Abstract

A 31-year-old man referred to our hospital for treatment of his chronic myeloid leukemia (CML) in the first chronic phase by bone marrow transplantation. We pretreated him with cyclophosphamide and total body irradiation and bone marrow transplantation (BMT) was carried out. On day 31, the engraftment was confirmed and on day 52, acute graft versus host disease (GVHD) was observed. On day 189, he lost consciousness due to cyclosporine A-induced leukoencephalopathy and 375 mg cyclosporine A was changed to 100 mg prednisolone. On day 199, liver dysfunction (AST 410 IU/L, ALT 557 IU/L, γGTP 385 IU/L, ALP 363 IU/L, D-Bil 0.3 mg/dl) developed and a liver biopsy was performed. PCR analysis of DNA from the liver biopsy specimen was positive for HHV-6 and immunostaining using anti-HHV-6 and anti-HHV-6b antibodies showed positive staining in the cytosol of hepatocytes. No other viruses were found to induce hepatitis. From these results, he was diagnosed as having HHV-6 hepatitis and it was successfully treated with gancyclovir (GCV) administration.

Key words: BMT, CML, HHV-6, hepatitis

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Introduction

Treatment of the adverse effects associated with bone marrow transplantation (BMT) is important to improve the quality of lives and prolong survival of the recipients. Cytomegalovirus (CMV), human herpesvirus-6 and 7 (HHV-6 and 7), are DNA viruses which belong to the β-herpesvirus family. While it is now widely accepted that CMV is associated with pathogen-related afflictions, such as pneumonitis, hepatitis, gastroenterocolitis, etc, which commonly present after BMT, the role of HHV-6 and HHV-7 in transplantation remains undisclosed. Here, we report a case of HHV-6 hepatitis associated with cyclosporine encephalitis after BMT for CML who has been successfully treated with GCV.

Materials and Methods

Histological analysis

Liver biopsy was performed by 18 G needle after informed consent. Samples were fixed in formalin and embedded in paraffin prior to sectioning. Sections of 7 μm were cut and stained with H&E. The anti-HHV-6 antibody (MAB 8537; Chemicon International, Temecula, CA) was used for the immunohistochemical detection of HHV-6 antigen and they were visualized by streptavidin-biotin immunoperoxidase method.
Figure 1. A magnetic resonance imaging study of the brain (T2). Arrows indicate diffuse hyper-intense lesions on the right temporal lobe.

**PCR analysis of HHV-6**

DNA was extracted from the liver biopsy specimen by QIAamp DNA Mini Kit (QIAGEN, Tokyo, Japan) according to the manufacture’s instructions. DNA was amplified by PCR using 5’-GTGTTTCCATTGTACTGAAACCGGT (sense) and 5’-TAAACATCAATGCGTTGCATACAG (antisense) consisting of 35 cycles (94°C for 60 sec, 60°C for 45 sec and 72°C for 45 sec) after the initial activation step (95°C for 7 min).

**Case Report**

A 31-year-old man presented to another hospital with abdominal fullness as the chief complaint. He had no relevant past or family history. He was diagnosed as having splenomegaly due to CML in an accelerated phase with leukocytosis, polycythemia, thrombocytosis and Philadelphia chromosome. Daily administration of 600 mg of imatinib mesylate was begun and a complete hematological response was obtained, although the bone marrow aspiration analysis showed positive results for Philadelphia chromosome and bcr/abl RT-PCR. He was referred to our hospital for treatment of his CML in the first chronic phase by bone marrow transplantation on January 5, 2004. We pretreated him with cyclophosphamide and total body irradiation as reported previously (1), and his older sister, with completely matching human leukocyte antigens, was chosen as the donor. On January 23 (day 0), bone marrow transplantation was performed, and on day 31, the engraftment was confirmed. On day 52, acute graft versus host disease (GVHD) was observed on his skin (stage 3), and so we began administration of 1 mg/kg/day of prednisolone. As there was no improvement, prednisolone was increased to 2 mg/kg from day 59 and the skin rash disappeared. He was discharged from our hospital, and prednisolone treatment for acute GVHD was gradually decreased, having a good clinical course until day 189. On July 30, the patient lost consciousness and was brought to a neurosurgery hospital. A magnetic resonance imaging study of the brain showed diffuse T2 hyper-intense lesions on the right temporal lobe (Fig. 1), and there was no particular finding in the cerebrospinal fluid obtained by lumbar puncture. Bacterial culture, antibodies against the human herpes virus, measles, rubella, and CMV in cerebrospinal fluid were negative. Concentration of the cyclosporine-A in the blood was elevated to 627 ng/ml (trough level). From these results, the patient was diagnosed with cyclosporine A-induced leukencephalopathy, and 375 mg cyclosporine A was replaced with 100 mg prednisolone on the day he lost consciousness. He was again admitted to our hospital for further treatment on day 193, August 3. Before the results of cerebrospinal fluid examination were obtained, we could not discount either herpes encephalitis or bacterial meningitis, so 500 mg/day acyclovir and 1 g/day meropenem administration were begun. However, on day 199, a recurrence of liver dysfunction (AST 410 IU/L, ALT 557 IU/L, γ GTP 385 IU/L, ALP 363 IU/L, D-Bil 0.3 mg/dl) was identified, which we suspected to be drug induced, so administration of acyclovir, meropenem, phenytoin, and lansoprazole were substituted with ranitidine, ursodeoxycholic acid and stronger Neo-Minofagen C; prednisolone treatment was continued. As the liver dysfunction persisted, a liver biopsy was performed on day 206. As shown in Fig. 2A, hematoxylin-eosin staining showed only mild lymphocyte infiltration in portal areas, possibly due to the 80 mg prednisolone administration. There were no signs of hepatocellular necrosis, fibrosis, eosinophil infiltration or lymphocyte infiltration to the bile ducts, and we could not determine the cause of liver dysfunction from the biopsy. Drug (acyclovir, meropenem, phenytoin, and lansoprazole)-induced liver dysfunction was not probable according to the scoring system used in Japanese Digestive Disease Week (2). We then suspected viral hepatitis, and a virological examination was carried out; peripheral blood tested negative for HBV-DNA, HCV-RNA, HEV-RNA, HGV-RNA, TTV-DNA and CMV antigenemia. In situ hibridization for the Epstein-Barr virus encoded small RNA-1, and immunostaining against the herpes simplex virus and CMV in a liver biopsy specimen showed negative results. PCR analysis of DNA from the liver biopsy specimen showed positive for HHV-6 but negative for adenovirus. Immunostaining of the liver biopsy specimen using anti-HHV-6 and anti-HHV-6b antibodies showed positive staining in the cytosol of hepatocytes (Fig. 2B-C). From these results we diagnosed the liver dysfunction was due to HHV-6 hepatitis. We suspected that the encephalitis was due to HHV-6 and performed HHV-6 PCR analysis using preserved cerebrospinal fluid. A negative result disproved this possibility. As shown in Fig. 3, ALT declined after glucagon-insulin therapy started, although ALT elevated.
Figure 2. Histological findings of liver biopsy performed on day 206. A, hematoxylin and eosin staining (×200). B, Immunostaining using normal mouse IgG (×200). C, Immunostaining using HHV-6 antibody (Chemicon, Temecula, CA, MAB8537; ×200). D, Immunostaining using HHV-6b antibody (Chemicon, MAB8535; ×200).

Figure 3. Clinical course after liver dysfunction developed.

again when we reduced prednisolone to 70 mg/day. Three days of γ-globulin administration stopped the ALT elevation, but again ALT worsened by when the dosage of prednisolone was reduced to 60 mg/day. Based on the diagnosis of HHV-6 hepatitis, we began administrating 200 mg/day GCV, and the liver dysfunction normalized. The patient was discharged from our hospital on day 230. CMV antigenemia remained negative after day 52. On day 300, he was readmitted to our hospital due to pneumonia. Again liver dysfunction developed due to levoflaxacin, and another liver biopsy was carried out, although we could not detect HHV-6 DNA in the specimen, and immunostaining for HHV-6 was negative.

Discussion

This is the second reported case of HHV-6 hepatitis associated with BMT. HHV-6 is a causative virus of exanthema
subitum, and more than 90% of adults are seropositive (3). In some cases, liver dysfunction is observed in the febrile phase of this disease and chronic hepatitis due to persistent infection of the virus, is observed in the others (4-6). It is also reported that HHV-6B genome was found in 75% of the children with a variety of liver diseases, indicating that the virus is capable of infecting hepatocytes (7). After primary infection, HHV-6 establishes latency similar to that of CMV, however it is not known whether HHV-6 establishes latency within the liver as it does in the salivary glands and lymph nodes. Like CMV, HHV-6 also reactivates in immunocompromised patients, including transplant recipients. It has been reported that HHV-6 was detected in the peripheral blood of 28 to 75% of BMT recipients but in only 0 to 30% in the normal population (8). Acute febrile with or without rash, interstitial pneumonitis, meningoencephalitis, hepatitis and pancytopenias are reported clinical manifestations due to this virus in transplanted patients (8, 9).

Ljungman et al monitored HHV-6 viral load in 74 subjects for 3 months after allogenic stem cell transplantation, the load peak being at 4 weeks and then decreasing from 8 weeks to 12 weeks after transplantation; higher DNA levels correlated with clinical symptoms (10). Three patients developed encephalitis, and one patient developed hepatitis, all developing within 12 weeks after transplantation. In the present case, hepatitis developed 199 days after BMT. It is speculated that changing the immunosuppressive agent (375 mg/day cyclosporine A to 100 mg/day prednisolone) made him more immune-compromised and induced HHV-6 reactivation. As to whether the liver injury was induced by the attack of infiltrated lymphocytes or by the direct effect of the infected virus it is necessary to consider the following: 1) previous reports show lymphocyte infiltration of the portal areas in HHV-6-associated liver failure patients (11) and 2) the fact that reducing the amount of prednisolone worsened the liver dysfunction in the present case. These findings seem to support the former possibility. Either of two scenarios can be supported from the case reported by Ljungman et al who developed HHV-6 hepatitis 3 weeks after transplantation (10), which is the time when recipient lymphocyte function is the most suppressed, although GVHD may develop while HHV-6 is in its highest duplication stage. Lymphocyte infiltration of portal areas was very faint in the present case, and combined with a very high ALT level, this case seems to support the latter possibility. In vitro studies have demonstrated that HHV-6 can infect and replicate in human hepatoma cell line HEPG2 (12, 13). In one study, hepatocyte markers (AST, ALT and γ GTP) in the media were elevated in HEPG2 cells infected with HHV-6 compared to the control (12). This indicates a direct cytopathic effect of the virus. Elucidation of the question remains the future task.

It is reported that GCV, cidofovir, and foscarnet show higher anti-HHV-6 activity than acyclovir in vitro (14). In fact, our patient developed liver dysfunction on day 199 while under acyclovir treatment and only GCV treatment normalized it. GCV should be the first choice for treating HHV-6-associated syndrome.

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Abbreviations: BMT: bone marrow transplantation, CML: chronic myeloid leukemia, CMV: Cytomegalovirus, GCV: ganciclovir, GVHD: graft versus host disease, HHV: human herpesvirus

References


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