□ CASE REPORT □

Rapidly Progressive Epstein-Barr Virus-associated Lymphoproliferative Disorder Unpredictable by Weekly Viral Load Monitoring

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Abstract

We report a case of Epstein-Barr virus (EBV)-associated lymphoproliferative disease (LPD) following unrelated bone marrow transplantation (UBMT) for severe aplastic anemia treated with a conditioning regimen that included anti-thymocyte globulin (ATG). The patient showed signs of EBV reactivation as early as 34 days after UBMT. Our weekly schedule for EBV monitoring failed to trace rapid changes in EBV viral load and the patient eventually developed EBV-LPD. However, early intervention with monoclonal antibody against CD20, rituximab, stopped the further progression of EBV-LPD. As several recent reports have suggested, the safety and efficacy of rituximab treatment for EBV-LPD is supported by our limited experience with post transplant EBV-LPD.

Key words: aplastic anemia, unrelated bone marrow transplantation, anti-thymocyte globulin, Epstein-Barr virus-associated lymphoproliferative disorders, rituximab

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Introduction

Epstein-Barr virus-associated lymphoproliferative disorder (EBV-LPD) is a rare but lethal complication in the setting of allogeneic hematopoietic stem cell transplantation (HSCT) (1). The overall cumulative incidence of EBV-LPD is low, occurring in approximately 1-3% of allogeneic HSCT, and most patients receive the diagnosis within the initial 6 months after transplantation. The major risk factor for EBV-LPD is the use of in vivo T-cell depletion with anti-thymocyte globulin (ATG) for prophylaxis of acute graft-versus-host disease (GVHD), or reduced intensity conditioning regimens (2, 3). EBV-LPD caused by the outgrowth of latently infected B cells under the absence of competent immune surveillance systems as a consequence of ATG treatment. Because of the rapid clinical course of EBV-LPD, immediate treatment is crucial to reduce mortality (4, 5). Recently, it has been suggested that frequent monitoring of

EBV-DNA load by quantitative real-time PCR makes early treatment possible, and there are several reports supporting this strategy (6, 7). We report a case of EBV-LPD following unrelated allogeneic bone marrow transplantation using a conditioning regimen including ATG. Our weekly schedule for EBV monitoring failed to predict the onset of EBV-LPD, but it was successfully treated with a single dose of rituximab.

Case Report

A 23-year-old man was diagnosed with severe aplastic anemia (SAA) in July 2006. The administration of cyclosporine was ineffective, and he became dependent on regular and frequent red blood cell transfusions. The patient was subsequently admitted to our hospital for unrelated bone marrow transplantation (UBMT) in August 2008. The physical findings included severe conjunctival anemia and several oral mucosal hematomas, but no superficial lympho-

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Figure 1. Photomicrographs of specimens from the bone marrow aspiration. Bone marrow aspiration specimen shows an increase in CD20 positive lymphocytes positive for Epstein-Barr virus (EBV)-encoded mRNA (EBER) by in situ hybridization. (A) Hematoxylin and Eosin staining, (B) CD20 immuno-staining, (C) in situ hybridization for EBER.

denopathy, hepatomegaly, or splenomegaly were noted. The laboratory findings demonstrated a white blood cell (WBC) count of $1,400/\mu\text{L}$, neutrophil count of $96/\mu\text{L}$, red blood cell count of $254 \times 10^4/\mu\text{L}$, hemoglobin (Hb) of 7.2 g/dL, hematocrit of 20.7%, platelet (Plt) count of $1.0 \times 10^4/\mu\text{L}$, reticulocytes 1%, and serum ferritin level of 2,269 ng/mL. A coagulation test was within normal limits. Bone marrow aspiration revealed severe hypo-cellular bone marrow ($0.1 \times 10^4/\mu\text{L}$) with no morphological dysplasia.

In February 2009, the patient underwent BMT from an HLA identical unrelated donor (total nuclear cells $3.2 \times 10^8/\text{kg}$). In our institution, patients with SAA usually receive total lymphoid irradiation (TLI) and cyclophosphamide as a conditioning regimen. But, this time, the patient was conditioned with fludarabine ($30 \text{ mg}/\text{m}^2/\text{day}$, day -10 to -5), cyclophosphamide ($60 \text{ mg}/\text{m}^2/\text{day}$, day -6 to -5) and thymoglobulin (rabbit, $2.5 \text{ mg}/\text{kg}/\text{day}$, day -4 to -1). This was mainly due to the unavailability of irradiation therapy equipment in the facility. The prophylaxis for acute graft-versus-host disease (GVHD) consisted of short-course methotrexate (10 , 7 , and $7 \text{ mg}/\text{m}^2$ on days 1, 3, and 6, respectively) and cyclosporine. Although the engraftment was observed 14 days after UMBT, the patient developed persistent high fever from day 33, which was refractory to antibiotics and antiviral agents. Moreover, his liver function tests worsened, including a total bilirubin of 1.9 mg/dL, aspartate

aminotransferase of 408 IU/L, alanine aminotransferase of 577 IU/L, γ -glutamyltranspeptidase of 779 IU/L, and alkaline phosphatase of 848 IU/L. In accordance with his liver dysfunction, the patient developed pancytopenia (WBC $4,200/\mu\text{L}$, Hb 8.4 g/dL, Plt $1.0 \times 10^4/\mu\text{L}$). Bone marrow aspiration revealed an increased number of lymphocytes, but no apparent hemophagocytic findings (Fig. 1A). Serum lactate dehydrogenase ($1,823 \text{ IU/L}$) and ferritin ($29,770 \text{ ng/mL}$) levels were also significantly elevated. A computed tomography scan of the abdomen obtained at this time verified splenomegaly.

Based on these clinical signs and the use of ATG in the conditioning regimen, EBV-LPD was highly suspected. Even though the result of plasma EBV-DNA load was not available at the time, a single dose of rituximab ($375 \text{ mg}/\text{m}^2$) was administered on day 42. On the following day, day 43, the patient became afebrile and hepatic dysfunction and ferritin level dramatically improved (Fig. 2). The belated result of peripheral plasma EBV-DNA (2.3×10^6 copies/ μg DNA) and pathological examination from bone marrow aspiration (demonstrating an increase in CD20 positive lymphocytes positive for EBV-encoded mRNA (EBER) by in situ hybridization (Fig. 1B and C)) confirmed the diagnosis of EBV-LPD. After the single administration of rituximab, the EBV-LPD stabilized with a low EBV-DNA load of 100 copies/ μg DNA level, as shown in Fig. 3.

Figure 2. Serial changes in ALT, LDH and ferritin levels.

Figure 3. Serial changes in EBV DNA levels. RQ-PCR assay was performed with the aid of TaqMan technology (PE Biosystems, Foster City, CA, USA). Primers and detection probes were as described previously. Assay was commercially available and actually performed at SRL Co., Ltd., Tokyo, Japan.

Discussion

In Europe and the United States, ATG has been used for more than 30 years as the principle prophylactic agent against GVHD, and numerous studies have confirmed its efficacy in reducing the incidence and severity of subsequent GVHD. By contrast, ATG has only recently been used in the setting of allogeneic transplantation in Japan (since December 2008). However, this medication has been commonly associated with several complications such as ongoing immune deficiency and opportunistic infections, including an increased risk of EBV reactivation (2). EBV-LPD is lethal, especially in allogeneic HSCT, and immediate treatment based on early diagnosis is crucial for patient survival. EBV-LPD could not be effectively controlled by the dose reduction or

discontinuation of immunosuppressants alone. An effective therapeutic option can be provided by EBV-specific cytotoxic T lymphocytes with donor lymphocytes infusion (DLI). However, the largest flaw of this strategy was the lack of ready availability especially in the setting of UBM. Moreover, DLI could be associated with an increased risk of severe GVHD. Recent studies have demonstrated the safety and efficacy of rituximab as a treatment for EBV-LPD (5, 8-10). Preemptive administration of rituximab before obvious EBV-LPD develops has received significant attention. When the viral load exceeds 1,000 DNA copies/ 10^5 cells, intervention with rituximab should be initiated promptly. This strategy appears to be highly effective in controlling viral proliferation and avoiding subsequent disease manifestation (2, 6, 11), although other reports have described different threshold values (Table 1) (7, 12-14).

Table 1. Studies Reported about the Preemptive Treatment with Rituximab in LPD

Patients population		PCR assay	Preemptive treatment			Reference
N	Patients group	Threshold	N	Therapy	Cure (%)	
77	HLA-mismatched, unrelated donor	1,000 gEq/mL	11	Rituximab once	93	2)
49	T-cell depleted	1,000 gEq/mL	15	Rituximab once	91	6)
NA	T-cell depleted, ATG	10 ⁴ gEq/10 ⁵ PBMCs	3	Rituximab once	100	7)
NA	T-cell depleted, HLA-mismatched	10 ⁴ gEq/10 ⁵ PBMCs	2	Rituximab, DLI	100	7)
NA	T-cell depleted	4000 gEq/ μ gDNA	2	Rituximab, CTLs	100	8)
115	T-cell depleted, ATG,	40,000 gEq/mL	19	Rituximab	89	13)
	HLA-mismatched, unrelated donor					
80	HLA-mismatched, unrelated donor	20,000 gEq/10 ⁵ PBMCs	9	Rituximab	89	14)

Abbreviations; Cure: no clinical sign of the recurrence of LPD, CTLs: EBV-specific cytotoxic T lymphocytes, DLI: donor leukocyte infusions, ATG: anti thymocyte globulin, PCR: polymerase chain reaction, gEq: genome equivalents, PBMCs: peripheral blood mononuclear cells, N: number of patients, NA: not available.

Certain obstacles which are inherent in performing EBV viral load monitoring need to be overcome, however. For example, the optimal EBV monitoring schedule is unclear at this time. In the present case, we examined EBV viral load every 7 to 10 days. The actual number of copies of EBV at day 34, 1 day after the onset of pyrexia, was 200 copies/ μ g DNA, but it was increased by more than 100,000 fold by day 42. Thus, our screening interval may not be adequate to trace rapid changes in the EBV viral load. Rather, more flexible monitoring is necessary, especially on the occasion of a clinical sign such as pyrexia, in order to administer prompt treatment with rituximab. In addition, it usually

takes several days until test results are available. Since some patients can become overwhelmed by EBV within a single day, it is not possible to withhold treatment until test results become available. Thus, collaboration between the clinical front and the laboratory is essential in order to ensure a prompt response on the same day. Finally, most of the previously reported series dealt with a limited number of patients with EBV-LPD, therefore, prospective multicenter trials are required to validate the optimal prophylaxis and efficacy of preemptive rituximab therapy, based on EBV DNA load monitoring.

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