Epstein-Barr Virus Lymphoproliferation After Bone Marrow Transplantation

By Mary M. Zutter, Paul J. Martin, George E. Sale, Howard M. Shulman, Lloyd Fisher, E. Donnall Thomas, and Diane M. Durnam

We review 15 cases of secondary B-cell lymphoproliferative disorders that occurred among 2,475 patients who received allogeneic bone marrow transplants (BMTs) at the Fred Hutchinson Cancer Research Center (Seattle) between 1969 and 1987. The histopathologic findings in 14 of the 15 patients spanned a wide spectrum of lymphoproliferative lesions. One patient had features characteristic of angioimmunoblastic lymphadenopathy. Epstein-Barr virus (EBV) genomic sequences were identified by Southern blot analysis in each of the 13 patients evaluated. Ten of the 12 lesions evaluated originated in donor cells. In two patients, who had mixed chimerism after transplantation, the lesions originated in host cells. The combined evidence from immunoglobulin light chain staining and the analysis of immunoglobulin heavy chain rearrangement indicated that the lesions in most patients represented polyclonal proliferations that gave rise to clonal subpopulations. The results indicate an overall actuarial incidence of 0.8% for this complication in BMT recipients. Anti-CD3 monoclonal antibody (MoAb) treatment of acute graft-vs-host disease (GVHD) and T cell depletion of the donor marrow were statistically significant risk factors, and GVHD appeared to play a contributing role, particularly in the setting of human leukocyte antigen (HLA) disparity. Two patients had no identifiable risk factors. Prophylaxis or treatment with acyclovir had no detectable effect in the patients; all but two died with uncontrolled lymphoproliferation.

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Case material and histologic studies. Tissue selected for study included autopsy material and surgical specimens from all patients reviewed by the Department of Pathology at the FHCRC between January 1969 and June 1987. The histology suggested the development of an immunoblastic or plasma cell proliferation or secondary lymphoma after BMT. During this period, 2,475 patients received allogeneic BMTs between January 1969 and June 1987 at the Fred Hutchinson Cancer Research Center (FHCRC) (Seattle). This report includes three cases published previously and 12 new cases. This report is focused on the clinical characteristics, histologic and immunologic features, the association with EBV, and the donor or host origin of the involved tissue.

MATERIALS AND METHODS

Case material and histologic studies. Tissue selected for study included autopsy material and surgical specimens from all patients reviewed by the Department of Pathology at the FHCRC between January 1969 and June 1987. The histology suggested the development of an immunoblastic or plasma cell proliferation or secondary lymphoma after BMT. During this period, 2,475 patients received allogeneic grafts. Of these, 1,906 (77%) grafts were for the treatment of hematologic malignancy, 326 (13%) for aplastic anemia, and 243 (10%) for other disorders. Donors were HLA-genotypically identical siblings for 2,037 patients (82.3%), haploidentical relatives for 2,037 patients (4%) with hematologic malignancy and an HLA-identical donor, and 243 (10%) for other disorders. Donors were HLA-genotypically identical siblings for 2,037 patients (82.3%), haploidentical relatives for 12 new cases. This report is focused on the clinical characteristics, histologic and immunologic features, the association with EBV, and the donor or host origin of the involved tissue.

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EBV LYPHOPROLIFERATION AND BMT

subclassified into two general histologic categories according to the
criteria established by Schneider et al19 based on the size distribution of
the lymphoid cells, the number of large immunoblasts, and the
degree of plasmacytoid differentiation. Immunophenotyping was
performed on frozen or formalin-fixed tissue from either surgical or
autopsy specimens. A panel of monoclonal antibodies (MoAbs)
including CD3, CD5, CD19, and CD20 that recognize pan-T and
pan-B cell surface antigens, and antibodies recognizing κ and λ light
chain immunoglobulins were used according to previously
established peroxidase-antiperoxidase or biotin-avidin techniques.18
A clonal population was judged to be present if the κ to λ ratio was 1 to
2 or > 3 to 1. 19

Southern blot analysis. DNA was analyzed from fresh or frozen
tissue obtained at the time of biopsy or autopsy. DNA extraction,
digestion, electrophoresis, Southern transfer, blot hybridization,
and autoradiography were performed as described.20,21 For EBV analysis,
DNA was digested with EcoRI and probed with the plasmid pDK14
that contains the 2.3 kilobase (kb) BamHI V fragment of the EBV
genome (kindly provided by Dr Elliott Kieff, University of Chicago).
To detect rearrangements of immunoglobulin heavy chain gene,
DNA was digested with EcoRI, BamHI, and BglII22,23 and
blots were probed with a 3.2 kb, EcoRI-HindIII, J1-containing
fragment provided by Dr Roger M. Perlmuter (University of
Washington, Seattle). To detect rearrangements of the κ gene, DNA
was digested with HindIII23 and blots were probed with a 2.5 kb
EcoRI-EcoRI, Cκ-containing fragment provided by Dr Philip Leder
(Harvard Medical School, Boston). For analysis of restriction frag-
ment length polymorphisms (RFLP), DNA was digested with either
EcoRI or HindIII and probed with the pAW 101 clone25 kindly
provided by Dr Ray White (University of Utah, Salt Lake City) or
with subclones of minisatellite regions described and provided by Dr
A. J. Jeffreys (University of Leicester, England). 26

EBV serology. Stored serum specimens from patients and
donors were assayed for the presence of IgG antibodies against the
EBV viral capsid antigen (VCA-IgG) and for antibodies against
Epstein-Barr nuclear antigen (EBNA). EBV-VCA antibody titers
were determined by indirect immunofluorescence.27 EBNA antibody
titers were determined by anticomplement immunofluorescence.28
Assays were performed in the Clinical Virology Laboratory of the
University of Washington under the direction of Dr Lawrence
Corey.

Statistical analysis. The association of EBV-LPS with HLA
disparity, T cell depletion, acute GVHD, and anti-CD3 MoAb
therapy was analyzed using statistical methods for failure time
data.29 Differences in the cumulative incidence of EBV-LPS were
assessed using the log rank Mantel-Haenzel approach. Because of
the small number of events the exact distribution (conditionally on
the marginal totals) for the number of events in each group was used
(Lloyd Fisher, unpublished algorithm, June 1984). The estimated
instantaneous relative risk was used to characterize the differences
between patient categories.

RESULTS

Patient and transplant protocol. Clinical features of the
15 patients are summarized in Table 1. Patients ranged in
age from 3 to 58 years and had hematopoietic disorders
representative of the diseases treated at the FHCRC. Unique patient no. (UPN) 3,439 received a BMT for aplastic anemia
after preparation with four doses of cyclophosphamide (50
mg/kg). Thirteen patients with malignant disease were
prepared with two doses of cyclophosphamide (60 mg/kg)
and total body irradiation (TBI) delivered from two opposing
60Co sources as 9.2 Gy in a single fraction (n = 4), 12.0
Gy in six fractions (n = 1), or 15.75 Gy in seven fractions
(n = 8). UPN 2,784 received cytosine arabinoside, 6 g/m2/d
for five days and 12.0 Gy fractionated TBI.

Nine patients received marrow from HLA identical
donors and six received marrow from related haploidentical
donors mismatched at one or more HLA loci.30 GVHD
prophylaxis included methotrexate (15 mg/m2/d on day 1, then
10 mg/m2/d on days 3, 6, and 11 and weekly thereafter),
cyclosporine (3 mg/kg/d intravenously [IV] or 12.5 mg/kg/d
orally when oral administration could be tolerated) or a
combined regimen of methotrexate (days 1, 3, 6, and 11) and
cyclosporine.31 UPN 2,764 and 2,779 received T-cell
depleted marrow in combination with methotrexate adminis-
tered on days 1, 3, and 6 after transplantation. Prophylactic
acyclovir for HSV was used in the two patients receiving
T-cell depleted marrow (UPN 2,764, 2,779) and in UPN
2,594. Acyclovir prophylaxis (500-1,000 mg/m2/d) was
administered four to 19 days before transplantation, con-
tinued for 42 to 67 days, and discontinued 116 to 130 days
before development of the lymphoproliferative disorder. In
UPN 3329, acyclovir prophylaxis was administered concomi-
tantly with antibody 64.1 (used for treatment of acute
GVHD32 and continued for six days until he died. UPN 2,594,
2,764, and 2,784 received acyclovir (500 mg/m2/d) to treat
HSV or VZV infection after transplantation. The drug was
discontinued eight to 84 days before the diagnosis of EBV-
LPS. UPN 2,481, 2,779 and 3,212 received acyclovir after
the diagnosis of EBV-LPS.

Post-transplant course. The time interval between
transplantation and development of the secondary neoplasm
ranged from 45 to 500 days (median, 77 days). All patients
had evidence of hematopoietic function when the second
neoplasm became apparent. In six patients, the hematopoietic
cells were shown to be entirely of donor origin by analysis
of informative chromosomal, HLA, or enzyme
markers. In two patients (UPN 1,134 and 2,779) cytogenetic
analysis showed evidence of mixed hematopoietic and/or
lymphoid chimerism before development of the lymphoprolif-
erative disorder. No informative markers were available to
distinguish donor and host cells in the other seven patients.
Twelve of the 15 patients had grade II-IV GVHD. In ten of
the 12 patients, multiple immunosuppressive agents were
required to control GVHD due to failure of initial treatment.
Four patients (UPN 1,847, 1,863, 2,784, and 3,329) received
IV infusions of the anti-CD3 MoAb 64.1.3

Presentation and autopsy findings. In eight patients, a
secondary neoplasm was unsuspected clinically and the diag-
osis was made only at the time of autopsy. The suspected
causes of death in these eight patients had been acute
respiratory failure (n = 1), severe GVHD (n = 4), or infec-
tion (n = 3). UPN 2,762 had submandibular and preauricular
swelling with spiking fever before the onset of respiratory
and renal failure but died without a diagnosis. In five
patients, lymphoma was complicated by infection and/or
GVHD: septicemia (UPN 304); disseminated aspergillosis
(UPN 2,784); CMV pneumonia and disseminated VZV
(UPN 2,764); GVHD, disseminated CMV and adenovirus
infection (UPN 3,329); or aspergillosis and CMV pneu-
monia (UPN 3,439).
<table>
<thead>
<tr>
<th>UPN</th>
<th>Age</th>
<th>Pretransplant Diagnosis</th>
<th>HLA-Disparity*</th>
<th>GVHD</th>
<th>Response to Initial GVHD Therapy</th>
<th>Acyclovir†</th>
<th>Onset Day‡</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>304</td>
<td>11</td>
<td>ALL-remission</td>
<td>0 MTX</td>
<td>IV</td>
<td>ATG x 2</td>
<td>No</td>
<td>—</td>
<td>102</td>
</tr>
<tr>
<td>1,085</td>
<td>5</td>
<td>ALL</td>
<td>1 MTX</td>
<td>III</td>
<td>ATG, pred</td>
<td>No</td>
<td>—</td>
<td>56</td>
</tr>
<tr>
<td>1,134</td>
<td>25</td>
<td>Acute myelofibrosis</td>
<td>0 MTX</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>500</td>
</tr>
<tr>
<td>1,437</td>
<td>22</td>
<td>ALL-1st relapse</td>
<td>0 MTX</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>54</td>
</tr>
<tr>
<td>1,847</td>
<td>19</td>
<td>ALL-2nd remission</td>
<td>0 MTX</td>
<td>III</td>
<td>CSP, pred, 64.1</td>
<td>No</td>
<td>—</td>
<td>81</td>
</tr>
<tr>
<td>1.863</td>
<td>3</td>
<td>AML-2nd relapse</td>
<td>1 MTX</td>
<td>III</td>
<td>pred, 64.1</td>
<td>No</td>
<td>—</td>
<td>69</td>
</tr>
<tr>
<td>2,481</td>
<td>17</td>
<td>ALL-2nd remission</td>
<td>0 MTX</td>
<td>III</td>
<td>CSP, ATG</td>
<td>Yes</td>
<td>d (4-10)</td>
<td>56</td>
</tr>
<tr>
<td>2,584</td>
<td>58</td>
<td>CML-allymphoidated phase</td>
<td>2 MTX</td>
<td>III</td>
<td>CSP, ATG</td>
<td>No</td>
<td>—</td>
<td>148</td>
</tr>
<tr>
<td>2,762</td>
<td>6</td>
<td>ALL-2nd relapse</td>
<td>3 CSP</td>
<td>II</td>
<td>pred</td>
<td>No</td>
<td>—</td>
<td>45</td>
</tr>
<tr>
<td>2,764</td>
<td>23</td>
<td>AML in 1st relapse</td>
<td>0 TCD + MTX</td>
<td>II</td>
<td>CSP, pred</td>
<td>No</td>
<td>d (4-10)</td>
<td>151</td>
</tr>
<tr>
<td>2,779</td>
<td>38</td>
<td>AML in 2nd remission</td>
<td>0 TCD + MTX</td>
<td>0</td>
<td>—</td>
<td>d (4-10)</td>
<td>143-148</td>
<td>145</td>
</tr>
<tr>
<td>2,784</td>
<td>25</td>
<td>AML-2* to Hodgkin’s</td>
<td>0 CSP→pred</td>
<td>II</td>
<td>pred, CSP, 64.1</td>
<td>d (2-20)</td>
<td>167-168</td>
<td>77</td>
</tr>
<tr>
<td>3,212</td>
<td>29</td>
<td>Hodgkin’s disease</td>
<td>0 MTX, CSP</td>
<td>III</td>
<td>pred</td>
<td>Yes</td>
<td>d (70-98)</td>
<td>67</td>
</tr>
<tr>
<td>3,329</td>
<td>17</td>
<td>CML-2nd chronic phase</td>
<td>2 MTX, CSP</td>
<td>IV</td>
<td>pred, 64.1</td>
<td>No</td>
<td>d 74-90†</td>
<td>52</td>
</tr>
<tr>
<td>3,439</td>
<td>6</td>
<td>AA</td>
<td>2 MTX, CSP</td>
<td>III</td>
<td>ATG, pred</td>
<td>No</td>
<td>—</td>
<td>81</td>
</tr>
</tbody>
</table>

*Table indicates the number of HLA-loci that were mismatched between the donor and the recipient.
†Numbers indicate days on which acyclovir was administered. The day of transplant is designated day 0.
‡Numbers indicate the day that symptoms attributable to secondary lymphoproliferation were first observed. The day of transplant is designated day 0.
§Acyclovir therapy was given for documented herpes simplex or varicella zoster infection.
¶Acyclovir was given because of proven or suspected EBV infection.
‖Acyclovir prophylaxis was given simultaneously with monoclonal antibody 64.1.
#Died with relapse leukemia on day 90 after marrow transplantation.

Abbreviations: AA, aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, BC, chronic myelogenous leukemia, blast crisis; CSP, ciclosporine, dosage as described in text; MTX, methotrexate, dosage as described in text; ATG, antilymphotropin globulin; pred, prednisone; 64.1, monoclonal antibody 64.1 (anti CD3); TCD, T-cell depleted marrow, see text.
In seven patients, clinical symptoms and biopsy led to a premortem diagnosis. UPN 1,847, 2,481, and 2,779 presented with massive hepatosplenomegaly, and the diagnosis was established by needle biopsy of the liver, exploratory laparotomy, and liver biopsy or splenectomy, respectively. Two patients (UPN 1,134 and 2,594) presented with neurologic symptoms referable to spinal cord lesions and in each one the diagnosis was established by biopsy. Two patients (UPN 1,437 and 3,212) presented with isolated findings of high spiking fever and cervical lymphadenopathy on days 54 and 67, respectively. In each patient, the diagnosis was established by cervical lymph node biopsy, and there was no evidence of disease outside the neck.

Thirteen patients died of lymphoma, and in 12 patients the cause of death was either lymphoma and/or superimposed infection (Table 1). Ten of the 11 autopsied patients had extensive disease. The sites of involvement included: abdominal and thoracic lymph nodes, liver, spleen, GI tract, and lungs (Table 2). Individual patients had involvement of the lacrimal glands, conjunctiva, pericardial fat, prostate gland, and fallopian tubes. There was considerable variation in the morphology of the lesions. For example, in UPN 2,779 and 2,764, hepatic lymphoproliferative lesions both diffusely expanded into the portal tracts and formed large discrete nodules. The splenic manifestations were similar to both diffuse replacement of the white pulp and focal mass lesions up to several centimeters in diameter. In UPN 304 the lymphoproliferative lesion, limited to a single kidney nodule, was an incidental finding at autopsy.

The two patients with disease limited to the cervical lymph nodes were the only patients to recover. UPN 1,437 received no treatment other than excisional biopsy. He later died of CMV pneumonia and recurrent acute lymphocytic leukemia with no evidence of EBV-LPS. In UPN 3,212, immunosuppressive therapy was discontinued, the lymph node group was resected, 34.0 Gy irradiation was administered to the tumor bed, and a 4-week course of acyclovir (500 mg/m²/d) was administered. He remains well more than 10 months after diagnosis of the lymphoproliferative disorder.

Histology. Fourteen of the lesions were classified as malignant lymphoma, large cell, immunoblastic type according to the classification system of the Working Formulation (equivalent to immunoblastic sarcoma in the Lukes-Collins system) (Table 2). The histopathology of these 14 patients with immunoblastic lymphoma encompassed a wide spectrum of morphologic characteristics. The unifying feature was the presence of large pyrinophilic immunoblasts interspersed with variable numbers of plasmacytoid lymphocytes.

**Table 2. Pathologic Features of Secondary Lymphomas**

<table>
<thead>
<tr>
<th>UPN</th>
<th>EBV</th>
<th>Donor/Host</th>
<th>Morphology*</th>
<th>Site of Involvement</th>
<th>Clonality Based on Light Chain Staining†</th>
<th>Heavy Chain Gene Analysis‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>304</td>
<td>NT</td>
<td>NT</td>
<td>I</td>
<td>Kidney</td>
<td>Polyclonal</td>
<td>NT</td>
</tr>
<tr>
<td>1,085</td>
<td>+</td>
<td>donor</td>
<td>II</td>
<td>Lymph nodes, liver, spleen, kidney, bone marrow, salivary gland, gut, prostate</td>
<td>Monoclonal lambda</td>
<td>2</td>
</tr>
<tr>
<td>1,134</td>
<td>+</td>
<td>host</td>
<td>I</td>
<td>Lymph nodes, gut</td>
<td>Monoclonal lambda</td>
<td>NT</td>
</tr>
<tr>
<td>1,437</td>
<td>+</td>
<td>donor</td>
<td>AILD§</td>
<td>Cervical, supraclavicular lymph nodes</td>
<td>Polyclonal</td>
<td>1</td>
</tr>
<tr>
<td>1,847</td>
<td>+</td>
<td>donor</td>
<td>I</td>
<td>Lymph nodes, gut, bone marrow</td>
<td>Polyclonal</td>
<td>NT</td>
</tr>
<tr>
<td>1,863</td>
<td>+</td>
<td>donor</td>
<td>II</td>
<td>Kidney, liver, spleen</td>
<td>Polyclonal NT</td>
<td>NT</td>
</tr>
<tr>
<td>2,481</td>
<td>+</td>
<td>NT</td>
<td>I</td>
<td>Lymph nodes, liver, spleen, kidney, adrenal heart, lung, ovary, bone marrow</td>
<td>Polyclonal</td>
<td>NT</td>
</tr>
<tr>
<td>2,594</td>
<td>NT</td>
<td>NT</td>
<td>II</td>
<td>Spine, lung, heart, liver</td>
<td>Polyclonal NT</td>
<td>NT</td>
</tr>
<tr>
<td>2,762</td>
<td>+</td>
<td>donor</td>
<td>II</td>
<td>Lymph nodes, spleen, heart, lung, adrenal, kidney, ovary, uterus</td>
<td>Polyclonal</td>
<td>2</td>
</tr>
<tr>
<td>2,764</td>
<td>+</td>
<td>donor</td>
<td>I</td>
<td>Lymph nodes, liver, lung, spleen, bowel, adrenal, bone marrow, kidney, lacrimal gland</td>
<td>Polyclonal</td>
<td>2</td>
</tr>
<tr>
<td>2,779</td>
<td>+</td>
<td>host</td>
<td>II</td>
<td>Lymph nodes, liver, spleen, bone marrow</td>
<td>Polyclonal NT</td>
<td>1</td>
</tr>
<tr>
<td>2,784</td>
<td>+</td>
<td>donor</td>
<td>I</td>
<td>Lymph nodes, liver, thymus, kidney, ileum</td>
<td>Polyclonal NT</td>
<td>NT</td>
</tr>
<tr>
<td>3,212</td>
<td>+</td>
<td>donor</td>
<td>II</td>
<td>Submandibular lymph node, salivary gland</td>
<td>Monoclonal kappa</td>
<td>2</td>
</tr>
<tr>
<td>3,329</td>
<td>+</td>
<td>donor</td>
<td>II</td>
<td>Mesenteric lymph nodes, peribronchial tissue, bowel</td>
<td>Polyclonal</td>
<td>0</td>
</tr>
<tr>
<td>3,439</td>
<td>+</td>
<td>donor</td>
<td>II</td>
<td>Lymph nodes, spleen, liver, kidney, thyroid</td>
<td>Polyclonal</td>
<td>1</td>
</tr>
</tbody>
</table>

*Roman numerals represent lymphoma morphology. I, a lesion composed of well differentiated lymphocytes with plasmacytoid features; II, a lesion composed of poorly differentiated large lymphocytes with atypical nuclear features.
†Monoclonality is based on a x to λ ratio less than the normal ratio of 1 to 2 or greater than 3 to 1 as described in Methods and Materials.  
‡Data indicate the number of non germ-line (or rearranged) bands detected. NT, not tested.
There were differences in the degree of pleomorphism, the size and maturation of the individual cells, the number of plasmacytoid or atypical large cells, and the extent of necrosis. Two patients best delineate the ends of the spectrum. UPN 2,481 had a well-differentiated lesion (grade I) composed of many small and intermediate sized lymphocytes with distinct plasmacytoid characteristics and many interspersed mature plasma cells (Fig 1a). In contrast, UPN 2,779 (Fig 1b) had a poorly differentiated lesion composed of a relatively monotonous population of large transformed lymphocytes with a moderate amount of pyroninophilic cytoplasm, large nuclei, and a central discrete nucleolus (grade II).

The histopathologic findings in the cervical lymph node of UPN 1,437 were not diagnostic of lymphoma. This lesion had features characteristic of angioimmunoblastic lymphadenopathy (Fig 2), a premalignant lesion not previously reported in association with post-transplant malignancy.

Clonality. All 15 lesions were composed primarily of B-lymphocytes as determined by surface marker analysis. Based on the results of staining for immunoglobulin κ and λ light chains, three patients had monoclonal lesions and 12 patients had “polyclonal” lesions (Table 2). Immunoglobulin gene rearrangements were examined in seven polyclonal lesions and one monoclonal lesion (Fig 3). In six of the seven “polyclonal” lesions, one or two immunoglobulin heavy chain gene rearrangements were detected, indicating the presence of clonal populations that could not be readily appreciated by immunoglobulin light chain staining (Table 2). The seventh “polyclonal” lesion did not show a clonal gene rearrangement of the immunoglobulin heavy chain (Fig 3) or the Cκ gene (data not shown). The lesion judged to be monoclonal by immunoglobulin light chain staining (UPN 3,212) also showed a clonally rearranged immunoglobulin heavy chain gene (Fig 3). Thus, a clonal element could be detected in all but one of the cases having the histology of lymphoma. A clonal element was also detected in the lesion that had the histologic appearance of angioimmunoblastic lymphadenopathy (Fig 3).

Donor host origin of lymphoid cells. In the three patients originally reported by Schubach et al3 and Martin et al.6 the malignant lymphoid cells were of donor origin. Tissue was available for analysis of RFLP in six additional patients. One sex-mismatched patient (UPN 1,437) was analyzed by cytogenetics, and one patient (UPN 2,764) was analyzed by enzyme polymorphism. Ten of the 12 lesions developed in lymphocytes of donor origin (Fig 4a; additional data not shown). In lesions from UPN 1,134 and 2,779 (Fig 4b), only

![Image](https://www.bloodjournal.org/content/107/9/524.fig1)

**Fig 1.** Examples of two immunoblastic lymphomas with differing morphology. A) A well-differentiated immunoblastic lymphoma from UPN 2,481. Small and intermediate-sized lymphocytes with distinct plasmacytoid characteristics are common (large arrow) and are mature plasma cells (small arrow). (Magnification, 400x). B) A poorly-differentiated immunoblastic lymphoma from UPN 2779. A monotonous population of large transformed lymphocytes (arrow) with a moderate amount of pyrinophilic cytoplasm form large nodules in the splenectomy specimen. (Magnification, 400x).
host cells were detected. Both patients had previous evidence of mixed lymphoid chimerism detected cytogenetically in the peripheral blood after transplantation.

**EBV.** The three lymphomas previously reported had contained EBV genomic sequences detected by Southern blot analysis. EBV genomic sequences were detected in all ten additional patients examined, including the case of angioimmunoblastic lymphadenopathy (Table 2). A representative autoradiogram demonstrating detection of EBV genome in tissue from UPN 2,779 is shown in Fig 5 (data from other patients not shown).

**Serology.** Serum samples from nine donor-recipient pairs were analyzed for the presence of anti-VCA and anti-EBNA antibodies (Table 3). Pretransplant specimens from both patient and donor were compared with samples collected from the patient during the week before the onset of symptoms due to EBV-associated lymphoproliferation. No consistent findings were identified. Only UPN 2,762 was seronegative before transplantation. The donors for UPN 2,481 and UPN 2,594 were also seronegative. In six seropositive patients, the specific titer of IgG anti-VCA decreased from the pretransplant value to a very low level at the time of lymphoma onset. UPN 1,437 showed a VCA-IgG titer rising from 1 to 320 at the time of the secondary lymphoma and UPN 1,847 showed the VCA-IgG titer rising to 1 to 640.

**Statistical analysis.** The overall cumulative incidence of EBV-LPS was 0.6% among patients receiving allogeneic BMT for treatment of hematologic malignancy, and 0.3% among those transplanted for aplastic anemia. Among patients transplanted for hematologic malignancy, the risk of developing EBV-LPS was greatly increased when anti-CD3 MoAb was used for treatment of severe acute GVHD or...
when T cells were depleted from the donor marrow (Table 4). The occurrence of grade II-IV acute GVHD appeared to be associated with an increased risk of EBV-LPS in patients receiving unmodified marrow and not treated with anti-CD3 antibody, but this difference was of borderline statistical significance. Although HLA disparity had no statistically significant effect, there was a statistically significant increase in the incidence of EBV-LPS among patients who developed GVHD after receiving HLA-mismatched marrow compared with those who did not develop GVHD after receiving HLA-identical marrow. However, it is noteworthy that EBV-LPS did develop in two patients (UPN 1,134 and 1,437) who had no identifiable risk factors.

**DISCUSSION**

We have studied 15 patients with B-cell lymphoproliferative disorder occurring among 2,475 BMT patients. The estimated actuarial incidence of this complication was approximately 0.6%, which is less than the 5% incidence reported after renal transplantation\(^{13,14}\) and the 20% incidence reported after cardiac transplantation.\(^15\) Among the BMT patients, three presented with high spiking fever and cervical lymphadenopathy; two presented with localized spinal cord involvement; the others presented with disseminated lymphoma involving many organs. In most cases, the EBV-LPS developed as a rapidly progressive and highly lethal disease.

**Table 3. EBV Serology Pretransplant and at Time of Onset of Lymphoma**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretransplant VCA IgG</th>
<th>Pretransplant EBNA</th>
<th>Disease Onset VCA IgG</th>
<th>Disease Onset EBNA</th>
<th>Donor Pretransplant VCA IgG</th>
<th>Donor Pretransplant EBNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPN 1,085</td>
<td>1:320 ++</td>
<td>1:20 +</td>
<td>1:80 +</td>
<td>1:40 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPN 1,437</td>
<td>1:80 +</td>
<td>1:320 +</td>
<td>1:40 +</td>
<td>1:20 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPN 1,847</td>
<td>1:320 +</td>
<td>1:640 nonspecific</td>
<td>1:320 +</td>
<td>1:20 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPN 2,481</td>
<td>1:40 +</td>
<td>1:20 nonspecific</td>
<td>1:10 +</td>
<td>1:20 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPN 2,594</td>
<td>1:80 +</td>
<td>1:40 +</td>
<td>1:10 +</td>
<td>1:20 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPN 2,762</td>
<td>1:10 +</td>
<td>1:10 +</td>
<td>1:80 +</td>
<td>1:20 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPN 2,764</td>
<td>1:40 +</td>
<td>NT</td>
<td>NT</td>
<td>1:20 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPN 2,779</td>
<td>1:10 +</td>
<td>1:10 +</td>
<td>1:60 +</td>
<td>1:20 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPN 2,784</td>
<td>1:320 +</td>
<td>1:20 +</td>
<td>1:80 +</td>
<td>1:20 +</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EBNA +, a titer of >1-2 or greater; EBNA -, a titer of <1-2; NT, not tested.
EBV LYMPHOPROLIFERATION AND BMT

Table 4. Risk of Epstein-Barr Virus Lymphoproliferative Syndrome

<table>
<thead>
<tr>
<th>Patient Categorya</th>
<th>Total at Risk§</th>
<th>Number of Cases</th>
<th>Actuarial Risk (%) at 500 days</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD-3 antibody</td>
<td>24</td>
<td>4</td>
<td>24.0</td>
<td>0.0002</td>
</tr>
<tr>
<td>GVHD O-II*</td>
<td>723</td>
<td>6</td>
<td>1.19</td>
<td></td>
</tr>
<tr>
<td>GVHD O-II†</td>
<td>1308</td>
<td>2</td>
<td>0.32</td>
<td>0.0503</td>
</tr>
<tr>
<td>HLA mismatched,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GVHD O-II‡</td>
<td>232</td>
<td>3</td>
<td>2.16</td>
<td>0.040</td>
</tr>
<tr>
<td>HLA matched</td>
<td>1173</td>
<td>2</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>HLA mismatched‡</td>
<td>386</td>
<td>3</td>
<td>1.41</td>
<td>0.1721</td>
</tr>
<tr>
<td>HLA matched‡</td>
<td>1868</td>
<td>5</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>T cell depletion</td>
<td>64</td>
<td>2</td>
<td>6.16</td>
<td>0.0342</td>
</tr>
</tbody>
</table>

aThe patient with aplastic anemia is excluded.
†Two-sided p values were calculated from the exact Mantel-Haenszel test.
‡Patients who received CD3 antibody or T-cell-depleted marrow were excluded from these categories. Not all patients had been assigned GVHD coding at the time of diagnosis.
§Patients who received marrow from syngeneic donors or who were transplanted for aplastic anemia were excluded from all analyses.

The morphology of the lymphoproliferative lesions that develop after renal transplantation has been extensively described.12,23 Based on histologic, immunologic, and cytogenetic criteria, the spectrum of described lesions has ranged from reactive lymphoid hyperplasia to immunoblastic B-cell sarcoma, with intermediate variants identified as polymorphic diffuse B-cell hyperplasia and polymorphic diffuse B-cell lymphoma. This spectrum implies an increasing malignant potential, beginning as a benign, reactive (and possibly reversible) process and terminating as a malignant, clonal, highly lethal lymphoma. The histologic features of increasing malignant potential include cellular monotonyness, decreased plasmacytoid differentiation, greater numbers of atypical immunoblasts, and increased nuclear atypia. This entire spectrum of lesions has been classified by the Working Formulation16 as large cell, noncleaved, immunoblastic lymphoma. Fourteen of the 15 patients fit the criteria of the Working Formulation for this diagnosis and further document the very diverse range of histopathologic findings encompassed within this diagnostic category. The case characterized as angioimmunoblastic lymphadenopathy appears to be unique and has not been previously described after organ transplantation.

Immunodeficiency represents a risk factor common to all clinical settings in which EBV-associated lymphoproliferative syndromes can occur. Reconstitution of the immune system proceeds slowly after BMT.34,35 Both T-cells and immunoglobulin producing B cells of donor origin repopulate the peripheral blood within 3 to 4 months, but restoration of normal function and immunoregulation does not occur until after 1 year. The humoral immune response to EBV infection is abnormal in BMT patients.3 During seroconversion, IgG-VCA titers increase to very high levels, but anti-EBNA responses are delayed in onset and titers remain at low levels. It was noteworthy that most of the patients with lymphoproliferative disorders showed no evidence of EBV seroconversion. Cellular immunity to EBV has been studied primarily by in vitro techniques.36,37 The response to EBV in BMT recipients has not been thoroughly characterized, and the defects responsible for allowing the emergence of EBV-LPS have not been identified.

EBV has been implicated in virtually all cases of post-transplant lymphoma. This virus is ubiquitous in humans and infects 95% to 98% of adults. Transmission is believed to occur by oropharyngeal spread.38 Infective replicating virus can be recovered from the saliva of all patients with infectious mononucleosis, in approximately 20% of healthy seropositive persons,39 and in 50% of seropositive patients receiving immunosuppressive drugs.40,41 Epithelial cells in the nasopharynx represent the principal reservoir of replicating virus.42 B lymphocytes represent another reservoir for EBV, but the infection in these cells is latent with no evidence of active replication in healthy individuals.

At least four sources of EBV can be postulated in BMT recipients: latently infected donor B lymphocytes, the host nasopharynx, latently infected host B lymphocytes, and blood transfusions. Most previously published cases of patients with secondary lymphoma after BMT occurred in B cells of donor origin,43 suggesting either that EBV-infected lymphocytes were transferred from donor to host at the time of transplantation or that transplanted cells became infected in the host. In two of the patients, the donor was seronegative and the lymphoproliferation occurred in donor lymphoid cells, indicating that the virus was transferred to donor cells after transplantation. Two of the 15 lymphomas occurred in cells of host origin, both in seropositive patients who had developed mixed lymphoid chimerism, suggesting either that EBV-infected B lymphocytes had survived the preparative regimen or that the virus was transferred to residual host B cells after transplantation.

The most clearly identifiable risk factor for developing EBV-LPS in the patient population was administration of anti-CD3 MoAb for treatment of acute GVHD. Anti-CD3 antibodies are known to interfere with T cell recognition by binding to structures associated with the T-cell receptor. Binding of antibodies also causes release of lymphokines that may facilitate EBV infection and B cell transformation. Either effect could account for the increased risk of EBV-LPS in patients with GVHD. In another study, it was found that EBV-LPS did not develop in any of 20 patients who received the OKT3 anti-CD3 antibody for GVHD prophylaxis.44 It is possible that anti-CD3 antibodies may differ in their propensity for inducing EBV-LPS.

From our results, T cell depletion of the donor marrow and GVHD, particularly in the setting of HLA-disparity, appear to represent risk factors for developing EBV-LPS. An association between T cell depletion and EBV-LPS has been observed previously in patients receiving HLA-mismatched marrow. In our study, the two patients who received T cell depleted marrow had HLA-identical donors. The increased risk of EBV-LPS may suggest a possible defect in the immune reconstitution of some patients given T cell depleted marrow, even though early studies have shown that removal of donor T cells from the graft has little impact on the pattern or kinetics of T cell repopulation as measured by cell surface phenotyping.45 The association of EBV-LPS with HLA-disparity and GVHD may reflect an impairment in immune function that increases susceptibility to viral infection. For
example, HLA-disparity and GVHD also represent risk factors for CMV and HSV infection.45

Efforts to treat or prevent post-transplant lymphoproliferative disorders have met with mixed success. Starzl et al46 has emphasized reversal of lymphomas after cardiac transplantation by reduction or discontinuation of immunosuppression. Acyclovir has been proposed for both prophylactic use in patients at risk of EBV-associated disease47 and also for treatment in patients with EBV-LPS.48 Acyclovir inhibits viral DNA polymerase, but this effect is limited to permissive infection as found in the nasopharynx.49 In clinical studies, acyclovir has been shown to decrease oropharyngeal shedding of EBV.46,49 Acyclovir does not affect the cellular DNA polymerase or the persistence of EBV in lymphoid cells and does not inhibit in vitro EBV transformation even when used at high concentrations.50

We have no data to support prophylactic or therapeutic use of acyclovir for EBV-associated lymphoproliferative disorders. Four of the patients received acyclovir either as prophylaxis and/or treatment of HSV or VZV infections before the development of lymphoma. Acyclovir (500 mg/m²/d) was also used specifically for the prophylaxis of EBV in one patient (UPN 3,329) and as treatment for EBV in three others (UPN 2,481, 2,779, and 3,212). Two patients (UPN 2,764 and 2,779) showed no response and died of extensive disease after 14 and 12 days of therapy, respectively. One patient who recovered, UPN 3,212, was treated with surgical resection, local irradiation, and discontinuation of immunosuppression, in addition to acyclovir (1 g/m²/d). The role of acyclovir in this case cannot be assessed reliably. The use of acyclovir for either prophylaxis or treatment of EBV-associated lymphoproliferation requires further assessment.

Analysis of IgH rearrangements represents a highly sensitive method for detecting small clonal populations.51,52 This method does not allow sensitive detection of nonclonal rearrangements. On the other hand, immunoglobulin light chain staining represents a highly sensitive method for detecting nonclonal populations, but is much less sensitive for detecting a clonal population in tissue sections. Immunoglobulin light chain staining from our patients demonstrated nonclonal populations, whereas DNA analysis demonstrated clonal populations in cells from all but one patient. Thus, it is possible that the lesions in most of our patients represent polyclonal proliferations that gave rise to clonal subpopulations. Secondary changes which might confer a proliferative advantage on individual cells in these lesions remain to be determined.

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LYMPHOPROLIFERATION AND BMT

529

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Epstein-Barr virus lymphoproliferation after bone marrow transplantation

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