Successful Immunosuppressive Therapy for Severe Infectious Mononucleosis in a Patient with Clonal Proliferation of EBV-infected CD8-positive Cells

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Abstract

A 30-year-old woman was diagnosed with severe infectious mononucleosis (IM). The Epstein-Barr virus (EBV) had infected both CD19- and CD8-positive cells, and clonal proliferation of EBV-infected cells and T-cells was detected. Although we suspected malignant lymphoma, her condition improved following immunosuppressive therapy. A similar case was recently reported; therefore, this case is the second case of IM with EBV-infected CD8-positive cells and clonal proliferation of EBV-infected cells. Our results demonstrate that the clonal proliferation of EBV-infected cells is not always an indication for chemotherapy in the primary infection phase and that monitoring the EBV viral load is useful for therapeutic decision-making.

Key words: EBV, infectious mononucleosis, CD8, T-LPD

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Introduction

Epstein-Barr virus (EBV) is associated with various benign and malignant hematological diseases. The clinical course of EBV-associated diseases differs depending on the type of infected lymphoid cell. For example, infectious mononucleosis (IM) is a self-limiting disease that results from primary EBV infection of B-cells, whereas fulminant EBV lymphoproliferative disorder following acute EBV infection of T-cells has been shown to rapidly progress to death within a few months (1). Meanwhile, extranodal natural killer (NK)/T-cell lymphoma is a refractory malignancy originating from EBV-infected NK/T-cells. Therefore, it is important to identify which lymphoid cells are infected in order to initiate the appropriate therapeutic strategy.

Lymphoid cells infected with EBV become targets of immune surveillance and are eliminated from the host by cytotoxic T-lymphocytes (CTL) (2). The immunological response

to EBV-infected cells may result in the development of pathological conditions, such as IM, multiple organ damage and hemophagocytic lymphohistiocytosis (HLH). Immunosuppressive therapy is indicated for patients with multiple organ damage or HLH in order to reduce the excessive immune response. In addition to immune-mediated diseases, EBV can cause malignant lymphoma. Patients with EBV-associated malignant lymphoma often exhibit rapid progression and a fatal course; thus, multi-drug chemotherapy or allogeneic hematopoietic stem cell transplantation should be considered in such cases. EBV-associated malignant lymphoma may also induce HLH. Therefore, making the differential diagnosis of HLH due to an excess immune response versus malignant lymphoma is crucial for properly managing EBV-associated HLH.

Molecular analyses of *T-cell receptor (TCR)* or *immunoglobulin gene (IG)* rearrangement are useful for detecting the clonal proliferation of certain lymphoid cells. Southern blot analyses using unique viral sequences can also be used

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to detect the clonal proliferation of virus-infected cells. Clonal proliferation is a characteristic of malignancy; therefore, the detection of clonal *TCR/IG* rearrangement or viral sequences on a Southern blot analysis suggests a malignant phenotype, which supports the indication for chemotherapy. However, previous studies have reported that the clonal proliferation of EBV-infected cells is not associated with prognostic outcomes; thus, the clinical significance of cell clonality in the management of EBV-associated diseases remains controversial (3, 4).

We herein describe a case of successful immunosuppressive therapy in a patient with severe IM exhibiting the clonal proliferation of EBV-infected CD8-positive cells. Our results indicate that monitoring the EBV viral load and EBV-specific CTL count is useful for therapeutic decision-making and that the detection of clonal proliferation on a Southern blot analysis is not always an indication for chemotherapy.

Analyses of EBV infection and cell clonality

Peripheral blood mononuclear cells (PBMCs) were sorted in sequence into the following fractions: CD19, CD4, CD8, CD56, CD14 and others. EBV DNA was quantified in each cell fraction using a real-time quantitative polymerase chain reaction (PCR) assay based on the TaqMan system (Applied Biosystems, Warrington, UK) (5). The EBV-specific CTL count was determined in the peripheral blood, as described elsewhere (6). Briefly, PBMCs were incubated for 30 minutes on ice with a mixture of an appropriate combination of fluorescently labeled monoclonal antibodies and PE-labeled EBV-specific HLA-A2402 tetramers (Medical and Biological Laboratories, Nagoya, Japan). After washing, a five-color flow cytometric analysis was carried out with a Cytomics FC500 analyzer (Beckman Coulter, Brea, USA). Southern blot analyses of bone marrow cells were subsequently performed at another laboratory (SRL, Chiba, Japan). DNA fragments that recognized the T-cell receptor C β region (C β), *immunoglobulin heavy chain joining region* (IGHJ) and EBV terminal repeats (EBV-TR) were used as probes. Double immunohistochemical staining for CD8 and Epstein-Barr virus-encoded small RNAs (EBER) was performed as described elsewhere (7).

Case Report

A 30-year-old woman was admitted to our hospital with a fever and sore throat. She and her family members had no history of hematological malignancies or HLH. However, a physical examination revealed lymphadenopathy in the right neck and splenomegaly. Her WBC count was $9.64 \times 10^9/L$, with 36% neutrophils, 14% monocytes, 45% lymphocytes and 5% atypical lymphocytes, as well as an Hb level of 13.3 g/dL and platelet count of $166 \times 10^9/L$ at the initial diagnosis. Blood biochemistry showed elevated liver enzymes [aspartate transaminase (AST): 155 IU/L, alanine aminotransferase (ALT): 197 IU/L, lactate dehydrogenase (LDH): 394 IU/L], and serological studies indicated primary EBV infection (Ta-

Table. EBV Serological Data.

	At diagnosis		After four months	
Anti-VCA IgM antibody	160	T	<10	T
Anti-VCA IgG antibody	160	T	160	T
Anti-EA IgG antibody	<10	T	ND	T
Anti-EBNA antibody	<10	T	<10	T

VCA: virus capsid antigen, EA: early antigen, EBNA: Epstein-Barr virus nuclear antigen, T: titer, ND: not determined

ble). Based on these clinical features, the patient was diagnosed with IM. Bone marrow aspiration performed 17 days after diagnosis showed few hemophagocytic histiocytes. Meanwhile, expansion of CD8-positive T-cells was detected on a flow cytometric analysis of bone marrow cells (Fig. 1A), although no chromosomal abnormalities were detected in these cells (data not shown). In order to distinguish between IM and lymphoma, a biopsy of the right cervical lymph node was performed 18 days after diagnosis.

The patient's platelet count progressively decreased during her clinical course, and sustained liver damage was noted (Fig. 2). Pancytopenia (WBC: $2.26 \times 10^9/L$, Hb: 11.0 g/dL, platelet count: $37 \times 10^9/L$) and a high serum ferritin level (4,979 ng/mL) suggested HLH. Temporary improvements were achieved with the administration of prednisolone (PSL); however, the thrombocytopenia and liver damage became exacerbated during PSL tapering. We initiated treatment with immunosuppressive therapy (PSL and cyclosporine), which subsequently reduced the lymphadenopathy and splenomegaly, and the pancytopenia and liver damage improved. The EBV viral load in the serum was undetectable four months after diagnosis. Five months later, the patient stopped taking the medications, without any recurrence of fever or elevated liver enzymes. In addition, the anti-EBV virus capsid antigen (VCA) IgM antibody titer became negative, and anti-Epstein-Barr virus, nuclear antigen (EBNA) antibodies were no longer positive four months after diagnosis (Table). We did not follow her condition further because she spontaneously stopped coming to our clinic.

Monitoring of the viral load and EBV-specific CTL

The EBV viral load in the serum and numbers of CD19-positive cells and CD8-positive cells were 1.5×10^5 copies/mL, 4.4×10^4 copies/ μ g DNA and 1.6×10^5 copies/ μ g DNA, respectively, 35 days after diagnosis. EBV was not detected in the CD4-positive T-cells or CD56-positive NK/T-cell fraction. These results indicate that the EBV had infected not only CD19-positive, but also CD8-positive, T-cells in the patient. Previous studies have demonstrated the importance of monitoring the serum EBV load during the management of EBV-associated diseases; therefore, we evaluated the EBV load in the peripheral blood (6, 8, 9). Consequently, the patient's condition improved after the administration of immunosuppressive therapy, while the EBV load decreased

Figure 1. Flow cytometric analyses. (A) Flow cytometry of the bone marrow cells showed expansion of CD8-positive T-cells. (B) EBV-specific CTL was noted in the peripheral blood at 45 (left) and 95 (right) days after the initial diagnosis.

Figure 2. Clinical course. The medications are shown in the top panel. The platelet count (PLT) and AST and ALT levels are indicated in the middle panel. The serum ferritin level and EBV viral load in the peripheral blood are shown in the lower panel.

during immunosuppressive treatment and EBV-specific CTL was detected (Fig. 1B). This result suggests that the EBV-infected cells were eliminated by EBV-specific CTL, even under treatment with immunosuppressive therapy. Therefore, we continued the immunosuppressive therapy. After the patient's organ damage was controlled, we tapered and discontinued the immunosuppressive therapy.

Southern blot analysis and immunohistochemical staining

The clinical course of the current patient differed from that observed for typical IM; i.e., a persistent fever, progressive organ damage and pancytopenia. Therefore, we suspected a complication of EBV-associated malignant lymphoma. A Southern blot analysis using the EBV-TR probe

Figure 3. Southern blot and histological analyses. (A) Left: Southern blot analysis of the EBV-terminal repeat in the patient's bone marrow cells showed a monoclonal band. Lane 1: positive control, lane 2: negative control, lane 3: the patient's bone marrow cells. Right: Southern blot analysis of the EBV-terminal repeat in the CD8-sorted cell fraction of peripheral blood cells revealed monoclonal expansion. Lane 1: the patient's cells, lane 2: negative control. (B) Southern blot analysis of the T-cell receptor C β region of bone marrow cells. Lane 1: BamHI-digested DNA, lane 2: EcoRV-digested DNA, lane 3: HindIII-digested DNA. The arrows indicate rearranged bands. (C) Hematoxylin and Eosin staining of the right cervical lymph node showed a pleomorphic lymphoproliferative disorder. (D) Double immunohistochemical staining of CD8 and EBER revealed EBV-infected CD8-positive T-cells. The CD8-positive and EBER-positive cells stained red and brown, respectively. The arrows represent double-positive cells.

revealed monoclonal proliferation of EBV-infected cells in the bone marrow (Fig. 3A), and a Southern blot analysis of CD8-sorted cells in the peripheral blood indicated the clonal expansion of EBV-infected cells (Fig. 3A). In addition, a Southern blot analysis using the C β probe also showed clonally rearranged bands (Fig. 3B). However, no *IGHJ* rearrangement was detected in whole blood cells, indicating the lack of clonal proliferation of B-cells (data not shown). A lymph node biopsy of the right cervical node showed expansion of lymphocytes mimicking pleomorphic lymphoproliferative disorder (Fig. 3C). The expanded cells were positive for both CD8 and EBER, as detected on double immunohistochemical staining (Fig. 3D). These results indicate the presence of clonal proliferation of EBV-infected cells and T-cells in this patient.

Discussion

IM is a benign self-limiting disease, the symptoms of which typically improve within a few weeks (10). This disease is attributed to primary EBV infection of B-cells. Our

patient exhibited clinically more severe sustained symptoms than typical IM patients, and we found EBV-infected CD8-positive T-cells and CD19-positive B-cells in this case. The clinical symptoms have been shown to be more severe when EBV infects T-cells versus B-cells (11, 12). Therefore, we suspect that our patient's symptoms may have been influenced by the infection of CD8-positive T-cells with EBV.

EBV-associated T-lymphoproliferative disease (T-LPD) is a malignant lymphoma that originates from the clonal proliferation of EBV-infected T-cells. Clinically, this disease is often complicated by inflammatory symptoms, rapidly progressing organ damage and HLH (13, 14). This disease may also develop after a diagnosis of IM. Pancytopenia mimicking HLH developed in the current case, and the clonal proliferation of EBV-positive cells and T-cells was detected on Southern blot analyses. Therefore, we suspected a complication of T-LPD in this patient and considered chemotherapy. However, previous studies have reported that the clonal proliferation of EBV-positive cells or certain lymphoid cells is not associated with the clinical outcomes of EBV-associated HLH (3, 4). These findings suggest that the presence of clo-

nal proliferation of cells alone does not fully define a malignant phenotype in patients with EBV-associated diseases. In the present case, we continued immunosuppressive therapy, instead of chemotherapy, with careful monitoring of the EBV load and EBV-specific CTL. Notably, the condition of our patient improved with immunosuppressive therapy; therefore, she did not have T-LPD.

Arai et al. recently reported a similar case of IM to that observed in our patient and documented their case as being the first case of IM accompanied by the clonal proliferation of EBV-infected cells and EBV-infected CD8-positive cells (15). Their and the current patient shared clinical and virological characteristics; thus, these two cases appear to represent a unique subset of IM. Because clonal cell proliferation was observed in both patients, it is a crucial issue for distinguishing these cases from EBV-associated T-LPD. Arai et al. reported that the detection of positivity for anti-IgM-VCA antibodies at diagnosis and subsequent positivity for anti-EBNA antibodies may be beneficial for making the differential diagnosis. Although we did not confirm positivity for anti-EBNA antibodies in the present patient, our results suggest that monitoring the EBV load and EBV-specific CTL count may be useful for decision-making regarding the appropriate therapeutic course.

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

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