Hepatitis B Virus-related Immune Reconstitution Inflammatory Syndrome in Two Patients Coinfected with Human Immunodeficiency Virus Diagnosed with a Liver Biopsy

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Abstract

Hepatic flares occurred in two patients with HBV/HIV coinfection following the commencement of antiretroviral therapy (ART). At that time, the HIV RNA and HBV DNA levels had decreased. The results of liver biopsies showed lymphocytic infiltration that was diffusely positive for CD8⁺ T cells in the portal areas and lobules. These findings suggested HBV-related immune reconstitution inflammatory syndrome (IRIS). The alanine aminotransferase levels of both patients gradually decreased with the continuation of ART. Because there are few reports of the liver histology of HBV-related IRIS, these cases provide a better understanding of the pathogenesis of HBV-related IRIS.

Key words: hepatitis B virus, human immunodeficiency virus, immune reconstitution inflammatory syndrome, liver biopsy

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Introduction

Coinfection with hepatitis B virus (HBV) and human immunodeficiency virus (HIV) is common, primarily due to the shared routes of transmission. It is estimated that approximately 10% of patients with HIV infection worldwide are chronically coinfecting with HBV (1). In Japan, HBV/HIV coinfection has been reported in approximately 6% of patients with HIV infection (2). The current guidelines regarding the treatment of HIV infection recommend that patients with HBV/HIV coinfection receive antiretroviral therapy (ART) with activity against HBV, regardless of the number of CD4⁺ T cells. However, it has been reported that hepatic flares [defined as an alanine aminotransferase (ALT) level >5 times the upper limit of normal or >100 IU/L higher than that observed at baseline] frequently occur in

patients with HBV/HIV coinfection after the start of ART due to numerous causes, including opportunistic infections, the use of new hepatotoxic medications, excessive alcohol consumption and the development of immune reconstitution inflammatory syndrome (IRIS). Although liver biopsies are helpful for elucidating the cause of hepatic flares, there are few reports concerning the pathological findings of such biopsies in this patient population. We herein report two cases of HBV/HIV coinfection in which hepatic flares were observed following the commencement of ART. These flares were thought to reflect HBV-related IRIS based on the patients' clinical and liver biopsy findings.

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Figure 1. Histopathological findings of the first patient obtained at the time of the hepatic flare. **a:** The biopsy results revealed mild chronic inflammation in the enlarged portal areas (Metavir score, A1, F0) (Hematoxylin and Eosin staining, $\times 200$). **b:** The infiltrating lymphocytes were diffusely positive for CD8 on immunohistochemical staining ($\times 400$). **c:** The CD4-positive cells were focal ($\times 400$). **d:** Few CD56-positive cells were noted ($\times 400$).

Case Reports

Case 1

A 30-year-old bisexual Japanese man with HBV/HIV co-infection developed a perianal abscess, stomachache, diarrhea and hematochezia over the seven months before his initial visit. He also experienced weight loss of 7 kg within the past year. Colonoscopy demonstrated that he was suffering from amoeba and cytomegalovirus (CMV) enterocolitis, as confirmed by the pathologic findings of the colon. Following the diagnosis of HBV and HIV coinfection, he was referred to our hospital for treatment of the enterocolitis and HBV/HIV coinfection. On admission, the patient was found to be positive for HIV-1 on a Western blot analysis, with an HIV RNA load of 4.4×10^5 copies/mL. The CD4⁺ and CD8⁺ T cell counts were 19 and 286/ μ L, respectively, while the aspartate aminotransferase (AST) and ALT levels were both within the normal limits (19 and 27 IU/L, respectively). Hepatitis B surface antigens (HBsAg) were positive. Hepatitis B e antigens (HBeAg) were positive ($>1,600$ C.O.I), whereas antibodies to HBeAg were negative. In addition, IgM antibodies against hepatitis B core antigens (anti-HBc

IgM) were negative. The plasma HBV DNA level was more than 9.53 log copies/mL, and the HBV genotype was A. Both antibodies to hepatitis C virus (HCV) and hepatitis A virus were negative. Anti-Entamoeba histolytica IgG antibodies were positive. CMV antigenemia was observed (C7-HRP: 27/80,200 cells). Metronidazole and ganciclovir were administered to treat the amoeba and CMV colitis, while sulfamethoxazole/trimethoprim and azithromycin hydrate were prescribed to prevent pneumocystis pneumonia and disseminated mycobacterium infection, respectively. Furthermore, treatment with alprazolam and lorazepam was started because the patient developed depression and insomnia after receiving the diagnosis of HIV infection. Because a colonoscopy re-examination showed a prominent improvement in the colonic ulcers 18 days later, the dose of metronidazole was discontinued, and ganciclovir was changed to valganciclovir as maintenance therapy for CMV colitis. Accordingly, ART was started with raltegravir (RAL) and tenofovir disoproxil fumarate/emtricitabine (TDF/FTC), as indicated for HBV and HIV coinfection. At that time, the ALT level was slightly elevated at 40 IU/L (normal range: 6-30 IU/L), although the AST and bilirubin levels were still normal, at 24 IU/L and 0.4 mg/dL, respectively. Six weeks after the initiation of ART, the AST and ALT levels became

Figure 2. Clinical course of Case 1. MNZ: metronidazole, GCV: ganciclovir, ART: antiretroviral therapy, RAL: raltegravir, TDF: tenofovir disoproxil fumarate, FTC: emtricitabine

elevated (AST: 72 IU/L, ALT: 127 IU/L). Ten weeks after the start of ART, the AST level had increased to 280 IU/L, while the ALT level had increased to 546 IU/L. The total bilirubin level remained normal at 0.6 mg/dL, and the prothrombin time was 12.8 seconds (INR: 1.09, 81%). Due to the administration of ART, the HIV RNA level decreased to 93 copies/mL and the CD4⁺ and CD8⁺ T cell counts increased from 19 to 138 cells/μL and 286 to 736 cells/μL, respectively. Meanwhile, the HBV DNA level had decreased from >9.53 to 3.63 log copies/mL, and anti-HBc IgM were strongly positive (29.9 S/CO). No CMV antigenemia or eosinophilia was observed, and antinuclear antibodies were negative. The patient was a non-drinker and had not taken any additional medications. The appearance of the liver, as noted on ultrasonography, was normal. Because the cause of the hepatic flare was unknown, a liver biopsy was performed, the results of which showed mild chronic inflammation in the enlarged portal areas (A1, F0). Scattered single-cell necrosis and mild lobular hepatitis were also observed; however, no interface hepatitis or bridging fibrosis was evident, and no CMV-related nuclear inclusions or CMV-positive cells were found. Infiltrating lymphocytes were diffusely positive for CD3 and CD8, whereas CD4⁺ cells were focal, and few CD56⁺ cells were detected. Such observations have been reported as being compatible with a diagnosis of HBV infection, thus they were unlikely to be the result of drug-induced liver injury (Fig. 1). The patient's clinical, laboratory and histological findings therefore suggested HBV-related IRIS rather than drug hepatotoxicity. Because his general condition was good despite the liver enzyme elevation, his medication regimen, including ART was continued. Subsequently, the AST and ALT levels gradually decreased to normal 18 weeks after the start of ART (Fig. 2). Eighteen months after the initiation of ART, HBeAg were negative and HBV DNA was undetectable.

Case 2

A 20-year-old Japanese man with HBV infection was diagnosed with condyloma acuminatum and rectal carcinoids. A preoperative screening test found HIV infection, and the patient was referred to our hospital for the treatment of HBV/HIV coinfection. On his first visit, he was found to be positive for HIV-1 on a Western blot analysis, and his HIV RNA load was 8.7×10^4 copies/mL. The CD4⁺ and CD8⁺ T cell counts were 484 and 735/μL, respectively. The AST and ALT levels were slightly increased at 54 and 77 IU/L, respectively. HBsAg and HBeAg (>1,600 C.O.I) were both positive, whereas antibodies to HBeAg were negative. The plasma HBV DNA level was 9.22 log copies/mL, and the HBV genotype was A. The degree of liver stiffness was 8.8 kPa, as measured on transient elastography (FibroScan, Echosens, Paris, France). Because the number of CD4⁺ T cells continued to be high, he was given pegylated-interferon (IFN)α-2a for chronic HBV infection. However, the HBV DNA level did not decrease, and no seroconversion of HBeAg was noted during this period. Treatment with ART was therefore started with RAL and TDF/FTC. At that time, the AST and ALT levels were both within the normal limits (29 and 23 IU/L, respectively). However, elevated levels of AST and ALT were observed 25 weeks after the initiation of ART (AST: 326 IU/L, ALT: 168 IU/L), and at 27 weeks, the AST level increased to 206 IU/L, while the ALT level increased to 389 IU/L. In contrast, the total bilirubin level and prothrombin time remained within the normal limits. Following the administration of ART, the HIV RNA level decreased to less than 20 copies/mL, although the CD4⁺ and CD8⁺ T cell counts did not change (441 cells/μL and 465 cells/μL, respectively). The HBV DNA level decreased from 9.22 to 2.56 log copies/mL, and anti-HBc IgM were negative. The appearance of the liver, as

Figure 3. Histopathological findings of the second patient obtained at the time of the hepatic flare. **a:** The biopsy results revealed mild lymphocytic infiltration in the portal areas and lobules (Metavir score, A1F0) (Hematoxylin and Eosin staining, $\times 200$). **b:** Infiltrating lymphocytes were diffusely positive for CD8 on immunohistochemical staining ($\times 100$). **c:** The CD56-positive cells were focal ($\times 100$).

observed on ultrasonography, was normal. The patient's general condition was good. He underwent a liver biopsy to elucidate the cause of the hepatic flare, and the results of the biopsy showed mild lymphocytic infiltration in the portal areas and lobules. In addition, scattered single-cell necrosis and mild lobular hepatitis were observed. Infiltrating lymphocytes were diffusely positive for CD3 and CD8 but negative for CD20 and CD56. These features indicated a diagnosis of chronic hepatitis, likely related to the HBV (A1, F0), rather than drug-induced liver injury (Fig. 3). The patient's clinical, laboratory and histological findings suggested HBV-related IRIS, and the ART regimen was continued. Subsequently, the AST and ALT levels gradually decreased, as did the titer of HBeAg (Fig. 4).

Discussion

We herein reported two cases of HBV/HIV coinfection in which hepatic flares were observed following the commencement of ART. Both cases were thought to involve HBV-related IRIS based on the patients' clinical and liver biopsy findings.

It has been reported that 20-25% of patients with HBV/HIV coinfection experience HBV-related IRIS after the start of ART (3, 4). The introduction of ART results in the reconstitution of various HBV-specific CD4⁺ and CD8⁺ T cell re-

sponses in association with restoration of the CD4⁺ T cell count (5). It is considered that the hepatic flares of patients with chronic HBV infection alone occur secondary to the recruitment of both HBV-specific CD8⁺ T cells and non-HBV specific mononuclear cells to the liver, a process that is primarily mediated by proinflammatory and antiviral cytokines, such as tumor necrosis factor (TNF)- α and IFN- γ (6-8). However, it is currently unclear whether the immunopathogenesis of HBV-related IRIS observed in patients with HBV/HIV coinfection is the same as that underlying the hepatic flares of patients with HBV infection alone. The pathological findings of the liver in the present cases are summarized in Table. These findings revealed the presence of lymphocytic infiltrates in the portal areas and lobules. The infiltrating lymphocytes were diffusely positive for CD3 and CD8, although CD4-positive cells were focal, and few CD56-positive cells were detected. This liver histology is similar to that of the hepatic flares seen in patients with chronic HBV infection alone, and no differences between these features were found in our two patients.

A previous study of cytokines and chemokines reported that the occurrence of hepatic flares in patients with HBV infection alone is associated with the production of IFN- α and interleukin (IL)-8, which leads to the recruitment of activated TNF-related apoptosis-inducing ligand (TRAIL)-expressing NK cells to the liver, with and subsequent hepa-

Figure 4. Clinical course of Case 2. ART: antiretroviral therapy, RAL: raltegravir, TDF: tenofovir disoproxil fumarate, FTC: emtricitabine

Table. Summary of the Histopathological Findings of the Liver

	Hematoxylin and Eosin staining	Immunohistochemical staining
Case 1	<ul style="list-style-type: none"> • Chronic hepatitis, A1, F0. (Mild lymphocytic inflammation in the enlarged portal areas) • Single cell necrosis and mild lobular hepatitis are scattered. • Interface hepatitis and bridging fibrosis are not evident. 	<ul style="list-style-type: none"> • Diffusely positive for CD3 and CD8. • Focally positive for CD4. • CD 56 positive cells are few.
Case 2	<ul style="list-style-type: none"> • Chronic hepatitis, A1, F0. (Mild lymphocytic inflammation in the portal areas and lobules) • Single cell necrosis and mild lobular hepatitis are few. • Interface hepatitis and bridging fibrosis are not evident. 	<ul style="list-style-type: none"> • Diffusely positive for CD3 and CD8. • Focally positive for CD4. • Negative for CD20 and CD56.

toocyte damage (9). A recent report showed that increased plasma levels of CXCL-10 and soluble CD30 are associated with hepatic flares in patients with HBV/HIV coinfection (10). CXCL-10 is a chemoattractant for T cells, NK cells and monocytes to the liver, and persistent elevation of the CXCL-10 level in the setting of a rising CD4⁺ T cell count may therefore drive enhanced T cell recruitment to the liver. However, in contrast to the findings for hepatic flares in patients with HBV infection alone, no clear associations have been detected between TRAIL-expressing NK cells and HBV-related IRIS in patients with HBV/HIV coinfection. Therefore, the immunopathogenesis of HBV-related IRIS in HIV-coinfected patients appears to be different from that underlying the hepatic flares seen in patients with HBV infection alone. However, further studies including more patients are needed to confirm these findings.

Both of our patients were asymptomatic at the time of HBV-related IRIS onset, and their ALT levels decreased in association with the continuation of ART. The first patient

exhibited seroconversion of HBeAg 16 months after the onset of HBV-related IRIS. In addition, the HBeAg titer in the second patient began to decrease after the hepatic flare. In cases of HBV-related IRIS, ART may be continued under close monitoring of the hepatic function, as the onset of IRIS may be followed by seroconversion of HBeAg or HBsAg (11).

Generally, patients who develop IRIS have a low CD4⁺ T cell count (<50 cells/μL) and high HIV RNA level at the commencement of ART. The Avihingsanon group showed that hepatic flares that seem to reflect HBV-related IRIS are observed at a median of 56 days (3). However, in the present case 2, the hepatic flare was observed 25 weeks after the start of ART, and the patient's CD4⁺ T cell count was greater than 400 cells/μL prior to the initiation of ART. The second case was atypical for HBV-related IRIS. This may be due to the immunomodulatory effects of pegylated-IFNα-2a therapy observed before the start of ART. It is well known that IFN has an immunomodulatory effect in addition to its

antiviral actions, and some patients with HBV infection experience HBeAg or HBsAg seroclearance after the end of IFN treatment (12-14). We speculate that IFN may have influenced the patient's immune response to HBV. For example, when his HBV DNA and HIV RNA levels decreased after the start of ART, his cellular immune response to HBV was reconstituted by treatment with IFN and ART. Therefore, he may have developed the hepatic flare as a form of HBV-related IRIS despite his CD4⁺ T cell count being more than 400 cells/ μ L prior to the initiation of ART. Furthermore, according to previously described criteria for IRIS issued by the International Network for the Study of HIV-associated IRIS (INSHI) and the AIDS Clinical Trials Group (ACTG), data regarding the CD4⁺ T cell count at the commencement of ART and its improvement are not necessary for a diagnosis of IRIS, although there is no universally accepted definition for the condition (15, 16).

In conclusion, conducting a liver biopsy is helpful for elucidating the cause of hepatic flares following the commencement of ART. If a hepatic flare is thought to be due to HBV-related IRIS, treatment with ART can be continued. HBV-related IRIS occurs in the recovery process of immunocompetence, and its pathological findings are similar to those of the hepatic flare seen in patients with HBV infection alone. Because there are few reports of the liver histology of patients with HBV-related IRIS, our cases may have implications with respect to improving knowledge regarding the pathogenesis of HBV-related IRIS.

The authors state that they have no Conflict of Interest (COI).

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