between September 2001 and May 2008 following rituximab-containing systemic chemotherapy [4]. These data include information gleaned retrospectively from medical practices, spontaneous reports to the company, reports at academic meetings and results of several investigational studies and clinical trials. The HBsAg status of 97 of these 111 patients prior to rituximab treatment was available: 47 (42%) were HBsAg-positive, and 50 (45%) were HBsAg-negative. The characteristics of HBV reactivation in these 50 HBsAg-negative patients are summarized below.

1. Of the 50 HBsAg-negative patients, only 11 also had a known anti-HBc status before rituximab therapy. All 11 were anti-HBc-positive, of which one and six patients were anti-HBs-positive and -negative, respectively.
2. Following treatment using steroid-containing regimens, such as R-CHOP, 40 patients developed HBV reactivation. Only four and three patients treated with regimens not containing steroids or receiving autologous peripheral blood stem cell transplantation, respectively, experienced HBV reactivation.
3. Among the HBsAg-negative patients, the incidence of fulminant hepatitis (20/50 patients, 40.0%) and mortality (25/50, 50.0%) was higher than that among the HBsAg-positive patients (10/47, 21.3% and 13/47, 27.7%, respectively).
4. Median time to onset of hepatitis from the last administration either of rituximab or chemotherapy was 9.6 weeks. Most of the HBsAg-negative patients developed hepatitis after completion of systemic chemotherapy, as anticipated. The most delayed case was reported to occur at 8.5 months in this cohort.

More recently, Fukushima et al. [45] conducted a prospective cohort study to monitor HBsAg on a monthly basis and HBV-DNA every 3 months in HBsAg-negative but anti-HBc-positive patients with malignant lymphoma both during and after systemic chemotherapy. They found that one of 24 patients developed HBV reactivation, which was diagnosed by an elevated HBV-DNA level—when the HBsAg was still negative. To the best of our knowledge, this study is the first clinical trial of HBV-DNA monitoring aimed at preventing HBV reactivation, and even with a limited number of cases at a single institution, it documents the potential benefit of early diagnosis by HBV-DNA monitoring, and thus earlier treatment.

Prevalence of the HBsAg-negative high-risk group for HBV reactivation (anti-HBc-positive and/or anti-HBs-positive)

According to a study of 3874 specimens collected consecutively over a 2-year period (2005, 2006) for the screening of viral infections before blood transfusion at the Nagoya City University Hospital, Japan, the frequency of HBsAg, anti-HBc and anti-HBs seropositivity was 1.5, 20 and 22%, respectively [4]. In this cohort, anti-HBc-positivity and/or anti-HBs-positivity reached 23.2% (899/3874 patients). In other countries, according to data mostly from single institutions, the frequency of HBsAg and anti-HBc seropositivity is 0.1 and 4.6% in the USA [46], 2.7–5.1 and 17.6–26.2% in Italy [47–49], 7.2 and 34.3% in Singapore [50], 23.2 and 44.2–62.0% in Hong Kong [2, 3] and 15.6 and 20.1% in China [51].

When interpreting data from other countries, it must be borne in mind that the prevalence of HBV infection varies greatly from country to country and in different areas. In Japan, if these anti-HBc-positive and/or anti-HBs-positive patients (23.2%) were actually to be at high risk for HBV reactivation following systemic chemotherapy including rituximab-plus-steroid combinations for malignant lymphoma, it would be necessary to follow up tenfold more patients than HBsAg-positive patients (1.5%), which represent the conventional high-risk group.

The characteristics of HBV reactivation in HBsAg-positive and -negative patients are summarized in Table 2. To establish an optimal strategy for hepatitis prevention and treatment, it is very important to recognize the difference between HBsAg-positive and -negative patients with HBV reactivation.

Management of HBV reactivation following systemic chemotherapy

Initiating antiviral treatment after hepatitis onset may be insufficient to control HBV reactivation. Yeo et al. [42] reported the results of a clinical trial in 32 patients given lamivudine as an antiviral drug for hepatitis due to HBV reactivation. Five patients (16%) died, and 22 (69%) needed to have their chemotherapy schedule modified. Based on the results of a retrospective study in Japan, Umemura et al. [40] reported that the incidence of fulminant hepatitis and mortality following HBV reactivation is high compared to the occurrence of acute hepatitis B. Therefore, it is necessary to identify high-risk groups in advance of chemotherapy, and it is crucial to start antiviral treatment immediately upon HBV reactivation—before hepatitis develops.

There are two current options to prevent HBV reactivation (1) prophylaxis with antiviral drugs, and (2) preemptive therapy starting at the time of detection of HBV-DNA in the blood. For HBsAg-positive patients undergoing systemic chemotherapy, prophylaxis with antiviral drugs is essential, as recommended by the latest American and Japanese guidelines [52, 53]. Antiviral drugs should also be administered to HBsAg-negative but HBV-DNA-positive

Table 2 Characteristics of HBV reactivation in HBsAg-positive and -negative patients

Characteristics	HBsAg-positive	HBsAg-negative high-risk group (anti-HBc-positive and/or anti-HBs-positive)
Seroprevalence of HBV infection	1.5% in Japan (Nagoya)	23.2% in Japan
	0.1% in USA (MDACC)	4.6% in USA[a]
	2.7–5.1% in Italy	17.6–26.2% in Italy[a]
	7.2% in Singapore	34.3% in Singapore[a]
	15.6% in China	20.1% in China[a]
	23.2% in Hong Kong	44.2–62.0% in Hong Kong[a]
Diagnosis and definition	A one log or greater increase in HBV-DNA level compared to baseline, with hepatitis	Reappearance of HBsAg and/or detection of HBV-DNA by RTD-PCR
Risk of HBV reactivation	20–50% on conventional chemotherapy	1.0–2.7% on conventional chemotherapy
	80% on rituximab-containing chemotherapy	12.2–23.3% on rituximab-plus-steroid combination
	>50% on HSCT	14–20% on HSCT
Risk factors on prechemotherapy	High viral load of HBV	Anti-HBs-negative
	HBeAg-positive	
	Liver cirrhosis or hepatocellular carcinoma	
Time of HBV reactivation	Most cases occur during and after chemotherapy, but HBsAg-positive cases with high viral loads may often occur at early stage of chemotherapy	Most cases occur at the end of chemotherapy and after completion of chemotherapy
		Median time to onset of hepatitis was 9.6 weeks
		The most delayed case was reported to occur at 8.5 months after chemotherapy (Zenyaku Company data)
		Some cases occurred at several years after HSCT
Rise in HBV-DNA prior to hepatitis	There are several patterns	Rising HBV-DNA occurred at a median of 18.5 weeks (range 12–28) prior to hepatitis due to HBV reactivation

HSCT Hematopoietic stem cell transplantation, *MDACC* MD Anderson Cancer Center, *RTD-PCR* real-time detection PCR

[a] The percentage shows the frequency of seropositivity for anti-HBc (not including cases seronegative for anti-HBc but seropositive for anti-HBs) in each cohort

patients who are potentially at an even greater risk for HBV reactivation. No standard management to prevent HBV reactivation has yet been established for HBsAg-negative patients seropositive for anti-HBc and/or anti-HBs. Nonetheless, an early diagnosis of HBV reactivation is critical to enable early initiation of active antiviral therapy. Preemptive therapy guided by serial HBV-DNA monitoring (monthly during and after chemotherapy for at least 1 year) is a reasonable strategy recommended by the latest Japanese guidelines [53]. If HBV-DNA becomes detectable, antiviral therapy should be started as soon as possible.

The latest CDC and Japanese guidelines recommend that patients receiving cytotoxic or immunosuppressive therapy should be tested for serologic markers of HBV infection, including HBsAg, anti-HBc and anti-HBs [53, 54]. HBV status should be established before any chemotherapy or immunosuppressive therapy is initiated because antibody titers may be reduced by the immunosuppressive action of the treatment. For patients positive for any HBV serological markers, the presence of HBV-DNA should be confirmed by RTD-PCR.

To date, most cases of HBV reactivation in HBsAg-negative patients have occurred in those who were anti-HBc-positive [2–4]. However, there is a report of an anti-HBc-negative but anti-HBs-positive patient developing HBV reactivation following rituximab-plus-steroid combination chemotherapy [2]. Therefore, for routine screening of these patients, both anti-HBc and anti-HBs should be tested as well as HBsAg.

Future perspectives regarding HBV reactivation

Most data on HBV reactivation in HBsAg-negative patients are limited to retrospective studies, so the exact risk of HBV reactivation has not been estimated precisely, and the

viral and host risk factors associated with HBV reactivation have not been analyzed thoroughly.

For HBsAg-negative patients, HBV reactivation is a complication not only of lymphoma treatment but also of treatments for other cancers [26, 55] as well as for autoimmune diseases [56]. Thus, establishing a standard strategy to prevent HBV reactivation is a very important and urgent issue.

A multicenter clinical trial in Japan is now ongoing to evaluate the efficacy and safety of preemptive therapy based on serial HBV-DNA monitoring in HBsAg-negative untreated B cell lymphoma patients seropositive for anti-HBc and/or anti-HBs during rituximab-plus-steroid combination chemotherapy (UMIN000001299).

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Virological and Clinical Characteristics on Reactivation of Occult Hepatitis B in Patients With Hematological Malignancy

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The virological characteristics of hepatitis B virus (HBV) implicated in the reactivation of occult hepatitis B in patients who have received hematopoietic stem-cell transplantation or chemotherapy for the hematological malignancy are not well defined. Twenty-eight HBsAg-negative patients who received hematopoietic stem-cell transplantation and 138 HBsAg-negative patients treated for malignant lymphoma with chemotherapy including rituximab were enrolled. Three of the 28 patients (10.7%) received hematopoietic stem-cell transplantation and one of the 138 (0.72%) patients treated for malignant lymphoma with chemotherapy developed de novo HBV hepatitis. Anti-HBc was detected in four and anti-HBs in two patients. Genotype Bj was detected in two and C in two of they all possessed wild-type sequences in the core promoter region. A precore stop mutation (A1896) was detected in a patient with genotype Bj who developed fulminant hepatic failure. HBV DNA was detected in pretreatment HBsAg-negative samples in two of four patients, and the HBV genome sequence identified from sera before chemotherapy and at the time of de novo HBV hepatitis showed 100% homology. In an in vitro replication model, genotype Bj with the A1896 clone obtained from a fulminant case had a replication level much higher than clones obtained from de novo hepatitis B patients with genotype Bj or C with G1896. In conclusion, this is the first report demonstrating de novo hepatitis B from the reactivation of occult HBV infection confirmed by molecular evolutional analysis. The fulminant outcome of HBV reactivation can be associated with genotype Bj exhibiting high replication due to the A1896 mutation. ***J. Med. Virol. 83:412–418, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: Hepatitis B virus; occult infection; reactivation; De novo; HBsAg

INTRODUCTION

Approximately 3 billion people, one half of the world population, have been exposed to hepatitis B virus (HBV), of whom approximately 350 million are infected persistently [Lee, 1997]. Reactivation of hepatitis B is a well-recognized complication in hepatitis B surface antigen (HBsAg)-positive patients when they undergo cytotoxic chemotherapy for malignant disease [Hoofnagle et al., 1982; Lok et al., 1991]. HBV reactivation has also been reported in patients with resolved HBV infection, as evidenced by the clearance of circulating HBsAg and the appearance of antibodies to hepatitis B core antigen (anti-HBc) with or without antibodies to hepatitis B surface antigen (anti-HBs) [Hui et al., 2006; Umemura et al., 2008; Kusumoto et al., 2009; Yeo et al., 2009]. In these patients, a low level of HBV replication has been shown to persist in the liver and in

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peripheral-blood mononuclear cells for decades [Kuhns et al., 1992; Fong et al., 1993]. HBV reactivation has been reported in this setting after transplantation, immunosuppressive therapy, and allogeneic and autologous hematopoietic stem-cell transplantation, with the reappearance of HBsAg [Dhedin et al., 1998; Seth et al., 2002]. De novo hepatitis B is of particular concern in this subset of patients because it commonly results in severe liver dysfunction and fatal hepatitis [Sarrecchia et al., 2005].

Many HBV carriers exist in Asian countries and anti-HBc-seropositive occult carriers have been estimated at 20%–60% in their populations [Kiyosawa et al., 1994; Kusumoto et al., 2009]; however, the incidence, effective monitoring, and risk assessment for reactivation of HBV during hematopoietic stem-cell transplantation or cytotoxic chemotherapy need further investigation. In addition, the virological characteristics of HBV on the reactivation of occult hepatitis B are not well defined. In this study, we investigated the incidence of HBV reactivation and influences of HBV genotype and viral mutations on the reactivation of occult hepatitis B in patients who received hematopoietic stem-cell transplantation or cytotoxic chemotherapy for hematological malignancy.

METHODS

Patients

In this retrospective survey, 28 HBsAg-negative patients who received hematopoietic stem-cell transplantation for hematological malignancy and 138 HBsAg-negative patients treated for malignant lymphoma with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) at Nagoya City University Hospital were analyzed.

Hepatitis B virus reactivation was diagnosed when the HBsAg status changed from negative to positive after the initiation of chemotherapy or hematopoietic stem-cell transplantation, with an elevation of serum HBV DNA to more than 10^5 copies/mL. HBV DNA was retrospectively quantified by real-time polymerase chain reaction (PCR) using stored samples from patients with HBV reactivation as described below.

Serological Markers of HBV Infection

HBsAg, HBeAg, and the corresponding antibody (anti-HBe) were determined by enzyme immunoassay (EIA) (AxSYM; Abbott Japan, Tokyo, Japan) or chemiluminescence enzyme immunoassay (CLEIA) (Fujirebio, Tokyo, Japan). Anti-HBc of IgG classes was determined by radioimmunoassay (Abbott Japan).

Quantitation of Serum HBV DNA

Hepatitis B virus DNA sequences spanning the S gene were amplified by real-time detection polymerase chain reaction (RTD-PCR) in accordance with the previously described protocol with a slight modification; it has a detection limit of 100 copies/mL [Sugiyama et al., 2009].

Sequencing and Molecular Evolutionary Analysis of HBV

Nucleic acids were extracted from serum samples (100 μL) using the QIAamp DNA extraction kit (Qiagen, Hilden, Germany) and subjected to PCR for amplifying genomic areas bearing enhancer II/core promoter/precore/core regions [nt 1628–2364], as described previously [Sugauchi et al., 2002]. Amplicons were sequenced directly using the ABI Prism Big Dye ver. 3.1 kit in the AMI 3100 DNA automated sequencer (Applied Biosystems, Foster City, CA, USA). All sequences were analyzed in both forward and reverse directions. HBV genotypes were determined by molecular evolutionary analysis. Reference HBV sequences were retrieved from the DDBJ/EMBL/GenBank database and aligned by CLUSTAL X, and then genetic distances were estimated with the 6-parameter method in the Hepatitis Virus Database (http://s2as02.genes.nig.ac.jp/) [Shin et al., 2008]. Based on obtained distances, phylogenetic trees were constructed by the neighbor-joining (NJ) method with the mid-point rooting option. To confirm the reliability of the phylogenetic trees, bootstrap resampling tests were performed 1000 times.

Construction of Plasmid and Site-directed Mutagenesis of HBV DNA

Serum samples were obtained from three patients infected with genotype Bj and a patient with Ce. HBV DNA was extracted from 100 μL serum using QIAamp DNA blood kit (Qiagen). Four primer sets were designed to amplify two fragments covering the entire HBV genome. Amplified fragments were inserted into pGEM-T Easy Vector (Promega, Madison, WI) and cloned in DH5a competent cells (TOYOBO, Osaka, Japan). At least five clones of each fragment were sequenced and the consensus sequence determined. Among them, those containing the consensus sequence were identified and adopted as templates for further construction. Finally, a 1.24-fold amount of the HBV genome (nt 1413–3215/1–2185), sufficient to transcribe oversized pregenome and precore mRNA, was constructed into pUC19 vector (Invitrogen Corp., Carlsbad, CA). For site-directed mutagenesis, wild-type HBV was digested by *HindIII* and *EcoO65I* and ligated with the fragment carrying T1762/A1764 to produce 1.24-fold the amount of the genome carrying the precore stop-codon mutation.

Cell Culture and DNA Transfection

For the standard replication assay, 10-cm-diameter dishes were seeded with 1×10^6 Huh7 cells each. After 16 hr of culture, cells were transfected with 5 μg DNA construct using the FuGENE 6 transfection reagent (Roche Diagnostics, Indianapolis, IN) and harvested 3 days later. Transfection efficiency was measured by cotransfecting with 0.5 μg reporter plasmid expressing secreted alkaline phosphatase and estimating its enzymatic activity in the culture supernatant.

Southern Blot Hybridization

Hepatitis B virus DNA samples from cells at day 3 in culture were separated on 1.2% w/v agarose gel, transferred to a positive-charged nylon membrane (Roche Diagnostics), and hybridized with full-length HBV DNA labeled with alkaline phosphatase. Detection was performed with CDP-star (Amersham Biosciences, Piscataway, NJ), and signals were captured using the LAS-1000 image analyzer (Fuji Photo Film, Tokyo, Japan). Viral replication was compared between genotypes by quantifying dots of the single-strand band.

RESULTS

Clinical and Virological Features of Patients who Developed De Novo HBV

Three of the 28 patients (10.7%) received hematopoietic stem-cell transplantation and one of the 138 (0.72%) patients treated for malignant lymphoma developed de novo hepatitis B. The demographic and clinical features of the four patients who experienced HBV reactivation are summarized in Table I (cases A–D). All had normal liver functions before initiation of treatment. The mean age of the four patients with de novo hepatitis B was 49 ± 10 years and three were male. Two patients (cases A and C) were positive for anti-HBs, and three (cases A, B, and D) were positive for anti-HBc at the baseline of hematopoietic stem-cell transplantation or chemotherapy. Dynamics of serial serum alanine aminotransaminase (ALT), total bilirubin (T.Bil), prothrombin time (PT), HBV DNA levels, and the HBV serological status of the four patients are shown in Figure 1. Three patients except case A had abnormal ALT levels and all received an oral dideoxynucleotide (Lamivudine or Entecavir) as soon as HBV reactivation was suspected. Three patients (A, C, and D) recovered from the HBV reactivation, but patient B died from severe progressive liver failure despite intensive care.

Virological features are summarized in Table I. HBV genotype Bj was detected in 2 (50%) and C in 2 (50%) patients who all possessed wild-type sequences (A1762/G1764) in the core promoter region, although the precore stop mutation (G1896A) was detected in only one patient with genotype Bj who developed fulminant hepatic failure. None of the patients had PreS deletion or escape mutations in the S-region of HBV.

DNA Sequencing and Phylogenetic Analysis

Hepatitis B virus DNA was quantified retrospectively by RTD-PCR in stored samples of the four patients with HBV reactivation. Evidence of occult HBV infection at the time of the HBsAg-negative status (before commencement of chemotherapy and hematopoietic stem-cell transplantation) was detected by RTD-PCR in patients B and C. To determine the source of HBV infection, sera from patients B and C before commencement of chemotherapy or hematopoietic stem-cell transplantation (case B-1 and C-1) and at the time of HBV reactivation (case B-2 and C-2) were subjected to HBV core promoter and precore region (481 bp) sequencing. Sequences encompassing the core promoter to precore region obtained from sera before commencement of

TABLE I. Demographic, Biochemical and Virological Characteristics of Four Patients With HBV Reactivation

	Cases			
	A	B	C	D
Age (years)	36	47	60	52
Sex	Male	Male	Female	Male
Hematological malignancy	Malignant lymphoma	Acute myeloid leukemia	Multiple myeloma	Malignant lymphoma
Source of HSCT	Cord Blood (allogenic)	Bone marrow (allogenic)	Peripheral Blood (autologous)	None
Immune suppression therapy	Cyclosporin A	Tacrolimus, Prednisolone	Prednisolone	None
Chemotherapy	None	None	MCP	Rituximab-CHOP
HBV serology and DNA before therapy				
HBsAg	−	−	−	−
Anti-HBs	+	−	+	−
Anti-HBc	+	+	−	+
HBV-DNA	Negative	2.8 log copy/ml	2.2 log copy/ml	Negative
Peak levels of				
HBV-DNA	6.0 log copy/mL	8.6 log copy/mL	6.2 log copy/mL	6.3 log copy/mL
ALT (U/mL)	31	1435	938	1040
T. Bilirubin	0.6	18.5	0.9	4.8
ETV or LAM given	+	+	+	+
Outcome	Recovered	Died	Recovered	Recovered
HBV genotype	Bj	Bj	Ce	Ce
Core Promoter (1762/1764)	Wild	Wild	Wild	Wild
Pre Core (1896)	Wild	Mutant	Wild	Wild
PreS deletion	None	None	None	None

HSCT, hematopoietic peripheral stem cell transplantation; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; MCP, ranimustine, cyclophosphamide, prednisolone; ETV, entecavir; LAM, lamivudine.

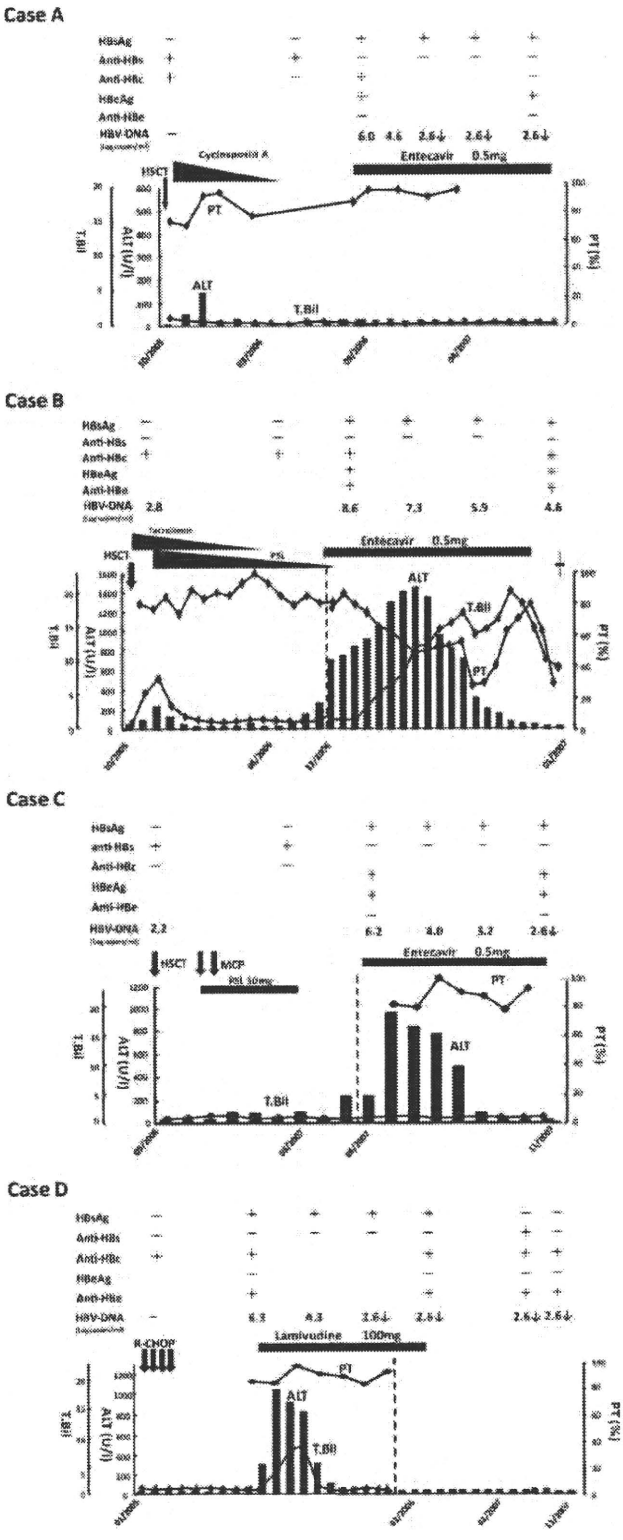

Fig. 1. Clinical course of 4 patients who developed reactivation of HBV. Changes of serial serum ALT, T.Bil, PT, HBV DNA, and HBV serology are shown. Hematopoietic stem-cell transplantation, hematopoietic peripheral stem cell transplantation; PSL, prednisolone; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; MCP, ranimustin, cyclophosphamide, prednisolone.

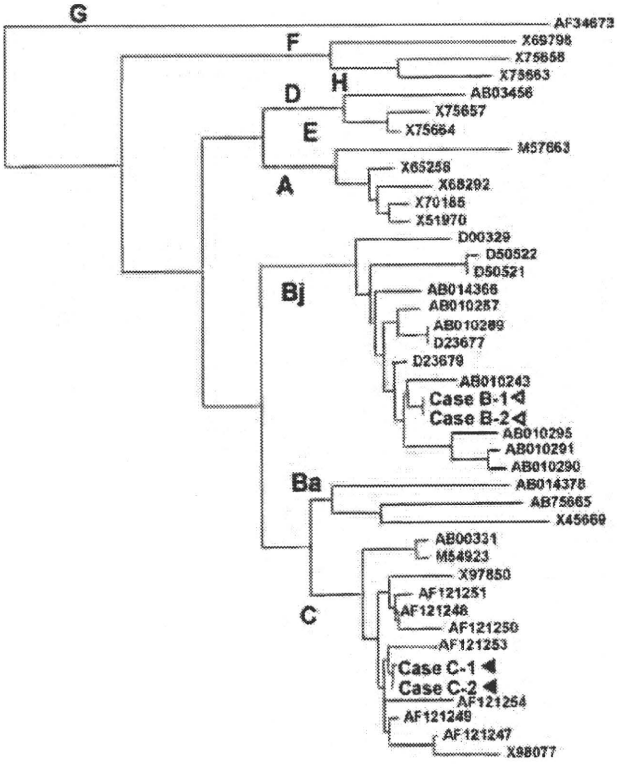

Fig. 2. Sera from cases B and C before chemotherapy or hematopoietic stem-cell transplantation (case B-1 and C-1) and at the time of HBV reactivation (case B-2 and C-2) were sequenced from the core promoter to precore region (481 bp). The phylogenetic tree demonstrated that patients B and C had de novo HBV hepatitis from reactivation of occult HBV infection.

chemotherapy and at the time of de novo HBV hepatitis showed 100% homology, and on the pylogenetic tree they were clustered together (Fig. 2). These results demonstrated that patients B and C had de novo HBV hepatitis from reactivation of occult HBV infection.

Wild-type HBV and Precore and Core Promoter Mutant Replication In Vitro

Figure 3 compares Southern blotting densities of HBV replicons constructed on bases of the following isolates: genotype Bj with PC wild-type clone obtained from case A (Bj: PC Wild), genotype Bj with PC mutant-type clone obtained from case B (Bj: PC Mutant) and genotype C with PC wild-type clone obtained from case C (C: PC Wild). The densities of the single-strand band were far higher for the Bj: PC Mutant clone obtained from patient B who had a fulminant outcome, indicating greatly enhanced replicative activity of the precore mutant in vitro.

DISCUSSION

The present study highlighted HBV reactivation in four patients who had resolved HBV infection evidenced by clearance of circulating HBsAg and the appearance of antibodies to anti-HBc with or without anti-HBs. Two patients had occult HBV infection at the time of the

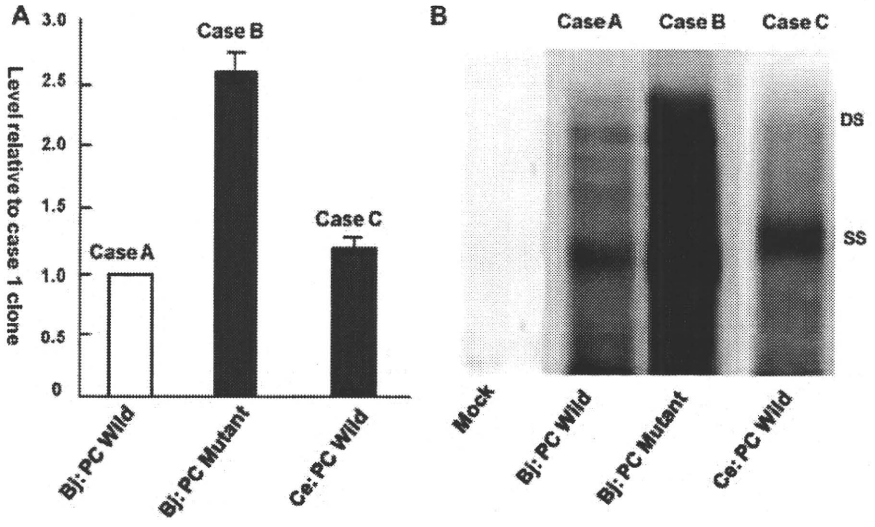

Fig. 3. **A**: Comparison of viral replication among patients in vitro. Southern blot analysis for replicative activity (intracellular HBV DNA) of genotype Bj with precore (PC) wild-type clone obtained from case A (Bj: PC Wild), genotype Bj with PC mutant-type clone obtained from case B (Bj: PC Mutant) and genotype C with PC wild-type clone obtained from case C (C: PC Wild). **B**: Quantitation of single-stranded HBV-DNA. DS, double strand; SS, single strand.

HBsAg-negative status before chemotherapy, as detected retrospectively by RTD-PCR. Sequencing of the HBV core promoter and precore region indicated complete matching between isolates from sera before commencement of chemotherapy and at the time of the manifestation of de novo hepatitis B. To our knowledge, this is the first report demonstrating de novo hepatitis B from the reactivation of occult HBV infection confirmed by molecular evolutionary analysis.

Hepatatis B Virus reactivation is known to occur in patients after solid organ transplant, allogenic, and autologous hematopoietic stem-cell transplantation, and after immunosuppressive therapy. Recently, HBV reactivation has been reported to occur in HBsAg-negative patients receiving rituximab-containing chemotherapy. In this study, one of the 138 (0.72%) lymphoma patients treated by R-CHOP developed de novo hepatitis B. As 20–25% of hospitalized HBsAg-negative patients were positive for anti-HBc and/or anti-HBs in our hospital, around 4% developed HBV reactivation, suggesting a high risk for this group. Hui et al. reported that 8 of 244 HBsAg-negative lymphoma patients receiving systemic chemotherapy developed new-onset hepatitis B (3.3%) [Hui et al., 2006]. These 8 patients were seropositive for either HBc or HBs antibody. Furthermore, the incidence of HBV reactivation in this cohort was higher in those receiving rituximab-plus-steroid combination than in those receiving other combination therapy: 12.2% (6 of 49) and 1.0% (2 of 195), respectively [Hui et al., 2006]. Multivariate analysis demonstrated that rituximab-plus-steroid combination chemotherapy is a risk factor for HBV reactivation. In another report Yeo et al. [2009] investigated 80 HBsAg-negative patients with diagnosed diffuse large B-cell lymphoma who were receiving R-CHOP or CHOP and had HBV reactivation as high as 6.25% (5/80). All five patients receiving R-CHOP had an anti-HBc-positive and anti-HBs-negative status at the treatment baseline. Thus, of 21 anti-HBc-positive lymphoma patients receiving R-CHOP in that study, five (23.8%) developed HBV reactivation [Yeo et al., 2009]. These observations strongly suggest that not only HBsAg-positive patients, but also HBsAg-negative patients with anti-HBc and/or anti-HBs and/or detectable serum HBV-DNA must be considered as a group at high risk for HBV reactivation following rituximab-plus steroid combination chemotherapy.

Eight genotypes have been classified by sequence divergence of >8% in the entire HBV genome composed of approximately 3200 nucleotides (nt), and designated A to H in the order of documentation [Miyakawa and Mizokami, 2003]. The genotypes have distinct geographical distribution and are associated with the severity of liver disease as well as response to antiviral therapies [Miyakawa and Mizokami, 2003]. Furthermore, subgenotypes have been reported for genotype A, B, and C, and named Aa (Asian/African type) and Ae (European type) [Sugauchi et al., 2004a], Bj (Japanese type) and Ba (Asian type) [Sugauchi et al., 2003; Sugauchi et al., 2004b], as well as Ce (east Asian type) and Cs (southeast Asian type) [Tanaka et al., 2005]. There are increasing lines of evidence that Aa and Ae, as well as Ba and Bj, influence the replication of HBV and have clinical relevance [Sugauchi et al., 2002; Tanaka et al., 2005]. In this study, the precore stop mutation (G1896A) was detected only in a patient with genotype Bj who developed fulminant hepatic failure. A recent cross-sectional study in which 23 patients with reactivation were compared with 529 acute hepatitis B patients in Japan, revealed that genotype B is more frequent among patients with HBV reactivation [Umemura et al., 2008]. Ozasa et al. [2006] compared 40 patients with fulminant and 261 with acute self-limited hepatitis, revealing that subgenotype Bj is associated

with the development of fulminant hepatitis. Furthermore, the frequency of both precore (G1896A) and core-promoter (A1762T/G1764A) mutations was significantly higher in patients with fulminant hepatitis than in those with acute self-limited hepatitis. These results suggested that HBV genotypes as well as gene mutations could be associated with the onset of fulminant hepatitis caused by HBV reactivation.

In this study, in vitro replication analysis demonstrated that the intracellular HBV DNA level of the wild-type genotype Bj was comparable with that of wild-type Ce, and precore (G1896A) mutation enhanced the replication capacity of genotype Bj (Fig. 3). Sugiyama et al. [2006] reported that the extracellular HBV DNA level of the genotype Bj clone replicating in vitro was much higher than other genotypes (even those with comparably high intra-cellular expression; i.e., subgenotype Ba and genotype C), suggesting that the Bj subgenotype might have a stronger inclination toward extracellular secretion. Such a high concentration of secreted viruses during subgenotype Bj infection in patients might cause the rapid and extensive infection of intact hepatocytes. Extremely high intracellular expressions of viral DNA were observed for the genotype Bj replicon with a precore stop-codon mutation reconstructed on the basis of an isolate from a patient with fulminant hepatitis, in agreement with previous in vitro and in vivo observations. Together, these clinical observations and experimental results strongly implicate the mutation in the precore region in fulminant manifestations of hepatitis.

In our patients, entecavir was chosen as the initial treatment rather than lamivudine. Entecavir achieves better HBV-DNA suppression and has a higher resistance barrier, which is important in a long treatment course in order to avoid the emergence of drug resistance; therefore, entecavir may be a promising drug for first-line treatment of the reactivation of occult hepatitis B.

Reactivation of HBV has also been described in the setting of bone marrow transplantation [Dhedin et al., 1998; Seth et al., 2002]. In the present study, de novo hepatitis B developed in 10.7% (3/28) of patients received hematopoietic stem-cell transplantation. In the recent report of a study on 137 consecutive patients (23 positive for HBsAg, 37 positive for anti-HBs, and 77 negative for HBV) who underwent hematopoietic stem-cell transplantation, hepatitis developed in 32 patients (23%), considered to be due to hepatitis B reactivation in 13 of the 32 patients [Kusumoto et al., 2009]. Hepatitis due to HBV reactivation was more common in HBsAg-positive patients than in HBsAg-negative patients (11 of 23 versus 2 of 114). It is concluded that in HBsAg-negative patients, the risk of HBV reactivation is higher for those receiving hematopoietic stem-cell transplantation (14–20%) than for those on conventional chemotherapy (1.0–2.7%) [Kusumoto et al., 2009].

In conclusion, this study showed that the fulminant outcome of the reactivation of occult hepatitis B in patients receiving hematopoietic stem-cell transplantation or cytotoxic chemotherapy for hematological malignancy was associated with genotype Bj, accompanied by high replication due to G1896A mutation.

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CORRESPONDENCE

Clinical Significance of Hepatitis B Virus (HBV) -DNA Monitoring to Detect HBV Reactivation After Systemic Chemotherapy

TO THE EDITOR: In *Journal of Clinical Oncology*, Niitsu et al[1] recently reported a prospective study to evaluate the risk of hepatitis B virus (HBV) reactivation in 51 HBV-recovered patients with diffuse large B-cell lymphoma who received rituximab-containing chemotherapy. Although no standard management of HBV reactivation was established for these patients, the study documented the potential benefit of early diagnosis by HBV-DNA monitoring, and thus, earlier treatment.

However, several issues concerning management of HBV reactivation are described in this report. First, Niitsu et al[1] reported that among patients who were positive for antibodies to the hepatitis B surface antigen (anti-HBs) and antibodies to the hepatitis B core antigen, the level of anti-HBs titer was checked once per week, and when the anti-HBs decreased to a level of less than 200 mU/mL, serum HBV-DNA was measured monthly. HBV reactivation in most patients who are negative for hepatitis B surface antigens and who have high anti-HBs titers has been reported to occur during periods after chemotherapy when anti-HBs titers are decreased. However, in a few cases, HBV reactivation may have occurred in patients with sustained high titers of anti-HBs, for instance, the patient who was reported to have maintained high titers of anti-HBs after rituximab-containing chemotherapy.[2] In this situation, escape mutants were demonstrated by sequence analysis, and that might have induced HBV reactivation, which suggests that simply monitoring anti-HBs titers may be insufficient to diagnose HBV reactivation at an early stage. Therefore, to prevent HBV reactivation, HBV-DNA monitoring is necessary in all patients who have recovered from HBV infection, both those without anti-HBs (or with low titers) as well as those with high anti-HBs titers.

Second, Niitsu et al[1] reported that the serum HBV-DNA load was determined by a quantitative reverse transcriptase polymerase chain reaction (detection value of 1.8 to 8.8 log copies/mL;COBAS AmpliPrep/COBAS TaqMan HBV-Test, Roche Diagnostics Japan, Tokyo, Japan). However, in October, 2006, when this study began, it was only possible to measure the HBV-DNA load in plasma with the TaqMan HBV-Test version 1.0.[3] Then, in 2009, a new version, TaqMan HBV-Test version 2.0,[4] became available worldwide, and the HBV-DNA load became measurable in serum. Therefore, it is prudent to note that there is an inconsistency with respect to sample selection for HBV-DNA measurements in the report by Niitsu et al.

Niitsu et al[1] also reported that patients were enrolled onto the trial for 3 years, and that HBV-DNA was determined during and after chemotherapy for 2 years. However, given that the median follow-up period for the patients who were enrolled onto this study was not indicated, it is likely that the evaluation of the risk of HBV reactivation was affected because the dropout rate during follow-up and in the event of death are competing risks.

This prospective study demonstrated the possibility of using HBV-DNA monitoring to make an early diagnosis of HBV reactivation. Thus, for patients who have recovered from HBV infection, preemptive antiviral therapy that begins when HBV-DNA is detected in the blood is a reasonable strategy.[5-7] However, the clinical evidence to date does not provide enough information to determine the optimal frequency and duration of such HBV-DNA monitoring. Well-designed clinical trials are needed to investigate the efficacy and safety of preemptive therapy guided by serial HBV-DNA monitoring.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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ORIGINAL ARTICLE

Reactivation of hepatitis B virus in HBsAg-negative patients with multiple myeloma: two case reports

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Abstract It was recently reported that hepatitis B virus (HBV) reactivation had occurred in HBsAg-negative lymphoma patients who received rituximab plus steroid combination chemotherapy. HBV reactivation in myeloma patients have not been reported extensively. We describe here two cases of HBV reactivation in HBsAg-negative myeloma patients receiving systemic chemotherapy: one from the medical records of 40 patients and another from 61 patients with prospective HBV-DNA monitoring. In the first case positive for anti-HBs, HBV reactivation was diagnosed when hepatitis developed during conventional chemotherapy such as MP and MCP regimen in a relapsed patient after autologous stem cell transplantation (APBSCT); in the second case positive for anti-HBc and anti-HBs, elevation of HBV-DNA was recognized by serial HBV-DNA monitoring performed prospectively following APBSCT. Interestingly, these two cases had the reduction of the titer of anti-HBs during the treatment, followed by HBV reactivation. These clinical data suggest that the HBV-DNA monitoring is necessary for not only HBsAg-positive but also HBsAg-negative myeloma patients with anti-HBc-positive and/or anti-HBs-positive following transplantation and after conventional chemotherapy in the salvage setting. Establishment of a standard strategy to prevent HBV reactivation is important for myeloma patients receiving systemic chemotherapy.

Keywords Reactivation · HBV · Myeloma · Transplantation

Abbreviations

HBV	Hepatitis B virus
HBsAg	Hepatitis B surface antigen
Anti-HBc	Hepatitis B core antibody
Anti-HBs	Hepatitis B surface antibody
AST	Aspartate transaminase
ALT	Alanine aminotransferase
RTD-PCR	Real-time detection polymerase chain reaction
APBSCT	Autologous peripheral blood stem cell transplantation
VAD	Vincristine, doxorubicin, dexamethasone
MP	Melphalan, prednisolone
MCP	Ranimustine, cyclophosphamide, prednisolone
MMCP	Melphalan, ranimustine, cyclophosphamide, prednisolone
BD	Bortezomib, dexamethasone
TD	Thalidomide, dexamethasone
CHOP	Cyclophosphamide, doxorubicin, vincristine, prednisolone
R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone

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1 Introduction

Most cases of hepatitis B virus (HBV) reactivation have been reported in hepatitis B surface antigen (HBsAg)-positive cancer patients receiving systemic chemotherapy [1]. It was recently reported, however, that HBV reactivation also occurred in HBsAg-negative lymphoma patients who received rituximab plus steroid combination chemotherapy [2–5].

The clinical data on HBV reactivation in myeloma patients have not been reported extensively; therefore, we have performed retrospective and prospective analyses of HBV reactivation in 101 myeloma patients who received systemic chemotherapy at Nagoya City University Hospital. Based on these analyses, we report here two cases of HBV reactivation in HBsAg-negative myeloma patients.

2 Patients and methods

Between January 2001 and July 2009, 101 patients were diagnosed as multiple myeloma at Nagoya City University Hospital. We retrospectively analyzed the medical records of 40 patients for the development of hepatitis B who were diagnosed as multiple myeloma between January 2001 and December 2005. In 2006, we instituted the strategy described below to prevent HBV reactivation, and carried it out prospectively in 61 patients between January 2006 and July 2009. The serological markers for HBsAg, hepatitis B core antibody (anti-HBc) and hepatitis B surface antibody (anti-HBs) were tested to establish HBV infection status before the initial chemotherapy. HBsAg and anti-HBs were determined by enzyme immunoassay (EIA) (AxSYM; Abbott Japan, Tokyo, Japan) or chemiluminescence enzyme immunoassay (CLEIA) (Fujirebio, Tokyo, Japan). Anti-HBc of IgG classes was determined by radioimmunoassay (Abbot Japan) or CLEIA (Fujirebio). If the patient was positive for any of the serological markers, plasma HBV-DNA was measured by real-time detection polymerase chain reaction (RTD-PCR). If the patient was HBsAg-positive and/or had HBV-DNA before chemotherapy, prophylactic therapy with an antiviral drug was administered during and for at least 6 months after the chemotherapy. On the other hand, if the patient was HBsAg-negative, but seropositive for anti-HBc and/or anti-HBs (defined as resolved HBV infection), a serial monitoring of HBV-DNA was performed monthly by RTD-PCR during and for at least 1 year after the chemotherapy. If plasma HBV-DNA levels became detectable, antiviral therapy was started as soon as possible.

In this prospective HBV-DNA monitoring, each case of plasma HBV-DNA was measured at SRL Inc, using methods with the highest sensitivity available at the time in clinical practice; the assays included the following: TaqMan PCR assay (Roche Molecular Systems Inc, between April 2008 and July 2009), or Amplicor-PCR assay (Roche Molecular Systems Inc, between January 2006 and March 2008). The cutoff values of the TaqMan PCR assay and Amplicor-PCR assay were set at 1.8 log copies and 2.6 log copies/mL, respectively. In this retrospective analysis, serum HBV-DNA was measured at our laboratory of Nagoya City University using preserved specimen, and HBV-DNA sequences spanning the S gene were amplified by RTD-PCR in accordance with the previously described protocol with a slight modification; it has a detection limit of 2.0 log copies/mL [6].

The two patients with HBV reactivation provided written informed consent to the publication of this report.

3 Treatment for multiple myeloma

In patients younger than 65 years, autologous peripheral blood stem cell transplantation (APBSCT) was performed using high-dose melphalan (200 mg/m^2) following three courses of a VAD (vincristine, doxorubicin, and dexamethasone) regimen as induction therapy and a high-dose cyclophosphamide regimen as stem cell harvest therapy.

In patients who did not choose the transplantation treatment option, or from whom we could not collect enough hematopoietic stem cells, the initial treatment for symptomatic multiple myeloma was MP (melphalan plus prednisolone) or MMCP (melphalan, ranimustine, cyclophosphamide, prednisolone combination chemotherapy).

Patients over 65 years of age were not candidates for transplantation. The initial treatment regimens for these patients were MP, VAD, MMCP or VAD following MP. In relapsed and refractory patients, BD (bortezomib, dexamethasone: after December 2006), or TD (thalidomide, dexamethasone: after December 2008) or other regimens (MP, VAD, etc.) were administered as salvage treatments for all patients.

4 Results

4.1 A case with HBV reactivation on the basis of the medical records

Based on the retrospective analyses, only one patient developed HBV reactivation among 40 multiple myeloma patients diagnosed from January 2001 to December 2005. The clinical course is shown in Fig. 1. A 59-year-old woman diagnosed as symptomatic multiple myeloma (BJP-κ type) received APBSCT as initial treatment. Before APBSCT, she was seronegative for HBsAg, but no

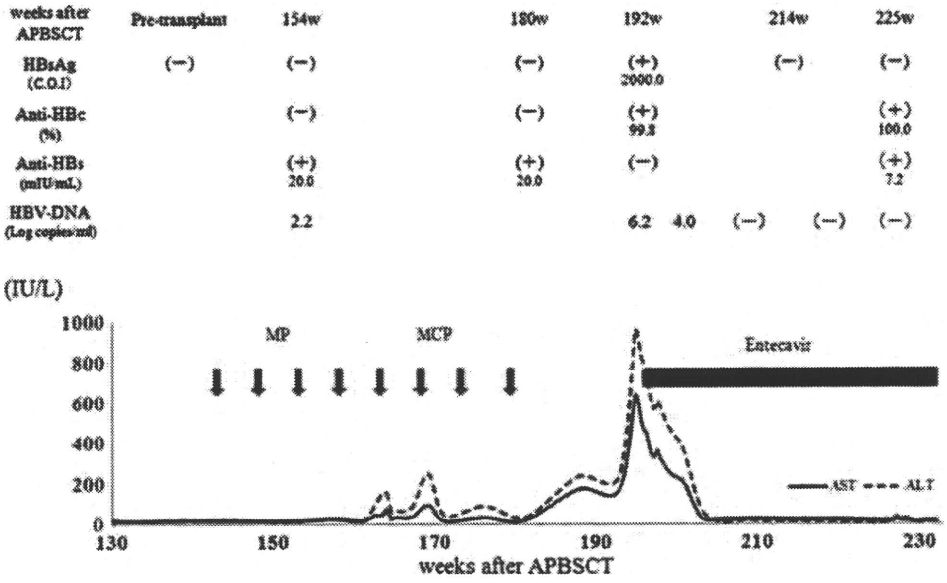

Fig. 1 Clinical course of Case 1. *AST* aspartate transaminase, *ALT* alanine aminotransferase

screening tests for anti-HBc or anti-HBs were performed. Multiple myeloma recurred about 3 years after APBSCT, and MP was administered as salvage treatment. When MP therapy was started, she was seronegative for both HBsAg and anti-HBc, but seropositive for anti-HBs. Because MP could not control the disease, MCP (ranimustine, cyclophosphamide, prednisolone) therapy was administered as the next salvage regimen. Liver damage occurred 32 weeks after the initial salvage chemotherapy was started, and at that time HBsAg changed from negative to positive, and serum HBV-DNA was detectable at 6.2 log copies/mL, so we concluded that the liver damage was caused by hepatitis B virus.

Analyses of specimens preserved during and after salvage therapy showed that serum HBV-DNA was detectable at 2.2 log copies/mL at base line when MP therapy was started, as shown in Fig. 1. In other words, the patient had an occult HBV infection (defined as HBsAg-negative, but HBV-DNA detectable) before salvage chemotherapy.

Furthermore, the HBV gene sequences before and after salvage chemotherapy were confirmed identical in Case 1, so we judged that the liver damage was caused by HBV reactivation. HBV reactivation was reduced after entecavir (0.5 mg, once daily) was administered as an anti-HBV nucleotide analog, and HBV-DNA levels decreased to below the limit of detection.

4.2 HBV-DNA monitoring to prevent HBV reactivation (Fig. 2)

Among 61 patients with symptomatic multiple myeloma diagnosed between January 2006 and July 2009, 1 patient was seropositive for HBsAg, 15 patients were seropositive for anti-HBc and/or anti-HBs (indicating resolved HBV infection), and the remaining 45 patients were seronegative for either anti-HBc or anti-HBs according to the screening tests shown in Fig. 2. One HBsAg-positive patient was given an antiviral drug for prophylaxis before initial treatment. On the other hand, we prospectively performed serial HBV-DNA monitoring during and after myeloma treatment in the 15 patients with resolved HBV infection who had no occult infection.

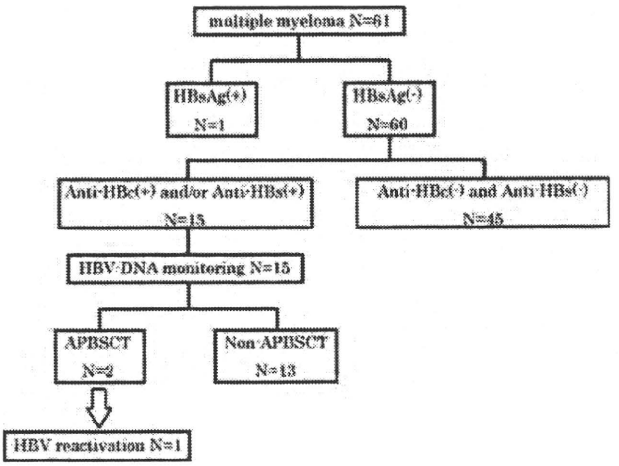

Fig. 2 Screening tests for serological markers of HBV infection and the strategy to prevent HBV reactivation in 61 myeloma patients diagnosed between January 2006 and July 2009 at Nagoya City University Hospital. One patient was seropositive for HBsAg, 15 patients were seropositive for anti-HBc and/or anti-HBs (defined as resolved HBV infection), the remaining 45 patients were seronegative for either anti-HBc or anti-HBs. We prospectively performed serial HBV-DNA monitoring during and after myeloma treatment in 15 patients with resolved HBV infection: following APBSCT, 1 of 15 patients developed HBV reactivation without hepatitis and prior to liver damage because elevation of HBV-DNA was confirmed at an early stage

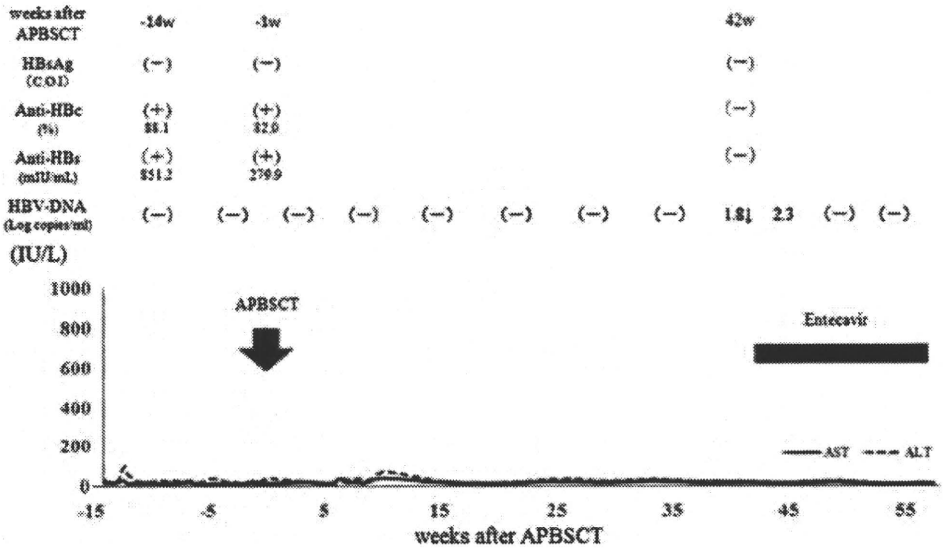

Fig. 3 Clinical course of Case 2

In 8 of these 15 patients, the initial treatment was MP. Following VAD therapy, three patients received MP. Two patients underwent APBSCT. Each of the remaining two patients received MMCP therapy but no further treatment. For salvage treatment, 4 of the 15 patients received BD and/or TD.

One of the 15 patients developed HBV reactivation without hepatitis following APBSCT, after elevation of HBV-DNA was confirmed at an early stage prior to liver damage (Fig. 3). A 61-year-old woman was diagnosed as symptomatic multiple myeloma (BJP-λ type). In screening tests before treatment, HBsAg was negative, both anti-HBc and anti-HBs were positive, and plasma HBV-DNA was below the limit of detection. Therefore, the patient's HBV status was confirmed as a resolved infection. Prospective serial HBV-DNA monitoring was performed monthly, but the testing was sometimes postponed up to 3 months on account of the patient.

Forty-two weeks (about 10 months) after APBSCT, the plasma HBV-DNA level was less than 1.8 log copies/mL but an amplification signal was detectable by the TaqMan PCR assay, and during the following month the HBV-DNA level became detectable with up to 2.3 log copies/mL, as shown in Fig. 2. HBV reactivation was diagnosed at that time, and entecavir (0.5 mg, once daily) was administered immediately as an anti-HBV nucleotide analogue. The plasma HBV-DNA decreased to an undetectable level without liver damage. At the time of HBV reactivation, all HBV serological markers (HBsAg, anti-HBc and anti-HBs) were negative, which suggested that the antibody titers may be reduced by the myeloma treatment. We performed a retrospective search of blood transfusions (red cells and platelets) received by this patient during the previous chemotherapy using the stored specimens from all the blood donors. As a result, it was concluded that the possibility of HBV infection through blood transfusion was extremely low.

5 Discussion

We reported two cases of HBV reactivation in myeloma patients who were seronegative for HBsAg before treatment. HBV was reactivated in a patient with occult infection and definitely diagnosed by a retrospective analysis of preserved specimens when the onset of liver damage occurred after salvage treatment 3 years after APBSCT. In another patient with resolved HBV infection, HBV reactivation at an early stage was detected by the serial HBV-DNA monitoring performed prospectively, and an antiviral drug was administered before liver damage had occurred.

Some HBsAg-negative patients have recently been reported to develop fatal hepatitis by HBV reactivation in the rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone (R-CHOP) or R-CHOP-like regimens, which combine rituximab and steroid for treatment of CD20-positive malignant lymphoma [2–5]. In 2006, Hui et al. [3] reported that 8 of 244 HBsAg-negative lymphoma patients receiving systemic chemotherapy developed hepatitis by HBV reactivation, and these eight patients were seropositive for either anti-HBc or anti-HBs. It was shown that rituximab plus steroid combination chemotherapy was a risk factor by multivariate analysis. Most recently, Yeo et al. [4] reported that 5 of 80 HBsAg-negative patients diagnosed as diffuse large B cell lymphoma and receiving R-CHOP or CHOP-like regimens had reactivated HBV. All five had received R-CHOP and all were positive for anti-HBc and negative for anti-HBs.

The HBV reactivation following APBSCT in patients with multiple myeloma has been reported sporadically.

Endo et al. [7] reported that 3 of 24 HBsAg-negative patients with resolved HBV infection developed new-onset hepatitis B following APBSCT, and all three patients were multiple myeloma patients. Uhm et al. [8] performed a retrospective analysis of the change of HBV serologic markers following APBSCT. Seven of 129 HBsAg-negative patients became HBsAg-positive after transplantation. All seven patients were seropositive for both anti-HBs and anti-HBc before treatment, and six of the seven patients had multiple myeloma. Furthermore, at reactivation after transplantation, it was shown that the titers of anti-HBs decreased in six of the seven patients. This phenomenon was also shown in our both cases. These data suggested that the decreased titer of anti-HBs may be associated with HBV reactivation, and that the pathophysiology of reactivation may be affected by the dysfunction of humoral immunity in multiple myeloma.

It is necessary to pay attention to the onset of HBV reactivation during salvage treatment; thus, if immunologic inhibition is strong over a longer period, the risk of HBV reactivation may be increased more in patients on second-line or third-line chemotherapy than in those undergoing chemotherapy for the first time [9]. As mentioned above, APBSCT may be one of the important risk factors for HBV reactivation in myeloma patients; however, the reactivation may occur even if the salvage treatment was performed with a mild myelosuppressive regimen such as MP and MCP after the autologous transplantation shown in Case 1. New molecular target drugs such as bortezomib, thalidomide, and lenalidomide improve the survival of myeloma patients remarkably [10–12], so the number of patients who will receive the immunosuppressive therapy for longer periods may increase in the future. Therefore, a standard strategy to prevent HBV reactivation may also become more important in myeloma treatment.

HBV reactivation may lead to fatal fulminant hepatitis, so we hematologists and oncologists should identify high-risk groups in advance before chemotherapy. The latest CDC and Japanese guidelines recommend that patients receiving cytotoxic or immunosuppressive therapy should be tested for serologic markers of HBV infection (i.e., HBsAg, anti-HBc, anti-HBs) [13, 14]. HBV infection status should be established before any chemotherapy or immunosuppressive therapy is initiated (when there is no immunologic inhibition), because antibody titers may be reduced by the treatment, as shown in Case 2. For patients positive for any of the HBV serological markers, the presence of HBV-DNA should be confirmed by RTD-PCR [5, 14].

Prophylaxis with antiviral drugs is essential for HBsAg-positive patients undergoing systemic chemotherapy as recommended by the latest American and Japanese guidelines [14, 15]. Because patients with serum HBV-DNA have more potential risk factors for HBV reactivation, they should be given antiviral drugs as well [5, 14].

If a patient is seropositive for anti-HBc and/or anti-HBs, no standard strategy to prevent HBV reactivation has been established, but making an early diagnosis of HBV reactivation is critical to enable early initiation of active antiviral therapy. Preemptive therapy by serial HBV-DNA monitoring is a reasonable strategy recommended by the latest Japanese guidelines [14]. If HBV-DNA levels become detectable, antiviral therapy should be started as soon as possible.

Only a few studies have reported on the optimal frequency and duration of HBV-DNA monitoring. Hui et al. [3] reported on malignant lymphoma patients that the median time from the elevation of serum HBV-DNA to hepatitis onset was 18.5 weeks (range 12–28 weeks). Most recently, Fukushima et al. [16] conducted a prospective study to monitor HBsAg monthly and HBV-DNA every 3 months during and after systemic chemotherapy in HBsAg-negative but anti-HBc-positive patients with malignant lymphoma; they found that 1 of 24 patients developed HBV reactivation, which was diagnosed by elevation of HBV-DNA level, while their HBsAg was still negative. In fact, as shown in Case 2, we were able to diagnose HBV reactivation at an early stage by the monthly HBV-DNA monitoring and avoid liver damage and decrease plasma HBV-DNA to below the limit of detection by starting the antiviral drug administration.

It is also necessary to make a differential diagnosis in order to distinguish transmission of HBV by blood transfusion from HBV reactivation, because blood transfusion may be received during systemic chemotherapy. In Case 2, we performed a retrospective search of blood transfusion (red cells and platelets) received during previous chemotherapy, using the stored specimens of all blood donors. It was concluded that the possibility of HBV infection through blood transfusion was extremely low.

In conclusion, these clinical data suggest that the HBV-DNA monitoring is necessary for not only HBsAg-positive but also HBsAg-negative myeloma patients with anti-HBc-positive and/or anti-HBs-positive following transplantation and after conventional chemotherapy in the salvage setting. Preemptive therapy by serial HBV-DNA monitoring may be a useful and cost-effective option for preventing HBV reactivation in patients with resolved HBV infection. Establishment of a standard strategy to prevent HBV reactivation is important for myeloma patients receiving systemic chemotherapy.

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モニタリングによるB型肝炎再活性化の予防

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今月のテーマ●B型肝炎ウイルス再活性化の問題点とその対策

モニタリングによるB型肝炎再活性化の予防

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要旨：がん化学療法・免疫抑制療法にともなうB型肝炎対策ガイドラインが厚生労働省研究班によって2009年1月に発表された．スクリーニング検査におけるHBs抗原に加えて，HBc抗体およびHBs抗体の測定は必要不可欠である．いずれか陽性の場合にはHBV-DNA定量検査を行うことによってB型肝炎再活性化リスクを判断する．HBs抗原陽性例およびHBs抗原陰性ハイリスク群の臨床経過の違いを理解し，抗ウイルス薬予防投与（prophylaxis）およびHBV-DNAモニタリングによる早期発見・早期治療（preemptive therapy）の対策法を行うことが重要である．

索引用語：HBV，再活性化，HBV-DNAモニタリング，preemptive therapy

はじめに

B型肝炎ウイルス（HBV）の再活性化は，がん化学療法・免疫抑制療法を行う患者において問題となり，一部の症例においては劇症肝炎に至り，致死的な経過をたどることが報告されている．従来，HBs抗原陽性例において多数報告されてきたが，最近，リツキシマブをはじめとする新規分子標的治療薬の導入によってHBs抗原陰性例からの再活性化が報告されるようになってきた[1~3]．

2009年1月，厚生労働省研究班による免疫抑制・化学療法にともなうB型肝炎対策ガイドラインが発表された[3]．HBs抗原陽性例に対する免疫抑制・化学療法時には抗ウイルス薬の予防投与を行うことが原則とされた．一方，HBs抗原陰性ハイリスク群（HBc抗体陽性and/or HBs抗体陽性）に対しては，HBV-DNAモニタリング（月1回，化学療法中および化学療法後少なくとも1年間）を行い，肝炎に先行するHBV-DNAの上昇をとらえ，陽性化した時点で抗ウイルス薬の投与を開始する対策が明記された．

本稿では，HBs抗原陰性例を中心に，B型肝炎再活性化の臨床経過，リスク因子および予後について現時点でのエビデンスをまとめ，HBV-DNAモニタリングによる対策のポイントを論じたい．

Ⅰ B型肝炎の自然経過とB型肝炎再活性化の臨床経過

HBVに感染すると，成人例の大半が急性肝炎の経過をたどり，HBs抗原は数週間で消失し，HBV-DNAも検出感度以下となる．なんらかの介入がない限り，通常この状態は維持され，HBV-DNAの増幅は認めないため，“既往感染”または“治癒”したと判断される．

しかしながら，HBVはHBs抗体（中和抗体）の出現後においても，肝臓や末梢血単核球内に微

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Preemptive therapy for HBV reactivation by serial HBV-DNA monitoring

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