Abstract

We herein report the case of a 22-year-old woman with severe aplastic anemia who underwent allogeneic hematopoietic stem cell transplantation (HSCT). After HSCT, the Epstein-Barr virus (EBV)-DNA load in the peripheral blood gradually increased, and the patient presented with a fever and lymphadenopathy on day 56 post-HSCT. Although we administered rituximab, her clinical condition worsened. After rituximab treatment, CD8 T-cells emerged and became dominant in the peripheral blood, some of which were positive on an EBV-specific tetramer analysis. However, an open biopsy of the lymphadenopathy lesions revealed the CD8 T-cells to be infected with EBV, exhibiting proliferation with oligoclonality. The patient ultimately died of multiple organ failure on day 99 post-HSCT.

Key words: Epstein-Barr virus, post-transplant lymphoproliferative disorder, CD8 T-cell, rituximab, hematopoietic stem cell transplantation

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Introduction

Post-transplant lymphoproliferative disorder (PTLD) is a fatal complication of allogeneic hematopoietic stem cell transplantation (HSCT) that is caused by reactivation of the Epstein-Barr virus (EBV). As most cases of PTLD arise among donor- or recipient-derived B-cells, one strategy to prevent the development of PTLD is to eliminate EBV-infected B-cells in the early phase after HSCT. Monitoring the EBV-DNA load in the peripheral blood in addition to administering preemptive rituximab therapy has recently been reported to be a successful approach to preventing the development of B-cell PTLD (1, 2). EBV-specific tetramer analyses using flow cytometry can also be useful for detecting EBV-specific cytotoxic T-cells (CTL), the actions of which are negatively associated with the development of PTLD (3). However, the occurrence of PTLD in T- or natural killer (NK)-cell lineages is rare, and the treatment and/or prevention of such cases have not yet been established. CD8 T-cell PTLD is especially difficult to diagnose because it has a similar phenotype to that of CTL. We herein report the case of a 22-year-old woman with aplastic anemia who was diagnosed with CD8 T-cell PTLD following the administration of rituximab for lymphadenopathy with a high level of EBV replication after HSCT.
Case Report

A 22-year-old Japanese woman with severe aplastic anemia was referred to our hospital for unrelated HSCT. She had received three course of standard immunosuppressive therapy (IST), including two courses with horse anti-thymocyte globulin (ATG) and one course with rabbit ATG. She did not respond to these IST treatments and was dependent on blood transfusions. The last course of IST of rabbit ATG (Thymoglobulin, Genzyme) was administered at 18 years of age. Her HLA DNA typing (A, B, Cw and DRB1) was completely matched with an unrelated male donor. The anti-EBV antibody titers measured immediately prior to HSCT were as follows: EBV viral capsid antigen (VCA) IgG=1:160, early antigen-diffuse and restrict complex (EA-DR) IgG<1:10, EA-DR IgA<1:10, EB nuclear antigen (NA)=1:40. The EBV-DNA load among the peripheral blood white blood cells (PBWBCs) was under 40 copies/10^6 WBCs, which was below the sensitivity of the test. The conditioning regimen for stem cell transplantation consisted of fludarabine (30 mg/m^2/day) from days -6 to -3, cyclophosphamide (25 mg/kg/day) from days -6 to -3, thymoglobulin (2.5 mg/kg/day) from day -4 to -3 and 200 centigray (cGy) of total body irradiation on day -1, a modified regimen of that reported by the EBMT-SAA Working Party (4). The transplanted mononuclear cell count was 1.31×10^8/kg and the CD34-positive cell count was 2.10×10^6/kg. The prophylaxis for graft-versus-host disease (GVHD) comprised the continuous infusion of tacrolimus (0.02 mg/kg/day) from day -1 and short-term methotrexate on day +1 (15 mg/m^2/day) as well as days +3 and +6 (10 mg/m^2/day). Granulocyte colony-stimulating factor was administered starting on day +5. Engraftment of neutrophils was achieved on day +15 (Fig. 1).

The EBV-DNA load was monitored once every two weeks after HSCT. The EBV-DNA load of PBWBCs was found to be elevated at 100 copies/10^6 WBCs on day +33 and 1,000 copies/10^6 WBCs on day +48. The patient presented with recurrent fevers and progressive lymphadenopathy in the left posterior cervical and supraclavicular fossa regions on day +56. The EBV-DNA load in the whole blood was elevated at 40,000 copies/mL on day +60; therefore, we reduced the dose of tacrolimus immediately. However, the recurrent fevers and lymphadenopathy did not improve. We subsequently administered 375 mg/m^2 of rituximab on day +64. Prior to the administration of rituximab, flow cytometry of peripheral blood lymphocytes showed that 60% of lymphocytes were CD56+CD3- cells and 30% were CD19+ cells, whereas no CD3+ cells were detected (Fig. 2A). Meanwhile, a short tandem repeat (STR) analysis revealed that the peripheral blood cell chimerism was of the complete donor type. Following the administration of rituximab, a flow cytometry analysis of peripheral blood lymphocytes showed that 70% of lymphocytes were CD56+CD3- cells and 30% were CD19+ cells, whereas no CD3+ cells were detected (Fig. 2A). A short tandem repeat (STR) analysis revealed that the peripheral blood cell chimerism was of the complete donor type. Following the administration of rituximab, a flow cytometry analysis of peripheral blood lymphocytes showed that 70% of lymphocytes were CD56+CD3- cells and 30% were CD19+ cells, whereas no CD3+ cells were detected (Fig. 2A). We then analyzed the chimerism of T-cells in the peripheral blood and found that it constituted mixed chimerism in which 66% of the T-cells were of recipient origin.

We administered rituximab again on day +78. However,
EBV-associated PTLD is a serious and often fatal post-transplant complication. In HSCT recipients, risk factors contributing to the development of PTLD include T-cell depletion of the donor marrow, ATG administration, unrelated or HLA-mismatched graft use and a recipient age of 50 years or older at transplantation (5). In particular, among patients with severe aplastic anemia, the prior administration of ATG as IST has a strong impact on the development of EBV-associated PTLD (6). Because our patient received ATG three times prior to undergoing HSCT, she was at high risk of developing EBV-associated PTLD. In addition, the conditioning regimen also included ATG for HSCT, which may have induced in vivo purging of donor-derived T-cells, thus allowing EBV-infected B cells to proliferate.

Most PTLDs are reportedly of B-cell origin, with only approximately 5% of T-cell and T/NK cell origin (7). One strategy for treatment is therefore to eliminate EBV-infected B-cells. Rituximab is an anti-CD20 monoclonal antibody that has been utilized for the treatment of various mature B-cell malignancies and has been shown to be a well-tolerated and effective treatment for PTLD after HSCT, with a response rate of 55% to 100% (8-10).

Rituximab has also been used as a preemptive therapy for PTLD. The EBV-DNA load detected according to PCR showed a correlation with the risk of PTLD. Preemptive therapy with rituximab in HSCT patients who develop a
Figure 3. Biopsy of the supraclavicular lymph node. Diffuse effacement of the nodal architecture with a large area of necrosis is observed. Atypical lymphocytes had infiltrated around the necrotic area (A). The immunostaining specimens showed the large lymphocytes to be positive for CD79a (B) and the middle-sized lymphocytes to be positive for CD3 (C). The majority of the lymphocytes were positive for EBER (D). (A) Hematoxylin and Eosin staining, ×400. (B, C, D) ×400

Figure 4. Molecular analyses of the lymph node. A Southern blot analysis of the T-cell receptor β-chain gene showed oligoclonal rearranged bands. DNA was digested with BamH I (B), Hind III (H) and EcoR I (E). R: rearranged band, GL: germ line (A). A polymerase chain reaction (PCR) analysis of the immunoglobulin heavy-chain (IgH) gene showed oligoclonal rearranged bands. The red marker indicates the positive peak appearance range. With the VH FR1 and JH primers, the positive peak appears at 310-360 bp. With the VH FR3 and JH primers, the positive peak appears at 110-170 bp (B).

persistent EBV-DNA load in the context of a poor T-cell recovery has been reported to be safe and highly effective in preventing PTLD (9, 11, 12). However, a limited number of studies have described the use of preemptive therapy with rituximab, and prospective controlled trials are therefore needed to evaluate the efficacy of this regimen.

In the present case, we administered rituximab for lymphadenopathy with a high rate of EBV replication. Following the administration of rituximab, flow cytometry of peripheral blood lymphocytes showed that CD8 T-cells had emerged and become dominant. In addition, some of the CD8 T-cells were positive on an EBV-specific tetramer analysis. We therefore considered these CD8 T-cells to contain cytotoxic T-cells against EBV-infected B-cells. However, an open biopsy of the supraclavicular lymph node revealed that the CD8 T-cells were infected with EBV, exhibiting proliferation with oligoclonality. We initially planned to administer donor lymphocyte infusion (DLI) immediately after the rituximab therapy was found to be ineffective; however, the patient’s disease progression was so rapid that there was not enough time to obtain donor lymphocytes. To our knowledge, only five cases of PTLD affecting both B- and T-cells simultaneously have been reported (13). A unique feature of this case is the finding that EBV-infected CD8 T-cell PTLD was diagnosed following the administra-
nation of rituximab. It is unknown at which time point EBV infected the CD8 T-cells. Because the T-cells exhibited mixed chimerism, EBV-infected CD8 T-cells may have been present prior to the administration of rituximab. On the other hand, EBV may have infected CD8 cytotoxic T-cells against EBV-infected B-cells after rituximab treatment. The administration of rituximab for tumor cells can potentially induce a CD8 cytotoxic T-cell response (14). In addition, it has been postulated that cytotoxic T-cells can be infected by EBV during the killing of EBV-infected target cells in patients with a high level of uncontrolled viral replication (15, 16). Therefore, providing earlier preemptive rituximab therapy in order to eliminate the initial EBV-infected B-cells at a lower level of EBV replication may have prevented the CD8 T-cells from being infected with EBV in this case.

This case suggests that performing a biopsy in the initial stage of the disease is essential for confirming the diagnosis of PTLD (17) and that a sequential analysis to identify the lineage of the EBV-infected cells should be conducted when administering rituximab in patients with a high level of EBV replication.

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

References


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