eight genotypes (A to H) (Arauz-Ruiz et al., 2002; Norder et al., 1994; Okamoto et al., 1988; Stuyver et al., 2000). The amplification products were sequenced directly or after cloning on both strands. The phylogenetic tree constructed based on the full genome sequences of HBV revealed that the HBV isolates from serum (BAJT2006-1S) and CSF (BAJT2006-1C) belonged to genotype C (Fig. 2). These isolates had mutations at nucleotides (nt) 1762/1764 (A1762T/G1764A) in

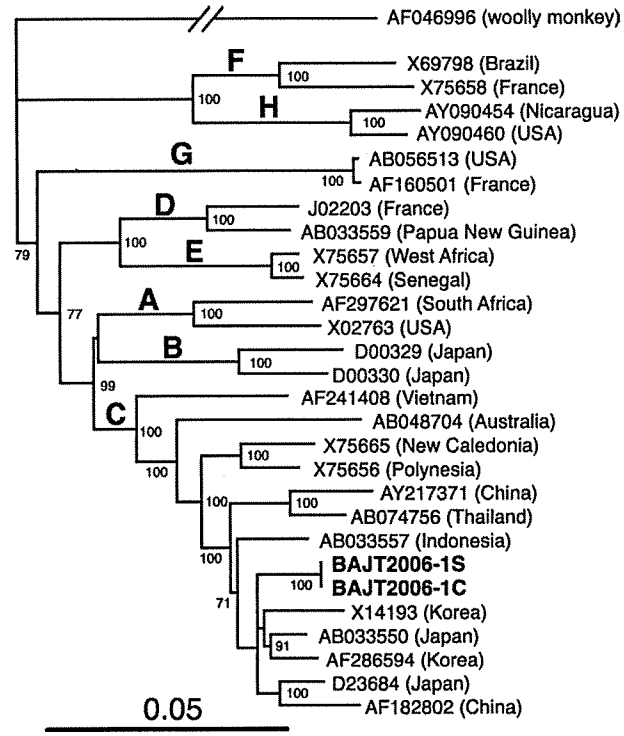

Fig. 2. A phylogenetic tree constructed based on the entire nucleotide sequences of 28 HBV isolates, using a woolly monkey HBV isolate (AF046996) as an out group. In addition to the two isolates obtained from serum (BAJT2006-1S) and CSF (BAJT2006-1C), 26 reported HBV isolates of genotypes A to H were included for comparison. The previously reported isolates are indicated with the accession no. followed by the name of the country where it was isolated. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1,000 resamplings.

(a)

```
BAJT2006-1S  2001 CCTCTGCTCTGTATCGGGAGGCCTTAGAGTCTCCGGAACATTGTTCACCT  2050
BAJT2006-1C  2001 ------------------R---------------------------  2050
GT C wild    2001 ----------------------------------------------  2050

BAJT2006-1S  2601 CTTACAGTTAATGAAAAAAGGAGATTAAAATTAATTATGCCTGCTAGGTT  2650
BAJT2006-1C  2601 ------------------------------W-----------------  2650
GT C wild    2601 ----------------------------------------------  2650
```

(b)

Sample	No. of clones	Nucleotide no. 2020	2119	2631
Serum	16	G	A	T
CSF	10	G	A	T
CSF	5	A	A	A
CSF	1	A	G	A

Fig. 3. (a) Alignment of partial sequences (nt 2001–2050 and 2601–2650) of BAJT2006-1S and BAJT2006-1C. Sequence of wild type HBV of genotype C (GT C wild) was included for comparison. R is G or A, and W is A or T. (b) Nucleotides at nt 2020, 2119 and 2631 after cloning of BAJT2006-1S (serum) and BAJT2006-1C (CSF). The numbers of clones which had each indicated nucleotide were shown.

the core promoter region and nt 1896 (G1896A) in the pre-core region, and were identical to each other except for two ambiguous codes at nt 2020 (R: G or A) and nt 2631 (W: A or T) in BAJT2006-1C (Fig. 3a). After cloning of the amplicons of nt 1673–3215/1–228 recovered from serum and CSF, G to A substitutions at nt 2020 (G2020A) and T to A at nt 2631 (T2631A) were found in 6 (38%) of the 16 CSF clones (Fig. 3b). One clone of the six CSF clones with G2020A and T2631A had an additional substitution at nt 2119 of A to G (A2119G). None of the 16 serum clones had these substitutions. G2020A and A2119G did not change the amino acid sequences, whereas T2631A converted amino acid 109 of Leu to Ile in HBV polymerase.

3. Discussion

Because there are few reports about HBV in the CNS, it remains unknown whether HBV is merely present in the CNS after blood stream dissemination or can replicate in the CNS of HBV-infected individuals, especially in patients with acute HBV infection. In this report, HBsAg and HBV DNA were detected in the CSF of a patient with acute hepatitis B and transverse myelitis. Evidence that this did not represent contamination of CSF with blood was provided by sequence analysis of blood and CSF HBV clones. The HBV clones obtained from CSF were heterogeneous and 38% (6/16) of the clones were different from homogeneous clones obtained from the serum. Although the virological significance of the substitutions at nt 2020, 2119, and 2631 is unclear, HBV with these substitutions might favor replication in the CNS. The presence of HBV clones unique to CSF suggests that the HBV DNA in the CSF was not from contamination of by blood, and that HBV was possibly the cause of the myelitis. Additionally, the HBV isolates recovered from serum and CSF had in common A1762T/G1764A and G1896A, which are known to be associated with fulminant hepatitis B (Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991; Sato et al., 1995). The strains with these mutations have heightened replicative activity (Baumert et al., 1996; Hasegawa et al., 1994). Although the patient in this study did not develop fulminant hepatitis, the concentration of HBV in the serum in the acute phase reached 1.1×10^{11} copies/mL. The high replicative potential of this patient's HBV, in addition to nt substitutions in the CSF clones, may have contributed its ability to replicate in the CNS and cause myelitis.

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Case Report

A case of HIV co-infected with hepatitis B virus precore/core deletion mutant treated by entecavir

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We report a case of a HIV and hepatitis B virus (HBV)-co-infected patient to whom entecavir (ETV) was administered initially before the notification regarding the potential mutagenesis effect on HIV against the nucleoside analog. Since initial evaluations indicated the advanced stage of chronic hepatitis B and preserved numbers of peripheral CD4+ lymphocytes without the manifestation of immunodeficiency, priority was given to the management of HBV. We started HBV therapy with ETV at a dose of 0.5 mg daily without using any HIV drugs. The viral loads of both HBV and HIV-1 decreased gradually during the 5 months following the initial administration of ETV. HBV was well controlled by the gradual replacement of ETV with highly-active antiretroviral therapy against HIV with a regimen including atazanavir, emtricitabine, and tenofovir. HBV was genotyped as A2 with the quasispecies pool consisting of the –1G precore/core deletion mutant strain.

Key words: co-infection, entecavir, hepatitis B virus, HIV

INTRODUCTION

ENTECAVIR (ETV), AN analog of 2′-deoxyguanosine, is regarded as an effective inhibitor of hepatitis B virus (HBV) and also inhibits HIV-1 replication both *in vitro* and *in vivo*. It selects for mutations (such as the M184V mutation) in HIV-1 reverse transcriptase, leading to lamivudine (LMV) and emtricitabine (FTC) resistance. Previous guidelines recommended ETV as the first-line treatment for patients with HIV-1 and HBV co-infection who do not require anti-HIV therapy.[1,2] In April, 2007, the US Food and Drug Administration (FDA) recommended avoiding the usage of ETV for HBV- and HIV-co-infected patients since the inhibitory effect of ETV against HIV may develop resistant HIV against nucleoside therapy.[3] A novel HBV precore/core deletion mutant referred to as the –1G deletion was identified in HIV-co-infected patients.[4] The –1G deletion is located in a homopolymeric string of guanosine nucleotides between HBV core nucleotides 185 and 190 and introduces a frameshift that terminated the coding sequence of the HBV core and precore genes, which leads logically to the production of the corresponding truncated core protein. The –1G deletion mutant was associated with a high HBV viral DNA concentration in these cases.

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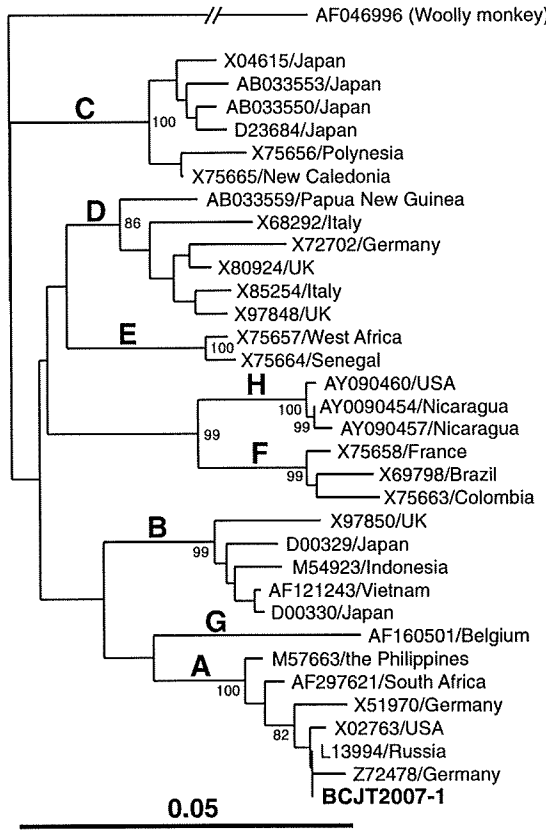
CASE REPORT

A 39-YEAR-OLD man visited us in December 2006 for the management of his hepatitis B. He also had carried HIV without any symptoms. The results of the initial examination of his serum were as follows: aspartate aminotransferase, 115 IU/l; alanine aminotransferase, 62 IU/l; total bilirubin, 2.2 mg/dL; prothrombin time (%, international normalized ratio), 32.2%, 2.22; number of peripheral lymphocytes, 1550/μL; CD4/CD8, 30.5%/60.0% (0.5); number of platelets, 50×10^3/μl; hepatitis B surface (HBs) antigen, positive; anti-HBs antibody, negative; hepatitis B e (HBe) antigen, positive; anti-HBe antibody, negative;

(a)



(b)

	Nt and aa sequences of nt 2063-2101	No.clones	
Genotype A wild	CTCAGGCAAGCCATTCTCTGCTGGGGGGAATTGATGACT LeuArgGlnAlaIleLeuCysTrpGlyGluLeuMetThr	Pre-ETV (23 Jan)	Post-ETV (3 Apr)
-1G deletion	CTCAGGCAAGCCATTCTCTGCTGGGGG/AATTGATGACT LeuArgGlnAlaIleLeuCysTrpGly Asn***	4/10	0/10

Figure 1 (a) Phylogenetic tree constructed based on the partial nucleotide sequence of the S gene (396 nt) of 33 hepatitis B virus (HBV) isolates, using a woolly monkey HBV isolate (AF046996) as an outgroup. In addition to the isolate obtained from the present case (BCJT2007-1), 32 representative HBV isolates of genotypes A–H were included for comparison. Previously reported isolates are indicated with the accession number followed by the name of the country where it was isolated. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1000 resamplings. (b) Sequence alignments and numbers of the subcloned HBV −1G precore/core deletion mutant from 10 clones at a pair of time points are shown. Wild sequence was identical to the reference sequence of genotype A HBV listed in DDBJ/EMBL/GenBank (accession number: Z72478). ***, stop codon.

HBV-DNA, 7.6 log (10) copies/mL; HBV genotype, A2 (Fig. 1a); immunoglobulin G 4670 mg/dL; antinuclear antibody, 320×; antismooth muscle antibody, 160×; HIV-1 RNA 23×10^3 copies/mL; and antihepatitis C antibody, negative. A further analysis of the HBV sequence revealed a −1G precore/core deletion mutant along with the wild strain (Fig. 1b). Liver biopsy was not performed because of impaired coagulation. We considered his liver function to be seriously impaired, probably due to the chronic hepatitis B or autoimmune hepatitis. With regard to HIV, antiviral therapy was not necessary because of the preserved numbers of $CD4^+$

lymphocytes and low titers of HIV RNA. Thus there were several concerns to be considered for the management of his hepatitis. Our decision was to start anti-HBV therapy with the oral administration of a nucleoside analog, ETV, which appeared to be the best option at that time in January 2007. His serum number of HBV-DNA and HIV RNA decreased gradually along with an improvement of liver function during the 5 months from the initial therapy with ETV at the dose of 0.5 mg daily (Fig. 2). Based on the FDA recommendation at the end of April 2007, highly-active antiretroviral therapy (HAART) against HIV and HBV, which was the only alternative option, was employed. HAART, with a combination of 200 mg atazanavir, 300 mg tenofovir (TDF), and 200 mg FTC was started in early in July 2007 with the patient's consent. In the mutational analysis, we did not detect M204V/I of HBV.

Laboratory testing procedures are performed as follows.The titers of serum HBs antigen, HBs antibody, HBe antigen, and the HBe antibody, or a number of copies of HBV-DNA and HIV RNA were evaluated by chemiluminescent immunoassay, enzyme-linked immunosorbent assay, or polymerase chain reaction (PCR) and reverse transcription PCR on consignment with commercially-available diagnostic kits. Nucleic acids were extracted from 50 μL serum with a QIAamp DNA blood mini kit (QIAGEN, Tokyo, Japan). For HBV genotyping and the mutational analysis of the core promoter and precore region, a nucleic acid sample was subjected to nested PCR with primer sets based on the well-conserved sequence in the S gene region and corresponding regions. A phylogenetic tree was constructed by the neighbor-joining method based on the 396-nt sequence identified by direct sequence with the ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA) for genotyping as described previously.[22]

DISCUSSION

THE JAPANESE MINISTRY of Health, Labor and Welfare approved interferon, LMV, and ETV as the first-line drugs for HBV infection. ETV has been a safe and effective modality for HBV therapy due to the rarity of resistant HBV strains compared to LMV,[5,6] and 8 years after the initial drug investigation, it is currently regarded as the most favorable medication for HBV infection in Japan.[7] The US Public Health Service's latest guidelines (http://AIDSinfo.nih.gov/contentfiles/AdultandAdolescentGL.pdf), the Japanese guidelines published by the Research Group for Therapy of HIV

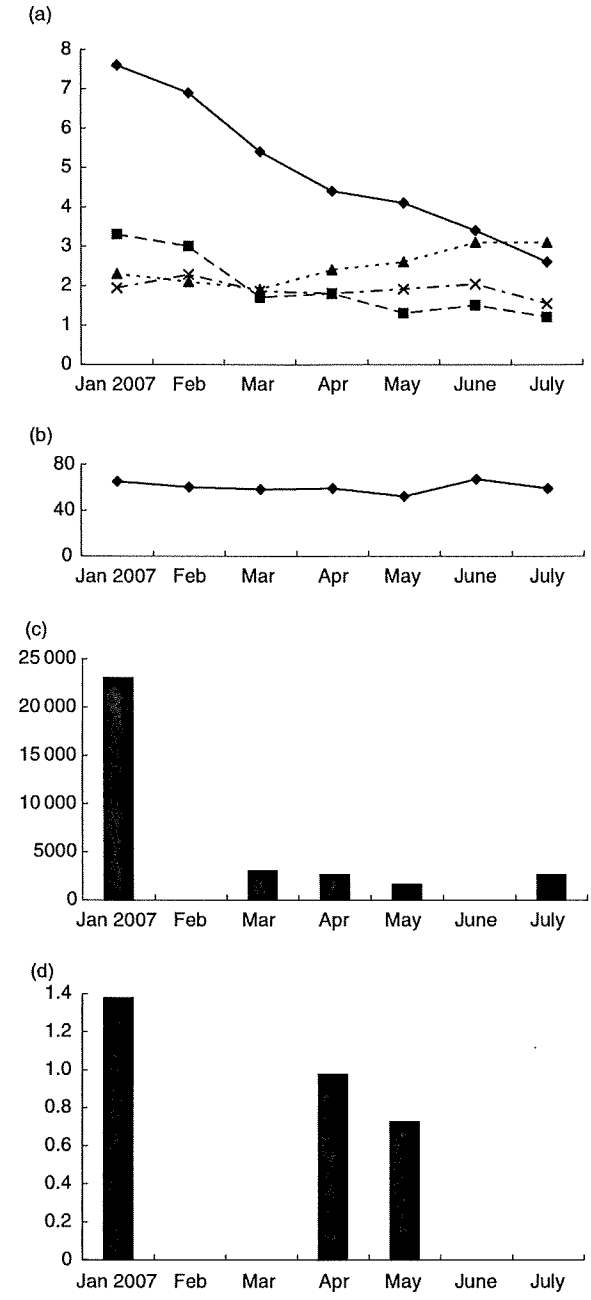


Figure 2 Time-courses of clinical parameters from the initial administration of entecavir on 23 January 2007 demonstrate improvement in liver function and a decrease in hepatitis B virus (HBV) and HIV in the serum. (a,b) HBV load and clinical parameters; (c) HIV load; (d), hepatitis B e antigen (HBe antigen). ALB, albumin; ALT, alanine aminotransferase; INR, international normalized ratio; OD, optical density; PT, prothrombin time; T-bil, total bilirubin. (a) —◆— HBV-DNA (log copies/mL), -■- T-BIL (mg/dL), ···▲··· ALB (g/dL), -✳- PT (INR). (b) —◆— ALT (IU/l). (c) ■ HIV RNA (copies/mL). (d) ■ HBe antigen (OD at 450 nm).

Infection in December 2006 (http://www.hivjp.org/), and other groups[8] have recommended ETV for hepatitis B treatment in HIV-1-infected people who do not meet the criteria for HIV-1 treatment.[9] The process of establishing the previous consensus should be reviewed. As for the HBV genotype, 85% of HBV-positive patients are genotype C, and 12% of patients are genotype B in Japan.[10] In contrast, only 1–10% of HBV-positive patients carry HBV of genotype A, which is increased mainly in urban districts, probably due to sexual transmission accompanied by chronic outcome.[11,12] The distribution of HBV genotypes in HIV-positive patients in Japan was A (50%), B (5%), C (24%), D (5%), E (2%), H (10%), A plus D (2%), and A plus G (2%).[13] Thus the current case represents the majority of cases of HBV and HIV co-infection in Japan. McMahon *et al.*[14] reported three cases showing an inhibitory effect on HIV by ETV with one case showing selection for the M184V mutation of HIV. They also confirmed that M184V conferred resistance to ETV by *in vitro* experiments. With regard to the current case, one log (10) reduction of HIV RNA load was observed during the 5 months of ETV monotherapy, and HIV mutation relevant to the therapy has been investigated, which will be published. As for HBV, ETV did not select for M204V/I, which is relevant to LMV resistance, and viral reduction of over two log was achieved during 5 month (over 7.6 to under 2.6 log copies/mL) even in the HIV-co-infected case. The genotype A HBV quasispecies pool consisted partly of the HBV −1G precore/core mutant which was revealed to have the higher prevalence in HIV–HBV-co-infected cases compared with the HBV mono-infected case, that is, 20% (5/26) versus 5% (3/62) before LMV therapy, and 40% (20/48) versus 0% (0/62) after LMV therapy.[4] The mutated HBV may need to be supplied with the HBV core protein in trans to form mature HBV particles.[15] An associated high HBV load may hypothetically lead to the progression of liver disease[16] due to the direct cytopathic effect against hepatocytes.[17] The current case suggests evidence of the prevalence of the −1G precore/core deletion mutant that prevails even in Japanese cases. This deletion mutant was originally found in Western countries among HIV–HBV-co-infected cases as a characteristic mutation related to high viral load. The −1G clone diminished during ETV therapy in accordance with the decrease of the viral load of wild strain. The biological and virological significance of the −1G deleted mutant still needs to be clarified. The patient's HBV is currently well managed by ETV and the succeeding combination of FTC and TDF. The numbers of either HBV- or HCV-co-infected HIV cases has risen to 8.8% and 4.3%, respectively, of all HIV-positive cases in Japan.[18] Thus the life-long strategy should be managed based on the latest information. Pegylated interferon, which induces seroconversion of HBe antigen at a higher rate among HBV genotype A-positive patients[19] with preserved liver function, might be an alternative option for ETV. However, interferon therapy was not applied to the current case for its potential of causing acute exacerbation of hepatitis B or underlying autoimmune hepatitis on nearly decompensated livers. Therefore, regardless of the HIV status, patients who need urgent care with severe, impaired liver function may need HAART, including plural anti-HBV nucleoside analogs. Moreover, for co-infected cases, especially for HBV/HIV-co-infected patients with a high HBV load and impaired immune/liver function,[20,21] concomitant or prior therapy against HBV to the anti-HIV therapy is recommended for the risk of flare based on the possible immune reconstitute. HBV and immunological status should be monitored closely in all cases under care in this context.

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免疫抑制・化学療法により発症するB型肝炎対策
—厚生労働省「難治性の肝・胆道疾患に関する調査研究」班劇症肝炎分科会および「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」班合同報告—

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田中　榮司　市田　隆文　溝上　雅史　鈴木　一幸　輿芝　眞彰
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梅村　武司　熊谷公太郎

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＜特別寄稿＞

免疫抑制・化学療法により発症するB型肝炎対策 —厚生労働省「難治性の肝・胆道疾患に関する調査研究」班 劇症肝炎分科会および「肝硬変を含めたウイルス性肝疾患の 治療の標準化に関する研究」班合同報告—

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索引用語：　劇症肝炎　HBV再活性化　*de novo* B型肝炎　核酸アナログ製剤　リツキシマブ

近年，化学療法，免疫療法，移植療法の進歩に伴い，多様な抗癌剤や免疫抑制剤を使用する機会が増加している．以前よりB型肝炎ウイルス（HBV）キャリアに合併した悪性腫瘍患者に対し，ステロイドを併用した化学療法を施行した場合，HBVの急激な増殖すなわちHBVの再活性化（reactivation）により致死的な重症肝炎が発症することが知られていた[1)2)]．HBV遺伝子にはglucocorticoid enhancement elementが存在するため[3)]，ステロイドにより直接的にウイルス複製が助長されるだけでなく，化学療法による免疫抑制や治療終了後に生じる免疫学的な均衡の破綻により，HBVの増殖とともに広範な感染肝細胞の破壊を伴う重症肝炎が惹起される．このようなHBVキャリアに対する化学療法時にはラミブジンなどの核酸アナログを予防投与してHBV再活性化を避けることが必要である[4)]．

一方，HBs抗原陰性でHBc抗体ないしHBs抗体陽性例は従来HBV既往感染とされ，臨床的には治癒の状態と考えられてきた．しかしこのような既往感染例でも肝臓や末梢血単核球中では低レベルながらHBV-DNAの複製が長期間持続することが明らかになっている[5)~7)]．最近，移植後やB細胞表面抗原CD20に対する抗体であるリツキシマブなど強力な免疫抑制剤の使用により，このような既往感染例からもHBV再活性化により重症肝炎が発症することが報告され，de novo B型肝炎と呼ばれている[8)~10)]．厚生労働省「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」班の全国調査によりこのようなde novo B型肝炎は通常のB型肝炎

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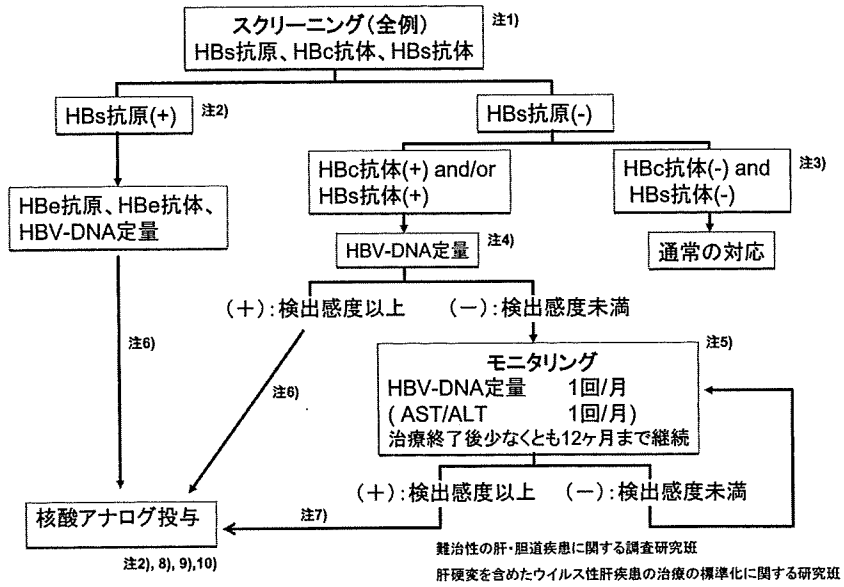

Fig. 1　免疫抑制・化学療法により発症するB型肝炎対策ガイドライン*

補足

*血液悪性疾患に対する強力な免疫・抑制化学療法中あるいは終了後にHBs抗原陽性あるいはHBs抗原陰性例の一部にHBV再活性化によりB型肝炎が発症し，その中には劇症化する症例があり，注意が必要である．その他の疾患においても治療によるHBV再活性化のリスクを考慮して対応する必要がある．また，ここで推奨する核酸アナログ予防投与のエビデンスはなく，劇症化予防効果を完全に保証するものではない．

注1)　CLIA法で測定することが望ましい．

注2)　HBs抗原陽性例は肝臓専門医にコンサルトすること．全ての症例で核酸アナログ投与にあたっては肝臓専門医にコンサルトするのが望ましい．

注3)　初回治療時にHBc抗体，HBs抗体未測定の再治療例では抗体価が低下している場合があり，HBV-DNA定量検査などによる精査が望ましい．

注4)　PCR法およびリアルタイムPCR法により実施する．より検出感度の高いリアルタイムPCR法が望ましい．

注5)　リツキシマブ・ステロイド使用例，造血細胞移植例はHBV再活性化の高リスクであり，注意が必要である．フルダラビンは強力な免疫抑制作用を有するが，HBV再活性化のリスクは不明であり，今後注意が必要である．

注6)　免疫抑制・化学療法を開始する前，できるだけ早期に投与を開始するのが望ましい．

注7)　免疫抑制・化学療法中はHBV-DNA定量検査が検出感度以上になった時点で直ちに投与を開始する．

注8)　核酸アナログはエンテカビルの使用を推奨する．

注9)　下記の条件を満たす場合には核酸アナログ投与の終了を検討して良い．
スクリーニング時にHBs抗原（+）例ではB型慢性肝炎における核酸アナログ投与終了基準を満たす場合．スクリーニング時にHBc抗体（+）and/or HBs抗体（+）例では，(1) 免疫抑制・化学療法終了後，少なくとも12カ月間は投与を継続すること．(2) この継続期間中にALT（GPT）が正常化していること．（但しHBV以外にALT異常の原因がある場合は除く）(3) この継続期間中にHBV-DNAが持続陰性化していること．

注10)　核酸アナログ投与終了後12カ月間は厳重に経過観察する．経過観察方法は各核酸アナログの使用上の注意に基づく．経過観察中にHBV-DNA定量検査が検出感度以上になった時点で直ちに投与を再開する．

に比して劇症化する頻度が高率で，死亡率も高いことが明らかになった[11)~13)]．また，厚生労働省「難治性の肝・胆道疾患に関する調査研究」班で実施している劇症肝炎・遅発性肝不全(LOHF)の全国調査でもここ数年，特に悪性リンパ腫に対しリツキシマブとステロイドを併用したR-CHOP治療例からの劇症化やde novo B型肝炎が増加傾向にあり，予後不良であった[14)15)]．以上のような経緯から，早急なHBV再活性化対策が必要となり，両研究班が合同でワーキンググループを立ち上げ，Fig. 1に示すガイドラインを作成した．

ガイドラインの要旨は以下のとおりである．まずHBV再活性化リスク群の同定を目的にスクリーニング検査として，全ての症例にHBs抗原およびHBc抗体，HBs抗体を測定する．HBs抗原が陽性の場合はさらにHBe抗原，HBe抗体，HBV-DNA定量検査を実施する．HBs抗原陽性例では，無症候性キャリアだけではなく，慢

性肝炎，肝硬変例が含まれる可能性があるので肝臓専門医にコンサルトする必要がある．HBs抗原陽性例での再活性化のリスクは大きいので，基本的に核酸アナログの予防投与を実施する．但し，HBV再活性化のリスクが少ない悪性疾患以外の若年HBe抗原陽性無症候性キャリアに対するステロイド治療例などでは，核酸アナログ予防投与の有効性に関するエビデンスはなく経過観察など他の選択肢があり，適応は慎重に判断する必要がある．HBs抗原陰性でHBc抗体，HBs抗体いずれも陰性の場合は通常の対応とする．HBs抗原陰性でHBc抗体ないしHBs抗体が陽性，すなわち感染既往例と判断される場合は更にHBV-DNA定量検査を実施し，HBV-DNAが陽性の場合は核酸アナログの予防投与を行う．一方，HBV-DNAが陰性の場合はHBV-DNAを毎月モニタリングしながら，陽性化した時点で直ちに核酸アナログを投与する．特にリツキシマブ・ステロイド使用例，造血細胞移植例は再活性化のリスクが高いので慎重な対応が必要である．核酸アナログ予防投与例の投与中止時期に関する明確なエビデンスはないが，HBs抗原陰性，HBc抗体ないしHBs抗体陽性例では免疫抑制・化学療法終了後も12カ月間は投与を継続し，この継続期間中に一定の基準を満たせば投与終了も可能とした．以下にガイドライン作成にあたり論点になった事項を補足する．①スクリーニングにあたってはHBs抗原だけではなくHBc抗体，HBs抗体をできるだけ感度の高い検査法で実施する必要がある．HBs抗原陰性でHBc抗体，HBs抗体いずれも陰性の場合でも，患者が既に免疫抑制状態にある場合には抗体が検出されないことがあり，HBV-DNA定量検査まで測定することが望ましい．②B型キャリア例の急性増悪では発症後早期の核酸アナログ治療が有効であるが，HBV再活性化による劇症化例は発症後の核酸アナログ治療では予後不良であり，発症前の予防投与が必要である．しかし既往感染例でのHBV再活性化率は明らかでなく，また本邦におけるHBc抗体ないしHBs抗体陽性の既往感染例の頻度は高率であることより，全ての症例に核酸アナログの予防投与を実施するのは医療経済的にも困難である．Huiらの報告[16]ではHBs抗原陰性例のHBV再活性化では，HBV-DNAが陽性化し，肝炎が発症するまでに12～28週（平均18.5週）を要しており，したがってHBV-DNAをPCR法またはリアルタイムPCR法で毎月モニタリングし，検出感度以上になった時点で直ちに核酸アナログを投与しても肝炎の重症化は予防可能と推測される．③核酸アナログ製剤はB型慢性肝炎の治療ガイドライン[17]に準拠して，エンテカビル投与を推奨している．しかし，投与期間が長期に及ばない場合など，より安価なラミブジンへの代用も検討の余地がある．④核酸アナログ投与終了に関する明確な基準はない．HBs抗原陽性例では使用する各核酸アナログの投与終了基準に準ずる．HBs抗原陰性，HBc抗体ないしHBs抗体陽性例では免疫抑制・化学療法終了後も12カ月間は投与を継続し，この継続期間中にALTの正常化とHBV-DNAの持続陰性化が見られる場合は投与終了の検討も可能である．但し，HBV以外にALT異常の原因がある場合はALTの正常化は必須ではない．また，核酸アナログ予防投与終了後のHBV再活性化例の報告もあり，投与終了後も更に12カ月間は厳重な経過観察が必要である[18]．

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Prevention of immunosuppressive therapy or chemotherapy-induced reactivation of hepatitis B virus infection —Joint report of the Intractable Liver Diseases Study Group of Japan and the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis—

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