

Case Study

Unrelated Bone Marrow Transplantation Induced Long-Term Remission in a Patient with Life-Threatening Epstein-Barr Virus-Associated Hemophagocytic Lymphohistiocytosis

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We present a case of life-threatening Epstein-Barr virus (EBV)-associated hemophagocytic lymphohistiocytosis (HLH) with severe hepatitis that was successfully treated by allogeneic stem cell transplantation from an unrelated donor. A 26-year-old woman was admitted to hospital with a high fever and liver dysfunction. Laboratory tests, including bone marrow aspiration, revealed severe HLH that occurred after EBV infection. High-dose methylprednisolone and etoposide therapy did not control the disease. We could control the HLH, but the EBV viremia continued following the CHOPE (cyclophosphamide, doxorubicin, vincristine, prednisone, and etoposide) chemotherapy regimen. Therefore, the patient underwent allogeneic bone marrow transplantation from an HLA-matched, unrelated donor. The patient has remained in good condition without disease recurrence for 2 years after bone marrow transplantation. Although there is no consensus regarding allogeneic stem cell transplantation for EBV-HLH, it is the treatment of choice for aggressive EBV-HLH when the patient is refractory to intensive chemotherapy. [*J Clin Exp Hematopathol* 51(1) : 57-61, 2011]

Keywords: Epstein-Barr virus, hemophagocytic lymphohistiocytosis, bone marrow transplantation

INTRODUCTION

Epstein-Barr virus (EBV) infection commonly occurs in early childhood. EBV is the causative agent of infectious mononucleosis, which is usually self-limiting and resolves spontaneously after the emergence of EBV-specific immunity. In a limited number of individuals, infectious mononucleosis develops into life-threatening, EBV-associated hemophagocytic lymphohistiocytosis (EBV-HLH). In this syndrome, patients present with prolonged fever, hepatosplenomegaly, pancytopenia, and disseminated intravascular coagulation; hemophagocytosis is found on bone marrow examination.¹⁻⁵

A review of published cases of 219 children diagnosed with infection-associated hemophagocytic syndrome before 1996 revealed that more than half were from the Far East. EBV was the triggering virus in 74% of the children in whom an infectious agent was identified. Overall mortality in patients with EBV-HLH was 73%.⁶ Peripheral blood T cells harbor EBV in life-threatening EBV-HLH, leading to high mortality rates with multiple organ failure, including hepatic failure.

Despite immunochemotherapy with a corticosteroid, cyclosporin A (CyA), and etoposide (ETP), as used in the HLH-94 regimen developed by a study group of the Histiocyte Society, some patients with aggressive EBV-HLH have a poor prognosis.^{4,5,7} Questions remain about how to define refractory patients with EBV-HLH and to improve the clinical outcome. Here, we present a case of EBV-HLH with severe hepatitis that was successfully treated by allogeneic bone marrow transplantation (BMT) from an unrelated donor.

CASE REPORT

A 26-year-old Japanese woman complaining of a persistent fever lasting for 3 days was admitted to Komaki City

Received : September 8, 2010

Revised : December 12, 2010

Accepted : February 4, 2011

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Hospital. This patient had been healthy until the disease onset. There was no family history of immunohematologic disorders. Peripheral blood counts showed pancytopenia (white blood cells [WBC], $0.7 \times 10^9/L$; hemoglobin, 10.3 g/dL; hematocrit, 28.9%; and platelets, $23 \times 10^9/L$) with atypical lymphocytes (26%). Coagulation studies revealed a prothrombin time of 20.2 sec (control, 11.2 sec), an activated partial thromboplastin time of 74.8 sec (control, 34 sec), a fibrinogen level of 279.0 mg/dL, and a D-dimer level over 100.0 $\mu\text{g/mL}$. Transaminase (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]), lactate dehydrogenase (LDH), and total bilirubin were increased to 569 IU/L, 133 IU/L, 3,961 IU/L, and 10.1 mg/dL, respectively. A bone marrow study showed hemophagocytic histiocytes. The EBV titers based on enzyme-linked immunosorbent assay (ELISA)

indicated that this was the first infection by the virus (EBV viral capsid antigen [VCA] immunoglobulin G [IgG], 1.3; VCA IgM, 7.4; EBV nuclear antigen [EBNA], 0.2). The quantitative polymerase chain reaction (PCR) result for EBV DNA was high (22,000 copies/ 10^6 cells) in the peripheral blood lymphocytes (PBL). The patient was diagnosed with EBV-HLH. Consecutive and combined administration of antibiotics and methylprednisolone (mPSL) only transiently abated the fever. Because transaminase (AST and ALT), LDH, and total bilirubin were increased to more than 11,160 IU/L, 2,670 IU/L, 30,570 IU/L, and 16.4 mg/dL, respectively, and pancytopenia remained, twice weekly ETP administration was started on day 27 after admission (Fig. 1).

The patient was referred to Meitetsu Hospital on day 45 after admission because of persistent fever and pancytopenia.

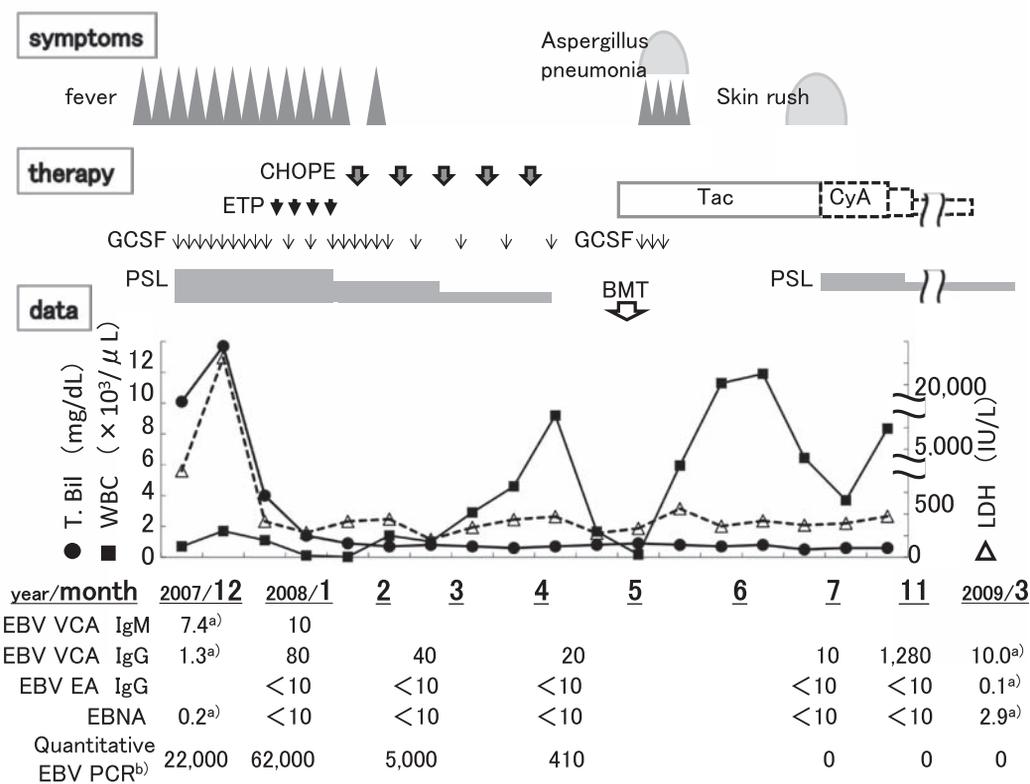


Fig. 1. Clinical course of a patient with life-threatening Epstein-Barr virus (EBV)-associated hemophagocytic lymphohistiocytosis. The time course of intravenous infusion of etoposide (ETP, *black bold arrow*), and subcutaneous injection of granulocyte colony-stimulating factor (GCSF, *black fine arrow*), CHOPE (cyclophosphamide, doxorubicin, vincristine, prednisolone, and ETP; *gray arrow*) chemotherapy, and bone marrow transplantation (BMT, *black blank arrow*), is shown. The administration of prednisolone (PSL, *gray bar*), tacrolimus (Tac, *gray blank bar*), and cyclosporine A (CyA, *black blank bar*) is also shown. WBC indicates white blood cell; T. Bil, total bilirubin; LDH, lactate dehydrogenase. The EBV titers are based on folic acid assay except a).

Abbreviations: EBV VCA IgM, EBV viral capsid antigen immunoglobulin M; EBV VCA IgG, EBV viral capsid antigen immunoglobulin G; EBV EA IgG, anti-early antigen IgG against EBV; EBNA, EBV nuclear antigen; PCR, polymerase chain reaction.^{a)} The EBV titers are based on enzyme-linked immunosorbent assay; ^{b)} quantitative PCR for EBV DNA (copies/ 10^6 cells) in the PBL.

Peripheral blood counts with daily administration of granulocyte colony-stimulating factor showed a WBC count of $0.1 \times 10^9/L$, a hemoglobin level of 9.4 g/dL, a hematocrit of 28.3%, and a platelet count of $60 \times 10^9/L$. Blood chemistry findings were as follows: AST, 49 IU/L; ALT, 39 IU/L; LDH, 737 IU/L; total bilirubin, 0.4 mg/dL; and ferritin, 2,127 ng/mL. A clot specimen obtained by bone marrow aspiration to determine the cause of the pancytopenia confirmed the pathologic diagnosis of remaining EBV-HLH (*in situ* hybridization for EBV-encoded RNA [EBER] positive) and bone marrow suppression by ETP (Fig. 2). The quantitative PCR result for EBV DNA remained high (62,000 copies/ 10^6 cells) in the PBL. Southern blotting of bone marrow blood mononuclear cell-derived DNA probed with the terminal repeats of EBV showed polyclonal bands. Surface marker analysis of the bone marrow blood revealed that the rates of cells positive for CD2, CD3, CD4, CD8, CD19, CD20, and CD56 were 92.9%, 71.5%, 26.4%, 48.4%, 11.3%, 6.5%, and 46.5%, respectively. To determine which cells were infected with EBV, peripheral blood mononuclear cells were fractionated into CD3⁺ T cells, CD4⁺ T cells, CD8⁺ T cells, CD19⁺ B cells, and CD56⁺ NK cells using an immunobead method (DynaBeads, Dynal AS, Oslo, Norway) and analyzed by quantitative PCR as previously described.⁸ The results indicated that EBV infected predominantly B cells and CD8⁺ T cells.

Chemotherapy with CHOPE (cyclophosphamide, doxorubicin, vincristine, prednisolone [PSL], and ETP) was promptly started for refractory EBV-HLH. After the first CHOPE therapy, the patient's pancytopenia improved, but EBV-HLH remained evident on the smear findings from the bone marrow aspiration, and the quantitative PCR result for EBV DNA remained high (27,000 copies/ 10^6 cells) in the PBL. Therefore, we considered that the EBV-HLH was so

severe that allogeneic BMT was necessary to achieve remission.

We chose a reduced-intensity regimen for BMT because the patient's cardiac function was reduced without coronary disease after 4 cycles of CHOPE. Five months after the disease onset, the patient was placed on a reduced-intensity conditioning regimen comprising 1,500 mg/m² cyclophosphamide for 2 days and 25 mg/m² fludarabine for 4 days and she was transplanted with bone marrow stem cells from an unrelated HLA-matched male donor ($3.47 \times 10^6/kg$ nucleated cells). The EBV titers of her donor, based on folic acid assay, indicated that the donor had a past infection (EBV VCA IgG, $\times 40$; VCA IgM, < 10 ; EBV EBNA, $\times 10$; anti-early antigen IgG against EBV, < 10). The prophylaxis for graft-versus-host disease (GVHD) comprised a combination of tacrolimus (Tac) and mini-dose methotrexate. Absolute neutrophil and WBC counts exceeded $0.5 \times 10^9/L$ and $1.0 \times 10^9/L$, respectively, on day 12 after transplantation. Chromosomal analysis using fluorescence *in situ* hybridization indicated complete engraftment on day 29 of transplantation, and EBV in the PBL disappeared on day 30 of transplantation. Complications of Aspergillus pneumonia, acute grade 2 GVHD of the skin, and asymptomatic reactivation of cytomegalovirus infection were all controlled, although Tac was changed to CyA and PSL owing to skin eruption indicative of drug allergy. We gradually decreased PSL and CyA, and stopped administration of CyA 9 months after BMT. The change in EBV-specific antibody titers showed a seroconversion (VCA IgG, from 1.3 to 10.0; EBNA, from 0.2 to 2.9, both using ELISA) when she took 5 mg/day PSL 10 months after BMT. The data for the EBV titer and quantitative PCR for EBV DNA are shown in Fig. 1. The patient has been doing well in the 2 years since the BMT.

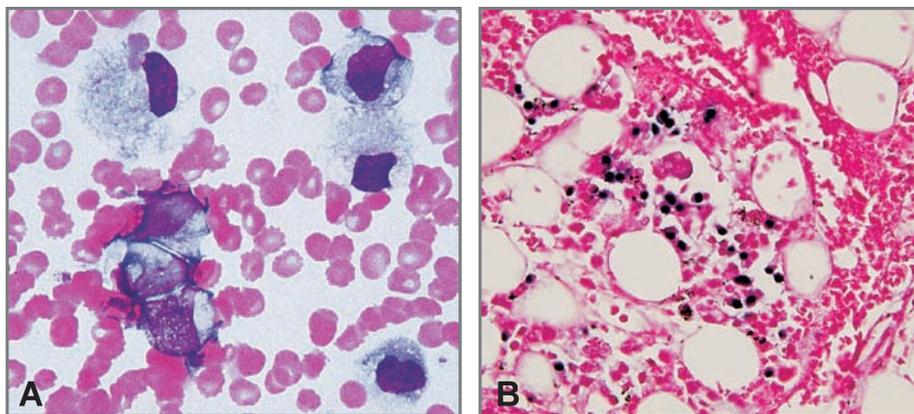


Fig. 2. Histological findings of bone marrow aspiration. (2A) The smear of bone marrow aspirate showed histiocyte with phagocytosis and reactive lymphocytes (May-Giemsa staining). (2B) *In situ* hybridization for Epstein-Barr virus-encoded RNA (EBER) in bone marrow clot sections. EBER expression was identified in nuclei of bone marrow cells.

DISCUSSION

EBV-HLH occurs in both children and adults, and the heterogeneity of its clinical course is striking, ranging from self-limiting in some patients to severe/aggressive and fatal in others. Previous data suggest that adult age, EBV reactivation, severe-type disease, and multidrug chemotherapy are all associated with a poor clinical outcome in patients with this HLH subtype.⁹ One of the most important life-threatening complications and prognostic factors in EBV-HLH cases is hepatic failure as a consequence of severe hepatitis. Hepatitis due to primary EBV infection is usually mild and self-limited. Severe hepatitis is infrequent at the time of primary EBV infection in immunocompetent individuals. The severity of the liver damage seems to be linked not only to elevated EBV viremia, but also to preponderant infection with CD8 T cells infiltrating the liver.¹⁰ Although we could not perform a liver biopsy because the patient suffered severe pancytopenia and coagulation disorders, prompt immunotherapies such as mPSL, ETP, and CHOPE were keys to successful treatment for the patient to control severe hepatitis. In particular, the prompt use of ETP greatly improves the outcome in both pediatric and adult patients. The efficacy of ETP was confirmed by findings from a larger series of 47 patients with EBV-HLH in which none of 33 patients who received more than 4 doses of ETP died.¹¹ The addition of ETP from day 27 might have partially controlled the severe hepatitis and life-threatening EBV-HLH in our case.

Hematopoietic stem cell transplantation (HSCT) is recommended for patients with genetic forms and for patients with recurrent, persistent, or refractory severe disease. BMT recipients have a significantly superior outcome compared with non-transplanted patients with a 66% versus 10.1% survival rate. When combining chemotherapy and BMT, the probability of survival at 3 years is 55% for all cases.⁶ It is reported that 8 of 10 patients with EBV-HLH received allogeneic HSCT within 1 year of onset in a nationwide survey in Japan. The event-free survival rate following allogeneic HSCT is 0.614 ± 0.186 .¹² Thus, HSCT for severe EBV-HLH cases is effective and potentially curative, but the strategies for making the decision regarding HSCT in the treatment of EBV-HLH are not well established. The appropriate timing and criteria for HSCT in EBV-HLH treatment remain to be determined. Some treatment strategies and prognostic factors or scores have been proposed.^{10,13} There are also some retrospective studies and case reports of HSCT for EBV-HLH,^{5,14-18} but most cases that received HSCT were refractory to primary immunochemotherapy or had disease relapse. In addition, the choices of conditioning regimens and stem cell sources for HSCT for EBV-HLH remain unclear. Caution must be taken regarding long-term complications of HSCT, such as sterility and growth disturbances, because many cases are in children and adolescents. There is currently no consen-

sus about the optimal stem cell source; nevertheless, this disorder is an active viral infection in the background of immune dysregulation. There are some reports of successful cord blood transplantation for this disease, and cord blood might serve as a stem cell source, especially if viral memory has not been established.^{15,16,18} Quantitative PCR for EBV DNA can be used to decide on the treatment because the detection of EBV-DNA by PCR is an important prognostic factor.¹⁹ On the basis of the smear findings from bone marrow aspiration and high level of EBV-DNA in PBLs in our case, we assessed the presence of EBV-HLH after the first CHOPE therapy. HSCT therapy was successful and the patient has been in remission for 2 years. Clear criteria for HSCT treatment in this disease are lacking, which adds to the confusion, and more EBV-HLH cases need to be accumulated to analyze the prognostic factors and results of each treatment. HSCT is associated with a high risk of therapy-related mortality and a reduced quality of life; thus, the therapeutic options should be carefully considered. Nevertheless, HSCT is a promising option for patients refractory to immunotherapy and/or intensive chemotherapy.

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