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Case Report

Failure to Detect Epstein-Barr Virus (EBV) DNA in Plasma by Real-Time PCR in a Case of EBV-Associated Posttransplantation Lymphoproliferative Disorder Confined to the Central Nervous System

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Abstract

We report here a patient who developed multiple central nervous system (CNS) space-occupying lesions 6 months after bone marrow transplantation from an HLA-matched unrelated donor. He had extensive chronic graft-versus-host disease and severe thrombocytopenia. Posttransplantation lymphoproliferative disorder (PTLD) was diagnosed after biopsy of the lesion was facilitated by the transfusion of 40 units of platelets. Epstein-Barr virus (EBV) DNA was not initially detected in the peripheral blood by real-time polymerase chain reaction, and the blood became positive for EBV at a low level only after more than 6 weeks had passed since the initial identification of detectable intracranial lesions. The patient died of cerebral herniation while donor leukocyte infusion was being prepared, and an autopsy confirmed the diagnosis of EBV-associated PTLD restricted to the CNS. *Int J Hematol.* 2002;75:416-420.

Key words: Bone marrow transplantation; Posttransplantation lymphoproliferative disorder; Epstein-Barr virus; Real-time PCR; Central nervous system

1. Introduction

Posttransplantation Epstein-Barr virus (EBV)–associated lymphoproliferative disorder (PTLD) is a fatal complication of stem cell transplantation (SCT). According to a recent multi-institutional study, the cumulative incidence of PTLD at 10 years is about 1%, and the associated risk factors are unrelated or HLA-mismatched related donors, T-cell depletion of donor marrow, use of antithymocyte globulin or anti-CD3 monoclonal antibody, and chronic graft-versus-host disease (GVHD) [1]. PTLD can present as localized lymphadenopathy or disseminated disease involving lymph nodes, gastrointestinal tract, liver, kidney, central nervous system (CNS), or bone marrow [2]. Although up to 28.6% of patients with disseminated PTLD have CNS involvement [3], to our knowledge, only 1 pediatric case with isolated CNS PTLD following SCT has been reported [4].

Quantitative real-time polymerase chain reaction (PCR) to detect EBV DNA in the plasma or the peripheral blood cells has been claimed to be a good indicator of EBV-associated PTLD [5,6]. Such a noninvasive examination is

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of diagnostic value, especially when biopsy is difficult to perform.

The case reported here is one with isolated CNS PTLD after bone marrow transplantation (BMT) from an unrelated donor, the diagnosis of which was confirmed by postmortem examination. Real-time PCR failed to detect EBV DNA in the plasma when multiple CNS lesions were demonstrated by magnetic resonance imaging (MRI), although biopsy and postmortem examination confirmed the diagnosis of EBV-associated PTLD.

2. Case Report

A 38-year-old man with chronic myeloid leukemia (CML) in the first chronic phase received an HLA-matched BMT from an unrelated male donor in August 1999. At the pre-transplantation examination, the titers for EB-related antigens in the recipient were as follows: viral capsid antigen (VCA)–immunoglobulin G (IgG) $\times$80, VCA-IgM $<\times$10, early antigen (EA)–IgG $<\times$10, and EB nuclear antigen (EBNA) $<\times$10. Conditioning consisted of cyclophosphamide

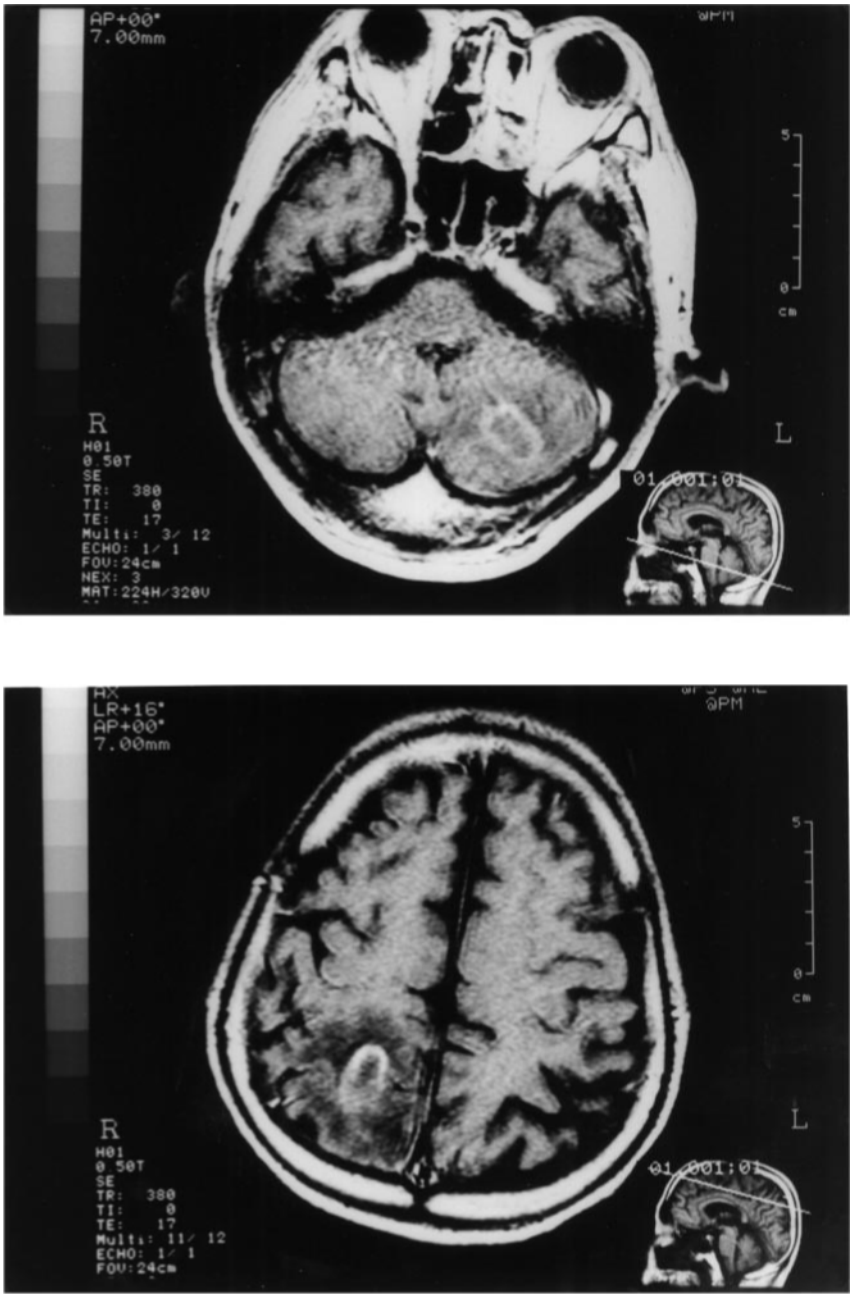

Figure 1. Magnetic resonance imaging (MRI) of the brain at the initial diagnosis demonstrated multiple ringed-enhanced space-occupying lesions. The upper panel shows a lesion in the left hemisphere of the cerebellum, and the lower panel shows one in the right occipital lobe of the cerebrum.

120 mg/kg intravenously (IV) in divided doses and total body irradiation 1200 cGy (Cy-TBI), and GVHD prophylaxis was cyclosporin plus methotrexate. The patient developed grade 3 acute GVHD with cytomegaloviral enteritis and microangiopathy and was treated with methyl-prednisolone 2 mg/kg IV in divided doses daily, with a partial response. The disease evolved to a chronic extensive GVHD with mucocutaneous and ocular involvements that required persistent immune suppression by the administration of corticosteroid and tacrolimus instead of cyclosporin. Platelet concentrates were frequently infused after day 81 because of severe thrombocytopenia and moderate bleeding tendency.

On day 196 after transplantation, when the patient was taking 1 mg of tacrolimus and 25 mg of prednisolone for immunosuppression, the patient had a generalized seizure. At this time the patient's white blood cell count was 8500/mm^3, of which 2% was lymphocytes. Lymphocyte subset analysis of the peripheral blood on day 187 revealed 25/mm^3 CD4, 184/mm^3 CD8, 53/mm^3 CD56, and 3/mm^3 CD19 cells. The titers for EB-related antigens on day 187 were as follows: VCA-IgG $\times$40, VCA-IgM $<\times$10, EA-IgG $<\times$10, and EBNA $<\times$40. MRI of the brain demonstrated multiple, spatially separated, incompletely ringed-enhanced space-occupying lesions in the bilateral parietal lobes, right occipital lobe, and left hemisphere of the cerebellum on T1-weighted images, suggesting necrotic infectious lesions, such as toxoplasmosis and aspergillosis, or tumoral lesions, such as PTLD and metastatic malignancies including recurrent CML (Figure 1). Computed tomography (CT) scans of the chest, abdomen, and pelvis showed no abnormality other than a nodule with a cavity in the upper lobe of the right lung, which later proved to be aspergillosis at autopsy. Both brain biopsy and cytologic examination of the cerebrospinal fluid by lumbar puncture were considered difficult to perform because of low platelet counts, bleeding tendency, and risk of cerebral herniation. Real-time PCR of the plasma was negative for EBV (PCR performed by Otsuka Assay Laboratories, Osaka, Japan, with PCR primers to the BNRF1 region of the EBV genome and a TaqMan probe. The PCR product was detected and quantitated with ABI PRISM 7700 [Applied Biosystems, Foster City, CA, USA]. The number of DNA copies was calculated with the Sequence Detection System ver. 1.6.3. software.). The PCR for BCR-Abl of both peripheral blood and bone marrow cells was also negative on day 211 (15 days after the initial seizure). Because the lesions were considered to be infectious, antitoxoplasmosis treatment was started with pyrimethamine and clindamycin. Although the MRI taken 10 days later revealed progressive disease, invasive diagnostic procedures were still judged to be contraindicated, and amphotericin B plus itraconazole were initiated as antiaspergillosis therapy. The patient gradually developed left hemiparesis, and repeated brain MRIs demonstrated progressive enlargement of both the space-occupying lesions and the edema around them, suggesting antifungal therapy–refractory aspergillosis.

On day 241, a CT-guided stereotactic biopsy of the right parietal lobe lesion was safely performed with 40 units of platelet transfusion, although drainage of abscesses for decompression had been originally planned. Histopathological examination of the biopsy specimens demonstrated

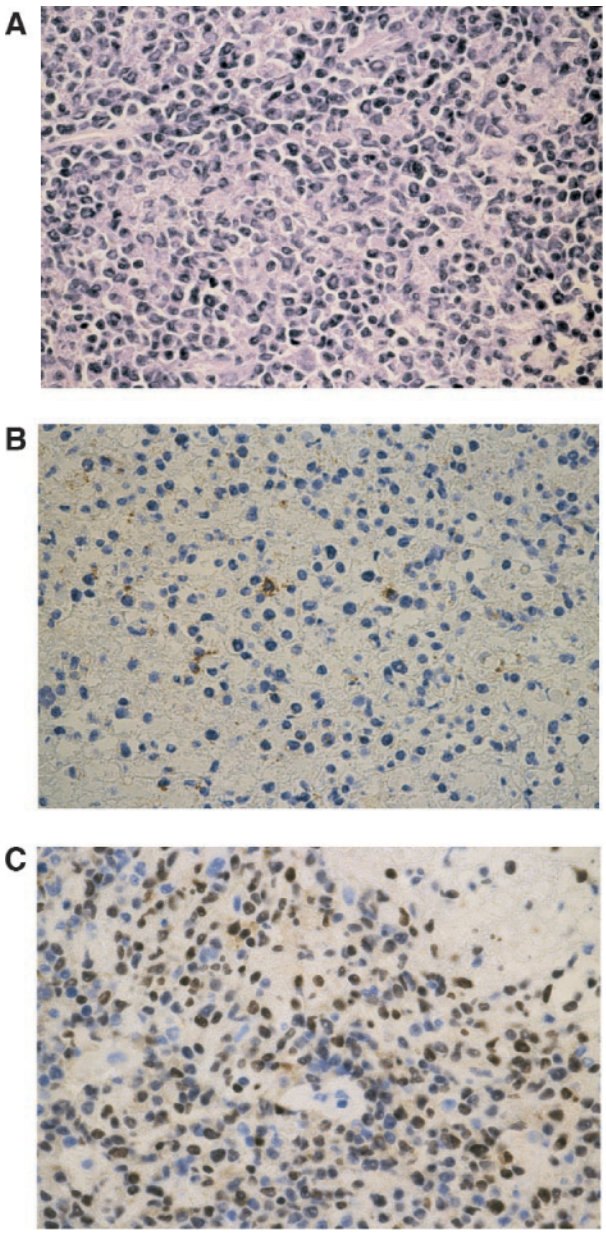

Figure 2. Histopathologic examination of the biopsy specimen. Immunohistochemical analysis was performed on formalin-fixed biopsy specimens using the 3-step indirect immunoperoxidase technique with monoclonal antibodies to Epstein-Barr virus (EBV) nuclear antigen (EBNA-2) and EBV latent membrane protein 1 (LMP-1). A, Hematoxylin-eosin staining: small lymphocytes are present and they show moderate plasmacytic differentiation. There are large areas of necrosis and mild cytologic atypia. B, EBNA-2 positive cells; C, LMP-1 positive cells.

polymorphic PTLD expressing CD19 and CD20. As for EBV-related antigens, latent membrane protein 1 (LMP-1) and EBNA-2 were positively stained, indicating type III latency of EBV infection (Figure 2). Real-time PCR of the serum was again negative for EBV, but that of CD3-negative (and thus B-cell–rich) peripheral blood mononuclear cells (PBMNCs) was positive at the level of 100

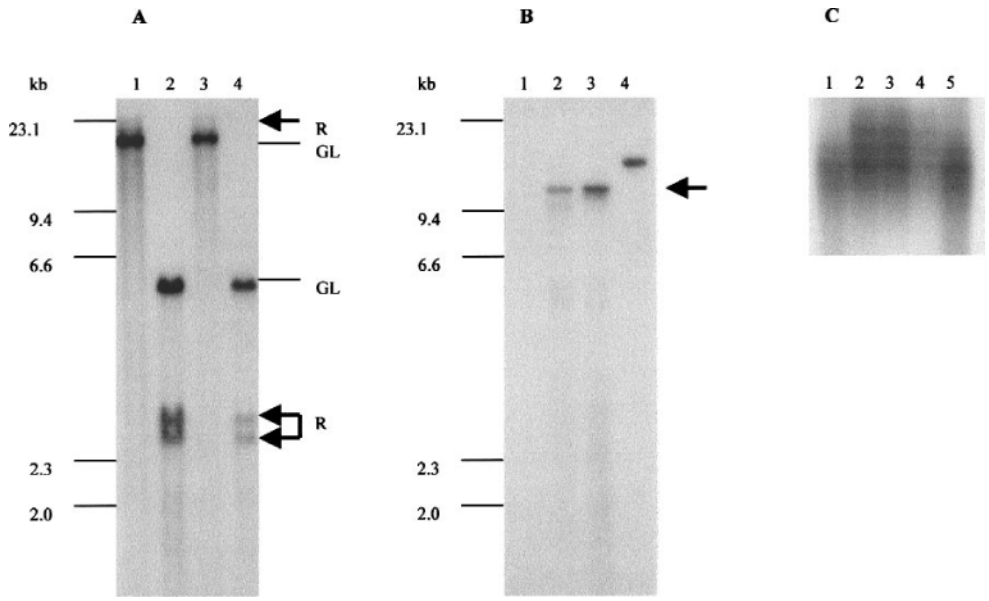

Figure 3. Molecular analyses of the tumors. High–molecular weight DNA was extracted from 2 spatially separated tumors (tumors 1 and 2). Five micrograms of DNA from each sample was digested with *Eco*RI or *Bam*HI, separated by electrophoresis through agarose gel, transferred to nylon membrane, and hybridized to ^{32}P-labeled human immunoglobulin heavy chain (IgH) joining region (J_H) probe or Epstein-Barr virus (EBV)–DNA terminal repeat (TR) probe. A, Southern blot analysis for IgH. Lane 1, *Eco*RI digestion of tumor 1; lane 2, *Bam*HI digestion of tumor 1; lane 3, *Eco*RI digestion of tumor 2; and lane 4, *Bam*HI digestion of tumor 2. R indicates rearranged band; GL, germ-line band. B, Southern blot analysis for EBV TR. Lane 1, normal control; lane 2, tumor 1; lane 3, tumor 2; lane 4, positive control (Raji cell). C, Short tandem repeat (STR) analysis: DNA from bone marrow mononuclear cells obtained before bone marrow transplantation (lane 1) and bone marrow cells (lane 2), tumor 1 (lane 3), tumor 2 (lane 4), and skin (lane 5) (lanes 2 to 5 obtained at autopsy) were amplified with primers for a polymorphic marker on chromosome 5 (D5S669) in the presence of ^{32}P-dCTP, electrophoresed through polyacrylamide gel, and exposed to a film. Lanes 1 and 5 show distinctly different patterns from lanes 2 to 4.

copies/μg DNA on day 242 (the day after biopsy). Despite the use of high-dose methyl-prednisolone, increasing mass effect necessitated emergent treatment with whole-brain irradiation (200 cGy per day). On day 253, before implementation of a donor leukocyte infusion (DLI), the patient died of cerebral herniation.

Autopsy confirmed isolated CNS PTLD, and no other lymphoproliferative lesion outside the CNS was revealed. Southern hybridization for Ig heavy chain (IgH) gene in 2 spatially separated tumors demonstrated the same monoclonal pattern, and hybridization for EBV terminal repeat also demonstrated the same monoclonal pattern (Figures 3A and 3B). Short tandem repeat (STR) analysis by PCR demonstrated that this tumor was of donor origin (Figure 3C).

3. Discussion

According to several observational historical studies on PTLD after SCT, CNS involvement in disseminated PTLD occurred in up to 28.6% of cases and was associated with poor prognosis [3]. Verschuur et al reported the first case with isolated CNS PTLD after BMT, which was not confirmed by postmortem examination [4]. This 4-year-old girl was treated with cerebral irradiation and died of progressive CNS lesions that did not spread outside the CNS.

Recently, adoptive immunotherapy with infusion of unselected donor leukocytes (DLI) or ex-vivo expanded donor-derived EBV-specific cytotoxic T-lymphocytes has proved to be effective for PTLD [7,8]. Emanuel et al reported a case of isolated CNS PTLD after lung transplantation that was successfully controlled with DLI [9].

The use of quantitative real-time PCR for EBV DNA has been suggested as a rapid indicator of PTLD development and a useful noninvasive diagnostic tool [5,6]. According to these studies, regardless of the different sources of DNA, either PBMNCs or plasma, EBV DNA was detected in all cases with PTLD, and the viral load was significantly higher than that of the control group. Davis et al reported a patient with EBV-associated meningoencephalitis and lymphoid interstitial pneumonitis who was examined by semiquantitative competitive PCR for EBV DNA load in PBMNCs [10]. In that case, although the viral load was relatively low (80 copies/μg DNA), the authors confirmed the potential utility of PCR examination in the diagnosis and monitoring of response to therapy.

The histopathologic and molecular analysis results of the biopsy and autopsy specimens, including monoclonality of both EBV and IgH genes, type III latency of EBV infection, and donor origin of the tumor, all indicate that our case is typical EBV-associated PTLD. Yet, the EBV DNA loads in plasma (day 211) and serum (day 242) were at undetectable levels and the load in the B-cell–rich fraction of PBMNCs (day 242) was low and only comparable to that of the immunocompetent EBV-seropositive control

group reported by Kimura et al [5]. These results suggest that this method might be of limited diagnostic value in some cases of PTLD, such as those confined to the CNS.

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