INTERNAL MEDICINE

□ CASE REPORT □

Recurrence of Chronic Active Epstein-Barr Virus Infection from Donor Cells after Achieving Complete Response Through Allogeneic Bone Marrow Transplantation

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Abstract

We report the case of a 35-year-old woman with chronic active Epstein-Barr virus (EBV) infection (CAEBV). She underwent allogeneic bone marrow transplantation (BMT) from an unrelated male donor and achieved a complete response. However, her CAEBV relapsed one year after BMT. EBV-infected cells proliferated clonally and revealed a 46XY karyotype. In addition, the infecting EBV strain differed from that detected before BMT. These findings indicated that her disease had developed from donor cells. This is the first report of donor cell-derived CAEBV that recurred after transplantation, suggesting that host factors may be responsible for the development of this disease.

Key words: chronic active Epstein-Barr virus infection, bone marrow transplantation, systemic lupus erythematosus

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Introduction

Epstein-Barr virus (EBV) can be detected not only in B-cell tumors but also in T- and NK-cell tumors, which are known as EBV-positive T/NK-cell lymphoproliferative diseases (EBV-T/NK-LPDs). EBV-T/NK-LPDs comprise extranodal NK/T-cell lymphoma nasal type (ENKL), aggressive NK-cell leukemia, and chronic active EBV infection (CAEBV). CAEBV is a rare disorder accompanied by the clonal proliferation of EBV-infected cells (1). Its T-cell infecting type is designated as "EBV-positive T-cell lymphoproliferative disease of childhood" in the WHO classification revised in 2008 (2). However, adult-onset cases have been reported (3, 4).

The pathogenesis of CAEBV is assumed to be due to the EBV infection of T or NK cells followed by their immortalization and expansion. However, the mechanisms responsible for the clonal expansion of infected cells remain unclear.

We report here the case of CAEBV in a female patient. In spite of achieving a complete response (CR) after bone marrow transplantation (BMT), CAEBV recurred. At recurrence, the infected cells were clonally proliferating donor cells, and the infecting virus differed from that originally causing the disease. We describe her clinical course and discuss the possible pathological mechanism responsible for the recurrence.

Methods

The detection and isolation of infected cells (5) and sequence analysis for *perforin* (6) were performed as de-

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scribed previously. For the sequence analysis of the variable region of *lmp1*, the genomic DNA extracted from infected cells was amplified by PCR. The following primers were used: 5-AAGGGAGTGTGTGCCATTAAG-3 (fwd) and 5-ACCCCCACTCTGCTCTCAA-3 (rev); their nucleotide positions in B95.8 (Genbank No.V01555) were 168052-168073 and 168619-168601, respectively. The conditions for PCR reactions were as follows: 94℃ for 5 minutes, 94℃ for 15 seconds, 60℃ for 30 seconds, and 72℃ for 60 seconds; 35 cycles. The amplicon was directly sequenced using the same primers. The ethics committee of Tokyo Medical and Dental University Hospital approved this study, and written informed consent was obtained from the patient.

Case Report

A 35-year-old woman developed fever and cervical lymphadenopathy and was transferred to our hospital. She had systemic lupus erythematosus (SLE) for the previous 13 years and was receiving prednisolone (PSL) at 5 mg/day. Her anti-EBV antibodies on admission were 1:20,480 for anti-VCA-IgG and 1:1,280 for anti-EA-DRIgG, which were extremely elevated. Anti-VCA-IgM was undetectable, and the titer of anti-EBNA was 1:40. EBV DNA copy numbers in peripheral blood (PB) were elevated to 1×10^7 copies/μg DNA.

EBV-positive T-cell lymphoproliferative disease was diagnosed by cervical lymph node biopsy (Fig. 1A). Infiltrating cells were positive for CD8, Granzyme B, and EBER (Fig. 1B-D). In addition, activated $CD8^+$ cells were increased in the PB (Fig. 1E). These cells were EBV-positive; they were clonally similar to those in the lymph node, which involved a *TCRJβ1* gene rearrangement (Fig. 1F-H).

Chemotherapy was administered followed by BMT as described by Koyama et al (7). The donor was a 29-year-old unrelated male. His HLA type was A2 (0207) / A26 (2602), B46 (1501) / B62 (4601), and DR14 (1403) / DR14 (1406). The patient's type was A2 (0207) / A26 (2602), B46 (1501) / B62 (4601), and DR14 (1401) / DR14 (1401). Their serologic HLA types were identical, whereas the DNA types displayed disparities in 2 HLA-DR alleles.

The conditioning regimen for transplantation comprised fludarabine (37.5 mg/m^2 intravenously, once daily from days -6 to -2), melphalan (60 mg/m^2 intravenously, once daily from days -6 to -5), and total body irradiation (4 Gy in 2 fractions on day -1). Cyclosporine (3 mg/kg, from day -1) and short-term methotrexate (5 mg, 10 mg, and 10 mg on days 1, 3, and 6, respectively) were administered for the prophylaxis of acute graft-versus-host disease. Engraftment was confirmed 1 month after BMT, and the EBV genome in PB became undetectable after 2 months and remained so for nearly 12 months.

Although graft-versus-host disease had not developed, administration of low-dose corticosteroid (hydrocortisone, 10 mg/day) was continued to compensate for her endogenous cortisol deficiency due to the long-term administration of PSL. One year later, her EBV DNA level began to increase and reached 1.7×10^4 copies/μg DNA. Three years after BMT, it was 1.0×10^5 copies/μg DNA, and the number of CD8-positive cells had increased among her PB mononuclear cells (PBMC; Fig. 2A).

Infected cells in PB were investigated again; these were identified as CD8-positive T cells. Their clonality was confirmed by detecting a *TCRJβ1* gene rearrangement, which revealed a difference from the original (Fig. 1I). EBV-infected cells (Fig. 2B) and a lymphoblastoid cell line (LCL) established from the patient's PBMC soon after engraftment (Fig. 2C) had XY karyotype, confirming that these were donor cells. Furthermore, sequence analysis of the variable region of *lmp1* showed that the infecting virus differed from that detected in CD8-positive cells before BMT and was identical to that detected in LCL (Fig. 2D). Although we did not examine whether the donor was seropositive for EBV, the virus obtained from LCL might have been of donor origin.

Liver dysfunction developed gradually 4 years after BMT. Liver biopsy was performed, and a significant sinusoidal infiltration of atypical cells (CD8- and EBV-positive) was detected (Fig. 3A-C). Her PBMC retained the 46XY karyotype (Fig. 3D) and mainly comprised activated CD8-positive cells. In addition, CD4-positive cells were detected (Fig. 3E).

The EBV DNA copy numbers, the chimerisms of nucleated cells and lymphocytes, and the percentage of CD4- and CD8-positive cells in peripheral blood are summarized in Table 1. The chimerism maintained the donor type during the clinical course. An abnormal XXYY clone suggesting donor origin appeared 4.5 years after BMT as the disease progressed. From these results, the diagnosis of CAEBV, which developed from donor cells infected with a different virus, was confirmed.

Discussion

The mechanisms responsible for CAEBV development have not been elucidated. Some investigators reported that EBV-infected T or NK cells could be detected during primary infection (8, 9), indicating that EBV could infect these cells under a high level of viral load. However, some factors leading to disease development may exist because CAEBV shows a marked geographic preference for East Asia. Although the strains identified in the present patient before and after BMT were not identical, the relationship between strains and disease development needs to be investigated. In addition, a patient's genetic background may be involved. In our patient, recurrence after BMT underlines the importance of non-hematological factors for disease development.

According to Ohshima et al, following infection with EBV, T, or NK cells can undergo poly-, oligo-, or monoclonal expansion, resulting in CAEBV (10). For the expansion of EBV-infected T or NK cells, suppression of cytotoxic T-cell (CTL) activity may play an important role. Sugaya et al

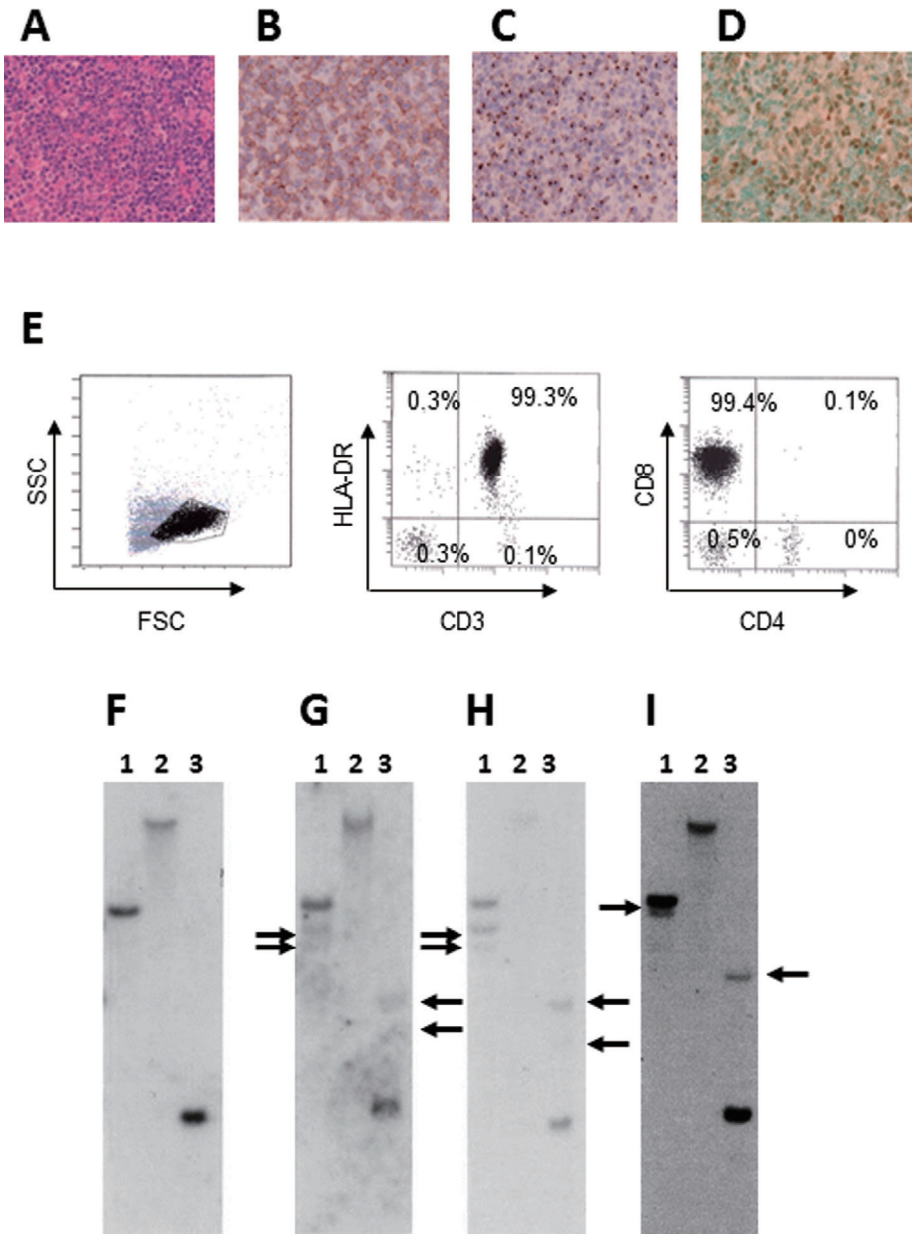

Figure 1. **Analysis of Epstein-Barr virus-infected cells at the onset of chronic active EBV infection. A-D: Biopsy specimens of a cervical lymph node (original magnification, ×200). A: Hematoxylin and Eosin staining shows diffuse infiltration of atypical cells. B: Stained with the anti-CD8 antibody. C: Stained with the anti-granzyme B antibody. D: *In situ* hybridization of Epstein-Barr virus-encoded mRNA. Neoplastic cells were positive for CD3 and CD5; these cells were negative for CD4, CD20, and CD56 (data not shown). E: Analysis of peripheral blood mononuclear cells by flow cytometry at disease onset. (F-I) Southern blot analysis for *T-cell receptor Jβ1 gene.* After digestion with *EcoR*I (1), *BamH*I (2), and *Hind*III (3), DNA was analyzed to detect gene rearrangements. Arrows show rearranged bands. F: Negative control. G: DNA extracted from peripheral blood (PB) at disease onset. H: DNA extracted from a cervical lymph node at disease onset. I: DNA extracted from PB at recurrence.**

demonstrated suppressed EBV-specific CTL activity in CAEBV patients using human leukocyte antigen (HLA)-A* 2402-restricted tetramers (11). In addition, Katano et al reported that mutations in both alleles of the *perforin* gene, which is indispensable for CTL activity, resulted in its reduced expression and could play a role in CAEBV development (12).

However, we were unable to detect *perforin* gene mutations in CAEBV cells from the present patient (data not shown). We previously reported suppressed CTL activity against EBV-infected B cells in an EBV-B-LPD patient who had been administered low-dose PSL for more than 7 years (13). The present patient and one in another report who had SLE developed CAEBV during PSL administration (14). Thus, PSL, even at low doses, may suppress CTL activity and trigger disease development.

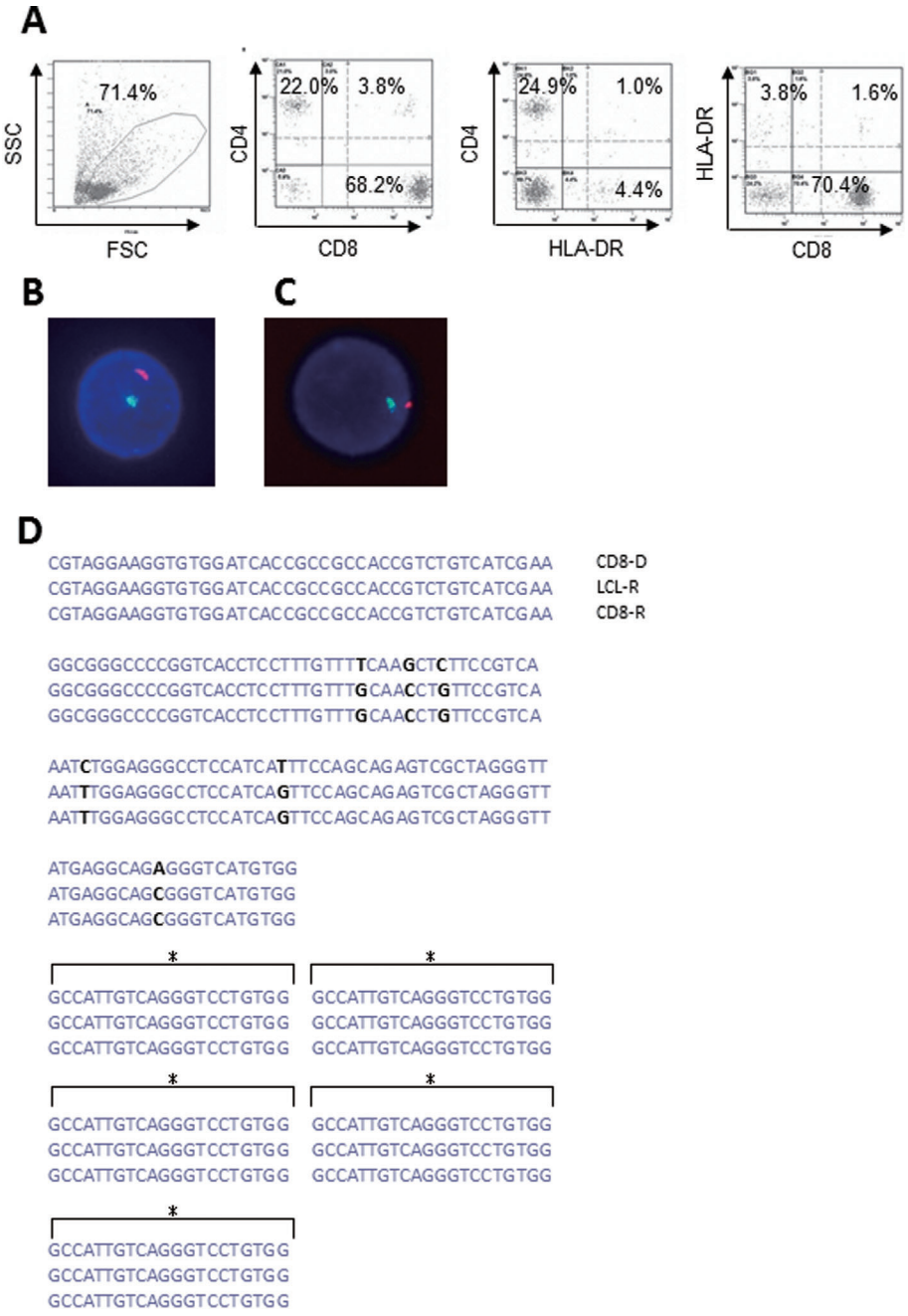

Figure 2. **Analysis of Epstein-Barr virus-infected T cells 3 years after BMT at recurrence of chronic active EBV infection. A: Analysis of peripheral blood mononuclear cells by flow cytometry at the diagnosis of recurrence. B, C: Fluorescence *in situ* hybridization (FISH) analysis. Red and green signals indicate X and Y chromosomes, respectively. B: Lymphoblastoid cell line (LCL) established from patient's PBMC soon after engraftment. The XY signal was positive in 96.8% of cells and was considered to be of donor origin. EBV-DNA titer, 1.4×10^6 copies/μg DNA. C: CD8-positive cells from PB at recurrence. The XY signal was positive in 98.4% of CD8-positive cells. EBV-DNA titer, 2.4×10^6 copies/μg DNA. D: *Lmp1* sequence analysis of CD8-positive T cells at diagnosis (CD8-D, upper lane) of LCL, established from patient's PBMC soon after engraftment (LCL-R, middle lane), and of CD8-positive T cells at recurrence (CD8-R, lower lane). The first nucleotide corresponds to nucleotide No. 168238 of B95.8 (Genbank No.V01555). Asterisks indicate repeat regions; black letters indicate distinctive nucleotides.**

EBV itself can contribute to the clonal proliferation of infected T or NK cells. NF-kB was constitutively activated in EBV-infected T or NK cells derived from CAEBV patients and protected them from VP-16-induced apoptosis, suggesting that EBV infection of T or NK cells could directly contribute to their immortalization (15). However, EBV-induced immortalization of infected cells may be insufficient for CAEBV development.

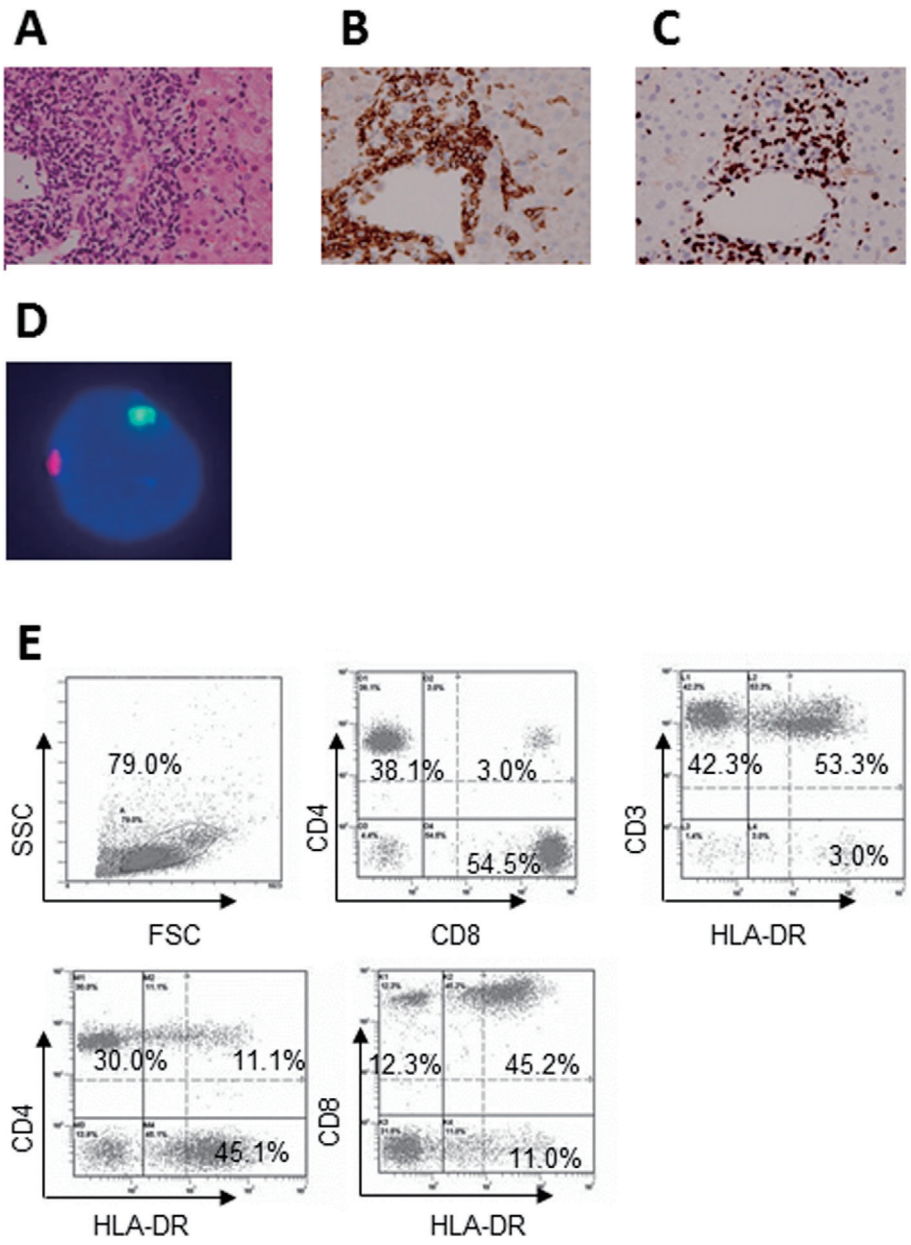

Figure 3. **Analysis of Epstein-Barr virus-infected T cells at liver dysfunction development. A-C: Biopsy liver specimens at recurrence (original magnification, ×200), showing severe sinusoidal infiltration of atypical cells. A: Hematoxylin and Eosin staining. B: Stained with the anti-CD8 antibody. C: *In situ* hybridization of Epstein-Barr virus-encoded mRNA. D: FISH analysis of peripheral nucleated cells. Red and green signals indicate X and Y chromosomes, respectively. E: Analysis of peripheral blood mononuclear cells by flow cytometry at the time of liver biopsy.**

We recently generated a xenograft model of CAEBV by transplanting a patient's PBMC to NOD/Shi-*scid*/IL-2Rγ-null strain mice (16). In this model, neither EBV-infected T and NK cell engraftment nor CAEBV development occurred without CD4-positive T cells. This indicates that both infected cells and CD4-positive T cell-associated mechanisms (e.g., interactions with CD4-positive T cells, CD4- positive T cell-related cytokines, and so on) may be necessary for CAEBV development. At recurrence, the present patient had activated CD4-positive cells that may have originated from the donor's PBMC (Fig. 3E and Table 1). Three other cases of CAEBV have been reported in patients with autoimmune diseases (14, 17, 18). Hyperactivated, uninfected T cells, including CD4-positive T cells, may facilitate the expansion of EBV-infected T or NK cells, as in our murine model.

In conclusion, the present case indicates that certain background host factors may predispose a patient to CAEBV development. Further studies should be conducted in order to determine these factors.

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

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Table 1. Chemerism and Lymphocyte Subsets of Peripheral Blood after Bone Marrow Transplantation

	Years after Bone Marrow Transplantation				
	0	1	3	4	4.5
Chimerism of nucleated cells (%)	XX 0.6% XY 99.4%	NE	NE	XX 0% XY 100%	XX 0% XY 100%
Chimerism of T cells		NE	XX 0.5%	NE	XX 0%
		NE	XY 99.5%	NE	XY 78.5%, XXYY 21.5%
The Percentage of CD4-positive cells in CD3-positive cells (in MC)		NE	25 % (24%)	NE	43% (41%)
The Percentage of CD8-positive cells in CD3-positive cells (in MC)		NE	75% (71%)	NE	57% (57%)
Epstein-Barr virus-DNA (copies/μgDNA)	ND	1.7×10^4	1×10^5	1×10^5	5.6×10^6

ND: not detected
NE: not examined
MC: monocuclear cells

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References

1. Kawa K. Epstein-Barr virus—associated diseases in humans. Int J Hematol **71**: 108-117, 2000.
2. Quintanilla-Martinez L, Kimura H, Jaffe ES. EBV-positive T-cell lymphoproliferative disorders of childhood. In: World Health Organization Classification of Tumors. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Jaffe ES, Harris NL, Stein H, et al, Eds. IARC Press, Lyon, 2008: 527-533.
3. Kimura H, Morishima T, Kanegane H, et al. Prognostic factors for chronic active Epstein-Barr virus infection. J Infect Dis **187**: 527-533, 2003.
4. Arai A, Imadome K, Watanabe Y, et al. Clinical features of adult-onset chronic active Epstein-Barr virus infection: a retrospective analysis. Int J Hematol **93**: 602-609, 2011.
5. Imadome K, Shimizu N, Arai A, et al. Coexpression of CD40 and CD40 ligand in Epstein-Barr virus-infected T and NK cells and their role in cell survival. J Infect Dis **192**: 1340-1348, 2005.
6. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science **286**: 1957-1959, 1999.
7. Koyama M, Takeshita Y, Sakata A, et al. Cytotoxic chemotherapy successfully induces durable complete remission in 2 patients with mosquito allergy resulting from Epstein-Barr virus-associated T-/natural killer cell lymphoproliferative disease. Int J Hematol **82**: 437-440, 2005.
8. Anagnostopoulos I, Hummel M, Kreschel C, Stein H. Morphology, immunophenotype, and distribution of latently and/or productively Epstein-Barr virus-infected cells in acute infectious mononucleosis: implications for the interindividual infection route of Epstein-Barr virus. Blood **85**: 744-750, 1995.
9. Kasahara Y, Yachie A, Takei K, et al. Differential cellular targets of Epstein-Barr virus (EBV) infection between acute EBV-associated hemophagocytic lymphohistiocytosis and chronic active EBV infection. Blood **98**: 1882-1888, 2001.
10. Ohshima K, Kimura H, Yoshino T, et al. Proposed categorization of pathological states of EBV-associated T/natural killer-cell lymphoproliferative disorder (LPD) in children and young adults: overlap with chronic active EBV infection and infantile fulminant EBV T-LPD. Pathol Int **58**: 209-217, 2008.
11. Sugaya N, Kimura H, Hara S, et al. Quantitative analysis of Epstein-Barr virus (EBV)-specific CD8+ T cells in patients with chronic active EBV infection. J Infect Dis **190**: 985-988, 2004.
12. Katano H, Ali MA, Patera AC, et al. Chronic active Epstein-Barr virus infection associated with mutations in perforin that impair its maturation. Blood **103**: 1244-1252, 2004.
13. Arai A, Imadome K, Fujiwara S, Miura O. Autoimmune hemolytic anemia accompanied by reactivation of an Epstein-Barr virus infection with suppressed CTL response to EBV-infected cells in an elderly man. Intern Med **49**: 325-329, 2010.
14. Ogawa J, Koike R, Sugihara T, et al. An autopsied case of chronic active Epstein-Barr virus infection complicated in systemic lupus erythematosus and antiphospholipid antibody syndrome. Nihon Rinsho Meneki Gakkai Kaishi **25**: 458-465, 2002 (in Japanese, Abstract in English).
15. Arai A, Takahashi M, Imadome K, et al. NF-kB is constitutively activated in EBV-infected T or NK cells and can be a molecular target for EBV-positive T/NK-cell lymphoproliferative disease treatment. Blood **116**: 1998, 2010 (Abstract).
16. Imadome K, Yajima M, Arai A, et al. Novel mouse xenograft models reveal a critical role of CD4+ T cells in the proliferation of EBV-Infected T and NK cells. PLoS Pathog **7**: e1002326, 2011.
17. Young KH, Zhang D, Malik JT, Williams E. Fulminant EBV-driven CD8 T-cell lymphoproliferative disorder following primary acute EBV infection: a unique spectrum of T-cell malignancy. Int J Clin Exp Pathol **1**: 185-197, 2008.
18. Tokuhira M, Hanzawa K, Watanabe R, et al. Co-existence of acute myeloid leukemia with multilineage dysplasia and Epstein-Barr virus-associated T-cell lymphoproliferative disorder in a patient with rheumatoid arthritis: a case report. J Hematol Oncol **2**: 27, 2009.