

[CASE REPORT]

Recurrent Enteritis Associated with Epstein-Barr Virus-positive CD4⁺ T-cell Lymphoproliferative Disorder after Autologous Stem Cell Transplantation

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Abstract:

We encountered a patient with multiple myeloma treated with autologous hematopoietic stem cell transplantation (HSCT) who developed repeated episodes of enteritis but regressed spontaneously. An endoscopic examination revealed no abnormalities, but biopsy specimens showed massive infiltration of CD4⁺ and Epstein-Barr encoding region (EBER⁺) abnormal lymphocytes in which a high copy number of Epstein Barr virus (EBV) genomes was detected by quantitative polymerase chain reaction (qPCR). EBV infection was exclusively detected in CD4⁺ T-cells, leading to a diagnosis of EBV-positive CD4⁺ T-cell lymphoproliferative disorder (LPD). This case suggests that an immediate biopsy and examinations, including qPCR for EBV DNA, should be considered for patients with recurrent enteritis after autologous HSCT, regardless of endoscopic findings.

Key words: EBV-associated LPDs, enteritis, double-balloon endoscopy, EBV, T-cell LPDs, acute abdomen

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Introduction

Lymphoproliferative disorders (LPDs) are associated with primary immune disorders, human immunodeficiency virus (HIV) infection, immunosuppressive drugs, and transplantation (1). Withdrawal of immunosuppressive drugs, such as methotrexate and calcineurin inhibitors, frequently leads to lymphoma regression, indicating that the dysregulation of host immune surveillance can play a central role in the pathogenesis of LPDs. Regardless of the etiology, the most common histological type is diffuse large B-cell lymphoma (DLBCL), followed by Hodgkin lymphoma. No specific clinical features to distinguish LPDs from *de novo* lymphomas have been identified, but extranodal involvement appears to be more common in LPDs than *de novo* lymphomas. Epstein-Barr virus (EBV) infection is more frequently detected in LPDs than *de novo* lymphomas, although its frequency is variable among histologies. T-cell and natural kil-

ler (NK)-cell LPDs have also been reported; however, they are extremely rare, so their clinical features are not well understood.

We herein report a case of multiple myeloma treated with high-dose melphalan and autologous hematopoietic stem cell transplantation (HSCT) who developed B-cell LPD (DLBCL) followed by T-cell LPD [peripheral T-cell lymphoma (PTCL)]. EBV infection was confirmed in both populations of lymphoma cells. The clinical presentation and disease course as well as the endoscopic findings were unusual, and it was difficult to determine the underlying cause of the symptoms.

Case Report

A 70-year-old woman, who had undergone conditioning with high-dose melphalan and autologous HSCT for multiple myeloma in with a good partial response, followed by 2-year lenalidomide maintenance therapy until 8 months ago,

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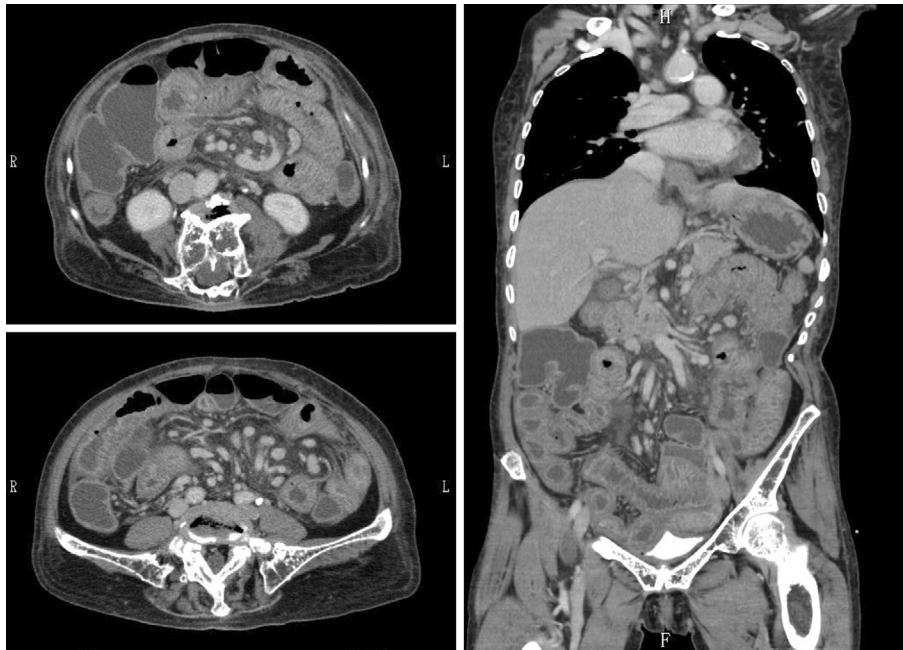


Figure 1. Contrast-enhanced CT of abdomen.

was admitted to our hospital due to abdominal pain. Physical and laboratory examinations did not reveal any evidence of infection or myeloma relapse. Computed tomography (CT) demonstrated lymphadenopathy in the neck, axilla, mediastinum, and mesentery along with tonsil enlargement. A biopsy of the tonsil showed revealed the massive infiltration of large atypical lymphoid cells in the mucosa. Immunohistochemical analyses showed that the lymphoid cells were positive for CD20, CD79a, and Epstein-Barr encoding region (EBER) and negative for CD5, CD10, cyclin D1, and BCL-2, with an MIB-1 index of 80%. The plasma EBV DNA load measured by quantitative polymerase chain reaction (qPCR) was 71,000 copies/ μg DNA. Thus, a diagnosis of EBV-positive DLBCL was made. At that time, the disease status of multiple myeloma was stringent CR (Complete Response) based on the International Myeloma Working Group (IMWG) criteria (2).

Three days after admission, she suddenly complained of nausea, vomiting, and shivering and rapidly went into shock. Contrast-enhanced CT showed uniformly dilated fluid-filled loop and a diffusely thickened wall with homogeneous enhancement of the small intestine, which was consistent with shock bowel (Fig. 1). She developed respiratory distress syndrome and was subsequently transferred to the intensive-care unit (ICU) for mechanical ventilation. A total small-bowel examination by double-balloon endoscopy (DBE) did not find any abnormalities. A histological examination of specimens randomly taken from the small intestine showed nonspecific inflammatory changes with no lymphoma cell infiltration. Therefore, we assumed that the enlarged abdominal lymph nodes had physically impaired the blood flow to the mesenteric arteries, thereby leading to intestinal ischemia and resulting in shock bowel. She was successfully treated with supportive therapy and left the ICU on day 9.

The patient then received four cycles of rituximab monotherapy for B-cell lymphoma and successfully achieved complete remission. At completion, the plasma EBV DNA load had decreased to 790 copies/ μg DNA. However, she later experienced a similar gastrointestinal (GI) episode and required intensive-care management five times over the next year. The peripheral lymphocyte count, which was within the normal range at the diagnosis of B-cell lymphoma, gradually but steadily increased and reached more than 7,000/ μL at 6 months after the diagnosis of B-cell lymphoma.

A flow cytometric analysis showed that nearly all of the lymphocytes in the peripheral blood were positive for CD3 (97.7%), and most of the cells were positive for CD8 (74.7%). To identify the EBV-infected cells, CD4⁺ T-cells, CD8⁺ T-cells, CD19⁺ B cells, CD14⁺ monocytes, and CD56⁺ NK cells were isolated from the peripheral blood by flow cytometry (Fig. 2), and the EBV DNA load was measured in each cell population. EBV DNA was detected in CD4⁺ T-cells (3.6×10^5 copies/ μg RNA) but not in other cells, including CD8⁺ T-cells, or in plasma. A Southern blot analysis confirmed monoclonal integration of the EBV genome as well as the rearrangement of the T-cell receptor C β 1 and J γ genes in lymphocytes. Again, the total small intestine examined by DBE was normal in appearance except for edematous mucosa (Fig. 3).

However, random biopsy specimens showed the diffuse infiltration of atypical lymphoid cells that were positive for CD4 and EBER but negative for CD8 on immunohistochemistry (Fig. 4). An increased copy number of EBV genome was detected in the extracted specimen (1.5×10^7 copies/ μg RNA), while EBV DNA was not detected in the plasma at that time. Positron emission tomography showed an abnormal fluorodeoxyglucose uptake in the stomach, small in-

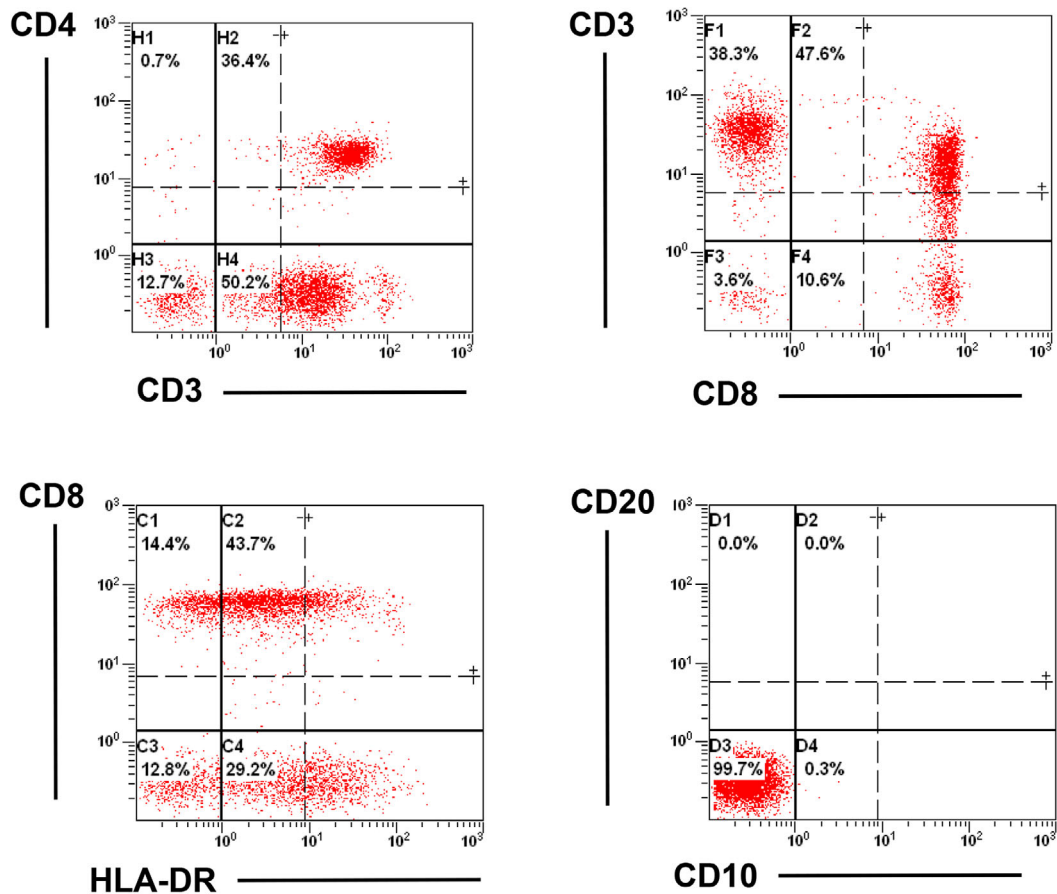


Figure 2. Flowcytometric analysis of peripheral blood.

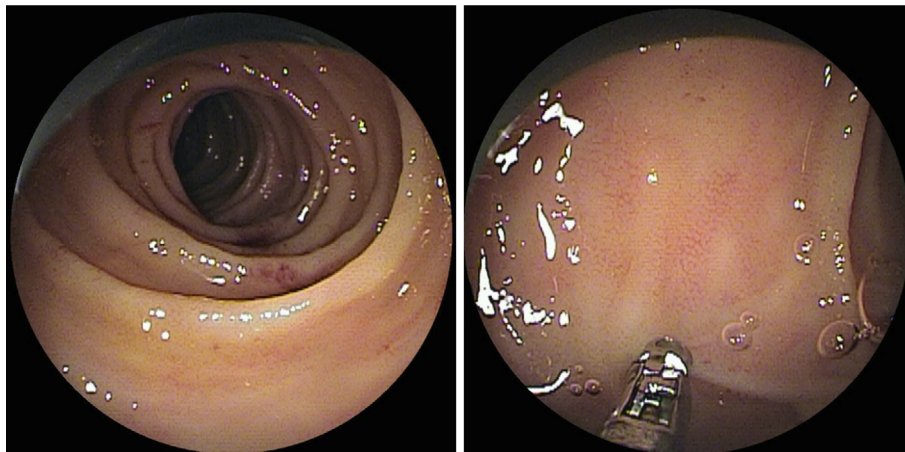


Figure 3. Small intestine examination of DBE.

testine, and spine. Therefore, we finally diagnosed this case as EBV-positive T-cell LPD (PTCL), not otherwise specified, that occurred during rituximab treatment for EBV-positive B-cell LPD (DLBCL) after autologous HSCT.

She showed no response to CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy, romidepsine, or forodesine and ultimately died on day 330 after the initial onset of acute abdomen.

Discussion

According to the 2017 World Health Organization classification, the term post-transplant lymphoproliferative disorders (PTLDs) refers to lymphoid and plasmacytic proliferation caused by severe immunosuppression in recipients who have received solid organ or hematopoietic stem cell allograft (1). LPDs following autologous HSCT are typically associated with the high-dose immunosuppressive regimens adminis-

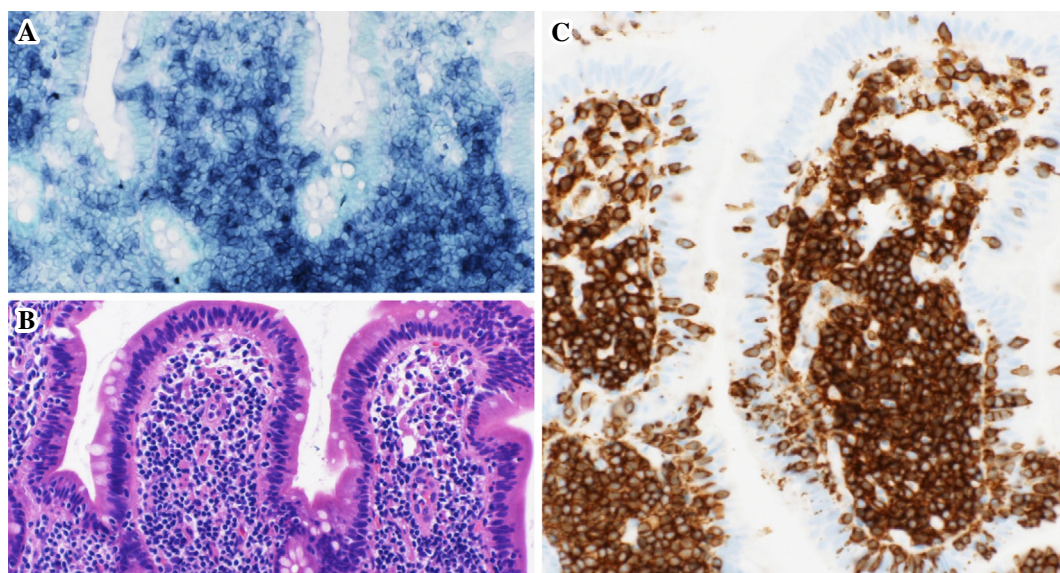


Figure 4. Pathology of small intestine mucosa. (A) EBER immunostain. (B) Hematoxylin and Eosin staining. (C) CD4 immunostain.

tered prior to transplantation; however, it is often difficult to determine to which category they belong due to the involvement of many other factors. Lenalidomide maintenance following high-dose melphalan, which has been shown to increase the risk of second malignancy, may have contributed to the disease progression in the present case.

The incidence of EBV-associated LPDs after allogeneic HSCT varies among studies, ranging from 0.5% to 17%, probably depending on the transplant conditions (3). The rate will most likely increase with the widespread use of *in vivo* or *ex vivo* T-cell depletion for human leukocyte antigen (HLA)-mismatched HSCT. EBV-associated LPDs post-autologous HSCT have been reported in 3.5% (5/165) and 3.6% (2/56) of patients with neuroblastoma and severe autoimmune diseases, respectively (4, 5). The median time from autologous HSCT to the onset of EBV-associated LPDs ranges from 1 to 5 months, which is comparable to the onset timing in allogeneic HSCT patients. EBV-negative LPDs generally develop relatively late (>1 year) after transplant. Regardless of EBV infection, T-cell LPDs also occur in the late post-transplantation period with a median interval of 72 months (range 1-324 months), which is consistent with our case (34 months) (4).

Extranodal involvement appears to be more common in LPDs than in *de novo* aggressive lymphomas (1, 7). Indeed, extranodal involvement was observed in as much as 93% (140/150) of T/NK-cell LPDs, while GI involvement was found in only 12% of extranodal lesions (6). Clinical features and endoscopic findings of LPDs are nonspecific and often difficult to distinguish from those of other benign and malignant disorders (8, 9). Strong similarities have been shown between chronic active EBV infective enteritis and inflammatory bowel disease (10-12). A fever (100%), diarrhea (73%), and abdominal pain (64%) were the most frequently observed symptoms (12). The main endoscopic find-

ings were numerous irregular ulcers involving the small intestine and/or colon (12). Our case is not consistent with these previous reports in terms of the clinical presentation or endoscopic findings.

The lymphoma cells in the present case were strongly positive for CD4 and EBER on immunohistochemistry. EBV DNA was detected exclusively in CD4⁺ T-cells. These findings led us to a diagnosis of EBV-positive CD4⁺ T-cell lymphoma, while CD8⁺ T-cells were predominant in the peripheral blood. A flow cytometric analysis showed that more than half of CD8⁺ T-cells were positive for the late activation marker HLA-DR (Fig. 2), suggesting that they were cytotoxic T-cells against lymphoma. Following the initial onset of acute abdomen, the number of peripheral blood lymphocytes increased with recurrent GI symptoms. EBV DNA was detected in CD4⁺ T-cells at an extremely high level but not in the plasma at the diagnosis of T-cell lymphoma, suggesting that the CD4⁺ lymphoma cells had evaded the cytotoxic T-cell response and had not undergone apoptosis. Recent reports have shown that EBV-associated malignancies, such as Hodgkin lymphoma, PTLN, and extranodal NK/T cell lymphoma, often overexpress programmed death (PD)-L1 on tumor cells through the latent membrane protein (LMP)-1 and nuclear factor (NF)- κ B pathways. Although the exact associations with immunodeficiency and EBV infection and T-cell lymphomas are unclear, it is conceivable that EBV-encoded latent genes in tumor cells lead to PD-L1 expression, resulting in evasion from the host immune responses and thereby boosting the tumor survival. In patients with specific HLA alleles, such as HLA A*2402, a tetramer-assisted assay can help evaluate the immune response of EBV-specific CD8⁺ T-cells (13).

The clinical presentation, treatment, and prognosis of immunodeficiency-associated LPDs, especially GI involvement, after autologous HSCT have not been fully elucidated

due to their low incidence. Cases similar to our own are likely to be found due to the widespread use of DBE. Our case also suggests that, regardless of the presence or absence of microscopically apparent lesions, a random biopsy can be a useful tool for the early diagnosis of immunodeficiency-associated LPDs in the GI tract, as has been suggested for cases of GI involvement in mantle cell lymphoma (14).

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

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