An Epstein-Barr Virus-Associated Leukemic Lymphoma in a Patient Treated with Rabbit Antithymocyte Globulin and Cyclosporine for Hepatitis-Associated Aplastic Anemia

Title:

An Epstein-Barr virus-associated leukemic lymphoma in a patient treated with rabbit anti-thymocyte globulin and cyclosporine for hepatitis-associated aplastic anemia

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Short title: EPV-LPD after rabbit ATG for aplastic anemia

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Abstract

Lymphoproliferative disorders (LPDs) are generally caused by uncontrolled B-cell proliferation induced by the Epstein-Barr virus (EBV) in the setting of impaired EBV-specific T-cell immunity, particularly when there is pharmacological immunosuppression including Anti-thymocyte globulin (ATG).   We herein present an unusual case of EBV associated with LPD (EBV-LPD) in which LPD occurred 3 weeks after the use of rabbit ATG administered for severe hepatitis-associated AA, and he died of fulminant leukemic lymphoma five days after the onset.   We also review the pertinent literature about EBV-LPD after immunosuppressive therapy, and document efficacy of EBV-viral load monitoring and the need for preemptive therapy.

Introduction

Epstein-Barr virus (EBV) associated Lymphoproliferative disorders (LPD) is

becoming a serious problem with a recent increase in the number of patients

with immunodeficiency.　In particular, patients who have undergone

allogeneic hematopoietic stem cell transplantation (HSCT) are predisposed

to EBV infection or reactivation and development of EBV-related diseases [1].

EBV monitoring is generally recommended for high risk patients such as

HSCT recipients of HLA mismatched donors, and patients receiving

Anti-thymocyte globulin (ATG) after HSCT.　The early detection of EBV

reactivation would make it possible to perform preemptive therapy with

rituximab if necessary, thus preventing the proliferation of EBV infected

B-cells and the evolution of B-cell lymphoma.

Acquired severe aplastic anemia (SAA) is a rare disease defined by

peripheral blood pancytopenia associated with hypocellularity of bone

marrow [2].　Because bone marrow failure is thought to result from an

immune-mediated mechanism, immunosuppressive therapy (IST) is the

treatment of choice in patients without a suitable donor for HSCT.　IST

including ATG and cyclosporine A (CsA) is the most effective treatment for

SAA [3].   Several studies have shown that the use of ATG increases the

frequency of EBV reactivation and the risk of EBV-LPD [4].   However, this

risk has not yet been sufficiently documented in patients treated with ATG

for SAA.   It is also unclear whether EBV-viral load monitoring during IST

for SAA and subsequent preemptive therapy are beneficial [5-6].

We herein report an unusual case of fulminant EBV-LPD that occurred in a

patient as a form of leukemic lymphoma three weeks after the first

administration of rabbit ATG and CsA for hepatitis-associated AA.

Case report

A 54-year-old male presented to the hospital with a 3 week history of general malaise and loss of appetite.   He showed jaundice and had severely deranged liver function tests, with a total bilirubin of 5.5 mg/dL (0.3-1.2 mg/dL, direct fraction: 3.1 mg/dL)), alkaline phosphatase (ALP) of 536 IU/L (115-359 IU/L), aspartate aminotransferase (AST) of 1021 IU/L (13-33 IU/L), alanine aminotransferase (ALT) of 2718 IU/L (8-42 IU/L) and gamma glutaryl transpeptidase (GGTP) of 288 IU/L (10-47 IU/L).   His blood tests showed a platelet count of 12×10^9/L, hemoglobin (Hb) of 13.1 g/dL and a white blood cell count (WBC) of 1.4×10^9/L, with neutrophils of 0.7×10^9/L. The absolute counts of CD4 and CD8 positive T-cells were 0.2×10^9/L and 0.1×10^9/L.   A blood film showed leukopenia and thrombocytopenia, with no abnormal morphology.   There was no history of recent travel, blood transfusions, and the use of medications or excess alcohol consumption. Subsequent investigations showed no evidence of a viral etiology.   Hepatitis A virus IgM, Hepatitis B virus (HBV) Ag, hepatitis B core IgM, HCV antibody, hepatitis C RNA PCR, hepatitis E virus IgM and IgG were all negative.   The HBs antibody was positive, but HBV-PCR was negative.

The cytomegalovirus (CMV)-IgG was positive and CMV-IgM was weakly

positive.   His anti-EBV antibody titers were VCA IgG positive, VCA IgM

negative, EA negative, and EBNA positive.   Parvovirus B19 IgM and IgG

serology were negative.   <u>The patient was also negative for HIV antibodies.</u>

The bone marrow was severely hypocellular, which was consistent with AA.

Immunophenotyping of bone marrow cells was normal, and there was no

evidence of paroxysmal nocturnal hemoglobinuria.   <u>A liver biopsy was not</u>

<u>performed due to the presence of severe thrombocytopenia.</u>   We diagnosed

him with hepatitis-associated AA.   <u>ATG therapy was put on hold until his</u>

<u>liver function tests improved, but his pancytopenia progressed without</u>

<u>normalization of his jaundice.   Rabbit ATG was started three weeks after</u>

<u>admission at a dose of 3.75 mg/kg on days 1-5, and CsA at a dose of 3 mg/kg</u>

<u>with methylprednisolone, which resulted in a rapid improvement in his liver</u>

<u>function.</u>   He was further treated with prednisolone for prophylaxis of

serum sickness, with normalization of liver function tests, but his

hematological data still showed pancytopenia.

Three weeks after the administration of ATG, the patient developed a

persistent high fever, which was refractory to antibiotics and antifungal

agents.   Moreover, his liver function tests worsened, including a total

bilirubin of 1.9 mg/dL, AST of 408 IU/L, ALT of 577 IU/L, γGTP of 779 IU/L,

and ALP of 848 IU/L.   <u>Blood tests showed a platelet count of 15×10^9/L, Hb of

9.2 g/dL and a WBC of 7.5×10^9/L.</u>   A peripheral blood smear revealed an

increased number of lymphocytes (2.0 x 10^9/L), but no apparent

hemophagocytic findings.   <u>The phenotype of the atypical lymphocytes was

CD3–, CD10–, CD19+, CD20+ and IgG light chain lambda+.</u>   The serum

ferritin (199,770 ng/mL) level was also markedly elevated.   Based on these

clinical signs, laboratory data, and the use of ATG, EBV-LPD was highly

suspected.   The administration of rituximab was considered, but severe

metabolic acidosis and cardio-respiratory failure developed the evening after

EBV-LPD was diagnosed and the patient died of the LPD on the following

day.

The belated results of the peripheral blood EBV-DNA (3.3×10^6 copies/

10^6WBC) and pathological examination from liver biopsy (demonstrating an

increase in CD20 positive lymphocytes that were positive for EBV-encoded

mRNA (EBER) by *in situ* hybridization) confirmed the diagnosis of

EBV-associated diffuse large B-cell lymphoma (DLBCL).   Infiltration of the

lymphoma was also detected in his bone marrow. We analyzed the patient's

peripheral plasma EBV-DNA retrospectively. His plasma showed an

elevation of the EBV-viral load to 700 copies/mL for the first time at 7 days

before the onset of pyrexia, and the EBV-viral load rapidly increased within

5 days (Figure 1).

Discussion

We herein describe the occurrence of fulminant EBV-LPD that was diagnosed following a sharp increase in the atypical B cell count in the peripheral blood three weeks after IST for hepatitis-associated AA. The patient died of cardio-respiratory failure associated with severe lactic acidosis due to rapidly progressive lymphoma. Lactic acidosis in association with hematologic malignancies normally shows an extremely poor prognosis [7].

It is well documented that EBV is an important complication of prolonged immunodeficiency. All patients who have a limited number of circulating T cells and retain B cells are at risk of developing EBV reactivation, as the interplay among EBV replication, latency, and immune control is not as balanced as in the healthy host. When there is pharmacological immunosuppression, EBV reactivation can lead to LPD because T cell function is severely impaired and B cells can evade T cell attack and expand. This is particularly common in patients undergoing HSCT with an ATG-containing conditioning regimen [4].

Treatment with ATG combined with CsA is the standard therapeutic

approach to SAA.   Scheinberg et al. showed the risk of EBV disease in patients treated with ATG for AA to be low [5].   In their study of 78 patients with SAA who had received four different immunosuppressive regimens, including ATG, even though EBV reactivation occurred in most patients, none developed symptomatic EBV-LPD.   To determine the incidence of clinically-significant EBV-LPD, we searched Medline for published articles about LPD after IST for AA.   As shown in Table 1, a total of 8 cases of LPD that occurred after IST have been reported [6,8-14].

Viola et al. recently reported a patient who developed LPD 1 month after the use of horse ATG for the treatment of SAA [14].   Although the interval between ATG therapy and the onset of LPD in this case was short, similar to our case, he had received chemotherapy and autologous HSCT for the treatment of NHL 3 years prior to the ATG therapy, both of which may have predisposed the patient to develop LPD.

Wondergem et al. described a patient who received a higher dose rabbit ATG for SAA after failing to respond to horse ATG.   The patient then developed life-threatening EBV-related lymphoma [6].   In our patient, LPD occurred after his first ATG therapy.   To the best of our knowledge, this is

the first report of an EBV-related DLBCL in a patient treated with a single course of rabbit ATG.

Recently, the viral load has been shown to be a significant predictor of EBV related post-transplant lymphoproliferative disorders [15-16], and early treatment with an anti-CD20 antibody is recommended as preemptive therapy in patients undergoing an alternative donor transplant [1]. Because of the rapid clinical course of EBV-LPD, immediate treatment is crucial to reduce mortality [6]. No lymphocytosis or pyrexia was observed when the EBV-DNA level began to increase in our patient, (Figure 1). Preemptive therapy with rituximab may improve the treatment outcome of EBV-LPD, not only after HSCT [17], but also after IST for SAA. However, the EBV copy number in the plasma of the current patient at day 19 of ATG therapy, 1 week before the onset of pyrexia, was 700 copies/mL, but it increased by more than 20 fold on day 24 (16,000 copies/mL). Therefore, once a week screening of EBV would have been useless for this patient. The prompt examination of blood for EBV copy number in response to clinical signs such as pyrexia may be more practical than surveillance for appropriately starting rituximab. The preventive administration of

rituximab would be a possible option for AA patients with a high risk of developing EBV-LPD.

Several risk factors for susceptibility to EBV-related lymphoma have been identified. Dierksheide et al. showed that an IFN-gamma polymorphism affects a likelihood of EBV reactivation [18]. Genotyping of the IFN-gamma gene may be useful for identifying patients at greater risk of developing EBV-LPD. The percentage of $CD4^+$ T cells in hepatitis-associated AA patients is reported to be significantly lower than that in non-hepatitis-associated AA patients [19]. Therefore, the presence of hepatitis-associated AA may have predisposed our patient to developing EBV-LPD.

The short time interval between ATG treatment and diagnosis and the fulminant course of EBV-LPD in our case and in the case reported by Viola et al. may be related to the profound immunosuppressive state associated with hepatitis and precedent chemotherapy in the cases.

The present case indicates that fulminant EBV-associated lymphoma can occur in patients with AA even after a single course of rabbit ATG therapy. Close monitoring of the EBV-viral load is therefore prerequisite for rabbit

ATG therapy of AA.

Tables

Table 1. Reports of Lymphoproliferative disorders (LPD) after immunosuppressive therapy for aplastic anemia (AA)

References	Case	Type of LPD	Immunosuppression
Dorr, 1996	17 F	Lymphoma	ATG, CsA
Sarangi, 1999	22 M	T-ALL	CsA
Takeuchi, 2000	54 F	B-ALL	CsA, PSL etc
Hirose, 2001	24 M	T-ALL	CsA
Calistri, 2006	38 M	Infectious mononucleosis	rATG, CsA, M-PSL → hATG
Wondergem, 2008	42 F	EBV(+)DLBCL	hATG, CsA → rATG, CsA
Suzuki, 2009	63 F	EBV(–)DLBCL	ATG, CsA
Viola, 2010	55 M	EBV(+) plasma cell hyperplasia	hATG
Our case	54 M	EBV(+)DLBCL	rATG, CsA, PSL

ALL = acute lymphoblastic leukemia, DLBCL = diffuse large B-cell

lymphoma, EBV = Epstein-Barr virus, ATG = anti-thymocyte globulin, hATG

= horse ATG, rATG = rabbit ATG, CsA = cyclosporine, PSL = prednisolone

Figure legends

Figure 1. The changes in plasma Epstein-Barr virus (EBV)-DNA and his

body temperature.   Open and closed circles represent EBV-negative and

positive states.   Increase in EBV-DNA was observed two weeks after

administration of Anti-thymocyte globulin (ATG).

Acknowledgements

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