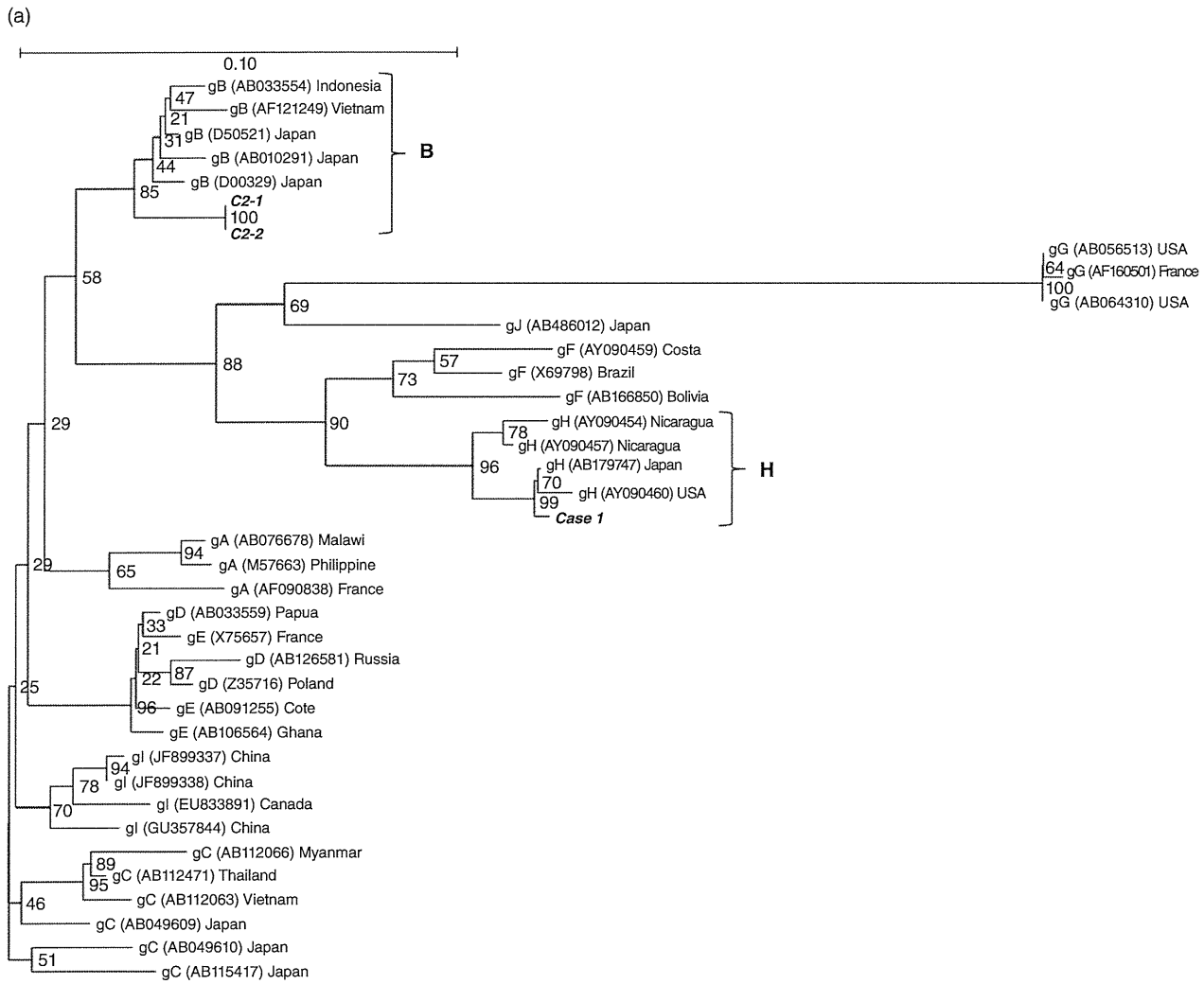

Figure 4 A phylogenetic tree constructed based on the sequence of the hepatitis B virus (HBV) strain isolated from case 2 in comparison with that of 35 reference strains. The bootstrap values are indicated at each tree root and the genotypes are on the right. The horizontal bar provides a genetic distance. The regions included in the analysis were: (a) nucleotide sequence between DR2 (1590 nt) and DR1 (1834 nt) in the X region, (b) between 2023 and 2262 nt in the precore/core region.

acute liver diseases caused by HBV, especially in urban areas as compared to the countryside,[29] suggesting that globalization and diversification of the sex industry may change the distribution pattern of the HBV genotypes in Japan, including in Saitama Prefecture, the area around our institution.

To our surprise, HBV genotype H strains, which are mainly prevalent in Central America, were isolated from two patients, one each with chronic and acute liver diseases. The HBV strain isolated from the patient with acute liver disease (case 1) showed a nucleotide sequence with 99.8% identity to the Thailand strain (EU498228), which has recently been reported to be isolated from Japan as well as Central America.[30] Considering that case 1 was a bisexual male with HIV co-infection contracted as a result of sexual activities with a number of unspecified Japanese partners, the HBV strain isolated from this patient may be resident in Japanese persons engaging in unusual sexual activities. On the other hand, HBV genotype A strains, especially the genotype A2/Ae strain, have been isolated increasingly frequently from patients with HBV and HIV co-infection.[31] These observations prompted us to postulate that HBV genotype H strains as well as genotype A

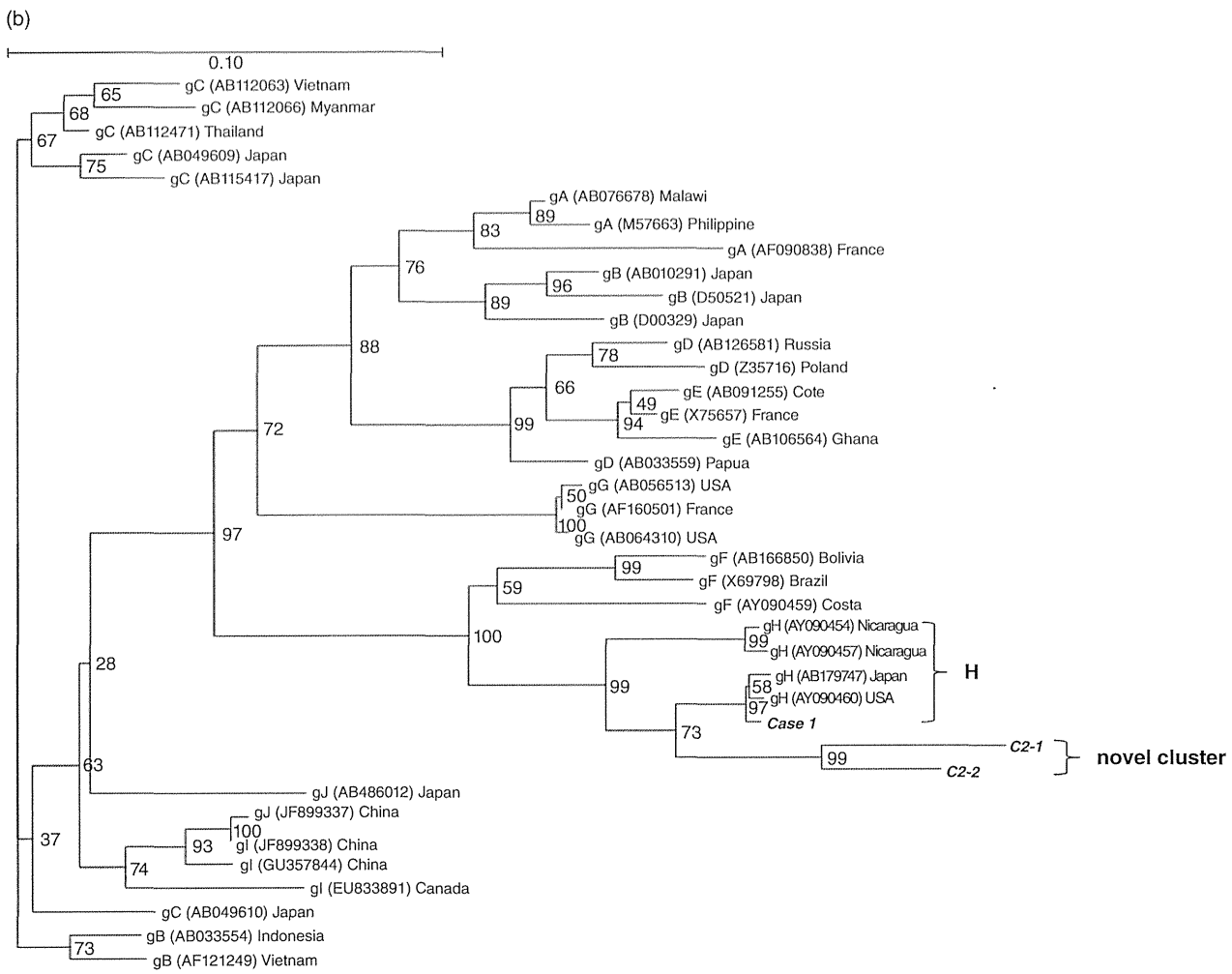

Figure 4 *Continued*

strains seem to spread among Japanese persons with unusual sexual habits. Previously, Tanaka *et al.* reported a HIV-infected patient in whom co-infection of both HBV genotype H and G strains was observed.[32] In case 1, however, co-infection of HBV genotype G strain was not detected.

It is noteworthy that HBV genotype H strains were isolated even from a Japanese patient with chronic liver disease (case 2), which showed recombination with a genotype B strain. The recombination breakpoint was estimated at positions 1590 and 1834 nt, located between DR2 and DR1 in the X region (Fig. 5): the nucleotide sequence in the X region of this strain showed an identity of 97.2% to that of genotype B strains in Malaysia (JQ027316) and Indonesia (JQ429079) despite the full-length nucleotide sequence showing 97.1% identity to a genotype H strain isolated from Mexico (AB375164). In the present study, nucleotide sequences were analyzed using two fragments (WA2 and gN2), suggesting that the possible recombination points exist in the overlapping regions of both fragments. However, the possibility that both genotypes B and H HBV strains existed as quasispecies in case 2 was neglected, because the sequences of the overlapping regions (1702–1780 and 1908–2081 nt) showed 100% identity between WA2 and gN2 fragments. It is well known that a HBV genotype B2/Ba strain, widely prevalent in Asian countries, shows nucleotide sequences identical to genotype C strains in the precore/core region due to the inter-genotype recombination

Table 1 Percentages of differences in the nucleotide and amino acid sequences of hepatitis B virus (HBV) strains isolated from case 2 (C2-1 and C2-2) and representative strains of genotypes A–J HBV

		Percentages of differences to representative HBV strains of genotypes									
		A (3)	B (5)	C (6)	D (3)	E (3)	F (3)	G (3)	H (4)	I (4)	J (1)
C1-1	Nucleotide	25.9–30.0	25.6–28.6	24.4–26.9	26.9–29.6	28.5–29.8	17.6–17.9	26.2–26.7	9.6–13.0	24.8–26.5	26.1
	Amino Acid	18.6–25.7	21.3–25.1	23.8–27.9	22.8–25.5	24.2–25.7	18.1–18.2	22.8–24.2	18.1–19.4	22.7–27.3	24.6
C2-2	Nucleotide	24.4–28.5	24.1–27.1	22.9–25.4	25.4–28.1	27.0–28.3	16.1–16.4	24.7–25.2	8.1–11.5	23.3–25.0	24.6
	Amino Acid	17.6–24.7	20.3–24.1	22.8–26.9	21.8–24.5	23.3–24.7	17.1–17.2	21.8–23.3	17.1–18.4	21.7–26.3	23.6

Values in parenthesis indicate the number of HBV strains.

between B and C strains.[33] Also, HBV strains developing as a consequence of the inter-genotype recombination between A and D, A and E, A and C, C and D, and C and G have been reported from Africa, Vietnam, Tibet and Thailand.[34–37] Moreover, recombination among HBV strains of the same genotype, the so-called intra-genotype recombination, has been proposed to occur especially in HBV genotype A, D, F and H strains.[38] However, HBV genotype H strains showing recombination with other genotype strains have not ever been reported. Considering the fact that the father of case 2 had lived in Brazil in his youth, the sequences of genotype H in case 2 strains might have originated in Brazilian strains. In Brazil, genotypes A and D HBV strains are predominantly distributed with frequencies of 49.5% and 24.3%, respectively, while genotype B HBV strains are only 2.9%.[39] Thus, the recombination event with the genotype B HBV strain might have developed following the emigration of his father to Japan. To clarify the area and era in which the recombination developed, the full-length nucleotide sequence of the HBV strain isolated from the elder brother of case 2 needs to be evaluated, but, unfortunately, the brother, receiving medical examination at another institution, rejected further viral genome analysis.

Although the mechanisms involved in the development of inter-genotype and intra-genotype recombination of the HBV genomes remains unclear, several observations reported in previous publications prompted us to postulate the "non-random pathway"; DR1 (1830 nt) in the X gene, a possible origin of viral replication, is considered to be a hot spot that may be responsible for recombination of HBV genomes among different strains.[40,41] Hino *et al.* reported, based on *in vitro* recombination assay, that HBV DNA fragments containing the region spanning DR1 increased the recombination events reproducibly in the presence of extracts from actively dividing HCC cells.[40] Also, Pineau *et al.* revealed that the integration sites of covalently closed circular HBV DNA were usually located in the nucleotide sequence between 1600 and 2000 nt, when the HBV genomes chromosomally integrated in the host genomes were evaluated in human HCC tissues.[41] These *in vitro* and *in vivo* observations were consistent with the results obtained from the analysis of the HBV strains isolated from case 2, showing that the genome of the HBV genotype B strains were integrated in that of the HBV genotype H strain between DR2 and DR1.

Hepatitis B virus strains isolated from case 2 were classified as quasispecies in accordance with the nucleotide sequence between 2023 and 2262 nt in the precore/

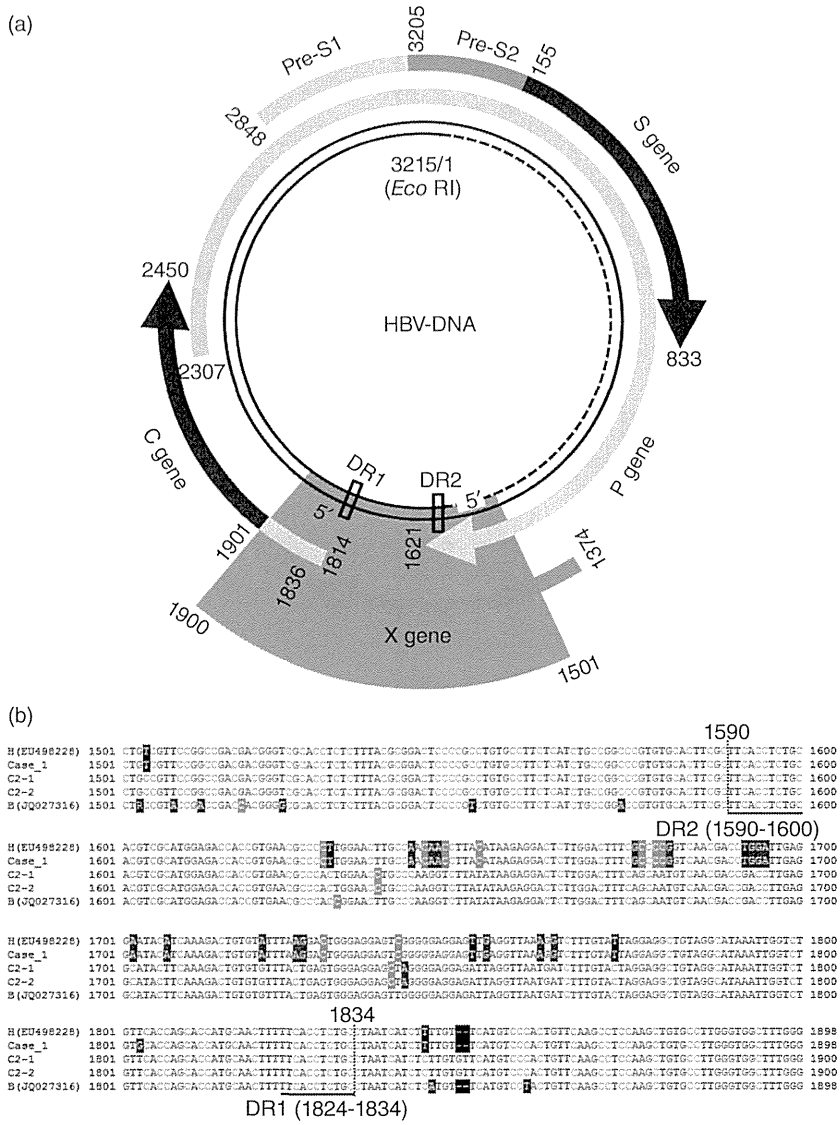

Figure 5 Hepatitis B virus (HBV) genome and the open reading frame. (a) The sequence region (shaded in red) includes the recombination breakpoint at position 1590 and 1834 nt, located between DR2 and DR1 in X region. (b) Nucleotide alignments over the sequences spanning 1501–1900 nt in case 1, C2-1, C2-2 and reference strains of HBV genotype H (accession no. EU498228) and B (JQ027316). Dashed lines at 1590 and 1834 nt represent the recombination breakpoint.

core regions. Thus, the nucleotide sequences were analyzed following cloning of the HBV genome, and two major clones, C2-1 and C2-2, were isolated. Neither clone showed any similarity to any of the previously reported strains in the precore/core regions, and a phylogenetic tree constructed based on these regions revealed that these strains may be classified into the novel cluster of HBV; sequence divergences of nucleotides in the range of 8.1–30.0% and of amino acid in the range of 17.1–27.9% as compared to previously reported genotype A–J strains. The possibility that intergenotype recombination of the HBV genome between H and B strains may provoke mutation of the nucleotide sequence in the precore/core regions leading to development of a possible novel genotype HBV strain needs to be evaluated in the future.

In conclusion, HBV genotype H strains, which are prevalent in Central American countries, were isolated from Japanese patients with chronic as well as acute liver diseases. HBV strains isolated from the chronic liver disease patient showed recombination of the genome between genotype H and B strains, and no similarity was found in the nucleotide sequences of the precore/core regions in comparison with those of the previously reported HBV strains. Thus, globalization may promote development of a possible novel genotype of HBV through recombination between Central American and East Asian strains.

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found in the online version of this article at the publisher's website:

Table S1 Hepatitis B virus DNA-specific oligonucleotide primers used in the study.

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REVIEW ARTICLE

A proposal for management of rheumatic disease patients with hepatitis B virus infection receiving immunosuppressive therapy

Masayoshi Harigai · Satoshi Mochida · Toshihide Mimura · Takao Koike · Nobuyuki Miyasaka

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Abstract Reactivation of hepatitis B virus (HBV) and de novo HBV hepatitis in patients with rheumatic diseases given intensive and long-term immunosuppressive therapy with or without biological disease-modifying antirheumatic drugs is of great concern, especially in regions where the virus is endemic, including Japan. To ascertain a better benefit–risk balance for immunosuppressive therapy for patients with rheumatic diseases, the Japan College of Rheumatology developed this proposal. All patients with rheumatic diseases commencing immunosuppressive therapy should be screened for hepatitis B surface antigen (HBsAg); those who are negative for HBsAg should be screened for hepatitis B core antibody (HBcAb) and hepatitis B surface antibody (HBsAb) as well. HBV carriers and serum HBV DNA positive patients with resolved infection should receive nucleoside analog as soon as possible, prior to commencing immunosuppressive therapy. For serum HBV DNA negative patients with resolved infection, careful monthly monitoring using serum levels of aspartate and alanine aminotransferases and HBV DNA is recommended during and at least 12 months after withdrawal of immunosuppressive therapy. If serum HBV DNA becomes positive, patients should receive nucleoside analog treatment as soon as possible, while ongoing immunosuppressive therapy should be continued to avoid severe or fulminant hepatitis development. To facilitate proper management of patients with HBV infection, collaboration between rheumatologists and hepatologists is strongly encouraged.

Keywords Hepatitis B virus · Reactivation · Rheumatic diseases · Immunosuppressive therapy · Glucocorticoid

M. Harigai (✉)
Department of Pharmacovigilance, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
e-mail: mharigai.mpha@tmd.ac.jp

M. Harigai · N. Miyasaka
Department of Medicine and Rheumatology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

S. Mochida
Department of Gastroenterology and Hepatology, Faculty of Medicine, Saitama Medical University, Saitama, Japan

T. Mimura
Department of Rheumatology and Applied Immunology, Faculty of Medicine, Saitama Medical University, Saitama, Japan

T. Koike
NTT Sapporo Medical Center, Hokkaido, Japan

N. Miyasaka
Global Center of Excellence Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo, Japan

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Introduction

Epidemiological data have indicated that about 350 million people worldwide (6 % of the world population) are infected with hepatitis B virus (HBV) and that 200 million of those live in Asian countries [1, 2]. Previous studies estimated that the number of HBV carriers who are positive for hepatitis B surface antigen (HBsAg) in Japan is 1.0–1.5 million and that 23.2 % of the total Japanese population has been previously infected with HBV [3, 4]. Once hepatocytes are infected with HBV, replication-competent covalently closed circular DNA (cccDNA) is formed in the nuclei of the infected hepatocytes during the viral

replication process. The cccDNA serves as the main template for transcription of viral pregenome RNA, as well as messenger RNA (mRNA), and persists permanently in the cells [5]. Hence, HBV carriers and patients with resolved HBV infection [HBsAg negative and hepatitis B core antibody (HBcAb) and/or hepatitis B surface antibody (HBsAb) positive] are equivalent in terms of the presence of replication-competent HBV genome in their hepatocytes. Chemotherapy- or immunosuppressive therapy-associated immunosuppressed status may increase the risk for reactivation of HBV, both in patients who are HBV carriers and in patients with resolved HBV infection. Hepatitis following viral reactivation in patients with resolved HBV infection is called "de novo HBV hepatitis" and often leads a fatal and fulminant course, especially in patients with malignant lymphoma given chemotherapy containing rituximab, i.e., anti-CD20 chimeric antibody, and corticosteroids [6, 7].

Recent advances in treatment for rheumatoid arthritis (RA) have improved outcomes for patients. In Japan, six biological disease-modifying antirheumatic drugs (DMARDs) have been approved for RA since 2003, and the maximum approved dosage of methotrexate for RA was increased to 16 mg/week in February 2011. Together with these changes in medications, goal-oriented early aggressive therapy has been introduced in clinical practice, aiming at remission and maintenance of remission of the disease [8]. Similar therapeutic strategies have also been introduced for other rheumatic diseases, such as systemic lupus erythematosus and systemic vasculitides [9, 10]. As a result, patients with rheumatic diseases receive intensive remission-induction treatment with long-term maintenance therapy using corticosteroids, immunosuppressants, and/or biological DMARDs, which have potential risk for reactivation of HBV and de novo HBV hepatitis. During the past few years, several investigators reported reactivation of HBV in patients with rheumatic diseases given biological DMARDs, especially tumor necrosis factor inhibitors [11–16]. Development of HBV reactivation and fatal fulminant hepatitis was also reported in patients with rheumatoid arthritis given low-dose methotrexate [17–19]. These data strongly suggest that appropriate screening for HBV infection and monitoring for reactivation in HBV-infected patients are mandatory in rheumatology clinical practice (Fig. 1).

In this proposal we summarize epidemiological data on reactivation of HBV in Japan and in patients with rheumatic diseases. Based on the latest evidence and expert opinions, we indicate methods of proper management for HBV-infected patients with rheumatic diseases who will receive immunosuppressive therapy. The diagnosis of HBV infection and prophylaxis of reactivation are in accordance with the "Guidelines for prevention of immunosuppressive therapy or chemotherapy-induced reactivation of hepatitis B virus infection" that were jointly developed by the Intractable Hepatobiliary Disease Study Group of Japan and the Study Group for the Standard Antiviral Therapy for Viral Hepatitis in the Health and Labour Sciences Research [20, 21]. This proposal is subject to changes as advances occur in research in this and related medical fields. This proposal was originally published in Japanese on the website of the Japan College of Rheumatology on September 6, 2011, and revised on October 17, 2011 and September 5, 2012.

Fulminant hepatitis and late-onset hepatic failure in Japan

Since 1998, the Intractable Hepatobiliary Diseases Study Group of Japan has conducted an ongoing nationwide annual survey for fulminant hepatitis and late-onset hepatic failure (LOHF). By 2009, 1,186 patients with these hepatic disorders [19–21], 39 % of which were HBV related, had been accumulated. In Japan, HBV-related acute liver failure is classified into transient infection, acute exacerbation in HBV carrier, and indeterminate infection patterns; de novo HBV hepatitis due to viral reactivation in patients with resolved HBV infection is classified as one of the subgroups of acute exacerbation in HBV carriers [22]. According to this classification, the causes of HBV-related fulminant hepatitis and LOHF in Japan are transient infection (55 %), acute exacerbation in HBV carrier including reactivation in patients with resolved infection (35 %), and indeterminate infection pattern (10 %) [19–21]. The percentage of HBV carriers who developed fulminant hepatitis or LOHF gradually decreased from 1998 to 2004, but increased again in and after 2005 due to the increased number of patients with viral reactivation in resolved HBV infection [23–25]. Of 488 patients who developed fulminant hepatitis or LOHF during 2004 and 2009, 194 (40 %) were HBV related; causes of these infections were transient infection in 91 (47 %), acute exacerbation in HBV carrier including reactivation in patients with resolved infection in 72 (37 %), and indeterminate infection pattern in 31 (16 %). Among the 72 patients classified into acute exacerbation in HBV carrier, the investigators identified 17 patients with reactivation of HBV in patients with resolved infection; these patients had been initially classified as HBV carriers showing acute hepatitis exacerbation. Thirteen of these 17 patients were treated with rituximab-containing regimens, but some received other chemotherapy or immunosuppressive therapy. All of these patients died, pointing to an extremely unfavorable prognosis [21]. Although the "Guidelines for prevention of immunosuppressive therapy or chemotherapy-induced reactivation of hepatitis B virus infection" were first published in 2009 [26], more recent data from the Intractable Hepatobiliary Diseases Study Group of Japan

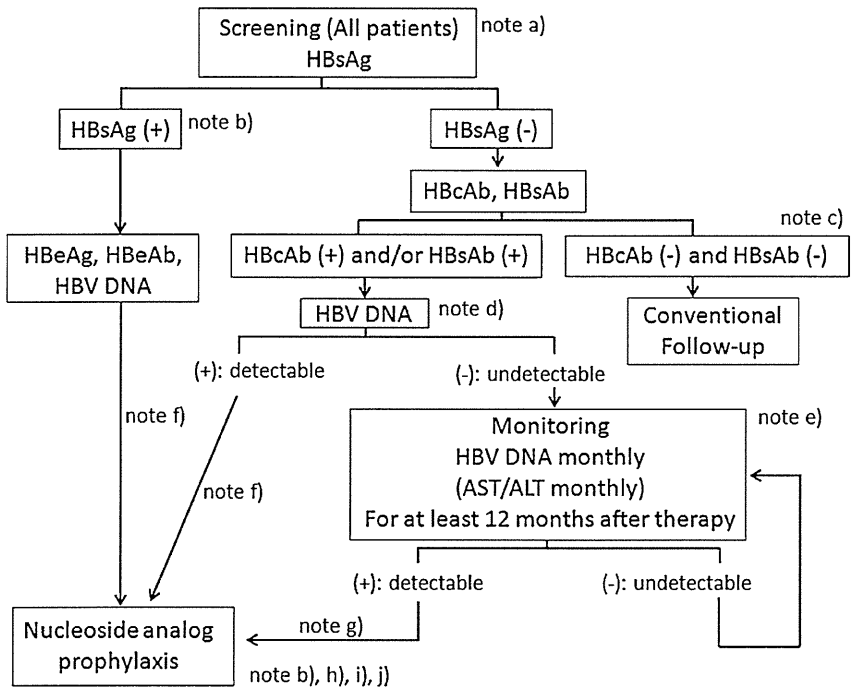

Fig. 1 Algorithm for screening and management of hepatitis B virus infection in patients with rheumatic diseases. All patients with rheumatic diseases who start immunosuppressive therapy should be screened for hepatitis B virus (HBV) infection using this algorithm. HBV carriers or patients with resolved HBV infection should be managed accordingly. Notes: *a* All patients with rheumatic diseases commencing immunosuppressive therapy should be screened for HBsAg. Those who are negative for HBsAg should be screened for HBcAb and HBsAb as well to identify patients with resolved infection. Chemiluminescent immunoassay/chemiluminescent enzyme immunoassay (CLIA/CLEIA) is highly recommended to measure HBsAg, HBcAb, and HBsAb. *b* HBsAg positive patients are subject to consultation with a hepatologist. Consultation with a hepatologist is desirable in all patients subject to administration of nucleoside analog. *c* Detection of serum HBV DNA is desirable in those patients who have previously received immunosuppressive therapy and have no results of HBcAb and HBsAb before the start of the therapy. *d* Detection by real-time polymerase chain reaction (PCR) method (Taq-Man™ PCR method) is recommended. *e* Patients receiving rituximab plus corticosteroid combination therapy for malignant lymphoma or patients receiving hematopoietic stem cell transplantation are at particular risk for HBV reactivation and deserve careful attention. *f* Prophylactic nucleoside analogs should be started as soon as possible before starting immunosuppressive therapy. *g* Nucleoside analogs should be administered immediately when HBV DNA becomes positive during and after immunosuppressive therapy. *h* Entecavir is recommended as the nucleoside analog. HBV DNA is monitored monthly during administration of nucleoside analogs. *i* Criteria for discontinuation of nucleoside analog treatment are described in the text. *j* Patients should be closely observed for 12 months after treatment with nucleoside analogs as described in the text. Nucleoside analog should be readministered immediately when HBV DNA becomes positive during observation. *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *HBcAb* hepatitis B core antibody, *HBsAg* hepatitis B surface antigen, *HBsAb* hepatitis B surface antibody, *HBeAg* hepatitis B envelope antigen, *HBeAb* hepatitis B envelope antibody. Adapted and modified from Oketani et al. [21]

shows that an even larger number of patients developed reactivation from a resolved HBV infection status [24], indicating a necessity for broadened publicity of the guidelines among physicians of related specialties.

Reactivation of HBV in patients with rheumatic diseases

The Health and Labour Sciences Research Group for "Clarification of current status for reactivation of hepatitis B virus associated with immunosuppressants and antineoplastics and establishment of the preventive measures" started a registry in 2009 for HBV-infected patients with solid cancers, hematopoietic malignancies, renal diseases, and rheumatic diseases [27, 28]. Rheumatic disease patients eligible for this study are those who are (1) positive for HBsAg, HBcAb or HBsAb, and (2) treated with corticosteroids (prednisolone equivalent dose $\geq$0.5 mg/kg body weight/day), immunosuppressive drugs or biological DMARDs approved in Japan, including infliximab, etanercept, adalimumab, tocilizumab, abatacept, and golimumab. As of March 2012, 127 patients from 19 medical institutions were enrolled in this study and were followed up according to the study protocol. An interim analysis of this prospective observation study found 11 of the 127 patients were HBV carriers; the remaining patients had resolved HBV infection. By the end of March 2012, nine patients with resolved

infection became positive for serum HBV DNA, two patients before and seven patients after commencing immunosuppressive therapy. Overall, 7.8 % of the 116 patients with resolved HBV infection had viral reactivation. All of these patients were successfully treated according to the guidelines developed by the Intractable Hepatobiliary Diseases Study Group of Japan [26], and none of them developed hepatitis.

Screening for HBV infection

Patients who should be screened for HBV infection

According to this proposal, all patients with rheumatic diseases who commence immunosuppressive therapy in clinical practice should be screened for HBV infection. At present, immunosuppressive therapy in this proposal includes moderate or high doses of corticosteroids, biological DMARDs, synthetic DMARDs with immunosuppressive potential, (e.g., methotrexate, tacrolimus, leflunomide, and mizoribine), and immunosuppressants (e.g., azathioprine, cyclophosphamide, cyclosporine A, and mycophenolate mofetil). Other immunosuppressants will be added to this list following their approval by the Japanese Ministry of Health, Labour, and Welfare.

Recommended methods for screening

All rheumatic disease patients commencing immunosuppressive therapy should be screened for HBsAg. Those negative for HBsAg should be screened for HBcAb and HBsAb as well. Among various methods currently available for measurement of these HBV-associated antigens and antibodies, chemiluminescent immunoassay/chemiluminescent enzyme immunoassay (CLIA/CLEIA) is highly recommended because of its sensitivity and specificity. An assay system for HBsAg with even higher sensitivity is under development; application of such an assay system for clinical practice should be considered in the future. Rheumatologists are encouraged to consult hepatologists regarding HBV carriers and patients with resolved HBV infection with rheumatic diseases prior to commencing immunosuppressive therapy. Patients positive for HBsAb alone due to previous HBV vaccination are not subject to the following management.

Management of high-risk patients with rheumatic diseases

Management of HBV carriers

Hepatitis B envelope antigen (HBeAg), anti-HBe antibody, and serum HBV DNA should be measured for HBV carriers. The real-time polymerase chain reaction (PCR) method (Taq-Man™ PCR method) is highly recommended for quantification of HBV DNA in sera because of its high sensitivity and specificity. Analyses for genotype of HBV and precore and core promoter gene mutation may also be indicated.

HBV carriers should receive nucleoside analog as soon as possible prior to commencing immunosuppressive therapy and should be concurrently followed up by both rheumatologists and hepatologists. Entecavir hydrate, lamivudine, and adefovir pivoxil are currently approved nucleoside analogs in Japan. We recommend 0.5 mg of entecavir hydrate, once a day at fasting as a first choice because emergence of entecavir hydrate-resistant HBV variants has been reported at a very low rate [29–33]. Nucleoside analog treatment should be continued during and at least 12 months after withdrawal of immunosuppressive therapy with careful monitoring of patients using alanine aminotransferase, HBeAg, HBeAb, and serum HBV DNA [21, 34, 35]. If copy numbers of serum HBV DNA do not significantly decrease with nucleoside analog treatment, resistance to the drug is suspected and consultation with hepatologists is needed.

Discontinuation of nucleoside analog treatment is based on the status of serum viral markers: negative for HBeAg and positive for HBeAb, and low levels of HBV DNA, HBV core-related antigen, and HBsAg [36]. Consultation with hepatologists is recommended before discontinuing nucleoside analog treatment and for monitoring patients afterwards. Patients who discontinue nucleoside analog treatment should be strictly followed up for at least 12 months and restarted on the drug if serum HBV DNA levels increase.

Management of patients with resolved HBV infection

Serum HBV DNA should be measured using the Taq-Man™ PCR method for patients with resolved HBV infection. If serum HBV DNA of a patient is positive (i.e., detectable with agarose gel electrophoresis or equal to or more than 2.1 log copies/ml), the patient should receive nucleoside analog treatment as soon as possible before commencing immunosuppressive therapy, as described for HBV carriers. Duration, monitoring, and discontinuation of nucleoside analog treatment for these patients are the same as those for HBV carriers. It should be mentioned that reactivation of HBV cannot be predicted by HBsAb titers at baseline or changes over time [21].

If serum HBV DNA levels in a patient are <2.1 log copies/ml and undetectable with agarose gel electrophoresis, careful monthly monitoring of patients using serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and HBV DNA is recommended during and for at least 12 months after withdrawal of immunosuppressive therapy. The median lag period

between elevation of serum HBV DNA and alanine aminotransferase levels was 18.5 weeks (range 12–28 weeks) [37]; starting nucleoside analog after the onset of hepatitis could not prevent progression to fatal hepatitis [21]. Therefore, if serum HBV DNA of a patient becomes positive, the patient should receive nucleoside analog treatment as soon as possible, as described above. Duration, monitoring, and discontinuation of nucleoside analog treatment for these patients are the same as those for HBV carriers. Ongoing immunosuppressive therapy should be continued to avoid restoration of host immunity against HBV, which may result in an immunological attack on infected hepatocytes and cause hepatitis.

Differential diagnosis for patients with abnormal hepatic function test

If a patient with a rheumatic disease shows abnormal results of hepatic function tests during or after immunosuppressive treatment, major differential diagnoses include, in addition to reactivation of HBV, drug-induced liver disease, hepatic involvement of rheumatic diseases, alcoholic or nonalcoholic fatty liver disease, autoimmune liver diseases (e.g., autoimmune hepatitis and primary biliary cirrhosis), diseases of the bile duct and pancreas, acute hepatitis due to hepatitis A, B, C or E virus, acute hepatitis due to other viruses (e.g., *Epstein–Barr virus, cytomegalovirus, herpes virus, adenovirus, coxsackie virus, rubeola virus, rubella virus, human immunodeficiency virus*, and *parvovirus*), abnormal thyroid function, and other hepatic diseases, including malignancy.

Points to consider for patients with rheumatic disease developing HBV reactivation or de novo hepatitis

Reactivation of HBV or de novo HBV hepatitis in rheumatic disease patients without previous screening and monitoring for HBV

If reactivation of HBV or de novo HBV hepatitis develops in a patient with rheumatic disease who had not been screened or appropriately monitored for HBV infection, the patient should receive nucleoside analog as soon as possible and hepatologists should be consulted.

Discontinuation and reintroduction of immunosuppressive therapy after reactivation of HBV or de novo HBV hepatitis

Discontinuation of immunosuppressive therapy in rheumatic disease patients with HBV reactivation or de novo HBV hepatitis should be carefully discussed with hepatologists because abrupt withdrawal of the therapy may induce severe or fulminant hepatitis. Based on currently available evidence and expert opinions, we recommend continuation of immunosuppressive therapy together with nucleoside analog treatment. Prospective observational studies are being implemented to address this issue in Japan [28]. For a patient who has successfully discontinued immunosuppressive therapy, benefit–risk balance should be carefully assessed before restarting immunosuppressive therapy for rheumatic diseases.

Collaboration with board-certified hepatologists

In-house and regional collaborations between rheumatologists and hepatologists are encouraged and required to facilitate prompt and proper management of HBV carriers and rheumatic disease patients with resolved HBV infection. Lists of board-certified rheumatologists and board-certified hepatologists are available on the websites of the Japan College of Rheumatology (http://pro.ryumachi-net.com/index.php?option=com_content&view=article&id=49&Itemid=57) and the Japan Society of Hepatology (http://www.jsh.or.jp/specialist/list.html).

Summary

Reactivation of HBV and subsequent de novo HBV hepatitis are preventable serious adverse events associated with immunosuppressive therapy for patients with rheumatic diseases. Before starting immunosuppressive therapy, it is highly recommended that all patients be thoroughly screened for current and resolved HBV infection according to the procedures described in this proposal. HBV carriers and patients with resolved HBV infection who are positive for serum HBV DNA should be treated with nucleoside analog prior to commencing immunosuppressive therapy. Close monitoring for reactivation of HBV is necessary for prompt intervention with nucleoside analog to mitigate subsequent hepatitis. Collaboration with hepatologists is encouraged and required to facilitate these management processes for patients with rheumatic diseases infected with HBV.

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Screening for and management of hepatitis B virus reactivation in patients treated with anti-B-cell therapy

Shigeru Kusumoto[1] and Kensei Tobinai[2]

[1]Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan; and [2]Department of Hematology, National Cancer Center Hospital, Tokyo, Japan

Reactivation of hepatitis B virus (HBV) is a potentially fatal complication after anti-B-cell therapy. It can develop not only in patients seropositive for hepatitis B surface antigen (HBsAg), but also in those with resolved HBV infection who are seronegative for HBsAg but seropositive for antibodies against hepatitis B core antigen (anti-HBc) and/or antibodies against HBsAg (anti-HBs). The risk of HBV reactivation depends on the balance between replication of the virus and the immune response of the host. Anti-CD20 monoclonal antibody—rituximab in combination with steroid-containing chemotherapy (R-CHOP: rituximab + cyclophosphamide + hydroxydaunorubicin + vincristine + prednisone/prednisolone)—is an important risk factor for HBV reactivation in HBsAg-negative patients. More obviously, HBsAg-positive patients are considered to be at very high risk for HBV reactivation and, in the rituximab era, 59%–80% of these patients develop HBV reactivation after R-CHOP-like chemotherapy. Patients with resolved HBV infection should also be considered at high risk of HBV reactivation, the incidence of which is reported to be 9%–24% in such lymphoma patients. All patients should be screened to identify risk groups for HBV reactivation before initiating anti-B-cell therapy by measuring serum HBV markers including HBsAg, anti-HBc and anti-HBs. To prevent the development of hepatitis due to HBV reactivation after anti-B-cell therapy, antiviral prophylaxis is recommended for HBsAg-positive patients and/or patients in whom HBV DNA is detectable at baseline, whereas regular monitoring of HBV DNA-guided preemptive antiviral therapy is a reasonable and useful approach for patients with resolved HBV infection.

Learning Objectives

- To become familiar with the risk of HBV reactivation in patients who receive anti-B-cell therapy
- To manage such high-risk patients successfully using antiviral prophylaxis or by HBV DNA-monitoring-guided preemptive antiviral therapy

Introduction

Reactivation of hepatitis B virus (HBV) is a potentially fatal complication after immunosuppressive therapy. HBV reactivation has been found, not only in patients seropositive for hepatitis B surface antigen (HBsAg),[1-3] but also in those with resolved HBV infection who are seronegative for HBsAg but seropositive for antibodies against hepatitis B core antigen (anti-HBc) and/or antibodies against HBsAg (anti-HBs).[1,4-9]

Rituximab + steroid-containing chemotherapy has been identified recently as an important risk factor for HBV reactivation in patients with resolved HBV infection.[5,6] Rituximab is a chimeric mouse-human monoclonal antibody that targets the CD20 molecule. Approximately 70%–80% of malignant lymphomas are of B-cell origin and >90% of B-cell lymphomas express CD20 on the cell surface. The introduction of rituximab has markedly improved outcomes in patients with CD20-positive B-cell lymphomas.[10] R-CHOP (rituximab + cyclophosphamide + hydroxydaunorubicin + vincristine + prednisone/prednisolone) is the most widely used for these types of lymphoma. Moreover, the usefulness of rituximab has also been demonstrated in patients with certain refractory autoimmune diseases, including rheumatoid arthritis,[11] granulomatosis with polyangitis,[12] and antineutrophil cytoplasmic antibody-associated vasculitis.[13]

The great success of rituximab is being followed by the development of new monoclonal antibodies to CD20. Ofatumumab is a human anti-CD20 monoclonal antibody that has been shown to be effective in refractory chronic lymphocytic leukemia.[14] Obinutuzumab is a humanized monoclonal antibody, which has been demonstrated to improve outcomes in previously untreated chronic lymphocytic leukemia patients with coexisting conditions.[15]

More recently, the U.S. Food and Drug Administration has presented new boxed warning information regarding the risk of HBV reactivation in patients who receive rituximab or ofatumumab.[9] To decrease the risk of HBV reactivation, the safety announcement recommends screening all patients for HBV infection before starting treatment with these anti-B-cell antibodies and monitoring patients with prior HBV infection in consultation with hepatitis experts after immunosuppressive therapy.

In this review, we summarize the current evidence regarding HBV reactivation in patients with hematological malignancies after immunosuppressive therapy, including anti-B-cell therapy, and propose a strategy for managing HBV reactivation, especially in patients with resolved HBV infection.

Pathophysiology of HBV reactivation after anti-B-cell therapy

In most immunocompetent hosts, HBV infection manifests as acute hepatitis. The host immune response targets the infected hepatocytes, after which serum HBV DNA and HBsAg levels gradually

decrease to below the detection limit over several months or years. Most cases of acute hepatitis B completely resolve in adult patients, who become seronegative for HBsAg but seropositive for anti-HBc and/or anti-HBs. However, HBV replication may chronically persist in the liver,[16] even in patients with anti-HBs, for several years after acute hepatitis B. Because HBV covalently closed circular DNA remains present in hepatocytes and provides a stable template for replication of HBV, viral reactivation has been reported after immunosuppressive therapy even in patients with resolved HBV infection. All individuals with a history of exposure to HBV should therefore be considered at risk of HBV reactivation. Under immunosuppressive conditions, HBV is more likely to replicate rapidly and to infect many hepatocytes. Subsequently, the recovered immunocompetent cells can attack the HBV-infected hepatocytes, resulting in the recurrence of hepatitis B.

Clinical manifestations of HBV reactivation range from asymptomatic, self-limiting to fulminant hepatitis, which can be fatal in some patients despite best supportive care. It is difficult to predict individual patient outcome after HBV reactivation.

How does anti-B-cell therapy increase the risk of HBV reactivation? Immune control of HBV infection is considered to be maintained mainly by HBV-specific cytotoxic T cells,[16] and the role of B cells has not yet been clearly elucidated. Therefore, the mechanism of HBV reactivation associated with anti-B-cell therapy is not fully understood.

Risk of HBV reactivation after immunosuppressive therapy

The risk of HBV reactivation depends on the balance between replication of the virus and the immune response of the host. In patients who receive immunosuppressive therapy, including anti-B-cell therapy, the risk of HBV reactivation varies according to the HBV infection status at baseline, but also with the intensity of immunosuppression.

Important viral factors for HBV reactivation have been reported to be high HBV DNA levels and serum HBV markers such as HBeAg, HBsAg, and anti-HBc.[3,6,17,18] Occult HBV infection, which is defined as seronegativity for HBsAg, but with detectable of HBV DNA in the blood or liver, may be one of the important risk factors.[5] Genotypes and gene mutations of HBV, which are associated with the enhancement of HBV replication and fulminant hepatitis, have also been reported to be associated with outcome in patients with HBV reactivation.[19,20]

Conversely, steroid-containing chemotherapy has been reported to be an important host risk factor associated with HBV reactivation, partly because of glucocorticoid stimulation of a glucocorticoid-responsive element in the HBV genome leading to up-regulation of HBV gene expression. Actually, in the pre-rituximab era, a randomized controlled trial demonstrated that steroid-containing chemotherapy increased the incidence of HBV reactivation in HBsAg-positive patients; the relative risk of steroid-containing versus steroid-free was 1.9 (95% confidence interval = 1.1-3.4).[21] The rituximab + steroid-containing chemotherapy has recently been demonstrated to be a risk factor for HBV reactivation in HBsAg-negative patients,[5,6] HBV reactivation is a well-known complication after allogeneic hematopoietic stem cell transplantation (HSCT) because of the long-term use of immunosuppressive drugs and the gradual immune reconstitution after HSCT.[22,23] Additional host risk factors for HBV reactivation have been reported as male sex,[6] diagnosis of lymphoma,[3] absence of anti-HBs at baseline,[6,24] and decrease of anti-HBs titers after immunosuppressive therapy.[25] The risk classification for HBV reactivation according to serum HBV markers and the intensity of immunosuppressive therapy can be determined based on the current evidence (Figure 1).[7]

HBV reactivation in HBsAg-positive patients after anti-B-cell therapy

HBV reactivation often occurs in HBsAg-positive patients after immunosuppressive therapy even if steroid alone is given. In the pre-rituximab era, HBsAg-positive patients were considered to be at high risk for HBV reactivation and it was reported that 24%–53% of these patients developed HBV reactivation after immunosuppressive therapy. Yeo et al reported that HBV reactivation was observed in 47 of 193 (24%) lymphoma patients seropositive for HBsAg who received systemic chemotherapy.[3] Lok et al reported that 13 of 27 (48%) HBsAg-positive patients developed HBV reactivation after lymphoma treatment.[1] Lau et al conducted a randomized controlled trial to evaluate the efficacy of antiviral prophylaxis in 30 HBsAg-positive patients with malignant lymphoma after systemic chemotherapy.[2] No reactivation occurred in patients who received antiviral prophylaxis, but 8 of 15 (53%) patients without prophylaxis had HBV reactivation. In the rituximab era, there is limited evidence regarding the risk of HBV reactivation in HBsAg-positive patients after anti-B-cell therapy because it has been widely recognized that antiviral prophylaxis is necessary to prevent HBV-related hepatitis for such high-risk patients. Pei et al reported that 8 of 10 (80%) HBsAg-positive patients developed HBV reactivation in a retrospective analysis.[26] More recently, Kim et al conducted a multinational retrospective study to evaluate the incidence of HBV reactivation and its risk factors and found that 13 of 22 (59%) HBsAg-positive patients had HBV reactivation without antiviral prophylaxis.[24]

HBV reactivation in HBsAg-negative patients (with resolved HBV infection) after anti-B-cell therapy

In the pre-rituximab era, the risk of HBV reactivation in HBsAg-negative patients with lymphoma after systemic chemotherapy was considered to be low. Lok et al reported that only 2 of 72 (3%) HBsAg-negative patients developed HBV reactivation compared with far more HBsAg-positive patients (13 of 27, 48%).[1] However, it is likely that the introduction of rituximab has increased the risk of HBV reactivation, which has often been reported especially in HBsAg-negative patients who received rituximab-containing chemotherapy. Dervite et al first reported that fatal HBV reactivation occurred in HBsAg-negative but anti-HBs-positive patients who received R-CHOP in 2001.[4] In 2006, Hui et al conducted a retrospective study of 244 HBsAg-negative lymphoma patients receiving systemic chemotherapy, 8 of whom (3%) developed hepatitis due to HBV reactivation. All 8 of these patients were seropositive for anti-HBc and/or anti-HBs.[5] Multivariate analysis showed that rituximab + steroid-containing chemotherapy was an independent risk factor for HBV reactivation compared with other combined chemotherapy (6 of 49,12%, vs 2 of 195, 1%, respectively). In 2009, Yeo et al also reported that 5 of 80 (6%) HBsAg-negative patients who were diagnosed as having diffuse large B-cell lymphoma developed HBV reactivation after R-CHOP or CHOP-like regimens.[6] All 5 of these patients were anti-HBc-positive and received R-CHOP, meaning that 5 of 21 (24%) patients seropositive for anti-HBc had HBV reactivation after R-CHOP. In 2013, Kim et al also showed that 16 of 153 (10%) HBsAg-negative but anti-HBc-positive patients developed HBV reactivation after R-CHOP in a multinational retrospective analysis.[24]

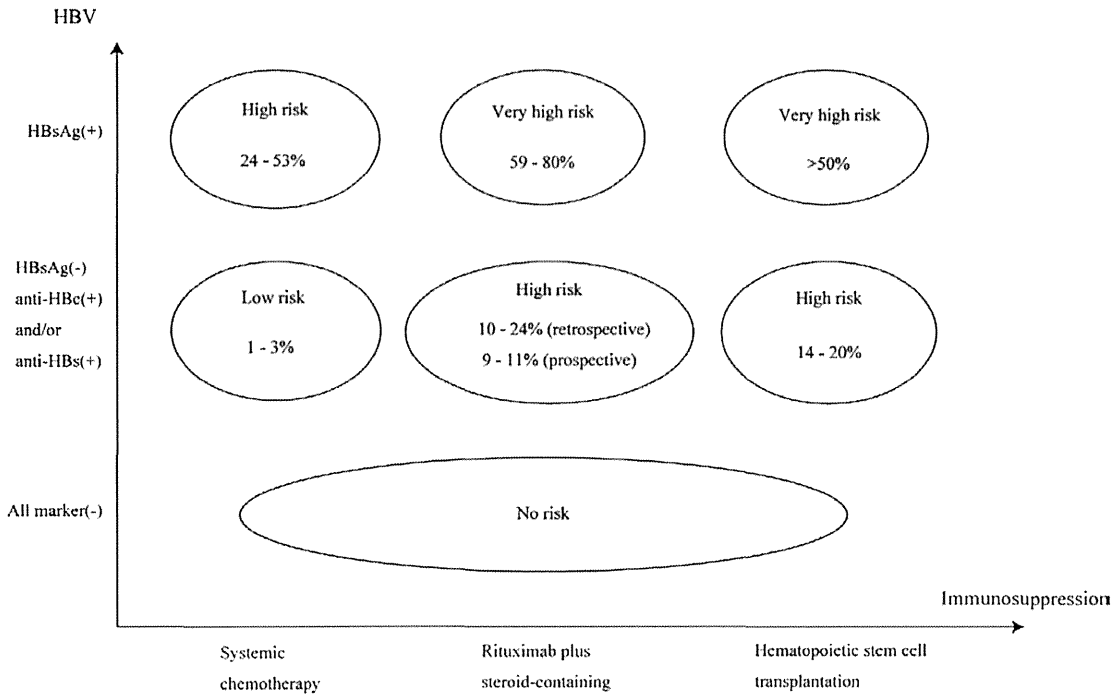

Figure 1. Risk classification of HBV reactivation after immunosuppressive therapy. The vertical axis shows the HBV infectious status at baseline according to serum HBV markers before immunosuppressive therapy. The horizontal axis shows the intensity of immunosuppression after immunosuppressive therapy. The incidence of HBV reactivation in HBsAg-positive patients who received systemic chemotherapy, rituximab + steroid-containing chemotherapy, or HSCT has been reported to be 24%–53%, 59%–80%, and >50%, respectively. In patients with resolved HBV infection (anti-HBc-positive and/or anti-HBs-positive in HBsAg-negative patients), the incidence of HBV reactivation has been reported to be 1%–3% after systemic chemotherapy, 10%–24% (by retrospective study) and 9%–11% (by prospective study) after rituximab + steroid-containing chemotherapy, and 14%–20% after HSCT. HBsAg-negative patients seronegative for anti-HBc and anti-HBs (all marker-seronegative at baseline) are considered to be at no risk for HBV reactivation after immunosuppressive therapy. This figure is modified and cited from Kusumoto et al[7] with permission from The Japanese Society of Hematology.

Summary of the characteristics and outcome of 211 Japanese patients developing serious hepatitis B after rituximab-containing chemotherapy

According to the data collected by the Zenyaku Kogyo Company and the Chugai Pharmaceutical Company of Japan between September 2001 and August 2013, 211 Japanese patients developed serious hepatitis B after rituximab-containing chemotherapy. These data included clinical information that were collected retrospectively from medical practices, spontaneous reports to the company, reports at academic meetings, and results from several investigational studies and clinical trials.

The HBsAg status before rituximab-containing chemotherapy was available in 175 patients: 66 (38%) were HBsAg-positive and 109 (62%) were HBsAg-negative. Of the latter, the anti-HBc status before initiating rituximab-containing chemotherapy was known in only 33 (30%). Of these, 32 (97%) were anti-HBc-positive and the remaining one was anti-HBc-negative, whereas 8, 13, and 12 were anti-HBs-positive, anti-HBs-negative, and with unknown anti-HBs status, respectively.

Of the 109 HBsAg-negative patients, 88 (81%) received rituximab + steroid-containing chemotherapies such as R-CHOP, 7 received a steroid-free regimen, 5 received HSCT, 1 received renal transplantation, 4 received rituximab alone, and 4 were not available for regimen information. Antiviral prophylaxis was administered in 21 of 66 HBsAg-positive and 2 of 109 HBsAg-negative patients. Of the HBsAg-negative patients, the incidence of fulminant hepatitis (32 of 109, 29%) and mortality (51 of 109, 47%) was higher in the HBsAg-positive patients (14 of 66, 21%, and 20 of 66, 30%, respectively). Median time to onset of hepatitis B from the last administration of either rituximab or other chemotherapy regimen in HBsAg-positive and HBsAg-negative patients was 5.5 and 9.1 weeks, respectively. Most of the HBsAg-negative patients developed hepatitis within 1 year after completion of chemotherapy, but 2 developed hepatitis >1 year after chemotherapy.

Screening for HBV reactivation after anti-B-cell therapy

HBV infection should be screened in all patients before initiating anti-B-cell therapy regardless of the presence of hepatitis, type of disease, and combined chemotherapy. To identify the 3 following risk groups for HBV reactivation, serum HBV markers including HBsAg, anti-HBc, and anti-HBs should be measured before initiating immunosuppressive therapy (Figure 2).[7,27,28] If an individual is seronegative for HBsAg but seropositive for anti-HBc and/or anti-HBs, then baseline HBV DNA levels should be measured in addition to the serum markers. The 3 risk groups are as follows.

Group 1 are HBsAg-positive patients. If an individual is seropositive for HBsAg, the additional following tests are recommended: HBV DNA levels, HBeAg, and anti-HBe.[7,28] Group 2 are HBsAg-negative but with HBV DNA detectable. These patients are considered to have occult HBV infection and may be at high risk for HBV reactivation similar to HBsAg-positive patients.[7,28,29] Because

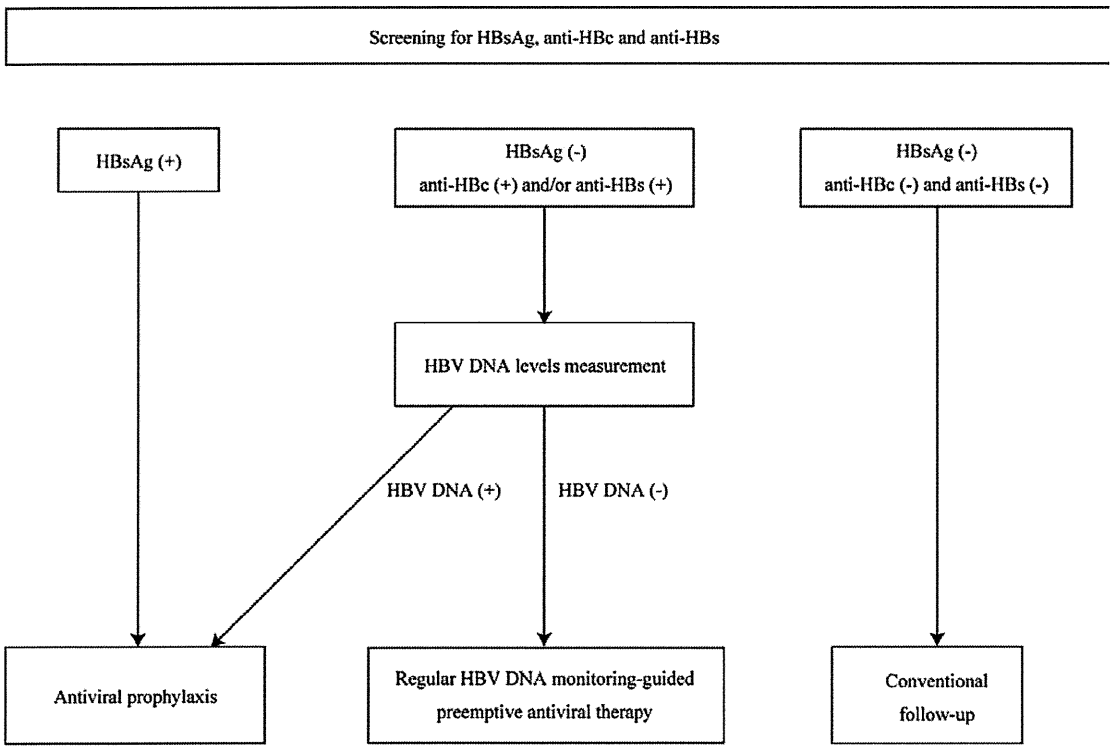

Figure 2. Flowchart illustrating the strategy for preventing hepatitis due to HBV reactivation. All patients are screened before starting anti-B-cell therapy by measuring serum HBV markers including HBsAg, anti-HBc, and anti-HBs to identify groups at risk of HBV reactivation. If an individual is seronegative for HBsAg but seropositive for anti-HBc and/or anti-HBs, baseline HBV DNA levels are measured in addition to the serum markers. To prevent hepatitis due to HBV reactivation after anti-B-cell therapy, antiviral prophylaxis is recommended for HBsAg-positive patients and/or patients in whom HBV DNA is detectable at baseline, whereas regular monitoring of HBV DNA-guided preemptive antiviral therapy is a reasonable approach for patients with resolved HBV infection who are seronegative for HBsAg but seropositive for anti-HBc and/or anti-HBs. This figure is modified and cited from Kusumoto et al[7] with permission from The Japanese Society of Hematology.

an individual with occult HBV infection is usually anti-HBc-positive and/or anti-HBs-positive, this risk group can be identified by additionally measuring HBV DNA levels if either HBV markers is seropositive. Group 3 are HBsAg-negative but anti-HBc-positive and/or anti-HBs-positive and HBV DNA is not detectable. HBsAg-negative patients who are anti-HBc-positive and/or anti-HBs-positive without detectable HBV DNA are considered to have resolved their HBV infection. However, an individual who is seropositive only for anti-HBs with a history of vaccination against hepatitis B should be excluded from the risk group for HBV reactivation.[28] Because HBV reactivation has also been reported in patients seropositive only for anti-HBs who were treated with anti-B-cell therapy,[5] such patients require careful attention and should not be excluded from the risk group for HBV reactivation, especially in countries that have adopted universal vaccination against hepatitis B.

The identification of these risk groups for HBV reactivation is strongly recommended before initiating immunosuppressive therapy, because this may decrease titers of serum HBV antibodies and make it difficult to evaluate the risk of HBV reactivation.[28] It is also recommended to use high-sensitivity testing kits when HBV-related markers are measured.[28]

Management of HBV reactivation after anti-B-cell therapy

Initiating antiviral treatment after the occurrence of overt hepatitis is insufficient to control HBV reactivation. Yeo et al conducted a prospective study showing that 5 (16%) patients died and 22 (69%) required modification of the planned chemotherapy schedule among 32 patients who received lamivudine as an antiviral drug for hepatitis due to HBV reactivation.[3] Umemura et al reported that the incidence of fulminant hepatitis and mortality in patients with HBV reactivation was higher than in acute hepatitis B in a retrospective analysis.[19] Therefore, it is necessary to identify these high-risk groups before initiating immunosuppressive therapy and to start antiviral treatment immediately before hepatitis onset after HBV reactivation.

Currently, there are 2 options to preventing hepatitis due to HBV reactivation: (1) antiviral prophylaxis, in which an antiviral drug is given before initiating immunosuppressive therapy, and (2) regular HBV DNA-monitoring-guided preemptive antiviral therapy, in which an antiviral drug is given if HBV DNA in the blood becomes detectable by regular monitoring.

Strategy to prevent HBV reactivation in HBsAg-positive patients

For HBsAg-positive patients (Risk Group 1) undergoing immunosuppressive therapy, antiviral prophylaxis is essential (Figure 2), as recommended by some guidelines.[28,30,31] Antiviral prophylaxis should also be given to patients who are HBsAg-negative but who have detectable HBV DNA (Risk Group 2), who are potentially at a higher risk for HBV reactivation (Figure 2).[7,28,29] The incidence of HBV reactivation in HBsAg-positive patients receiving rituximab-containing chemotherapy without antiviral prophylaxis has been

reported to be 59%–80%.[24,26] These patients are considered to constitute a very-high-risk group for HBV reactivation (Figure 1). Of the HBsAg-positive patients, most HBV reactivation occurs during and after chemotherapy, but patients with high HBV DNA levels at baseline potentially suffer HBV reactivation at an early stage of chemotherapy.[7] Therefore, antiviral prophylaxis is necessary to prevent hepatitis due to HBV reactivation in HBsAg-positive patients and/or patients in whom HBV DNA is detectable at baseline and should be started as soon as possible to reduce HBV DNA levels before immunosuppressive therapy.

Which drug is recommended in an antiviral prophylaxis setting to prevent HBV reactivation? Lamivudine is a first-generation nucleoside analog that can suppress HBV replication and improve hepatitis B. Some prospective studies have demonstrated the efficacy and safety of lamivudine for preventing HBV reactivation in HBsAg-positive patients who received immunosuppressive therapy.[3,32] However, it was reported that there is a high incidence of acquired viral resistance to lamivudine, resulting in breakthrough hepatitis, especially in patients who received long-term antiviral prophylaxis with this drug. Lamivudine resistance was reported as 24% at 1 year and >50% at 5 years in patients with chronic hepatitis B.[33]

Entecavir and tenofovir are new-generation nucleoside analogs that have greater potential to suppress HBV replication and to which there is a lower incidence of viral resistance mutation.[34,35] Entecavir resistance has been reported to be more likely to occur in patients with chronic hepatitis B who had prior lamivudine resistance compared with patients with previously untreated chronic hepatitis B.[36] There has been no prospective study directly comparing entecavir with lamivudine for antiviral prophylaxis after immunosuppressive therapy. Kim et al recently reported that no HBV reactivation was observed in 31 HBsAg-positive patients who received entecavir, whereas 30 of 96 (31%) who received lamivudine developed HBV reactivation after R-CHOP-like regimens.[24] Although the current evidence regarding prevention of HBV reactivation is insufficient for a definitive recommendation, it would seem that entecavir or tenofovir is better option as a first-line antiviral drug in view of the higher efficacy and less development of resistance.[28]

How long should we continue antiviral prophylaxis after anti-B-cell therapy? There is no consensus regarding the optimal duration of antiviral prophylaxis for HBsAg-positive patients. To establish when to discontinue the antiviral prophylaxis safely, we propose the following criteria as being essential: (1) planned immunosuppressive therapy completed, (2) undetectable HBV DNA levels by real-time PCR assay, and (3) both conditions 1 and 2 maintained at least for 1 year. More importantly, regular monitoring of HBV DNA for at least 6 months after discontinuation of antiviral prophylaxis is desirable to prevent HBV reactivation because reemergence of HBV was observed within 6 months after withdrawal of antiviral prophylaxis according to data collected by the Zenyaku Kogyo Company and the Chugai Pharmaceutical Company.

Strategy to prevent HBV reactivation in patients with resolved HBV infection (anti-HBc-positive and/or anti-HBs-positive in HBsAg-negative patients, Risk Group 3)

Although there is no consensus on a strategy to prevent HBV reactivation in patients with resolved HBV infection, regular HBV DNA-monitoring-guided preemptive antiviral therapy is a reason-

Table 1. Prospective studies on the prevention of HBV reactivation in lymphoma patients with resolved HBV infection

Principal investigator	Subjects	Lymphoma treatment	Planned sample size	Design	Start date	ID no.
Kusumoto S (Japan)	Anti-HBc(+) and/or anti-HBs(+), B-NHL	Rituximab + steroid-containing chemotherapy	321	HBV DNA monitoring single arm, 68 institutions	Aug 2008	UMIN000001299
Liu TW (Taiwan)	Anti-HBc(+), DLBCL or FL	R-CHOP	150	HBV DNA monitoring single arm, 14 institutions	June 2009	NCT00931229
Ji D (China)	Anti-HBc(+), DLBCL	R-CHOP	110	HBV DNA monitoring single arm, single institution	Oct 2010	NCT01210287
Yuen MF (Hong Kong)	Anti-HBc(+), B-cell lymphoma, CLL	Rituximab-containing chemotherapy	70	HBV DNA monitoring single arm, single institution	Dec 2011	NCT01502397
Huang YH (Taiwan)	Anti-HBc(+), B-NHL	NA	90	ETV prophylaxis vs therapeutic RCT, single institution	Apr 2009	NCT00926757
Zhu J, Song Y (China)	Anti-HBc(+), lymphoma	Chemotherapy	190	ETV prophylaxis vs therapeutic RCT, 13 institutions	Jan 2013	NCT01765231

B-NHL indicates B-cell non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; CLL, chronic lymphocytic leukemia; NA, not available; ETV, entecavir; and RCT, randomized controlled trial.

Table 2. Comparison of large-scale prospective studies on HBV DNA-monitoring-guided preemptive antiviral therapy in lymphoma patients with resolved HBV infection after rituximab-containing chemotherapy (preliminary results)

ID no.	NCT00931229	UMIN000001299
Locations	Taiwan	Japan
Study design	Prospective HBV DNA-monitoring-guided preemptive antiviral therapy	Prospective observational HBV DNA-monitoring-guided preemptive antiviral therapy
No. of patients enrolled	$n = 150$	$n = 187$ (interim analysis)
Lymphoma treatment	R-CHOP	R plus steroid-containing chemotherapy (R-CHOP, $n = 151$)
Definition of HBV reactivation	>10-fold increase in HBV DNA vs previous nadir levels	HBV DNA 1.8 log copies/mL or more
Cut-off value of HBV DNA measurement	3.0 log copies/mL	1.8 log copies/mL
Interval of HBV DNA monitoring	Every 4 weeks	Every 4 weeks
Antiviral drug	Entecavir	Entecavir
Incidence of HBV reactivation	11% (17 of 150)	9% (16 of 187)
HBV-reactivation-related hepatitis	7% (10 of 150)	0%
Severe HBV-related hepatitis*	5% (7 of 150)	0%
HBV-related death	0% (0 of 150)	0%
Reference	Hsu et al[38]	Kusumoto et al[39]

NA indicates not available.
*Severe hepatitis defined as alanine aminotransferase >10-fold of upper normal limit.

able approach for such patients.[7,28,37] Recently completed and ongoing prospective studies for preventing HBV reactivation in patients with resolved HBV infection are listed in Table 1. There are 2 study designs: preemptive antiviral therapy and antiviral prophylaxis.

What is the rationale for preemptive antiviral therapy? Hui et al reported that the median time from the elevation of serum HBV DNA to hepatitis onset was 18.5 weeks (range, 12–28) in a retrospective analysis,[5] suggesting that increased HBV DNA levels were observable at least 3 months before clinical hepatitis in patients with resolved HBV infection. We hypothesized that monthly HBV DNA-monitoring-guided preemptive antiviral therapy can prevent hepatitis due to HBV reactivation[7] and conducted a multicenter prospective study in Japan (Study UMIN000001299, Table 1).

A comparison of large-scale prospective studies on HBV DNA-monitoring-guided preemptive antiviral therapy in lymphoma patients with resolved HBV infection after rituximab-containing chemotherapy is shown in Table 2. These studies demonstrated that monthly monitoring of HBV DNA could prevent HBV-related death.[38,39] However, in the Taiwanese study, 10 of 150 (7%) HBV-resolved patients who received R-CHOP developed hepatitis due to HBV reactivation and 7 of 150 (5%) developed severe hepatitis defined as alanine aminotransferase >10-fold the upper normal limit. Patients with HBV reactivation were more likely to have a poorer prognosis.[38] The interim analysis of the Japanese study showed that serial monthly monitoring of HBV DNA is effective for preventing hepatitis due to HBV reactivation, with no hepatitis occurring in B-cell lymphoma patients with highly replicative HBV clones such as precore mutants.[39]

Although the different results of these 2 studies may have been related to the definition of HBV reactivation and the cutoff values of HBV DNA measurement (Table 2), the Taiwanese study might indicate that a more sensitive assay of HBV DNA is required to prevent hepatitis on such the setting of preemptive antiviral therapy. These 2 studies also showed that most HBV reactivation was observed within 1 year after completion of rituximab-containing chemotherapy, which again suggests that the monitoring of HBV DNA should be continued for at least 1 year after the completion of anti-B-cell therapy. Conversely, although antiviral prophylaxis is an alternative approach to preventing HBV reactivation in patients with resolved HBV infection,[40] there are some concerns, such as emergence of drug resistance and cost-effectiveness, that also need to be addressed.[41]

Conclusions

All patients should be screened before starting anti-B-cell therapy by measuring serum HBV markers including HBsAg, anti-HBc, and anti-HBs to identify groups at risk of HBV reactivation. To prevent hepatitis due to HBV reactivation after anti-B-cell therapy, antiviral prophylaxis is recommended for HBsAg-positive patients and/or patients in whom HBV DNA is detectable at baseline, whereas regular monitoring of HBV DNA-guided preemptive antiviral therapy is a reasonable approach for patients with resolved HBV infection.

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Disclosures

Conflict-of-interest disclosures: K.T. has received research funding from Zenyaku Kogyo and Chugai Pharmaceutical. S.K. has received research funding and honoraria from Chugai Pharmaceutical, Bristol-Myers Squibb, Zenyaku Kogyo, and Abbott. Off-label drug use: None disclosed.

Correspondence

Kensei Tobinai, MD, PhD, Department of Hematology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Phone: +81-3-3542-2511; Fax: +81-3-3542-3815; e-mail: ktobinai@ncc.go.jp.

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