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Case Report

NK-cell post-transplant lymphoproliferative disease with chronic active Epstein–Barr virus infection-like clinical findings

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ABSTRACT

A 69-year-old man who underwent cord blood transplantation seven years ago was admitted because of fever, elevated liver enzymes and thrombocytopenia. Bone marrow aspirate revealed hemophagocytic lymphohistiocytosis. Viral capsid antigen (VCA)-immunoglobulin (Ig) G, VCA-IgM, VCA-IgA, Epstein–Barr virus nuclear antigen-IgG, early antigen-diffuse-type and restricted-type (EA-DR) IgG, and EA-DR IgA titers were 2560, <10, 10, 40, 40, and <10, respectively. Real-time polymerase chain reaction assay of peripheral whole blood for Epstein–Barr virus-deoxyribonucleic acid (EBV-DNA) revealed 240,000 copies/μg DNA. Flow cytometric *in situ* hybridization assay confirmed that EBV-infected cells were NK-cells. Clonality evaluation by Southern blot assay of EBV-DNA terminal repeats proved to be bi-clonal. Accordingly, we made a diagnosis of NK-cell post-transplant lymphoproliferative disease with chronic active EBV infection-like clinical findings (CAEBV-like NK-cell PTLD). Although CAEBV-like PTLD is extremely rare, its prognosis seems to be very poor. The disease should be considered in such patients who present persistent or recurrent infectious mononucleosis-like symptoms after transplantation.

Case report

A 69-year-old man underwent cord blood transplantation (CBT) for mixed phenotype acute leukemia seven years ago. During CBT, viral capsid antigen (VCA)-immunoglobulin (Ig) G, VCA-IgM, and Epstein–Barr virus nuclear antigen (EBNA)-IgG titers were 80, <10, and <10. Real-time polymerase chain reaction (PCR) assay of Epstein–Barr virus-deoxyribonucleic acid (EBV-DNA) was negative. He completed immunosuppressive agents 7 months after CBT and had no signs of graft-versus-host disease or relapse thereafter. However, approximately 2 years ago, he began frequently complaining of recurrent aphthous ulcers, swelling after mosquito bites, and papules on the head. The mosquito bites caused only swelling but not fever or ulceration. The papules quickly collapsed, became crusted, and fell off. He experienced episodes of fever, mild elevation in the transaminase levels [maximum aspartate aminotransferase (AST) levels of 120 U/mL and alanine aminotransferase (ALT) levels of 154 U/mL], and mild thrombocytopenia (minimum 9.7×10^{10}/L) twice within a year, which spontaneously resolved. He was admitted to our hospital because of fever, elevated liver enzyme levels [AST 251 U/mL, ALT 439 U/mL, lactate dehydrogenase (LDH) 643 U/mL, γ-glutamyl transpeptidase (GTP) 1040 U/mL, and total bilirubin (T-bil) 3.8 mg/dL], and thrombocytopenia (4.5×10^{10}/L) 1 year ago. Cholangitis was suspected, and esophagogastro-duodenoscopy was performed. PCR assay of a biopsy specimen obtained from a duodenal ulcer tested positive for EBV-DNA of 6900 copies/μg DNA. However, histopathological examinations showed no malignancy. VCA-IgG, VCA-IgM, and EBNA-IgG titers were 1280, <10, and 10. Bone marrow aspirate (BMA) revealed hemophagocytosis. PCR assay of peripheral blood for EBV-DNA was not performed. He was treated with methylprednisolone (1 mg/kg) and completely recovered. One year later, he developed fever and anorexia. Laboratory examination revealed thrombocytopenia (6.3×10^{10}/L), elevated liver enzyme levels (AST 217 U/L, ALT 179 U/L, LDH 786 U/L, γ-GTP 140 U/L, and T-bil 1.0 mg/dL), and high serum ferritin value (9,831.4 ng/mL). PCR assay of peripheral whole blood for EBV-DNA revealed 240,000 copies/μg DNA. VCA-IgG, VCA-IgM, VCA-IgA, EBNA-IgG, early antigen-diffuse-type and restricted-type (EA-DR) IgG, and EA-DR IgA titers were 2560, <10, 10, 40, 40, and <10, respectively. He had hyperimmunoglobulinemia E at 5125 IU/mL. 18F-fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT) did not reveal any abnormal FDG accumulation. Mild

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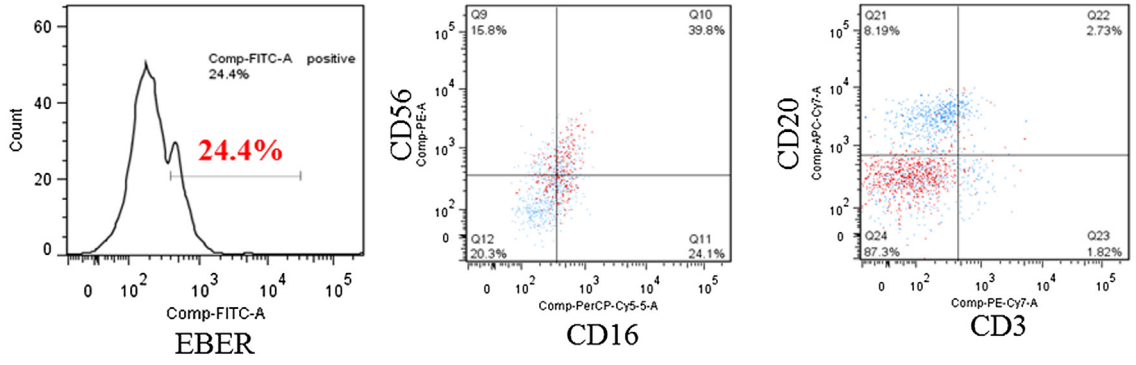
splenomegaly was observed. BMA revealed marked hemophagocytosis and increased large granular lymphocytes. A chimerism analysis of peripheral blood with short-tandem repeat polymorphisms showed complete CBT donor type. Flow cytometry of the bone marrow and peripheral blood revealed increased NK-cells (negative for surface CD3 and positive for CD2, CD7, CD16, CD56, and HLA-DR). T-cell receptor Cβ1 and Jγ gene rearrangements were not detected in Southern blot assay of peripheral blood cells. Clonality evaluation by Southern blot assay of EBV-DNA terminal repeats (EBV-DNA TRs) proved to be bi-clonal. EBV-encoded small RNA (EBER)-*in situ* hybridization assay of the BMA clot was focally positive. CD56 could not be stained because the sample detached from the slides during staining procedures. We performed flow cytometric *in situ* hybridization assay to identify the infected cells (tested at Nagoya University, Nagoya, Japan) (Kimura et al., 2009). In the lymphocyte region, 24.4% of cells were positive for EBER. EBER-positive lymphocytes were also positive for CD16 and CD56 (Figure 1A). To rule out familial hemophagocytic lymphohistiocytosis (HLH), we performed targeted next generation sequencing of 10 selected genes *AP3B1*, *BLOC1S6*, *LYST*, *PRF1*, *RAB27A*, *SH2D1A*, *STX11*, *STXBP2*, *UNC13D*, and *XIAP*. A heterozygous synonymous mutation in *UNC13D* (c. 2499C > T/p. Ala833Ala) was detected, but the clinical significance was not reported in ClinVar database (National Center for Biotechnology Information, 2019). Accordingly, we made a diagnosis of NK-cell post-transplant lymphoproliferative disease with chronic active EBV infection-like clinical findings (CAEBV-like NK-cell PTLD). We initiated treatment with methylprednisolone (1 mg/kg), following which hemophagocytosis subsided. We gradually reduced the methylprednisolone or prednisolone dose thereafter. However, EBV-DNA load persisted at high levels. HLH relapsed 7 months after initiating steroid therapy. Currently, we are treating this patient with etoposide and cyclosporin (Figure 1B).

PTLDs occur in patients after solid organ or hematopoietic stem cell transplantation. EBV is recognized as a causative factor of PTLDs. Although most of EBV associated PTLDs are B-cell types, T- and NK-cell types can rarely occur. Swerdlow (2007) reviewed 130 T-cell and NK-cell PTLDs. T-cell and NK-cell PTLDs occurred at a median of 66 months following transplantation. The commonest types reported were peripheral T-cell lymphoma, unspecified, and hepatosplenic T-cell lymphoma. Extranodal NK/T-cell lymphoma nasal-type or aggressive NK-cell leukemia were less frequently reported. CAEBV-like PTLD is extremely rare, and to our knowledge, only two similar cases have been previously reported. Yui et al. (2016) reported T-cell type CAEBV-like PTLD after CBT for AML. Arai et al. (2012) reported the recurrence of T-cell CAEBV



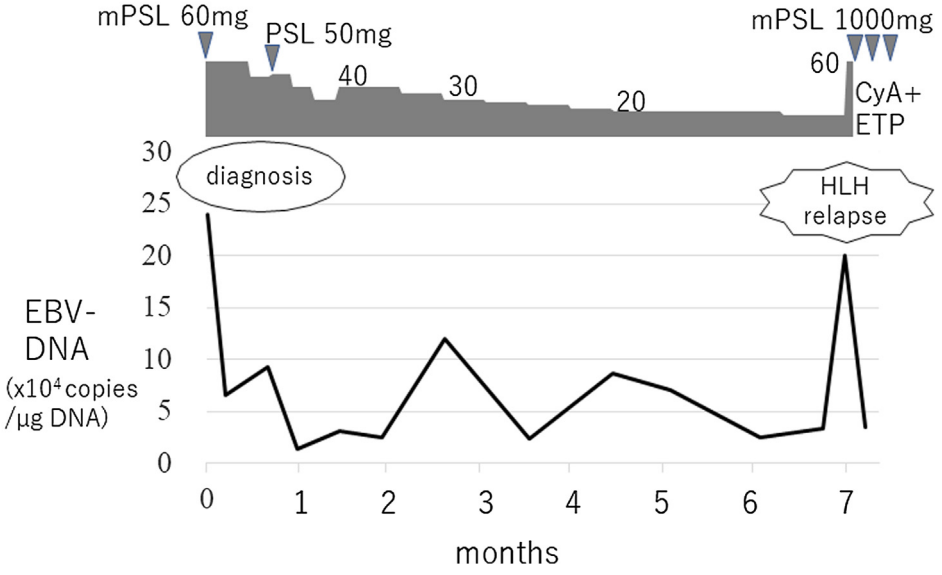


Figure 1. (A) Flow cytometric in situ hybridization assay of peripheral blood sample obtained 3 weeks after we initiated steroid therapy. Red dots show EBER-positive cells. In the lymphatic region, 24.4% of cells were EBER-positive cells. The EBER-positive cells were positive for CD16 and CD56 and negative for surface CD3 and CD20. (B) Clinical course. Abbreviations; mPSL, methylprednisolone; PSL, prednisolone; CyA, cyclosporin A; ETP, etoposide.

from donor cells after allogeneic bone marrow transplantation. We consider that it is possibly CAEBV-like PTLD.

CAEBV is characterized by chronic or recurrent infectious mononucleosis–like symptoms that persist over a long time and by an unusual pattern of anti-EBV antibody titers. Clonal expansion of EBV-infected cells is associated with the cause of CAEBV (Kimura et al., 2001). Because the subtypes of EBV-infected lymphocytes affect clinical manifestations and outcomes, the identification of EBV-infected cell type is important (Kimura et al., 2012).

Kimura et al. (2012) and Okano et al. (2005) proposed diagnostic criteria for CAEBV: (i) illness for >3 months in duration; (ii) increased amounts of EBV detected by Southern blot hybridization or EBER cells in affected tissues or peripheral blood ($>10^{2.5}$ copies/μg of EBV-DNA in peripheral blood mononuclear cells); and (iii) no evidence of previous immunological abnormalities or other recent infection that might explain the observed conditions. These criteria were compatible with the definition of CAEBV described by the World Health Organization in 2017 (Swerdlow et al., 2017). In our patient, primary EBV infection was ruled out because he already had been infected for at least one year before diagnosis, although we could not clarify whether EBV was newly re-infected or transmitted by the endogenous EBV after CBT. NK-cell leukemia/lymphoma was ruled out based on the morphology of expanding cells and no abnormal uptake on FDG-PET/CT examination. Besides abnormal immunological condition following transplantation, because the patient satisfied the diagnostic criteria, we made a diagnosis of CAEBV-like PTLD.

The expanding cells originated from CBT donor cells; however, we did not know the current condition of the CBT donor, although we consulted the cord blood bank. He had symptoms mimicking hypersensitivity to mosquito bites or hydroa vacciniforme-like eruptions, which sometimes accompany NK-cell CAEBV. Hyperimmunoglobulinemia E was also compatible with features of NK-cell CAEBV. As CAEBV is rare in the elderly, we considered that the immune-suppressed condition developed after transplantation and/or the CBT donor factor, or other factors were probably associated with the incidence of CAEBV-like disease. *PRF1* and *STXBP2* gene mutations were reported in CAEBV (Cohen et al., 2015), but we did not identify any mutation in the selected genes. Recently, frequent intragenic deletion of the EBV genome in CAEBV patients was reported (Okuno et al., 2019). Whether the mutation in the EBV genome is involved in the development of CAEBV-like PTLD is a very interesting issue that needs to be elucidated in the future.

The prognosis of adult-onset NK-cell CAEBV is poor (Kawamoto et al., 2018). Thrombocytopenia, HLH, and EBNA-IgG titers >40 were associated with poor prognosis. Patients treated with allogeneic stem cell transplantation (allo-SCT) had better survival. However, the risk for allo-SCT is very high. Moreover, there is no evidence whether CAEBV-like PTLD has an equally poor prognosis as sporadic adult-onset CAEBV; thus, whether we should perform allo-SCT needs to be carefully considered.

In summary, we encountered a patient who developed NK-cell PTLD with CAEBV-like clinical findings after CBT. Although it is a very rare type of PTLD, its cases should increase as the allo-SCT increases. We must further investigate its pathology and treatment.

Competing interests

The authors declare no conflict of interest in association with the present study.

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Ethical approval

Written informed consent was obtained from the patient for the publication of this case report.

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