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Case report

Reactivation of latently infected hepatitis B virus in a leukemia patient with antibodies to hepatitis B core antigen

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A 66-year-old man with chronic B-cell leukemia who had antibody to hepatitis B core antigen (anti-HBc) but not hepatitis B surface antigen (HBsAg) suffered from lethal hepatitis caused by hepatitis B virus (HBV) reactivation. He initially lacked circulating viral genomes in his sera and did not have a past history of liver dysfunction. In this patient, the immunosuppressive condition introduced by disease progression of leukemia induced reactivation of dormant HBV, and the withdrawal of chemotherapy resulted in fatal liver failure. Mutation-specific assay based on competitive polymerase chain reaction (PCR) and sequencing analyses revealed the predominant reactivation of an HBV strain with missence mutation (point mutation G-to-A at nucleotide 1896) in the precore regions, as well as point mutations in the core promoter regions. Therefore, it is important to note the risk of HBV reactivation, with resulting lethal hepatic failure, in anti-HBc-positive healthy individuals, even when they lack HBsAg, who receive immunomodulating therapy.

Key words: HBV, anti-HBc, latent infection, reactivation, immunosuppression, leukemia

Introduction

It has generally been accepted that clearance of hepatitis B surface antigen (HBsAg) in hepatitis B virus (HBV) carriers indicates recovery from viral infection. However, recent studies have demonstrated that traces of HBV infection are frequently detected many years after clinical recovery from acute hepatitis.[1–3] In addition, it was shown that HBV infection occurred in recipients who acquired a liver graft from healthy donors positive for antibodies to hepatitis B core antigen (anti-HBc), but negative for HBsAg, through liver transplantation.[4–7] Thus, it appeared that the dormant HBV maintained persistent infection in the liver of anti-HBc-positive but HBsAg-negative individuals without causing hepatic inflammation or clinical liver dysfunction. Recently, we have demonstrated that the majority of healthy individuals positive for anti-HBc were latently infected by the episomal form of HBV, with ongoing viral replication.[8] In addition, it was suggested that the predominant strain of latently infected HBV in these anti-HBc-positive healthy individuals was the wild type, without any mutations in the precore (C) and core promoter regions in the presence of circulating antibody to hepatitis B e antigen (anti-HBe).[8]

On the other hand, development of fatal liver failure was occasionally reported in HBsAg-positive carriers who were positive for anti-HBe, which was attributable to reactivation of chronically infected HBV.[9–13] In contrast to findings in these HBsAg-positive carriers, the risk of HBV reactivation in HBs-Ag-negative individuals has not been well characterized. Lok et al.[14] reported that reactivation of HBV occurred in patients with HBV-related serological markers during chemotherapy for malignant lymphoma, including two patients who were initially negative for HBsAg but positive for anti-HBc and who had antibodies to HBsAg (anti-HBs).[14] Evidence of possible HBV reactivation in anti-HBc-positive but HBsAg-negative individuals, because of immunosuppression, can also be seen in HIV-infected patients[15,16] and in the recipients of kidney transplants.[17,18] Although these findings suggest that HBV reactivation does occur in anti-HBc-positive but HBsAg-negative healthy individuals under certain immunosuppressive conditions, little is known about the mechanism of viral reactivation and the characteristics of the reactivated HBV in these individuals.

We encountered a patient with leukemia who was positive for anti-HBc and who developed fatal liver

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failure caused by reactivation of HBV brought about by the introduction of an immunocompromised condition. Notably, he initially lacked any of the serological markers for viral antigen, or circulating viral genomes in his sera, and he did not have a past history of liver dyfunction. To investigate the characteristics of the reactivated HBV strains, we employed a competitive polymerase chain reaction (PCR) with specific mutation-trapped oligonucleotide primers.

Case report

A 66-year-old man, who had been diagnosed as having chronic B-cell leukemia in January 1997, was admitted to our hospital in May 1997 for further treatment. On admission, he showed normal liver function, as confirmed by blood chemistry, and lacked a family history of liver disease; he had no prior history of liver dysfunction, blood transfusion, or intravenous drug abuse. The clinical course of the patient after admission is shown in Fig. 1. In September 1997, he began receiving chemotherapy with cyclophosphamide (750 mg/body × 1 day), vincristine (1.5 mg/body × 1 day), and prednisolone (60 mg/body × 4 days) every 14 days, and this was continued for nearly 5 months (nine courses in total). In March 1998, although he had no complaints, his blood chemistry tests showed an elevated level of alanine aminotransferase (ALT, 604 U/l). Thirteen days later, serum ALT had increased further, to 1566 U/l, in association with elevated total bilirubin (8.6 mg/dl) and a reduction of the prothrombin time to 38%, showing severe liver damage. Twenty-four days after the onset of liver dysfunction, he died of hepatic failure, despite having received intensive treatment. Liver histology carried out at autopsy was consistent with acute hepatitis with massive necrosis.

At the first visit to our hospital in May 1997, he was initially positive for anti-HBc, anti-HBs, and anti-HBe, but negative for HBsAg, hepatitis B e antigen (HBeAg), and anti-HBc shown in 200-fold dilution of his sera. He had no hepatitis C virus or hepatitis delta virus markers. With the progression of the leukemia, his serological level of anti-HBs began to decrease gradually, and finally anti-HBs in sera disappeared after the start of chemotherapy, which was accompanied by the appearance of HBsAg in his sera. Although HBV DNA was not detected in his sera by nested PCR[8] at the first visit to our hospital, it was detectable in September 1997 in association with the progression of leukemia (Fig. 1, lower panel). Before the onset of liver dysfunction, he had never received blood product transfusion, and he lacked any other risk factors for viral transmission, as evidenced by the absence of intravenous drug injection or chance of sexual transmission during hospitalization.

To clarify the characteristics of the reactivated viruses in this patient, the nucleotide sequences of viral strains in the serum after HBV exacerbation were ana-

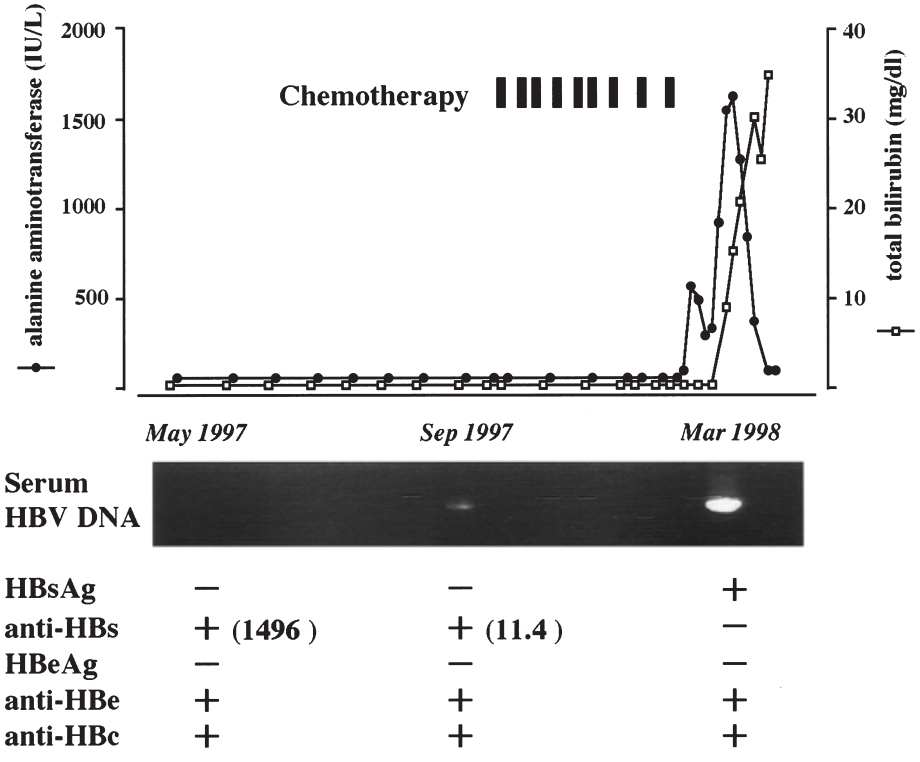

Fig. 1. Clinical course of leukemia patient with reactivation of latently infected hepatitis B virus (*HBV*). Diagram shows serial levels of alanine aminotransferase (*closed circles*) and total bilirubin (*open squares*). Serum HBV DNA was detected using polymerase chain reaction (PCR) with primers for the precore region. Serum titers of antibody to hepatitis B surface antigen (*anti-HBs*) were examined, using an enzyme immunoassay kit with a positive finding defined as 5 mIU/ml or greater (Dainabot, Tokyo, Japan). The patient received chemotherapy, including prednisolone (60 mg/body × 4 days) a total of nine times between September 1997 and February 1998. *HBsAg*, hepatitis B surface antigen; *HBeAg*, hepatitis B e antigen; *anti-HBe*, antibody to HBeAg; *anti-HBc*, antibody to hepatitis B core antigen

lyzed. After amplification of HBV DNA by PCR, using primer sets for the preC/C regions,[8] the nucleotide sequences of PCR products were determined directly, using a Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Cetus, Norwalk, CT, USA).[19] We found that this patient showed a dominant population of the preC stop codon mutant strain, with missence mutations from G-to-A at nucleotide (nt) 1896 in the preC region (nt position from the unique EcoRI site), and A-to-T and G-to-A mutations at nt 1762 and 1764, respectively, in the core promoter region.[20] The amounts of total HBV DNA and preC stop codon mutants were then determined, using competitive PCR with specific mutation-trapped oligonucleotide primers for the preC region (Otsuka Pharmacia, Tokushima, Japan).[21] It was found that, after HBV reactivation, this patient had high preC mutant HBV DNA titers (3×10^9 copies/ml), which were equal to the levels of total HBV DNA in his sera. These results confirmed the findings from direct sequencing that the preC defective mutant was the dominant population of circulating HBV.

Discussion

In the natural course of chronic HBV infection, the majority of HBsAg-positive carriers eventually lose HBeAg in association with seroconversion to anti-HBe, which is usually accompanied not only by reduction of viral replication but also by remission of hepatitis.[23] However, reactivation of HBV occasionally occurs in such HBsAg-positive and anti-HBe-positive individuals under certain immunosuppressive conditions, sometimes resulting in lethal hepatic failure.[9–13] Several recent studies have, however, demonstrated the reactivation of HBV in HBsAg-negative and anti-HBc-positive healthy individuals. Indeed, HBV reactivation was documented in patients positive for anti-HBc but negative for HBsAg after renal transplantation[17,18] and after intensive chemotherapy.[24] Confirming these previous findings, we showed here the reactivation of latently infected HBV in a leukemia patient who was positive for anti-HBc and anti-HBs in the absence of HBsAg under newly introduced immunocompromised conditions.

Similar to the findings in the present patient, we previously reported an HBsAg-negative and anti-HBc-positive patient with chronic myelocytic leukemia who died of fulminant hepatic failure caused by HBV reactivation after bone marrow transplantation.[25] There was no definite evidence, in either of our patients with leukemia or in patients in other studies, to show whether the HBV detected in their sera after deterioration of liver function was derived from their own liver tissue, because elucidation of the presence of HBV genomes in their liver before its reactivation was not possible. However, it appears reasonable that our patients had had reactivation of the dormant HBV in their liver, because they lacked any risk factors for new HBV infection, as evidenced by the absence of a history of blood product treatment or drug abuse after the onset of leukemia. The most important point to note is that they initially had anti-HBc and anti-HBs, which was replaced by HBsAg in their sera during the course of progression of liver dysfunction. Recently, we demonstrated that the majority of healthy individuals positive for anti-HBc, which had been assumed to denote a past history of transient HBV infection, were latently infected with the episomal form of HBV, associated with ongoing viral replication.[8] Therefore, it is likely that these patients with leukemia originally had had the latent HBV infection in their liver tissues before HBV exacerbation.

The majority of anti-HBc-positive and HBsAg-negative individuals were shown to be predominantly infected with wild-type strains without any mutations in the preC and core promoter region,[8] although only a small amount of preC stop codon mutant of HBV was also present in their liver tissues (our unpublished data). Thus, it appears reasonable that both our present and previous patients with leukemia[25] had been infected predominantly with wild-type HBV. However, in these patients, the predominant reactivation of viral strains with preC stop codon and core promoter mutations was found in the course of lethal hepatic damage. These findings are consistent with the findings in the patient reported by Grotz et al.,[18] who developed life-threatening liver failure after renal transplantation. Notably, the HBV reactivation in these patients is in sharp contrast to the findings on HBV reactivation in recipients of liver transplantation. Indeed, we have demonstrated that all recipients who received a liver graft from healthy donors positive for anti-HBc but negative for HBsAg either became asymptomatic carriers or developed chronic hepatitis with positivity for HBeAg.[7] The reason for this distinct pattern of HBV reactivation and clinical course in patients with leukemia and liver transplant recipients is not known at present. However, this distinct pattern may be due to the distinct immune responses to HBV caused by the different immunological conditions of the hosts. Indeed, the leukemia patients originally had antibodies to HBV, including anti-HBc, anti-HBs, and anti-HBe, while none of the liver transplant recipients possessed any immunity to HBV at the time of HBV transmission.[7] How and when the preC mutant is reactivated, instead of the wild-type strain, in patients with latent HBV infection should be further examined in a future study.

In conclusion, it is very important to note the possible risk of HBV reactivation in anti-HBc-positive individu-

als, even when they lack HBsAg. In particular, special attention should be paid to patients who receive certain immunosuppressive therapies and those who have immunodeficiency diseases, such as leukemia and AIDS, which may reduce the immunological pressure against HBV.

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References

1. Blum HE, Liang TJ, Galun E, Wands JR. Persistence of hepatitis B viral DNA after serological recovery from hepatitis B virus infection. Hepatology 1991;14:56–63.
2. Michalak TI, Pasquinelli C, Guilhot S, Chisari FV. Hepatitis B virus persistence after recovery from acute viral hepatitis. J Clin Invest 1994;93:230–9.
3. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patient's recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med 1996;2:1104–8.
4. Chazouilleres O, Mamish D, Kim M, Carey K, Ferrell L, Robert JP, et al. "Occult" hepatitis B virus as source of infection in liver transplant recipients. Lancet 1994;343:142–6.
5. Dodson SF, Issa S, Araya V, Gayowski T, Pinna A, Eghtesad B, et al. Infectivity of hepatic allografts with antibodies to hepatitis B virus. Transplantation 1997;64:1582–4.
6. Dickson RC, Everhart JE, Lake JR, Wei Y, Seaberg EC, Wiesner RH, et al. Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. Gastroenterology 1997;113:1668–74.
7. Uemoto S, Sugiyama K, Marusawa H, Inomata Y, Asonuma K, Egawa H, et al. Transmission of hepatitis B virus from hepatitis B core antibody-positive donors in living-related liver transplants. Transplantation 1998;65:494–9.
8. Marusawa H, Uemoto S, Hijikata M, Ueda Y, Koichi T, Shimotohno K, et al. Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen. Hepatology 2000;31:488–95.
9. Galbraith RM, Eddleston AL, Williams R, Zuckerman AJ. Fulminant hepatic failure in leukaemia and choriocarcinoma related to withdrawal of cytotoxic drug therapy. Lancet 1975;II:528–30.
10. Hoofnagle JH, Dusheiko GM, Schafer DF, Jones EA, Micetich KC, Young RC, et al. Reactivation of chronic hepatitis B virus infection by cancer chemotherapy. Ann Intern Med 1982;96:447–9.
11. Pariente EA, Goudeau A, Dubois F, Degott C, Gluckman E, Devergie A, et al. Fulminant hepatitis due to reactivation of chronic hepatitis B virus infection after allogeneic bone marrow transplantation. Dig Dis Sci 1998;33:1185–91.
12. Pinto PC, Hu E, Bernstein-Singer M, Pinter-Brown L, Govindarajan S. Acute hepatic injury after the withdrawal of immunosuppressive chemotherapy in patients with hepatitis B. Cancer 1990;65:878–84.
13. Yoshiba M, Sekiyama K, Sugata F, Okamoto H, Yamamoto K, Yotsumoto S. Reactivation of precore mutant hepatitis B virus leading to fulminant hepatic failure following cytotoxic treatment. Dig Dis Sci 1992;37:1253–9.
14. Lok AS, Liang RH, Chiu EK, Wong KL, Chan TK, Todd D. Reactivation of hepatitis B virus replication in patients receiving cytotoxic therapy. Report of a prospective study. Gastroenterology 1991;100:182–8.
15. Altfeld M, Rockstroh JK, Addo M, Kupfer B, Pult I, Will H, et al. Reactivation of hepatitis B in a long-term anti-HBs-positive patient with AIDS following lamivudine withdrawal. J Hepatol 1998;29:306–9.
16. Hofer M, Joller-Jemelka HI, Grob PJ, Luthy R, Opravil M. Frequent chronic hepatitis B virus infection in HIV-infected patients positive for antibody to hepatitis B core antigen only. Eur J Clin Microbiol Infect Dis 1998;17:6–13.
17. Marcellin P, Giostra E, Martinot-Peignoux M, Loriot MA, Jaegle ML, Wolf P, et al. Redevelopment of hepatitis B surface antigen after renal transplantion. Gastroenterology 1991;100:1432–4.
18. Grotz W, Rasenack J, Benzing T, Berthold H, Peters T, Walter E, et al. Occurrence and management of hepatitis B virus reactivation following kidney transplantation. Clin Nephrol 1998;49:385–8.
19. Marusawa H, Hijikata M, Chiba T, Shimotohno K. Hepatitis C virus core protein inhibits fas- and tumor necrosis factor alphamediated apoptosis via NF-kappaB activation. J Virol 1999;73:4713–20.
20. Okamoto H, Tsuda F, Akahane Y, Sugai Y, Yoshiba M, Moriyama K, et al. Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. J Virol 1994;68:8102–10.
21. Kinoshita M, Seno T, Fukui T, Shin S, Tsubota A, Kumada H. A detection method for point mutation in the precore region of human hepatitis B virus (HBV)-DNA using mutation-site-specific assay. Clin Chim Acta 1994;228:83–90.
22. Takeda S, Ichii S, Nakamura Y. Detection of K-ras mutation in sputum by mutant-allele-specific amplification (MASA). Hum Mutat 1993;2:112–7.
23. Realdi G, Alberti A, Rugge M, Bortolotti F, Rigoli AM, Tremolada F, et al. Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology 1980;79:195–9.
24. Ahmed A, Keeffe EB. Lamivudine therapy for chemotherapy-induced reactivation of hepatitis B virus infection. Am J Gastroenterol 1999;94:249–51.
25. Iwai K, Tashima M, Itoh M, Okazaki T, Yamamoto K, Ohno H, et al. Fulminant hepatitis B following bone marrow transplantation in an HBsAg-negative, HBsAb-positive recipient. Bone Marrow Transplant 2000;25:105–8.