23. Tanaka Y, Hasegawa I, Kato T, et al. A case–control study for differences among hepatitis B virus infections of genotypes A (subtypes Aa and Ae) and D. *Hepatology* 2004;40:747–55.
24. Sugauchi F, Mizokami M, Orito E, et al. A novel variant genotype C of hepatitis B virus identified in isolates from Australian Aborigines: complete genome sequence and phylogenetic relatedness. *J Gen Virol* 2001;82:883–92.
25. Shin IT, Tanaka Y, Tateno Y, et al. Development and public release of a comprehensive hepatitis virus database. *Hepatol Res* 2008;38:234–43.
26. Hannachi N, Fredj NB, Bahri O, et al. Molecular analysis of HBV genotypes and subgenotypes in the Central-East region of Tunisia. *Virol J* 2010;7: 302.
27. Sunbul M, Sugiyama M, Kurbanov F, et al. Specific mutations of basal core promoter are associated with chronic liver disease in hepatitis B virus subgenotype D1 prevalent in Turkey. *Microbiol Immunol* 2013;57:122–9.
28. Elkady A, Tanaka Y, Kurbanov F, et al. Virological and clinical implication of core promoter C1752/V1753 and T1764/G1766 mutations in hepatitis B virus genotype D infection in Mongolia. *J Gastroenterol Hepatol* 2008;23:474–81.
29. Sugiyama M, Tanaka Y, Sugauchi F, et al. Hepatitis virus genotype D1 specific mutations affecting viral replication and clinical course of hepatitis B patients. HBV Now in Asia: Single Topic Conference. 2009. 85.
30. Yu MW, Yeh SH, Chen PJ, et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005;97:265–72.
31. Guo X, Jin Y, Qian G, et al. Sequential accumulation of the mutations in core promoter of hepatitis B virus is associated with the development of hepatocellular carcinoma in Qidong, China. *J Hepatol* 2008;49: 718–25.
32. Tacke F, Gehrke C, Luedde T, et al. Basal core promoter and precore mutations in the hepatitis B virus genome enhance replication efficacy of Lamivudine-resistant mutants. *J Virol* 2004;78: 8524–35.
33. Liang TJ, Hasegawa K, Rimon N, et al. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *N Engl J Med* 1991;324: 1705–9.
34. Hasegawa K, Huang JK, Wands JR, et al. Association of hepatitis B viral precore mutations with fulminant hepatitis B in Japan. *Virology* 1991;185: 460–3.
35. Kao JH, Chen PJ, Lai MY, et al. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. *Gastroenterology* 2003;124: 327–34.
36. Fang ZL, Yang J, Ge X, et al. Core promoter mutations (A(1762)T and G(1764)A) and viral genotype in chronic hepatitis B and hepatocellular carcinoma in Guangxi, China. *J Med Virol* 2002;68:33–40.
37. Chu CJ, Keeffe EB, Han SH, et al. Prevalence of HBV precore/core promoter variants in the United States. *Hepatology* 2003;38: 619–28.
38. Fang ZL, Sabin CA, Dong BQ, et al. The association of HBV core promoter double mutations (A1762T and G1764A) with viral load differs between HBeAg positive and anti-HBe positive individuals: a longitudinal analysis. *J Hepatol* 2009;50:273–80.
39. Livingston SE, Simonetti JP, Bulkow LR, et al. Clearance of hepatitis B e antigen in patients with chronic hepatitis B and genotypes A, B, C, D, and F. *Gastroenterology* 2007;133:1452–7.

Journal of Medical Virology 85:1900–1906 (2013)

Reactivation of Hepatitis B Virus in Patients With Undetectable HBsAg Undergoing Chemotherapy for Malignant Lymphoma or Multiple Myeloma

Takeshi Matsui,[1,2]* Jong-Hon Kang,[2] Masanori Nojima,[3] Akiko Tomonari,[2] Hironori Aoki,[2] Hajime Yamazaki,[2] Kei Yane,[2] Kunihiko Tsuji,[2] Seisho Andoh,[4] Sachiko Andoh,[4] Hajime Sakai,[4] Masayo Maemori,[4] Hiroyuki Maguchi,[2] and Yasuhito Tanaka[1]

[1]*Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan*
[2]*Center for Gastroenterology, Teine-Keijinkai Hospital, Sapporo, Japan*
[3]*Department of Public Health, Sapporo Medical University School of Medicine, Sapporo, Japan*
[4]*Department of Hematology, Teine-Keijinkai Hospital, Sapporo, Japan*

Despite increasing reports of hepatitis B virus (HBV) reactivation in hematological malignancies, its incidence, and risk factors are still obscure. The aim of this study was to clarify the frequency and risk factors of HBV reactivation in hepatitis B surface antigen (HBsAg) undetectable patients with malignant lymphoma or multiple myeloma, during or after chemotherapy. A total of 109 patients with undetectable HBsAg undergoing chemotherapy for malignant lymphoma or multiple myeloma were enrolled in this study. Anti-hepatitis B surface (anti-HBs) and anti-hepatitis B core (anti-HBc) were checked before treatment, and HBV DNA in sera was quantified monthly during and after chemotherapy. Out of 109 patients, 42 (38.5%) had anti-HBs and 59 (54.1%) had anti-HBc. Among the 59 anti-HBc positive patients, four patients (4/59, 6.8%) showed HBV reactivation during 20.5 median follow-up months. In all four patients with HBV reactivation, peripheral lymphocyte counts before chemotherapy were lower than those without HBV reactivation ($P = 0.033$). HBV reactivation occurred during and after chemotherapy containing rituximab for non-Hodgkin lymphoma. Four patients, who had HBV reactivation, did not develop de novo hepatitis due to HBV reactivation and were able to undergo chemotherapy against malignant lymphoma as scheduled. Monitoring of HBV DNA in sera is useful for the early diagnosis of HBV reactivation, and preemptive therapy is an useful alternative to prevent hepatitis due to HBV reactivation. Patients must be monitored periodically for HBV-DNA levels during and after chemotherapy. ***J. Med. Virol. 85:1900–1906, 2013.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: reactivation; hepatitis B virus; chemotherapy; lymphocyte

INTRODUCTION

Reactivation of the hepatitis B virus (HBV) is a well-recognized complication following systemic chemotherapy for hematological malignancies [Francisci et al., 2010; Yagci et al., 2010; Sugauchi et al., 2011]. HBV infection has a wide clinical spectrum. Therefore different serologic markers or combinations of markers are used to identify different phases of HBV infection and to determine whether a patient has acute or chronic HBV infection, is immune to HBV as a result of prior infection or vaccination, or is susceptible. During acute or chronic hepatitis B infection, hepatitis B surface antigen (HBsAg) can be detected in high levels in serum. The presence of hepatitis B surface antibody (anti-HBs) is generally interpreted as an indication of recovery and immunity from HBV infection. Anti-HBs also develops in a person who has been successfully vaccinated against hepatitis B. Total hepatitis B core antibody (anti-HBc) appears at the onset of symptoms in acute hepatitis B and persists for life. The presence of anti-HBc indicates previous or ongoing infection with HBV in an undefined time frame. Therefore, in the past, anti-HBc and/or anti-HBs positive patients

*Correspondence to: Takeshi Matsui, Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan. E-mail: mtake0402@gmail.com

Accepted 6 June 2013

DOI 10.1002/jmv.23694
Published online 07 August 2013 in Wiley Online Library (wileyonlinelibrary.com).

without HBsAg was thought to be eradicated of HBV in the host after resolution of HBV infection. However, recently it has become known that a low level of virus replication continues in hepatocytes even after the end of the acute HBV infection [Yotsuyanagi et al., 1998]. The covalently closed circular DNA (cccDNA) persist for many years in the liver of patients, HBsAg and HBV DNA in the blood has fallen to an undetectable level. Immunosuppressive drugs interfere with the cellular and humoral arm of the immune system controlling hepatocellular HBV infection. In healthy patients, host immunity is able to control HBV in most of the cases. This changes in an immune impaired patient, whether it is due to immunosuppressive drugs or monoclonal antibodies like rituximab. The humoral arm is profoundly affected by rituximab which depletes the body's antibody producing B cells. Other immunosuppressive drugs also impair the innate immune response. Therefore, reactivation of HBV following systemic chemotherapy can develop not only in HBsAg positive patients with a sustained HBV infection, but also in HBsAg undetectable patients with a past history of HBV infection [Wu et al., 2009; Cheung et al., 2010; Matsue et al., 2010; Wursthorn et al., 2010]. Rituximab and corticosteroids are especially well known to cause reactivation of HBV in patients without HBsAg in sera [Kusumoto et al., 2011]. Rituximab is a human monoclonal antibody derived from chimeric mice that inhibits an immune response by attacking CD20 positive B cells [Hiddemann et al., 2005]. Corticosteroids block cytokine synthesis and act as immune-suppressing drugs [Auphan et al., 1995] and, in addition, stimulate HBV DNA, mRNA, and protein production in a stable expression system [Tur-Kaspa and Laub, 1990]. These agents are used for the treatment of malignant lymphoma or other hematological malignancies. Multiple myeloma is recognized as a disease with a risk of HBV reactivation, due to its treatment regime with a high dose of corticosteroids [Yoshida et al., 2010]. To date, there has been increasing reports of HBV reactivation in patients treated with chemo/immunosuppressive therapy including the agents noted above [Hui et al., 2006; Matsubara et al., 2009; Shinkai et al., 2010]. However, the incidence or risk factors of HBV reactivation remain unclear because only a few prospective cohorts have presented for this new clinical entity. This study aimed to assess the incidence and risk factors of HBV reactivation, and analyzing the clinical course of HBV reactivation that occurred in the patients with malignant lymphoma or multiple myeloma during and after treatment.

PATIENTS AND METHODS

Study Patients

Consecutive patients with undetectable HBsAg who received chemotherapy for malignant lymphoma or multiple myeloma from January 2007 to October 2010 were included in this study. After admission, all patients underwent a physical examination and blood chemistry analysis. The study patients consisted of 109 patients (60 male, 55%; 49 female, 45%). The median age was 68-years-old, with a range of 22–91 years. Ninety-six patients (88.1%) had malignant lymphoma and 13 (11.9%) had multiple myeloma. Diagnosis of subtypes in malignant lymphoma included Diffuse large B-cell lymphoma (n = 54, 56.3%), Follicular lymphoma (n = 22, 22.9%), Marginal zone B-cell lymphoma (n = 7, 7.3%), Mantle cell lymphoma (n = 2, 2.1%), Burkitt lymphoma (n = 2, 2.1%), Intravascular large B-cell lymphoma (n = 1, 1.0%), Lymphoplasmacytoid lymphoma (n = 1, 1.0%), Peripheral T-Cell lymphoma (n = 1, 1.0%), Angioimmunoblastic T-Cell Lymphoma (n = 1,1.0%), and Hodgkin lymphoma (n = 5, 5.2%).

Determination of HBV Serological Markers and HBV DNA Quantification

On admission, all patients were screened for HBsAg in sera using a commercially available kit (Architect, Abbott Japan, Tokyo, Japan). Patients with undetectable HBsAg were enrolled in this study. Before treatment of hematological malignancies, patients were tested for anti-HBc, anti-HBs and blood parameters, and were then followed up by monthly monitoring of HBV DNA loads in sera or plasma and blood parameters. All serial sera were stored at −40° C. HBV DNA levels were quantified using Amplicor (range from below 2.6 to 7.6 log copies/ml; Roche Diagnostics, Tokyo, Japan) up to December 2007 and real-time TaqMan PCR (range from below 1.8 to 8.8 log copies/ml; Roche Diagnostics) since then. HBsAg with a highly sensitive chemiluminescent enzyme immunoassay (CLEIA) [Shinkai et al., 2010] was checked in stored sera sampled from patients with HBV reactivation retrospectively.

Definition of HBV Related Hepatitis and HBV Reactivation

Hepatitis was defined as a serum level of alanine aminotransferase (ALT) threefold higher than the normal upper limit of two consecutive determinations, 5 days apart, in the absence of the clinical and laboratory features of acute hepatitis A, hepatitis C, hepatitis E, or other systematic infections [Matsue et al., 2010]. The definition of HBV reactivation was the detection of HBV DNA in sera, including when the DNA load was not quantifiable but a PCR signal was detectable.

Statistical Analysis

In order to assess the risk factors of HBV reactivation, Fisher's exact test was applied for categorical variables, and Mann–Whitney's *U*-test was used for numerical variables. Receiver operating characteristic (ROC) curve was constructed to evaluate the

TABLE I. Clinical Characteristics of HBsAg Undetectable Patients Undergoing Chemotherapy for ML or Multiple Myeloma

Sex (M/F)	60/49
Age of years, median (range)	67.9 (22–91)
Follow-up period, median (range)	20.5 months (1.0–58.5)
Anti-HBc positive	59
Anti-HBs positive	42
Diagnosis	
Multiple myeloma	13
Diffuse large B cell lymphoma	54
Follicular lymphoma	22
Marginal zone B cell lymphoma	7
Burkitt lymphoma	2
T-cell lymphoma	2
Hodgkin lymphoma	5
Others	4
No. of rituximab administration	81
No. of glucocorticoids administration	108

diagnostic ability of HBV reactivation using a measured variable. A *P*-value less than 0.05 was considered significant. The best cutoff was defined as the point on the ROC curve closest to the upper left corner. All statistical analyses were performed using SPSS18 (IBM).

RESULTS

Patient Characteristics

The background characteristics of patients are shown in Table I. Out of 109 patients, 59 (54.1%) had anti-HBc, 42 (38.5%) had anti-HBs, and 47 (43.1%) had neither. Thirty-nine (35.7%) had both anti-HBc and anti-HBs. The number of patients with multiple myeloma were 13, and 96 patients had malignant lymphoma. Of all patients with malignant lymphoma, Hodgkin lymphoma was diagnosed in 5 patients, and non-Hodgkin lymphoma was confirmed in 91 patients. Diffuse large B-cell lymphoma was the dominant subtype of lymphoma. Rituximab was administered in 81 patients and glucocorticoids were used in 108 patients. None were treated by autologous peripheral blood stem cell transplantation or allogenic stem cell transplantation.

Consequences of HBV Serology

Among the 109 patients with undetectable HBsAg at the follow-up period of 20.5 median months (1.0–58.5), 4 (3.7%) showed the emergence of HBV DNA in sera, and were therefore diagnosed as HBV reactivation. They had never received a blood transfusion. The background characteristics and clinical features in patients with HBV reactivation are shown in Table II. None of the 50 patients without anti-HBc revealed HBV reactivation. In contrast, out of the 59 anti-HBc positive patients, 4 (6.8%) became positive for HBV DNA in sera. Among 20 anti-HBc positive and anti-HBs negative patients, 3 (15.0%) patients developed HBV reactivation, and only 1 of the 39 (2.6%) positive for both had an emergence of HBV DNA in sera. Sufficient anti-HBs antibodies in sera among HBV-resolved patients might reduce the incidence of HBV reactivation.

All four patients who developed HBV reactivation had lymphoma and were treated with rituxmab and glucocorticoids containing chemotherapy (Table II).

TABLE II. Clinical Characteristics of HBV Reactivation Patients

	Case 1	Case 2	Case 3	Case 4
Age/sex	75/F	70/M	66/M	83/F
Diagnosis	DLBCL	DLBCL	FL	BL
Stage[a]	IIIB	IIIB	IIA	IVB
Anti-HBc/HBs	+/−	+/−	+/+	+/−
Treatment	R-CHOP like	R-CHOP like	R-CHOP	R-MTX/CPM/VCR/ ADM/DEXA/ETP
Period from initiation of treatment (days)	42	46	398	148
Period from last rituximab (days)	3	26	159	23
Frequency of rituximab administration	5	1	12	8
During or after treatment	During treatment	During treatment	After treatment	During treatment
HBV DNA on the reactivation point (log copy/ml)	3.6	<1.8+[b]	3.6	<1.8+[b]
Peak HBV DNA (log copy/ml)	7.6	2.7	3.6	<1.8+[b]
HBV genotype	C	Ba	Not detected	Not detected
HBV pre-core	Wild	Wild	Not detected	Not detected
HBV core promoter	Wild	Mutant	Not detected	Not detected
Antiviral treatment	+	+	−	−
Outcome	Alive	Alive	Alive	Alive

DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; BL, Burkitt lymphoma; R-CHOP, rituximab, pirarubicin, cyclophosphamide, vincristine, and prednisone; R-CHOP, rituximab, pirarubicin, cyclophosphamide, vincristine, and prednisone; R-MTX/CPM/VCR/ADM/DEXA/ETP, rituximab, methotrexate, cyclophosphamide, vincristine, adriamycin, dexamethasone, and etoposide; ALT, alanine aminotransferase.
[a]Ann Arbor staging.
[b]The DNA load was not quantifiable, but a PCR signal was detectable.

Reactivation of HBV occurred during the course of chemotherapy in three cases, and after maintenance therapy with rituximab in only one case (Fig. 1). Although there is no apparent correlation between HBV reactivation and complete blood count data prior to chemotherapy (data not shown), baseline counts of peripheral lymphocytes were associated with an incidence of HBV reactivation ($P = 0.033$). Nadir levels in peripheral lymphocytes for all subjects during treatment and baseline levels of immunoglobulin G were also assessed. However, none of the parameters were confirmed to be associated with the incidence of HBV reactivation, except for peripheral lymphocytes (Table III). The ROC analysis for the prediction of reactivation using lymphoid counts before treatment showed the area under the curve (AUC) to be 0.814, with the best cut-off to be 860/μl. In four cases with HBV reactivation, based on high sensitive HBsAg assay, HBsAg was examined from stored sera at the time of HBV reactivation, but none was detectable. The detailed clinical course are; (1) Case 1, initially negative for both HBsAg and anti-HBs, became positive for HBV DNA 42 days after the initiation of treatment. She had received chemotherapy using multiple agents such as rituximab, pirarubicin, cyclophosphamide, vincristine, and prednisone (R-CHOP like regimen). After an elevation in quantified HBV DNA in sera, the patient was treated with entecavir, at 1mg per day. HBV DNA was immediately undetectable without hepatitis. (2) Case 2 had detectable HBV-DNA 46 days after the initiation of an R-CHOP like regimen. One month after the transient emergence of HBV DNA in serum (signal positive, but not quantified), the HBV DNA became naturally undetectable. However, 6 weeks later, HBV DNA became detectable again, and after the confirmation of a sustained increase in the HBV DNA load, entecavir was continuously given. Thereafter, he showed a decrease in the HBV DNA load below the lower limit for detection without hepatitis, and ALT level became within normal range. (3) Case 3 had maintenance therapy with rituximab after the CHOP regimen. He showed an increase in the HBV DNA load at over 3 log copies/ml just once, 159 days after maintenance therapy with rituximab, but HBV DNA became undetectable again naturally. Although he had an anti-HBs titer of 601.2 mIU/ml before chemotherapy, the titer decreased to 500.8 mIU/ml at the showing of HBV reactivation. (4) Case 4 was not positive for quantified HBV DNA, but had a transient replication signal of HBV DNA in serum at day 148 by real time PCR. These two cases did not present with continuous viremia of HBV, and, as such, antiviral drugs were not administered. Although four cases showed HBV reactivation, they did not develop de novo hepatitis due to HBV reactivation and were able to undergo chemotherapy against malignant lymphoma as scheduled.

DISCUSSION

HBV reactivation is known as a significant complication of chemotherapy for hemodyscrasia [Francisci et al., 2010; Yagci et al., 2010; Sugauchi et al., 2011].

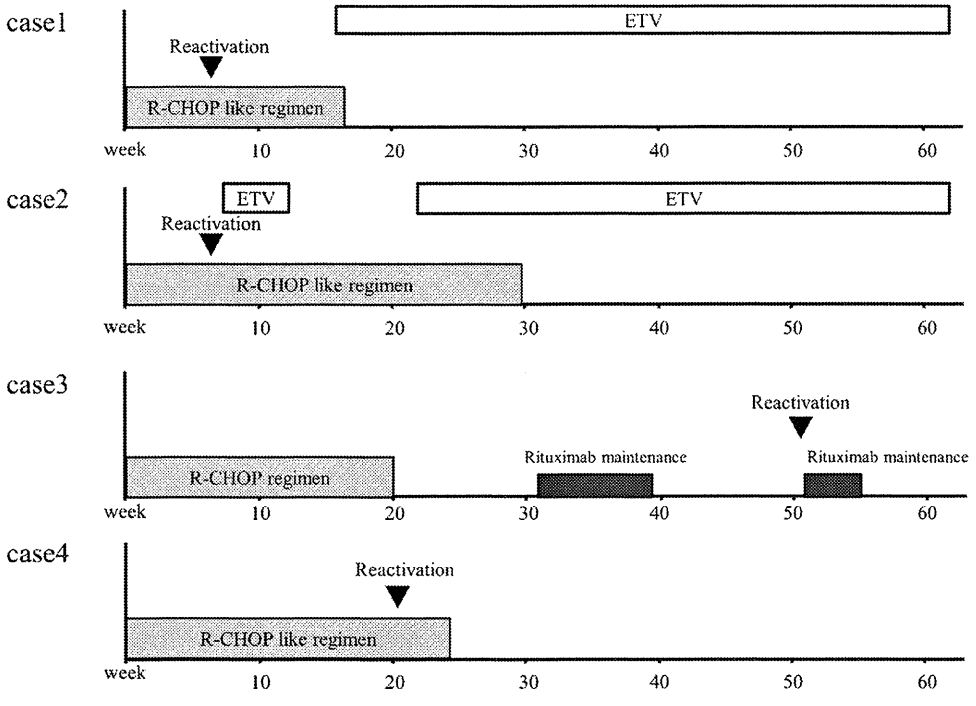

Fig. 1. Reactivation of HBV occurred during the course of chemotherapy in three cases, and after maintenance therapy with rituximab in only one case.

TABLE III. Comparison of Background Between Patients With or Without HBV Reactivation

			Reactivation proportion		
	Reactivation	Non-reactivation	Estimates(%)	95%CI(%)	*P*-value[a]
Number	4	105	3.7	(1.0–9.1)	
Sex(M/F)	2/2	58/47	3.3/4.1	(0.4–11.5)/(0.5–14.0)	1.000
Age[b], mean	73.5	67.7			0.420
Anti-HBc positive	4	55	6.8	(1.9–16.5)	0.123
Anti-HBs positive	1	41	2.4	(0.0–12.6)	1.000
Diagnosis					
Multiple myeloma	0	13	0.0	(0.0–24.7)	1.000
Diffuse large B cell lymphoma	2	52	3.8	(0.5–12.8)	1.000
Follicular lymphoma	1	21	4.8	(0.1–22.8)	1.000
Marginal zone B cell lymphoma	0	7	0.0	(0.0–41.0)	1.000
Burkitt lymphoma	1	1	50.0	(1.3–98.7)	0.072
T-cell lymphoma	0	2	0.0	(0.0–84.2)	1.000
Hodgkin lymphoma	0	5	0.0	(0.0–52.2)	1.000
Others	0	4	0.0	(0.0–60.2)	1.000
No. of rituximab administration	4	84	4.5	(1.3–11.2)	1.000
No. of glucocorticoid administration	4	104	3.7	(1.0–9.2)	1.000
Lymphocyte before chemotherapy median[b] (/μl) (range)	776 (460–1368)	1363 (274–10156)			0.033
Nadir lymphocyte during and after chemotherapy median[b] (/μl) (range)	133 (8–217)	247 (0–1729)			0.130
Immunoglobulin G before chemotherapy median[b] (mg/dl) (range)	1237 (1103–1479)	1421 (82–9085)			0.733

CI, confidence interval.
[a]Fisher's exact test.
[b]Medians and ranges are presented, compared by Mann–Whitney test.

Recently, the risk for development of HBV reactivation after chemotherapy in HBsAg undetectable patients has been reported [Wu et al., 2009; Cheung et al., 2010; Matsue et al., 2010]. Hui et al. [2006] described that 6 of 49 patients with undetectable HBsAg with malignant lymphoma receiving rituxmab plus corticosteroid chemotherapy developed new onset hepatitis B, and the risk factor was rituximab plus corticosteroid administration. Yeo et al. [2009] noted that 5 of 21 HBsAg undetectable, anti-HBc positive patients with diffuse large B-cell lymphoma who were treated with rituximab combination chemotherapy had reactivated HBV, and the risk factors were male, anti-HBs negative, and rituximab combination chemotherapy. It was recently reported that HBV reactivation had occurred in HBsAg undetectable multiple myeloma patients who underwent chemotherapy; 1 of 61 HBsAg undetectable multiple myeloma patients had reactivated HBV following chemotherapy [Yoshida et al., 2010]. However, additional prospective study would be required to know the precise frequency and risk factors for HBV reactivation among patients treated for malignant lymphoma or multiple myeloma. In addition, HBV reactivation was reported to be associated with the presence of anti-HBc and anti-HBs in baseline sera [Hui et al., 2006; Yeo et al., 2009], but the other factors associated with HBV reactivation have not yet been described.

In this study, all four cases with HBV reactivation were positive for anti-HBc before chemotherapy, and three of them were negative for anti-HBs (Table III). However, because of the limitation of size of samples in this study, this study could not evaluate the significance of anti-HBc for HBV reactivation ($P = 0.06$). This might be one of the key results of this study, but it could also be a chance finding. Therefore, serological markers including the titrations of anti-HBc and anti-HBs should be analyzed for the purpose of determining their relationship with HBV reactivation in larger scaled studies.

Out of four patients with HBV reactivation, two patients were treated with entecavir because they showed a persistent increase in the HBV DNA load. In contrast, in the remaining two patients, one patient showed a temporary replication signal of HBV DNA by real-time PCR, and another patient revealed a slight increase in the DNA load during a close follow-up. As HBV DNA of the latter two cases immediately became undetectable by real-time PCR, they were not given antiviral drugs. None of the four patients with HBV reactivation presented de novo hepatitis due to HBV reactivation. All cases were able to receive chemotherapy for underlying diseases as scheduled initially. Although there has been no proposal for the optimal time point for initiation of an anti-HBV treatment for this disease setting, preemptive therapy should be started immediately in patients with sustained viral replication quantified by real-time PCR.

In the present study, periodical quantitation of HBV DNA was useful for monitoring active

replication of HBV in patients receiving chemotherapy. HBsAg was also measured in serial sera in all cases of HBV reactivation, using novel CLEIA which was reported to be highly sensitive. However, HBsAg was not detected in any serum obtained from patients with HBV reactivation, indicating insufficient sensitivity of the assay for detecting HBV reactivation. As shown in the Japanese guidelines [Hirohito Tsubouchi et al., 2009], at present, routine measurement of HBV DNA levels would be preferable to an assay for HBsAg for the purpose of the early diagnosis of HBV reactivation.

Additionally, baseline lymphocyte counts in patients who had HBV reactivation were significantly less than those in patients who did not, although there was no difference in the nadir level of peripheral lymphocytes between patients who developed HBV reactivation and those who did not during or after chemotherapy. Based on these results, it is possible that lower levels of baseline peripheral lymphocytes might have a correlation with the occurrence of reactivated HBV in patients with malignant lymphoma or multiple myeloma. Although there is no similar data, further clinical studies are needed to evaluate the association between HBV reactivation and differential count of lymphocytes. In hepatocytes of chronic hepatitis B patients, cellular and humoral immunity could be associated with viral clearance, especially cytotoxic T lymphocytes (CTL) and natural killer (NK) cells which have roles to suppress proliferation of HBV. Gu et al. [2009] showed that serum HBV DNA levels in chronic hepatitis B patients were correlated to the frequency of HBV-specific CTL. Li et al. [2011] also reported that patients with acute hepatitis B possess a higher frequency of HBV-specific CTL than chronic hepatitis B patients. These reports may indicate that the HBV-specific CTL would be associated with suppression of HBV proliferation. This study could not evaluate differential counts of lymphocytes, and functional analyses of CTL. Further studies with CTL would provide a better understanding of the mechanism of this condition.

In conclusion, among the 59 anti-HBc positive patients with malignant lymphoma or multiple myeloma, four patients (6.8%) showed HBV reactivation during and after chemotherapy. HBV reactivation did not occur among patients without anti-HBc in this study. Monitoring of HBV DNA in sera is useful for the early diagnosis of HBV reactivation, and preemptive therapy is a useful alternative to prevent hepatitis due to HBV reactivation. Patients must be monitored periodically for HBV-DNA levels during and after chemotherapy.

REFERENCES

Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. 1995. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 270:286–290.

Cheung WI, Chan HL, Leung VK, Tse CH, Fung K, Lin SY, Wong A, Wong VW, Chau TN. 2010. Reactivation of hepatitis B virus infection with persistently negative HBsAg on three HBsAg assays in a lymphoma patient undergoing chemotherapy. J Clin Virol 47:193–195.

Francisci D, Falcinelli F, Schiaroli E, Capponi M, Belfiori B, Flenghi L, Baldelli F. 2010. Management of hepatitis B virus reactivation in patients with hematological malignancies treated with chemotherapy. Infection 38:58–61.

Gu XB, Yang XJ, Wang D, Hua Z, Xu YQ, Lu ZH. 2009. Relationship between serum HBV DNA level and HBV-specific, nonspecific cytotoxic T lymphocytes and natural killer cells in patients with chronic hepatitis B. Chin Med J 122:2129–2132.

Hiddemann W, Kneba M, Dreyling M, Schmitz N, Lengfelder E, Schmits R, Reiser M, Metzner B, Harder H, Hegewisch-Becker S, Fischer T, Kropff M, Reis HE, Freund M, Wormann B, Fuchs R, Planker M, Schimke J, Eimermacher H, Trumper L, Aldaoud A, Parwaresch R, Unterhalt M. 2005. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood 106:3725–3732.

Hirohito Tsubouchi HK, Kendo K, Satoshi M, Isao S, Eiji T, Takafumi I, Masashi M, Kazuyuki S, Shinsyo Y, Hisataka M, Toshifumi H, Norio H, Norihiro K, Tomoo F, Hiromi I, Yasuhiko S, Hiroshi Y, Akio I, Yasuhiro T, Kazuaki I, Makoto O, Hirofumi U, Nobuaki N, Takafumi N, Shinichiro T, Shinichi K, Koji Y, Ryujin E, Yasuhito T, Takeji U, Kotaro K. 2009. Prevention of immunosuppressive therapy or chemotherapy-induced reactivation of hepatitis B virus infection—Joint report of the intractable liver diseases study group of Japan and the Japanese study group of the standard antiviral therapy for viral hepatitis. Kanzo 50:38–42.

Hui CK, Cheung WW, Zhang HY, Au WY, Yueng YH, Leung AY, Leung N, Luk JM, Lie AK, Kwong YL, Liang R, Lau GK. 2006. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. Gastroenterology 131:59–68.

Kusumoto S, Tanaka Y, Ueda R, Mizokami M. 2011. Reactivation of hepatitis B virus following rituximab-plus-steroid combination chemotherapy. J Gastroenterol 46:9–16.

Li J, Han Y, Jin K, Wan Y, Wang S, Liu B, Liu Y, Lu S, Huang Z. 2011. Dynamic changes of cytotoxic T lymphocytes (CTLs), natural killer (NK) cells, and natural killer T (NKT) cells in patients with acute hepatitis B infection. Virol J 8:199.

Matsubara N, Kusano O, Sugamata Y, Itoh T, Mizuii M, Tanaka J, Yoshizawa H. 2009. A novel hepatitis B virus surface antigen immunoassay as sensitive as hepatitis B virus nucleic acid testing in detecting early infection. Transfusion 49:585–595.

Matsue K, Kimura S, Takanashi Y, Iwama K, Fujiwara H, Yamakura M, Takeuchi M. 2010. Reactivation of hepatitis B virus after rituximab-containing treatment in patients with CD20-positive B-cell lymphoma. Cancer 116:4769–4776.

Shinkai N, Tanaka Y, Matsuura K, Kani S, Naganuma H, Mizokami M. 2010. Evaluation and application of a newly developed highly sensitive HBsAg chemiluminescent enzyme immunoassay for chronic hepatitis B patients. Rinsho Byori 58:1078–1084.

Sugauchi F, Tanaka Y, Kusumoto S, Matsuura K, Sugiyama M, Kurbanov F, Ueda R, Mizokami M. 2011. Virological and clinical characteristics on reactivation of occult hepatitis B in patients with hematological malignancy. J Med Virol 83:412–418.

Tur-Kaspa R, Laub O. 1990. Corticosteroids stimulate hepatitis B virus DNA, mRNA and protein production in a stable expression system. J Hepatol 11:34–36.

Wu JM, Huang YH, Lee PC, Lin HC, Lee SD. 2009. Fatal reactivation of hepatitis B virus in a patient who was hepatitis B surface antigen negative and core antibody positive before receiving chemotherapy for non-Hodgkin lymphoma. J Clin Gastroenterol 43:496–498.

Wursthorn K, Wedemeyer H, Manns MP. 2010. Managing HBV in patients with impaired immunity. Gut 59:1430–1445.

Yagci M, Ozkurt ZN, Yegin ZA, Aki Z, Sucak GT, Haznedar R. 2010. Hepatitus B virus reactivation in HBV-DNA negative and positive patients with hematological malignancies. Hematology 15:240–244.

Yeo W, Chan TC, Leung NW, Lam WY, Mo FK, Chu MT, Chan HL, Hui EP, Lei KI, Mok TS, Chan PK. 2009. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. J Clin Oncol 27:605–611.

Yoshida T, Kusumoto S, Inagaki A, Mori F, Ito A, Ri M, Ishida T, Komatsu H, Iida S, Sugauchi F, Tanaka Y, Mizokami M, Ueda R. 2010. Reactivation of hepatitis B virus in HBsAg-negative patients with multiple myeloma: two case reports. Int J Hematol 91:844–849.

Yotsuyanagi H, Yasuda K, Iino S, Moriya K, Shintani Y, Fujie H, Tsutsumi T, Kimura S, Koike K. 1998. Persistent viremia after recovery from self-limited acute hepatitis B. Hepatology 27: 1377–1382.