Epstein-Barr virus (EBV) reactivation in a patient treated with ATG for severe aplastic anemia

American Journal of Hematology 81:355–357 (2006)

Epstein-Barr Virus Reactivation in a Patient Treated with Anti-thymocyte Globulin for Severe Aplastic Anemia

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Epstein-Barr virus (EBV) infection and reactivation is an increasing complication in immune deficient patients, particularly after allogeneic hematopoietic stem cell transplantation (HSCT). Therapy with anti-thymocyte globulin (ATG) is associated with higher incidence of EBV-related disease in HSCT patients, but this risk is not documented in patients receiving ATG for severe aplastic anemia (SAA). We describe the case of a patient who developed an EBV infection, with the clinical features of an infectious mononucleosis, after immune suppression with cyclosporine and two courses of ATG for SAA. Am. J. Hematol. 81:355–357, 2006. © 2006 Wiley-Liss, Inc.

Key words: Epstein-Barr virus; anti-thymocyte globulin; severe aplastic anemia; rituximab

INTRODUCTION

Epstein-Barr virus (EBV) disease has been increasingly observed in immune-deficient hosts. In particular, patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT) are predisposed to EBV infection or reactivation and development of EBV-related diseases [1]. Acquired severe aplastic anemia (SAA) is a rare disease defined as peripheral blood pancytopenia associated with hypocellularity of bone marrow [2]. Because bone marrow failure is thought to result from an immune-mediated mechanism, immunosuppression is the treatment of choice in patients without a suitable donor for HSCT. Anti-thymocyte globulin (ATG) is the single most effective drug in SAA [3,4]. Several studies have shown that the use of ATG in allogeneic HSCT reduces the incidence and severity of acute and chronic graft-versus-host disease but increases the risk of EBV infections and EBV-related lymphoproliferative disorders [reviewed in 5].

To our knowledge, however, this risk is not documented in patients treated with ATG for SAA. Here we report a case of infectious mononucleosis in a 39-year-old patient with SAA treated with rabbit ATG and horse ATG.

CASE REPORT

A 38-year-old man was diagnosed as having SAA in October 2003. He was admitted for the rapid development of a hemorrhagic syndrome with leg petechiae, epistaxis, and gum bleeding; blood count was hemoglobin (Hb) 10.4 g/dL, platelets 5×10^9/L, WBC 4.2×10^9/L with 13% neutrophils, 81% lymphocytes, and 6% monocytes. The bone marrow biopsy and aspirate were diagnostic for SAA (cellularity 10%); the cytogenetic analysis did not show any chromosomal abnormality. Serologic studies for HIV, HAV, HBV, HCV, CMV, parvovirus, and anti-nuclear antibodies were all negative. Anti-EBV antibody titers at diagnosis were VCA IgG positive, VCA IgM negative, EA negative, and EBNA positive.

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Received for publication 5 May 2005; Accepted 12 September 2005

Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.20560

The patient was treated with methylprednisolone (1 mg/kg/day, day 1–30), cyclosporine A (5 mg/kg/day) and rabbit ATG (3.5 mg/kg/day, day 1–5). Despite this treatment, no improvement was noted. On day +90 the patient was still anemic (Hb 8.8 g/dL) and thrombocytopenic (platelets 5×10^9/L); a biopsy confirmed a severe bone marrow hypocellularity (10–15%).

In February 2004 he received therapy with horse ATG (3.5 mg/kg/day). On day +19 he developed fever and elevated bilirubin and lactate dehydrogenase. Antibiotic therapy with meropenem was started, without appreciable effect. Enlarged and painful cervical lymph nodes (3 cm in diameter) were noted by day +20. WBC count was 6.5×10^9/L with 67% atypical lymphocytes. Flow cytometry of circulating lymphocytes revealed 85% CD3+, 28% CD4+, 58% CD8+, 6% CD20+, 6% CD19+, and 4% CD16/56+ cells. Most CD3+ cells expressed HLA-DR, and CD8+ lymphocytes co-expressed CD45RO (56% CD8/45RO+) and CD28 (54% CD8/28+). The presence of an active EBV infection was confirmed using real time PCR for the quantitative detection of EBV-DNA in plasma; on day +24 the EBV copy number resulted 30,000/150,000 cells. Based on these clinical and laboratoristic findings, a diagnosis of infectious mononucleosis was made. Cyclosporine therapy was stopped and, considering the high risk of developing an EBV-associated lymphoproliferative disease, the patient received two doses of anti-CD20 antibody rituximab (375 mg/sqm) on days +27 and +36. A decrease of viral load was observed early after the first infusion of anti-CD20 (EBV copy number by real time PCR: 300/150,000 cells on day +29; 30/150,000 cells on day +31; 1/150,000 cells on day +34). Concomitantly there was a progressive improvement of the clinical picture with a complete clinical response on day +46. Blood count was Hb 10.5 g/dL, platelets 30×10^9/L, WBC 6.5×10^9/L (lymphocytes 52%). Laboratory findings were normal and plasma EBV-DNA became negative. The bone marrow biopsy again showed a hypocellular marrow (cellularity 5%).

During the following months the plasma level EBV-DNA was repeatedly negative. Because of persistent anemia, thrombocytopenia, and bone marrow hypocellularity, the patient was considered a nonresponder to ATG. He refused an allogeneic HSCT from a matched unrelated donor and from August 2004 he was treated with androgens, achieving a partial hematologic response with transfusion independence (Hb always >8.0 g/dL), stable platelet count around 30×10^9/L, and an absolute neutrophil count greater than 1.5×10^9/L without G-CSF.

DISCUSSION

It is well documented that EBV is an important complication of prolonged immune deficiency. All patients who have few circulating T cells and still have 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. Particularly when there is pharmacological immunosuppression, EBV infection does not generally manifest as "classical" infectious mononucleosis, presumably because immunosuppressive drugs prevent the development of cytokine-secreting T cells that cause the symptoms [6]. In this situation, instead, EBV reactivation can lead to B-lymphoproliferative disease (BLPD) and lymphoma, because T cell function is severely impaired and B lymphocytes can evade T cell attack and expand. This is particularly common in patients undergoing HSCT with an ATG-containing conditioning regimen [5].

Treatment with ATG, combined with other immunosuppressive drugs (corticosteroids and cyclosporine A) or androgens, is also the therapeutic approach to SAA [3,4]. To the best of our knowledge, this is the first report of an EBV reactivation in a SAA patient treated with ATG. Of note, despite the in vivo purging of T cells due to ATG, EBV reactivation induced the clinical picture of an infectious mononucleosis. In fact, by real time PCR, EBV DNA copy number in plasma peaked at 30,000/150,000 cells. In addition, a high percentage of CD8+ cells with activated phenotype (CD8/45RO-positive and CD8/28-positive) were detected. This feature is peculiar of EBV-positive atypical lymphocytosis [7] and the activated CD8 cells were probably responsible for the characteristic signs and symptoms, as a result of massive cytokine production.

Because of bone marrow failure caused by SAA and the two lines of immunosuppressive therapy, our patient was considered at high risk of developing EBV-related lymphoma. Moreover, the viral load is a significant predictor of BLPD in the allogeneic transplant setting [8,9] and early treatment with an anti-CD20 antibody is recommended as pre-emptive therapy of EBV-related lymphoma in patients undergoing an alternative donor transplant [1].

Rituximab, alone or in addition to other therapies, promises a profound change in the landscape with regard to the treatment and perhaps the prevention of posttransplant lymphoproliferative disease.

Faye and co-workers reported a 66% complete response rate in 12 pediatric patients with BLPD after allogeneic HSCT. Early treatment with rituximab seems to be the most effective approach [10]. Milpied et al. [11] reported a multicentric experience with the use of rituximab in the treatment of 32 cases of

BLPD. Twenty-six patients had undergone solid organ transplants and 6 had received bone marrow transplantations. BLPD was associated with EBV in 22 of 26 tested cases. Overall response rate was 69%, with a remarkable 83% (5 of 6) in bone marrow transplanted patients [11].

Immediately after the first rituximab infusion, EBV DNA copies were significantly reduced and the clinical conditions improved greatly. To date, 12 months after viral reactivation, the patient remains negative for EBV DNA in plasma.

Our observation suggests that ATG treatment may represent a risk for EBV reactivation also in the SAA patients. In addition, our case is consistent with the efficacy of rituximab in preventing EBV-related lymphoma in immunocompromised hosts.

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