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Case Report

Epstein-Barr virus-associated NK-cell lymphoproliferative disorder treated with haploidentical hematopoietic stem cell transplantation

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Abstract: Epstein-Barr virus (EBV) is a ubiquitous herpes virus whose infection is usually asymptomatic and persists for a lifetime. It often causes symptomatic diseases, such as infectious mononucleosis (IM), but rarely leads to EBV associated-NK-cell lymphoproliferative disorder (EBV-NK-LPD) in non-immunocompromised individuals. No studies have described EBV-NK-LPD treated with haploidentical hematopoietic stem cell transplantation (haplo-HSCT). The present study reports a case of a 29-year-old man with recurrent fever and cervical lymph node enlargement. The result of sorting lymphocytes with immunomagnetic beads was diagnosis of EBV-NK-LPD. To stop progression of this disease, haplo-HSCT was employed, with a good prognosis. The patient has survived for one year with no signs of recurrence.

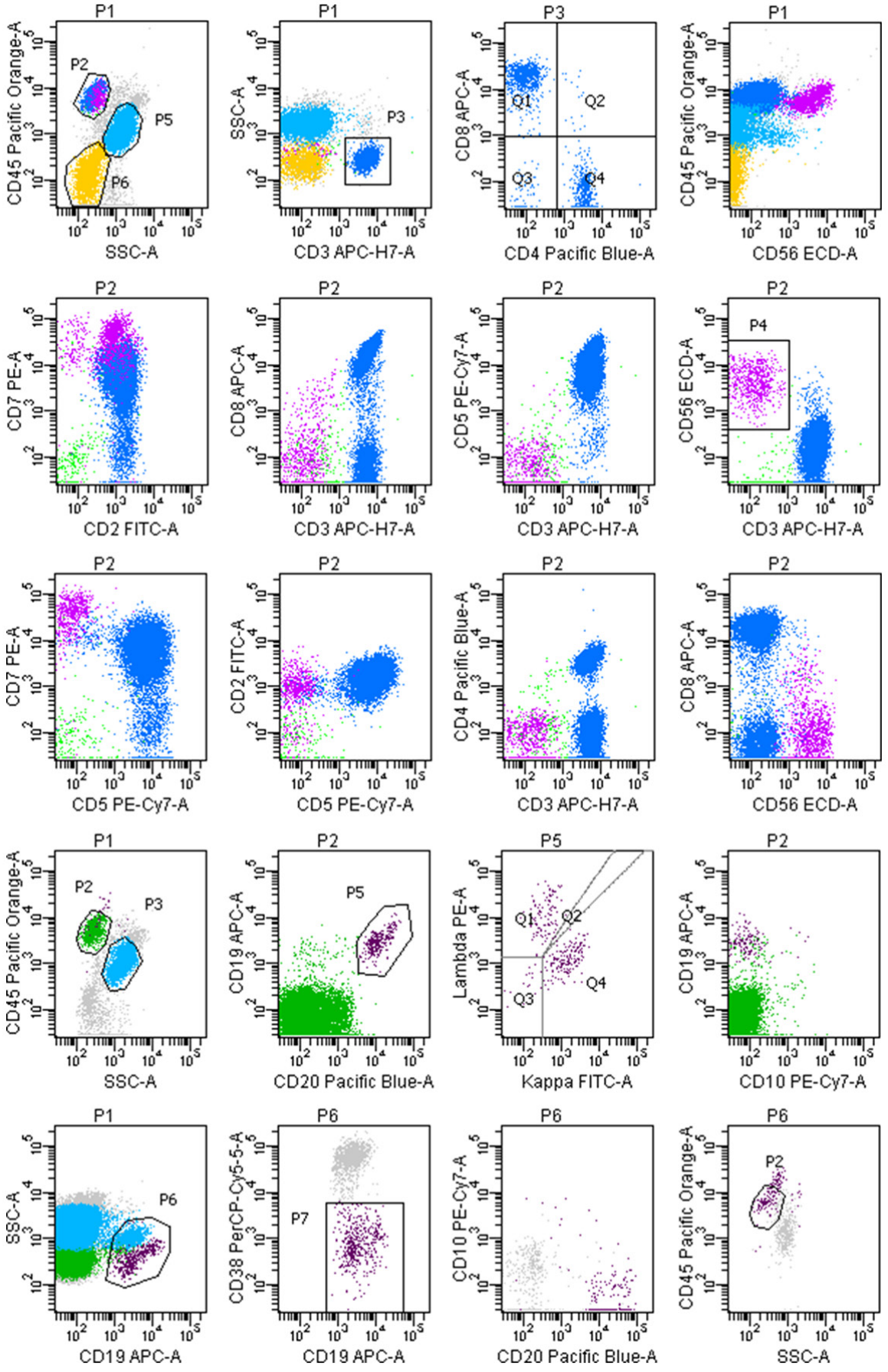
Keywords: Chronic active Epstein-Barr virus infection, Epstein-Barr virus-associated NK-cell lymphoproliferative disorder, EBV target cell sorting, haploidentical hematopoietic stem cell transplantation (haplo-HSCT)

Introduction

Epstein-Barr virus (EBV) is one of eight human ubiquitous herpes viruses, often causing symptomatic diseases, such as infectious mononucleosis (IM) and lymphoproliferative disorder (LPD), in immunocompromised individuals [1]. In addition, EBV has been linked with some human malignancies, including Burkitt lymphoma (BL), nasopharyngeal carcinoma, and gastric carcinoma. A small minority of individuals develop chronic EBV infections with persistent IM-like symptoms, without apparent immunodeficiency. These patients have high EBV-DNA loads in the peripheral blood and monoclonal T-cells or natural killer (NK) cells. Because of life-threatening complications, such as malignant lymphomas, hemophagocytic lymphohistiocytosis, and organ failure, they invariably have poor prognosis. To be unified, this disease has been generally named “chronic active EBV infection” (CAEBV) [2]. The number of reported cases with this entity have significantly increased in the last three decades. Furthermore, recent development of diagnostic procedures has enabled confirmation of certain diseases, especially malignant ones. Okano et.al proposed guidelines for diagnosing CAEBV, in 2005, to clarify this enigmatic disorder [3]. However, due to various clinical hallmarks, outcomes, and underlying diseases, including LPD derived from T-cell or NK-cell lineages, confusion persists concerning diagnosis of CAEBV [4-7]. Moreover, it should be emphasized that Epstein-Barr virus-associated NK-cell lymphoproliferative disorder (EBV-NK-LPD) is a very dangerous disease, with a high rate of early mortality, high misdiagnosis rate, and limited therapeutic regimen. Therefore, early diagnosis and treatment are of the utmost importance for these patients [8]. The present study presents an adult case of EBV-NK-LPD, with a timely diagnosis and good outcome.

Case report

A 29-year-old male patient was hospitalized, in August 2016, due to a year of recurrent fever and three months of cervical lymph node enlargement. Beginning in August 2015, the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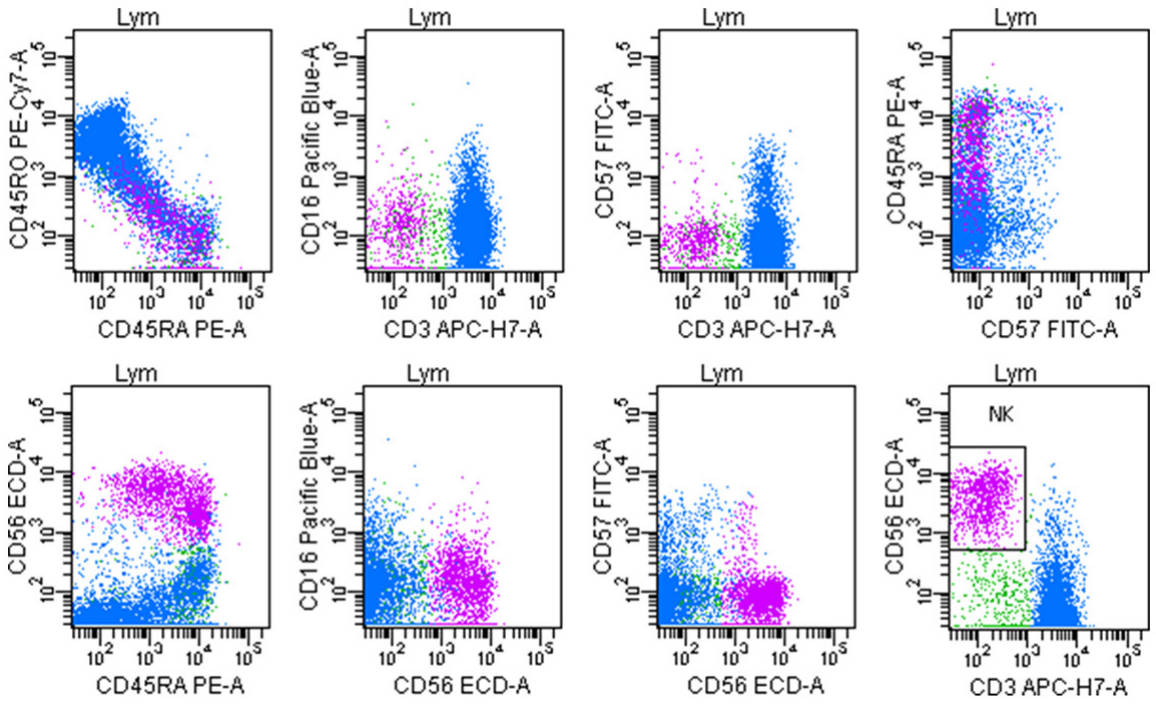

Figure 1. Bone marrow flow cytometry results revealed that 12% CD56 + NK cells, which presented as purple, were identified with CD2, CD7, CD45RA expression, and CD8 partial expression, without CD57, CD5, CD4, CD45RO, and CD16 expression.

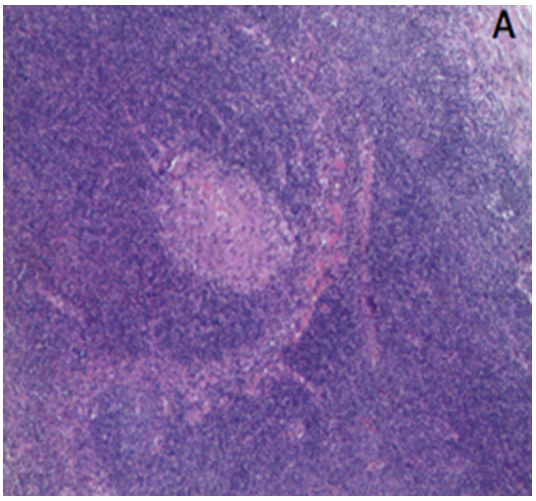

Figure 2. Sample from lymph node biopsy showing that parts of normal structures were reserved. T lymphocytes hyperplasia was found in the paracortex zone with slightly bigger lymphoid cells among them (hematoxylin and eosin staining; magnification, ×40).

patient began to suffer from irregular fever with no obvious cause. The highest temperature was 39.8°C during the entire course of the disease. Afterward, multiple swelling and painful lymph nodes were found in his cervical region. Thanks to treatment with antibiotics, the amount of enlarged lymph nodes was reduced and the pain was alleviated. However, the disease recurred within a short time. He was admitted to the hospital for further examination and treatment. The patient had no previous medical history or family history. He had a splenomegaly, with 3 cm under the costal region. Blood tests revealed that his leukocyte count was 1.52×10^9/L, with a hemoglobin concentration of 106 g/L, and a thrombocyte count of 83×10^9/L. Elevated transaminase (ALT 136 U/L, AST 220 U/L), bilirubin (TB 30.9 μmol/L), and decreased albumin (23.2 g/L) indicated liver dysfunction. The EBV genome load in his peripheral blood was notably high (6.27×10^5 copies/mL).

To identify whether there were malignant hematological diseases, he received bone marrow aspiration and cervical lymph node biopsy. The bone marrow smear showed that the lymphocyte percentage was low and immature lymphocytes occasionally appeared. Bone marrow flow cytometry results revealed that lymphocytes accounted for about 13.4% of total karyocytes. These lymphocytes included 86.8% CD3 + T

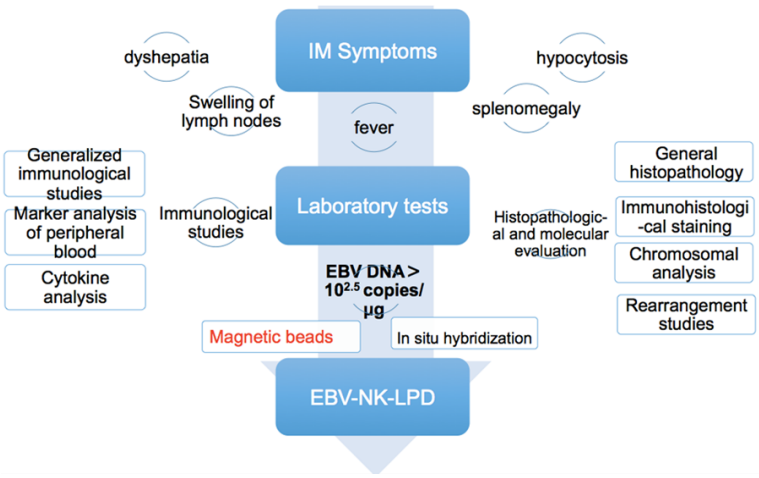

Figure 3. Diagnostic flow chart of EBV-NK-LPD.

lymphocytes, 12% CD56 + NK cells, and 1.2% CD19 + CD20 + B lymphocytes. T lymphocytes expressed CD2, CD5, and CD7, with partial expression of CD57 and CD56. NK cells were identified with CD2, CD7, and CD45RA expression, with CD8 partial expression and without CD57, CD5, CD4, CD45RO, and CD16 expression. There were no monoclonal abnormal B lymphocytes in the bone marrow (**Figure 1**). As results of the cervical lymph node biopsy did not identify the disease, T lymphocytes, B lymphocytes, and NK cells were sorted with immunomagnetic beads to clarify the target cells of EBV infection. Next, real- time probe method was conducted to quantify expression of EBV nucleic acid amounts in each type of lymphocytes. Eventually, quantitative test results could be represented by the ratio of EBV copy number and cell count. Test reports suggested that infected T lymphocytes, B lymphocytes, and NK cells amounts were 2.58×10^5, 1.69×10^5, and 1.95×10^7 ($/2\times10^5$), respectively, indicating NK cells as the main target cells. Finally, lymph node biopsy pathology showed parts of the normal structures were reserved while T lymphocytes hyperplasia was found in the paracortex zone, with slightly bigger lymphoid cells among them (**Figure 2**). Different shapes of multifocal red dye necrotic areas were also found in the lymph nodes. There were a group of infiltrated cells expressing CD3, CD56, Grb, and CD5, while the ratio of Ki-67-positive cells was estimated at 40%, according to immunohistochemistry (IHC). *In situ* hybridization targeted EBV-encoded RNAs (EBERs) of these cells were also positive. Combined with these results, it was considered that the patient suffered from chronic active Epstein-Barr virus disease-natural killer-cell lymphoproliferative disorder (CAEBV-N-K-LPD).

After the chemotherapy GEM-OX regimen, symptoms were briefly controlled, but still relapsed. Due to the lack of human leukocyte antigen (HLA) identical matched donors, haploidentical allogeneic hematopoietic stem cell transplantation (haplo-HSCT) was performed. The patient was treated with Ara-c/VP-16 (100 mg qod, twice)/Bu/cy/ATG regimen for preprocessing, on September 22. Ten days later, he was successfully injected with his father's bone marrow and peripheral blood stem cells. Bone marrow chimeric analysis on November 3rd showed that donor cells accounted for 96.94%, characterized by complete chimerism. Bone marrow flow cytometry results after haplo-HSCT showed that the percentage of lymphocytes was low and CD4/CD8 ratio decreased without antigen loss. This assay did not show obvious abnormal expression of perforin and granular enzyme in NK cells. Subsequently, this patient suffered from graft-versus-host disease (GVHD), mainly in the intestinal tract with cytomegalovirus infection. Thanks to active general treatment with glucocorticoids, immunosuppressors, basiliximab, and antivirals, his symptoms were well alleviated. On May 12, 2017, the EBV genome load was decreased to 2.32×10^3 copies/mL in peripheral blood mononuclear cells (PBMC) and was negative in the plasma, compared to previous results. The patient has remained in good condition for two years, with no evidence of recurrence.

Discussion

EBV is a ubiquitous virus infecting >90% of the adult population, worldwide. B lymphocytes are often the target cells, resulting in infectious mononucleosis. Active EBV infections are rarely prolonged, with abnormal expansion of polyclonal, oligoclonal, and monoclonal T or NK cells. These conditions were defined as EBV-associated T/NK lymphoproliferative diseases

that belong to CAEBV. In 2008, Ohshima et al. proposed a clinicopathological categorization of EBV-T/NK-LPD as category A1 (polymorphic LPD without clonal proliferation of EBV-infected cells), category A2 (polymorphic LPD with clonality), category A3 (monomorphic LPD with clonality), and category B (monomorphic LPD with clonality and fulminant course). It was based on pathological evaluations and molecular data, providing a guide for better understanding of this disorder [9].

Although diagnostic criteria and classifications have been articulated, it remains difficult to diagnose EBV-NK-LPD in clinical practice. Thus, the appropriate approach to diagnose this disease is necessary for clinicians. A detailed diagnostic process was shown in **Figure 3**. Currently, molecular techniques, such as polymerase chain reaction (PCR) and *in situ* hybridization, target EBV-encoded RNAs (EBERs), make it possible to detect EBV genomes. Thanks to these techniques, malignant pathological abnormalities in affected tissues are no more essential in patients with CAEBV at the time of diagnosis [3]. Furthermore, it has been demonstrated that patients with CAEBV have viral loads of more than $10^{2.5}$ copies/mg DNA in the mononuclear cells of peripheral blood [10, 11]. The most important thing in diagnosing CAEBV is to identify target cells in time, thus further examinations are necessary. It is now possible to use techniques, such as double-staining, *in situ*/immuno-histochemistry, or the magnetic beads procedure to differentiate target cells from human B lymphocytes or epithelial cells [12, 13]. As the pathology is often difficult to obtain, this has usually been accomplished with immunobead sorting of PBMC into lymphocyte subsets, followed by quantification EBV-DNA in each subset with PCR [14].

In the present case, the patient presented with repeated fever, organ enlargement, lymphadenopathy, and pancytopenia. These symptoms are typical but with low specificity for diagnosing EBV-NK LPD. To discover the kinds of this disease, PCR was used to detect EBV DNA loads in peripheral blood to screen for EBV infection. This patient showed unexpected high levels of EBV DNA load (6.27×10^5 copies/mL), prompting the diagnosis of CAEBV. Subsequently, routine bone marrow examinations and immunomagnetic beads procedure were used to sort lymphocytes. With these tests, NK cells were found to be the main target cells, eventually confirmed by pathology. Thus, this case was considered a rare adult EBV-NK-LPD category A2.

In the absence of HSCT, therapy for EBV-NK-LPD is often unsatisfactory, while momentarily delaying progression of disease. Antiviral therapy and immunomodulatory agents are usually invalid. Corticosteroids and other immunosuppressive agents only alleviate symptoms. Moreover, patients could develop progressive immunodeficiency, become refractory to therapy, and submit to occasional infections with extended time. Use of cytotoxic chemotherapy and autologous EBV specific CTLs have also been unsuccessful. Although these regimens may induce sustained complete remission, in some exceptional cases [15, 16], HSCT is still the only curative therapy for CAEBV [17]. Moreover, allogeneic HSCT has been successfully performed in several cases in Japan [18-20]. In 2011, Kawa et al. reported excellent results with HSCT, following a non-destructive pretreatment of reduced-intensity hematopoietic stem cell transplantation (RIST). There were 18 pediatric patients with CAEBV treated with RIST in the study. The 3-year event-free survival rate was 85.0 ± 8.0% and 3-year overall survival rate was 95.0 ± 4.9% [20]. In addition, if a patient had no family donor, unrelated cord blood could be an alternative source for RIST [21]. HSCT is, therefore, the best choice for CAEBV. However, it is still accompanied with high risk of transplantation-related complications [22]. Therefore, hope remains of developing novel therapies that are better than HSCT. Bortezomib [23] and valproic acid [24] are two candidate drugs for CAEBV that have been included in recent preclinical studies.

In the present case, the patient was initially treated with chemotherapy including glucocorticoids. Some symptoms, such as fever and lymphadenectasis, were briefly controlled, but there were no significant changes in EBV DNA loads. To achieve the purpose of a cure, it was suggested that he receive allogeneic HSCT as soon as possible. Since he had no HLA identical suited donors, his father was chosen as a donor to implement haplo-HSCT. In recent years, Haplo-HSCT has been confirmed to be a safe and effective option for hematological

malignancies in China [25, 26], though no literature reports of EBV-NK-LPD treated with Haplo-HSCT have been published. It has been reported that the number of life-threatening complications and plasma viral load may be useful factors in predicting outcomes of HSCT [2]. Thus, this study added VP-16 into pretreatment before haplo-HSCT to reduce side effects after transplantation. Although this patient experienced mild GVHD, cytomegalovirus infection, and liver damage after suitable treatment, he fortunately recovered. The EBV genome load decreased to 2.32×10^3 copies/mL in PBMC and was negative in plasma. The most gratifying thing is that he has survived for more than one year with no signs of recurrence. This patient has had a relatively good outcome, until now, but long-term follow up is still necessary.

Acknowledgements

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Disclosure of conflict of interest

None.

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