High Fatality Rate of Epstein-Barr Virus-Associated Lymphoproliferative Disorder Occurring after Bone Marrow Transplantation with Rabbit Antithymocyte Globulin Conditioning Regimens

E. Peres,* S. Savasan, J. Klein, M. Abidi, R. Dansey, and E. Abella

Bone Marrow Transplant Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan 48201

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Epstein-Barr virus (EBV)-associated lymphoproliferative disorder (EBV-LPD) following bone marrow transplantation can be fatal. The major risk factors for the development of EBV-LPD are ex vivo T-cell depletion or in vivo T-cell depletion with either antithymocyte globulin (ATG) or monoclonal anti-T-cell antibodies. Between March 1999 and January 2001, a total of 23 transplants with ATG of equine source (20 transplants) and ATG of rabbit source (3 transplants) used as part of the preparatory regimen were performed at the Barbara Ann Karmanos Cancer Institute in Detroit, Mich. The three patients who received rabbit ATG developed EBV-LPD between 60 and 90 days following bone marrow transplantation. However, there were no cases of EBV-LPD in the equine group. Treatment given in these cases consisted of tapering immunosuppression, antiviral therapy, unprocessed donor lymphocyte infusion, mobilized peripheral blood progenitor cell rescue infusion (one patient), and chemotherapy (one patient). All three patients died of complications from EBV-LPD. The association of rabbit ATG with the development of EBV-LPD suggests that patients receiving rabbit ATG as part of their preparatory regimens require close monitoring of the EBV viral load and possible early intervention with antiviral therapy.

CASE REPORTS

Case 1. A 1-year-old female with malignant osteopetrosis received a conditioning regimen with high-dose cyclophosphamide and rabbit antithymocyte globulin (ATG), at a dose of 5 mg/kg of body weight/day, for 4 days followed by an HLA-matched unrelated-donor umbilical cord transplant. Immunosuppression after transplantation consisted of cyclosporine, methotrexate, and corticosteroids. The patient did not receive any additional immunosuppression besides graft-versus-host disease (GvHD) prophylaxis with cyclosporine. On day 49, she developed low-grade fever, dyspnea, and rash. The fever, dyspnea, and rash persisted even after treatment with empirical antibiotic therapy and initiation of steroids for presumptive acute GvHD. The patient subsequently deteriorated and required mechanical ventilation. Bronchoalveolar lavage fluid was used in viral and bacterial cultures and Epstein-Barr virus (EBV)-PCR. Empirical antiviral therapy with ganciclovir was started. The patient further deteriorated and died on day 54 as a result of multiorgan failure. Autopsy findings revealed extensive multiorgan involvement, including the lungs, kidneys, liver, and multiple lymph nodes, and microscopy showed disseminated polymorphous B cells (posttransplant lymphoproliferative disease [PTLD]). These cells stained strongly positive for EBER, a nontranslated RNA (Fig. 1). PCR and EBV serology results, which were consistent with the diagnosis of PTLD, were subsequently available.

Case 2. A 28-year-old female with scleroderma received a conditioning regimen which included high-dose cyclophosphamide, total-body irradiation, and rabbit ATG at a dose of 5 mg/kg/day, followed by an autologous CD34-selected bone marrow transplant (BMT). The patient received acyclovir prophylaxis (800 mg orally twice a day) for a positive herpes simplex virus serology after transplantation. On day 54, she was readmitted with fatigue, adenopathy, and fever. Empirical antibiotics and antiviral therapy with ganciclovir were initiated. A reduction in her dose of steroids, which she had been taking for pulmonary toxicity, was immediately instituted. An infusion with unprocessed autologous peripheral blood progenitor cells was given on day 60 because of a presumptive diagnosis of EBV-associated lymphoproliferative disorder (EBV-LPD). The patient required mechanical ventilation and died of multiorgan failure on day 63. Subsequent studies were positive for EBV-PCR, and an immunohistochemical examination of the lymph node was positive for EBER. Autopsy findings were consistent with EBV-LPD (Fig. 2). This case was previously reported by Nash et al. (11).

Case 3. A 35-year-old female with Philadelphia chromosome-positive acute lymphoblastic leukemia in first complete remission received a conditioning regimen with cyclophosphamide, total-body irradiation, and rabbit ATG (10 mg/kg/day), followed by matched unrelated-donor stem cell transplantation. On day 58, the patient was readmitted with fever,
lymphadenopathy, night sweats, and dyspnea. A lymph node biopsy was performed and revealed a population of CD45-, CD19-, CD20-, and HLA-DR-positive cells. The patient was immediately weaned from immunosuppression therapy (corticosteroids). She had been receiving corticosteroids for a grade II acute GvHD of the skin. Bacterial and viral cultures were obtained along with peripheral blood for EBV-PCR and EBV serology. Multiorgan failure developed, and she died on day 62. Postmortem examination revealed infiltration of the lungs, heart, lymph nodes, and spleen by polymorphic lymphocytes and large-cell immunoblasts (Fig. 3).

PTLD is typically associated with an uncontrolled proliferation of B-lineage cells and occasionally T-lineage cells and can be fatal to the immunocompromised host. PTLD encompasses a heterogeneous group of EBV-initiated lymphoid proliferations ranging from a reversible polyclonal disorder to an oligoclonal or monoclonal lymphoproliferation that is irreversible (18). It is usually caused by EBV, which is a gammaherpesvirus with potent B-cell-transforming activity (16). EBV causes a self-limiting infection in the adolescent population and remains dormant in a subset of B cells (16). The virus persists in these B lymphocytes and is unrecognized by the EBV-specific HLA class I-restricted cytotoxic T cells (CTL), which are the effector cells in the nonimmunocompromised host (5). However, EBV-infected cells rarely cause disease in individuals who are not severely immunocompromised (5).

PTLD is a well-recognized complication occurring in patients with T-cell immune deficiencies (5). In the transplant setting, EBV-LPD in the majority of cases is a reactivation of the infected B cells that harbor the virus (5). It is characterized by the expression of eight viral genes, six of which encode nuclear proteins (EBNA-1, -2, -3A, -3B, -3C, and -LP) and two of which encode latent membrane proteins, LMP-1 and -2 (5). There are also two nontranslated RNAs (EBER-1 and EBER-2), which can be identified by histological staining of infected cells (5). Reactivation occurs after an appropriate stimulus, subsequent production of virions, and proliferation (5). This reactivation occurs secondarily to the suppression of cellular immunity after stem cell transplant (5). The pathogenesis seems to be an expansion of transformed cells that proliferate in the absence of T-cell immunity (10).

The NK-cell activity usually controls the proliferation of B cells via two target antigens, EBNA-2 and LMP-1 (5). CTL can usually eliminate the B cells in which the viral replication has been initiated and prevent further proliferation (5).

The risk for developing EBV-LPD is increased after solid-organ or bone marrow transplantation and usually occurs within the first 2 years, during T-cell function impairment (10). Primary EBV infection occurs in as many as 90% of PTLD patients, making seronegativity in the recipient a risk factor for the development of PTLD (10). PTLD has been reported to occur in both allogeneic and autologous BMTs (10). The reported incidence of PTLD in the bone marrow transplantation setting is 1%, with the majority occurring after allogeneic stem cell transplants (7). Clinical features of PTLD are usually nonspecific and may include fever, malaise, fatigue, lymphadenopathy, and diarrhea (7). PTLD can involve every organ system and may present as either localized or disseminated disease. PTLD may be rapidly progressive or may wax and wane for
several weeks to months (7). Here, we discuss the clinical characteristics, time to development of PTLD, treatment, and outcome for a subgroup of patients.

Between March 1999 and January 2001, a total of 539 patients received allogeneic (154 patients) and autologous (385 patients) BMTs at the Karmanos Cancer Institute of Wayne State University. Of the 539 patients, 23 received a conditioning regimen of ATG, with either horse or rabbit being the source. We report here on three patients who received rabbit ATG (Tables 1 and 2). All three patients had positive skin tests to horse ATG, and rabbit ATG was substituted. The three patients who received rabbit ATG went on to develop PTLD. There were no cases of PTLD following conditioning with equine ATG. PTLD occurred at a median of 70 days post-BMT (with a range of 60 to 90 days). The median dose of rabbit ATG was 5 mg/kg/dose (with a range of 2.5 to 10 mg/kg/dose). The diagnosis was based on EBV-PCR and EBV DNA quantification (Table 3), and immunohistochemical staining for EBER was included in this study. An autopsy was performed on each patient, and organs were sectioned and stained for EBER. Flow cytometry analysis was performed in one case.

Discussion. PTLD is a recognized complication known to occur after solid-organ transplantation and is now increasingly observed as a complication of bone marrow transplantation (3). EBV-LPD also occurs in patients with acquired or congenital immunodeficiency (19). Nontransplant patients who develop EBV-LPD include patients with severe combined immunodeficiency, X-linked lymphoproliferative disorder, and human immunodeficiency virus (19). All of these patients have impaired T-cell immunity and therefore are unable to control the proliferation of EBV-infected B cells (19). The proliferation of EBV infection can progress to a monoclonal proliferation with a spectrum of molecular and morphological varieties with unique individual presentations (15).

Multiple risk factors for the development of PTLD, in addition to immunosuppression, have been implicated in previous reports (12). Primary EBV infection is responsible for up to 90% of the cases occurring in the first 3 to 4 months after transplantation, eliminating previous infection as a risk factor for the development of LPD (20). The intensity and type of immunosuppression along with the development of chronic GvHD are known risk factors (20). Infection with cytomegalovirus has also been found to be a risk factor independent of immunosuppression (20). Other risk factors for allogeneic BMT patients include the use of T-cell-depleted grafts either in vivo or ex vivo, unrelated or HLA-mismatched donor, chronic GvHD, degree of immunosuppression therapy, and the use of either ATG therapy or monoclonal anti-T-cell antibodies (13). Nash et al. reported their experience with hematopoietic stem cell transplantation for severe autoimmune disease and the risk of developing EBV-PTLD in patients who received rabbit ATG (11) (the patient from case 2 is included in this subset of patients). Other risk factors in solid-organ transplant include EBV seronegativity, the number of rejection episodes requiring the use of OKT3, and the organ transplanted (2).

Here we have described three patients whose conditioning regimens included rabbit ATG. In two cases, the transplant was from an unrelated allogeneic donor, and in one case, the transplant was from a CD34+-selected autologous transplant. To date there have been 10 cases of autologous transplant-associated PTLD. Since PTLD results from immunosuppression, the initial approach with all of our patients was to withdraw immunosuppression. The withdrawal of immunosuppression alone can result in a response rate of 20 to 50% with a regression of the patient’s PTLD (14). The problem with the withdrawal of immunosuppression in earlier stages is graft rejection or increased GvHD in the allogeneic setting. The efficacy of treatment with antiviral agents is unclear at this time. Since EBV-PTLD is associated with a latent cycle of EBV infection and B-cell proliferation is independent of viral replication, these agents currently play little role in the treatment of established PTLD. However, they could possibly play a role in the prophylaxis of EBV-PTLD (21). Only one patient in this report was treated with antiviral therapy, with no significant impact on survival. Chemotherapy has been used in cases refractory to withdrawal of immunosuppression, and

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>Donor source</th>
<th>Preparatory regimen</th>
<th>Day of engraftment</th>
<th>Day of onset</th>
<th>No. of CD3+ cells</th>
<th>Outcome</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Female</td>
<td>Osteopetrosis</td>
<td>Matched unrelated umbilical cord blood</td>
<td>Rabbit ATG/cyclophosphamide</td>
<td>12</td>
<td>49</td>
<td>8</td>
<td>Fatal</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>Female</td>
<td>Scleroderma</td>
<td>Autologous peripheral blood</td>
<td>Rabbit ATG/TBI/cyclophosphamide</td>
<td>11</td>
<td>54</td>
<td>3</td>
<td>Fatal</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>Female</td>
<td>Acute leukemia</td>
<td>Matched unrelated peripheral blood</td>
<td>Rabbit ATG/TBI/cyclophosphamide</td>
<td>13</td>
<td>62</td>
<td>5</td>
<td>Fatal</td>
</tr>
</tbody>
</table>

* TBI, total-body irradiation.

### Table 2. Disease status of patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Acute GvHD</th>
<th>Transplant complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Autologous</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>Pulmonary toxicity</td>
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<tr>
<td>3</td>
<td>Grade II</td>
<td>GvHD</td>
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</table>

* None of the patients had any infection.

### Table 3. EBV test results

<table>
<thead>
<tr>
<th>Case or group</th>
<th>Amt of Epstein-Barr virus DNA</th>
<th>PCR result</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>6.6 log 10</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>5.2 log 10</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>4.6 log 10</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Control patients that received equine ATG

No DNA detected Negative
complete remissions have been reported with the use of multi-drug chemotherapy. Up to 70% of the patients from a previous study achieved a disease-free interval of 19 months (22). Rituximab has been used as a single agent and in combination with good response (9). One patient in our group was treated with multiagent chemotherapy without response. At the time of treatment of these patients, rituximab was not considered a therapeutic modality. Donor lymphocyte infusion has been reported to be effective in at least one patient after an allogeneic transplant (9). We treated the CD34⁺-selected autologous transplant patient with unselected autologous peripheral blood progenitor cells without success. EBV-generated CTL have been reported as a successful treatment for PTLD. They can be generated in vitro by the stimulation of T cells with EBV-transformed B cells; subsequently, clones of EBV-specific T cells can be generated to relatively high numbers. The infusion of these expanded T cells has been shown to be effective in the management and prophylaxis of EBV-PTLD (1, 4, 6). Several reports testify to the utilization of this procedure; however, the time involved to generate cell lines (weeks to months) and the expense limit this treatment modality (1, 4, 6).

Recently, there have been several approaches used to monitor and prevent EBV reactivation following bone marrow transplantation (17). Molecular monitoring of the EBV load in the peripheral blood using quantitative real-time PCR for EBV reactivation was one of those approaches (4). In that study, patients were treated preemptively with rituximab for viral loads that were greater than 1,000 genome equivalents/ml (8). Our current practice is to monitor these patients by EBV-PCR and to treat preemptively with rituximab for viral DNA levels that are >1,000 genome equivalents/ml.

We have presented three cases of EBV-LPD associated with rabbit ATG in the conditioning regimen. Rabbit ATG was used in this setting after the occurrence of a severe skin reaction from equine ATG. There was no other substitute for equine ATG besides rabbit ATG at our institution at the time patients were being treated with this conditioning regimen. One case occurred in an autologous BMT setting, and two cases occurred in an allogeneic BMT setting. Since no cases of EBV-LPD occurred in the equine ATG-conditioned group, we can speculate that rabbit ATG causes a much more potent T-cell depletion (Table 3). The number of T-cell subsets for the rabbit ATG-conditioned group of patients was significantly lower (CD4 and CD8) than that for the equine ATG-conditioned group, as was previously reported by Nash. The diagnosis of LPD should be considered for patients receiving prolonged immunosuppression or for patients receiving in vivo or ex vivo T-cell depletion and in the differential diagnosis of any BMT patient presenting with adenopathy, unexplained fever, or respiratory distress.

REFERENCES