Letter to the Editor

Epstein-Barr virus-induced polyclonal lymphoproliferative disorder of lymphoplasmacytic type in an autopsy case of aplastic anemia treated twice with anti-thymocyte globulin therapy

To the Editor:

Epstein-Barr virus (EBV) is a double-stranded DNA virus of the gamma herpesvirus group. Latent infection of EBV in the B-cell lineage is common in healthy individuals. EBV infection usually occurs during early childhood, but most individuals are asymptomatic. In immunosuppressed patients, EBV is reactivated to develop B-cell lymphoma or lymphoproliferative disorder (LPD). Young healthy individuals may develop a life-threatening chronic active EBV infection.

We report LPD seen in an autopsy case of aplastic anemia after immunosuppressive therapy using anti-thymocyte globulin (ATG) twice. ATG kills T-cells and stimulates T-cells to secrete hematopoietic cytokines. EBV-infected highly proliferative lymphoplasmacytic cells (suggestive of the latency level III) showed polyclonality. The patient died of enterococcal colitis, and at autopsy, LPD was found microscopically, the polyclonality may be related to early incidental detection of EBV-related lymphoproliferation.

A 68-year-old male complaining of general fatigue was hospitalized to the hematology ward, Fujita Health University Hospital in February, 2010. The peripheral blood revealed pancytopenia (leukocytes 2.9 × 10^9/L, hemoglobin 8.7 g/dL, reticulocytes 0.8% and platelets 9.0 × 10^9/L). Under the diagnosis of severe aplastic anemia, immunosuppressive therapy with cyclosporine, rabbit ATG (2.5 mg/kg) and prednisolone was given, together with granulocyte colony-stimulating factor infusion and erythrocyte and platelet transfusion. The patient experienced cytomegalic hepatitis and drug-induced thrombocytopenia slightly improved 3 months later. Consequently, he was followed up in an outpatient clinic. In May, 2011 when the patient general condition was stable but with an elevated serum prostate-specific antigen level (7.9 ng/dL), prostate adenocarcinoma was found by needle biopsy. In December, 2011, brachytherapy, implanting radioactive, iodine-125 seeds in the prostate, was done.

Aplastic anemia exacerbated in April, 2012, and the patient again received immunosuppressive therapy at the end of May, 2012. The second challenge of 5-days ATG infusion did not provoke acute allergic reactions. The patient’s condition gradually worsened with fever, gastrointestinal bleeding and renal dysfunction. The patient died on June 30, 2012. The total clinical course was 2 years and 4 months, half a year after brachytherapy and one month after the second ATG administration. Serum EBV antibodies were not evaluated.

Autopsy was performed 10 h after death. The bone marrow showed marked fatty marrow, with mucocutaneous petechiae and secondary hemosiderosis associated. The direct cause of death was multifocal colonic enterococcal infection with massive intestinal hemorrhage and serositis-induced retention of ascites (1500 mL). Pulmonary edema and renal acute tubular necrosis indicated eventual complication of shock. Gram-positive cocci colonizing the colonic wall were characteristic of colloidal iron-positive capsule formation and immunoreactive for Enterococcus antigens but not for Staphylococcus, Streptococcus and Pneumococcus antigens. No cytomegaly inclusions were seen.

The normal-sized prostate implanted with 80 small black seeds was carefully removed on the table. Several seeds were found in the extra-prostatic pelvic soft-tissue. Because of residual radioactivity, microscopic evaluation of the prostate was not allowed. No metastasis was noted.

The spleen and liver were normal-sized, and weighed 120 g and 1170 g, respectively. The enlarged splenic white pulp unexpectedly showed diffuse infiltration of lymphoplasmacytic cells. Some lymphoplasmacytic cells possessed enlarged nuclei. Mitoses were dispersed. In situ hybridization for EBV-related small nuclear RNA (EBER1) showed nuclear positivity, and Ki-67 labeling index was more than 80%. A small number of cells expressed latent membrane antigen-1 (LMP-1) and EBV nuclear antigen-2 (EBNA2). Polymerase-mediated immunoperoxidase staining after appropriate epitope retrieval disclosed diffuse membrane positivity of CD38 and CD45, focal (limited) reactivity to CD20 and cytoplasmic positivity of bcl-2. CD79a and CD138 were not expressed. Negative markers included CD10, CD30, CD56, CD57, cyclin D1 and p53 oncprotein. Reactive T-cells (CD3+, CD8+) and macrophages (CD68+) were dispersed. Immunoglobulin immunostaining revealed polyclonality with cytoplasmic positivity for both kappa and lambda chains. Regarding the heavy chain expression, gamma, alpha and mu chains were identified in this order (IgG->IgA -> IgM). The EBER1-positive polyclonal lymphoplasmacytic cells were also distributed in the liver, bone marrow, kidney, adrenal glands and colon. Representative microscopic features of the spleen are illustrated in Figs 1 and 2.

Southern blot analysis for EBV clonality was not applicable because of failure to extract intact DNA from the spleen. Clonality of the complementary determining region III of
immunoglobulin heavy chain was analyzed by polymerase chain reaction using DNA selectively extracted from clusters of lymphoplasmacytic cells in the paraffin-embedded spleen. A polyclonal pattern was demonstrated.

EBV transforms B-cells into lymphoblastoid cell lines showing a potential of unlimited growth.⁴ Cytotoxic T-cells and natural killer cells eliminate the infected B cells.⁵ EBV latently survives in B-cells and remains dormant in the host.² Once the patients become immunosuppressed secondary to immunosuppressive therapy or human immunodeficiency virus infection, EBV is reactivated, causing subsequent development of B-cell lymphoma or LPD. A life-threatening
condition, chronic active EBV infection, may occur in the youth without systemic immunosuppression.3

In our case of aplastic anemia, immunosuppressive therapy with cyclosporin and ATG provoked EBV reactivation. At autopsy, polyclonal and activated growth of EBER1-positive lymphoplasmacytic cells were incidentally observed in varied lymphoid organs, particularly in the spleen. Focal co-expression of LMP-1 and EBNA2 strongly suggested the latency level III of EBV infection.2,3 The lymphoplasmacytic cells were immunoreactive for both kappa and lambda chains and for three heavy chains such as IgG > IgA > IgM. Polyclonality was further confirmed by the clonal analysis of immunoglobulin heavy chain. CD38, CD45 and bcl-2 were expressed. Partial expression of CD20 and negativity of CD79a and CD138 may represent incomplete plasmacytic differentiation of the EBV-infected cells.

Lymphoplasmacytic appearance is a cytologic hallmark of lymphoplasmacytic lymphoma or Waldenström’s macroglobulinemia accompanying monoclonal IgM secretion, but Ki-67 labeling index of such low-grade B-cell malignancy is usually low. Lymphoplasmacytic LPD in our case is unique in showing polyclonality and high Ki-67 labeling index. The EBV-activated growth of B-cells should thus be regarded as non-neoplastic.

Lymphoplasmacytic growth was documented in a case of aplastic anemia after bone marrow transplantation.6 Rashid et al. reported reactive EBV-positive growth of lymphoplasmacytic cells in the lacrimal gland in a case of acute lymphoblastic leukemia after chemotherapy.7 Lin et al. described roles of EBV activation in overt transformation of low-grade lymphoplasmacytic lymphoma.8 Highly aggressive nature of post-transplantation EBV-related lymphoplasmacytic lymphoma has been clarified.9

Expectedly, EBV initially induces polyclonal B-cell growth, but with time, the clonality may be changed to oligoclonal and finally to monoclonal. In our case, enterococcal enterocolitis killed the patient, and LPD was microscopically found in grossly unremarkable lymphoid tissues. The death occurred just one month after the second challenge of ATG. The polyclonality may thus be related to the subclinical detection of EBV-related lymphoproliferation in an early stage.

We must discuss the relationship between EBV reactivation and brachytherapy for prostate cancer. Some radioactive implants migrated to the pelvic soft tissue outside the prostate, and the radioactivity may result in EBV reactivation. The radiation sources are enclosed in protective capsules, the irradiation affects localized areas just around them, and the photon energy of the seed is low.10 Hence, brachytherapy showed little effect on reactivating EBV in our case.

We should emphasize that ATG-induced immunosuppression may provoke EBV-associated LPD of highly proliferative lymphoplasmacytic type, with polyclonality detected in its early stage.

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DISCLOSURE

We have no conflict of interest in reporting the present case.

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