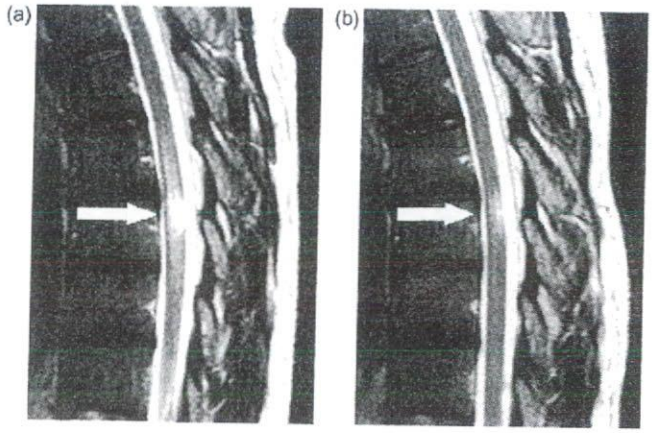

Fig. 1. (a) T2-weighted MRI of the thoracic spinal cord before the onset of acute hepatitis. A high intensity lesion at the Th7-8 level with spinal swelling is observed. (b) T2-weighted MRI 2 months after discharge. The lesion of the spinal cord is diminished.

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/ L 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

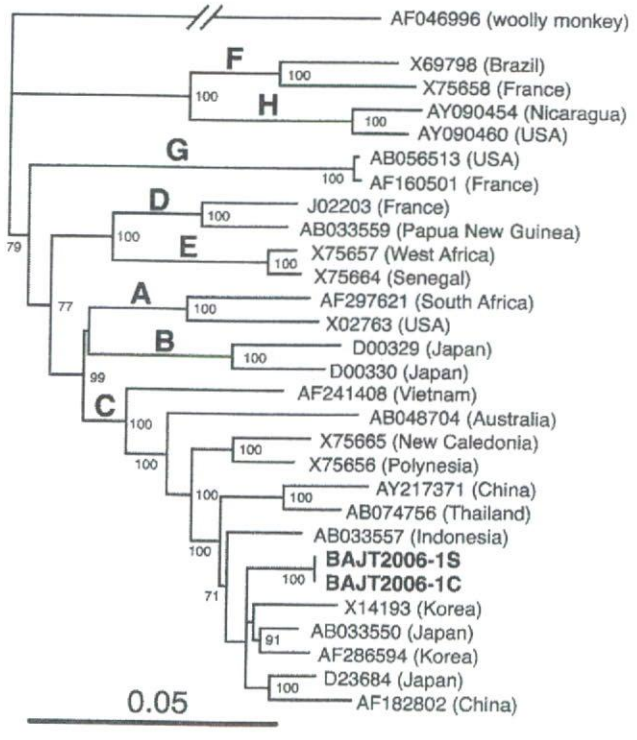

Fig. 2. A phylogenetic tree constructed based on the entire nucleotide sequences of 28 HBV isolates, using a woolly monkey HBV isolate (AF046996) as an out group. In addition to the two isolates obtained from serum (BAJT2006-1S) and CSF (BAJT2006-1C), 26 reported HBV isolates of genotypes A to H were 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)

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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 precore 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.

Acknowledgements

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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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The sequence of hepatitis B virus reported in this article has been deposited in the DDBJ/EMBL/GenBank databases under accession number AB353732.
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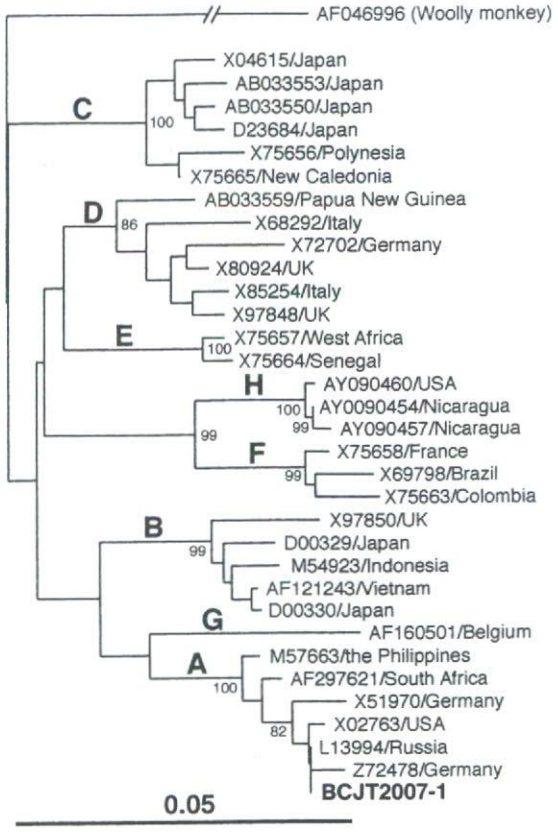
CASE REPORT

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/mL; CD4/CD8, 30.5%/60.0% (0.5); number of platelets, 50 ¥10^3/ml; 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 mL 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

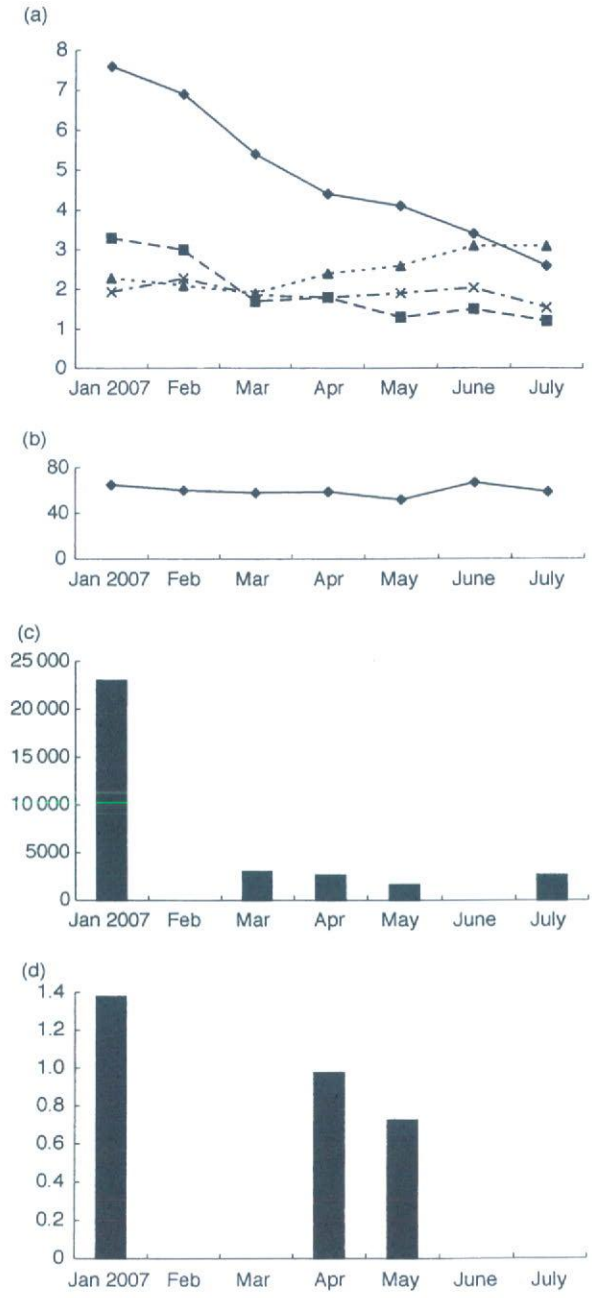


Figure 2 Time-courses of clinical parameters from the initial administration of entecavir on 23 January 2007 demonstrate improvement in liver function and a decrease in hepatitis B virus (HBV) and HIV in the serum. (a,b) HBV load and clinical parameters; (c) HIV load; (d), hepatitis B e antigen (HBe 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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