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 transplantations (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-versus-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.

© 1988 by Grune & Stratton, Inc.

HERPES VIRUS INFECTIONS represent a frequent cause of morbidity and mortality after bone marrow transplantation (BMT).1 Infections with cytomegalovirus (CMV), herpes simplex virus (HSV), and varicella zoster virus (VZV) are thought to result primarily from the reactivation of latent virus, although de novo infections have also been documented. Less attention has been given Epstein-Barr virus (EBV) infections after BMT. Oropharyngeal shedding of EBV and seroconversion have been documented to occur in most BMT recipients, but these events ordinarily have no associated symptoms.2,3 In the small number of patients who develop B-cell lymphoproliferative disorders post-transplant4-12 there is frequently an association with EBV infection.1-10

EBV-associated lymphoproliferative syndromes (EBV-LPS) represent a well-recognized complication of organ transplantation. The incidence of this complication can be as high as 5% in renal allograft recipients and 20% in cardiac allograft recipients.13-15 Thus, the morbidity and mortality associated with EBV infection in organ transplant recipients can be significant. Latent EBV infection together with impaired immune responses, chronic antigenic stimulation by the allograft, and direct oncogenic effects of immunosuppressive drugs may represent factors that increase the incidence of EBV-related disease after organ grafting.13

The incidence of lymphoproliferative disorders in BMT recipients has not yet been defined. Severe graft-versus-host disease (GVHD), immunosuppressive treatment, human leukocyte antigen (HLA) incompatibility between the donor and recipient, and T cell depletion of the donor marrow have been suggested as possible risk factors for this complication. EBV genomic sequences have been demonstrated in nearly all of the reported patients analyzed by DNA hybridization, but in one patient11 there was no evidence of EBV infection. Studies have shown that the infection usually involves cells of donor origin, but in two patients the cells were of host origin.14-11

In this report, we review the clinical and laboratory findings in the 15 cases of secondary B-cell lymphoproliferation recognized among the 2,475 patients who 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 and recipient, and T cell depletion of the donor marrow have been suggested as possible risk factors for this complication. EBV genomic sequences have been demonstrated in nearly all of the reported patients analyzed by DNA hybridization, but in one patient4 there was no evidence of EBV infection. Studies have shown that the infection usually involves cells of donor origin, but in two patients the cells were of host origin.4-11

© 1988 by Grune & Stratton, Inc.

© 1988 by Grune & Stratton, Inc.

© 1988 by Grune & Stratton, Inc.

© 1988 by Grune & Stratton, Inc.

© 1988 by Grune & Stratton, Inc.

© 1988 by Grune & Stratton, Inc.

© 1988 by Grune & Stratton, Inc.

© 1988 by Grune & Stratton, Inc.

© 1988 by Grune & Stratton, Inc.

© 1988 by Grune & Stratton, Inc.

© 1988 by Grune & Stratton, Inc.
subclassified into two general histologic categories according to the criteria established by Schneider et al15 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 recognizes pan-T and pan-B cell surface antigens, and antibodies recognizing $\kappa$ and $\lambda$ light chain immunoglobulins were used according to previously established monoclonal-antiperoxidase or biotin-avidin techniques.18 A clonal population was judged to be present if the $k$ to $\lambda$ 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 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).21 To detect rearrangements of immunoglobulin heavy chain gene, DNA was digested with EcoRI, BamHI, and BglII21,24 and blots were probed with a 3.2 kb, EcoRI-HindIII, J-$\kappa$ containing fragment provided by Dr Roger M. Perlmuter (University of Washington, Seattle). To detect rearrangements of the $\kappa$ gene, DNA was digested with HindIII21 and blots were probed with a 2.5 kb EcoRI-EcoRI, $\kappa$ containing fragment provided by Dr Philip Leder (Harvard Medical School, Boston). For analysis of restriction fragment length polymorphisms (RFLP), DNA was digested with either EcoRI or HindIII and probed with the pAW 101 clone23 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).20

### 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 indirect 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 FHRC. 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 twin opposing $^{60}$Co 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 $g/m^2/d$ 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/m$^2$/d on day 1, then 10 mg/m$^2$/d on days 3, 6, and 11 and weekly thereafter), cyclosporine (3 mg/kg/d intravenous [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 administered 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/m$^2$/d) was administered four to 19 days before transplantation, continued for 42 to 67 days, and discontinued 116 to 130 days before development of the lymphoproliferative disorder. In UPN 3,329, acyclovir prophylaxis was administered concomitantly with antibody 64.1 (used for treatment of acute GVHD30 and continued for six days until he died. UPN 2,594, 2,764, and 2,784 received acyclovir (500 mg/m$^2$/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 lymphoproliferative 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.

#### Presentation and autopsy findings
In eight patients, a secondary neoplasm was unsuspected clinically and the diagnosis 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 infection (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 pneumonia (UPN 3,439).
Table 1. Clinical Features of Patients With Secondary Lymphomas

<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</td>
<td>MTX 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</td>
<td>MTX 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</td>
<td>MTX 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</td>
<td>MTX 0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>54</td>
</tr>
<tr>
<td>1,847</td>
<td>19</td>
<td>AML-2nd remission 1</td>
<td>MTX III</td>
<td>CSP, pred, 64.1</td>
<td>No</td>
<td>—</td>
<td>81</td>
<td>Died of lymphoma</td>
</tr>
<tr>
<td>1,863</td>
<td>3</td>
<td>AML-2nd relapse 1</td>
<td>MTX III</td>
<td>pred, 64.1</td>
<td>No</td>
<td>—</td>
<td>69</td>
<td>Died of lymphoma</td>
</tr>
<tr>
<td>2,481</td>
<td>17</td>
<td>ALL-2nd remission 0</td>
<td>MTX III</td>
<td>CSP, ATG</td>
<td>No</td>
<td>—</td>
<td>d 62 + 63</td>
<td>56</td>
</tr>
<tr>
<td>2,594</td>
<td>58</td>
<td>CML-accelerated phase 2</td>
<td>MTX III</td>
<td>CSP, ATG</td>
<td>Yes</td>
<td>d (–4)–30</td>
<td>d 63–65</td>
<td>146</td>
</tr>
<tr>
<td>2,762</td>
<td>6</td>
<td>ALL-2nd relapse 3</td>
<td>CSP</td>
<td>II</td>
<td>pred</td>
<td>No</td>
<td>—</td>
<td>45</td>
</tr>
<tr>
<td>2,764</td>
<td>13</td>
<td>AML in 1st relapse 0</td>
<td>TCD + MTX</td>
<td>II</td>
<td>CSP, pred</td>
<td>No</td>
<td>d (–4)–21</td>
<td>d 88–105</td>
</tr>
<tr>
<td>2,779</td>
<td>38</td>
<td>AML in 2nd remission 0</td>
<td>TCD + MTX</td>
<td>II</td>
<td>pred, pred, 64.1</td>
<td>No</td>
<td>—</td>
<td>141</td>
</tr>
<tr>
<td>2,784</td>
<td>25</td>
<td>AML-2* to Hodgkin’s 0</td>
<td>CSP — pred</td>
<td>II</td>
<td>pred, pred, 64.1</td>
<td>No</td>
<td>—</td>
<td>167–168</td>
</tr>
<tr>
<td>3,212</td>
<td>29</td>
<td>Hodgkin’s disease 0</td>
<td>MTX, CSP II</td>
<td>pred</td>
<td>Yes</td>
<td>—</td>
<td>d 20–205</td>
<td>77</td>
</tr>
<tr>
<td>3,329</td>
<td>17</td>
<td>CML-2nd chronic phase 2</td>
<td>MTX, CSP IV</td>
<td>pred, ATG 64.1</td>
<td>No</td>
<td>d 74–80</td>
<td>d 63–69</td>
<td>87</td>
</tr>
<tr>
<td>3,439</td>
<td>6</td>
<td>AA</td>
<td>MTX, CSP II</td>
<td>ATG, pred</td>
<td>No</td>
<td>—</td>
<td>81</td>
<td>Died of Aspergillus, systemic CMV, adenovirus &amp; lymphoma</td>
</tr>
</tbody>
</table>

Abbreviations: AA, aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, BC, chronic myelogenous leukemia, blast crisis; CSP, cyclosporine, dosage as described in text; MTX, methotrexate, dosage as described in text; ATG, antithymocyte globulin; pred, prednisone; 64.1, monoclonal antibody 64.1 (anti CD3); TCD, T-cell depleted marrow, see text.

*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 therapy 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.
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 Formulation¹⁶ (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 pyroninophilic 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></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></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</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</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></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></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</td>
<td></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</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.¹⁸

‡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)\textsuperscript{17} 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).\textsuperscript{17}

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 $\kappa$ and $\lambda$ 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 $C_\kappa$ 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 al.\textsuperscript{4} and Martin et al.\textsuperscript{5} 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

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 pyrininophilic cytoplasm form large nodules in the splenectomy specimen. (Magnification, 400x).
Fig 2. Angioimmunoblastic lymphadenopathy from UPN 1,437. This cervical lymph node biopsy had features characteristic of angioimmunoblastic lymphadenopathy including marked small blood vessel proliferation, obliteration of the lymph node architecture, and a proliferation of plasma cells, atypical immunoblasts (arrow), and mature lymphocytes scattered throughout the lymph node. (Magnification, 160x).

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 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 3 (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
Fig 4. Southern blot analysis of the genetic origin of tumor cells. The RFLP patterns of DNA from patient peripheral blood drawn before transplant (patient); donor peripheral blood (donor); and post-transplant tumor tissue (tumor) are isolated and compared. A) Analysis of UPN 3,212 lymph node tumor DNA. DNA was digested with EcoRI and probed with pAW101. Tumor DNA pattern is identical to donor DNA pattern. Two bands are shared by the donor and tumor DNA. A single band is unique to patient DNA. B) Analysis of DNA from the tumor present in the liver of UPN 2,779. DNA was digested with Hinfl and probed with 15.1.114. Tumor DNA shows a pattern identical to patient. Bands present in donor DNA are absent in tumor DNA. In both panels, the size markers are indicated in kb.

Fig 5. Southern blot detection of EBV genomic sequences in the lymphoproliferative lesion from UPN 2,779. Tumor DNA was isolated from involved areas of liver and spleen. Control DNA was prepared from peripheral blood leukocytes of an individual not known to be infected with EBV. Aliquots (20 pg and 100 pg) of the (pDK14) plasmid containing the EBV genomic sequences used to probe the blot are shown as positive controls. All DNA samples were digested with EcoRI. EBV sequences were detected in both the liver and spleen specimens from this patient. The size markers are indicated in kb.

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretransplant</th>
<th>Disease Onset</th>
<th>Donor Pretransplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPN</td>
<td>VCA IgG</td>
<td>EBNA</td>
<td>VCA IgG</td>
</tr>
<tr>
<td>1,085</td>
<td>1:320</td>
<td>+</td>
<td>1:20</td>
</tr>
<tr>
<td>1,437</td>
<td>1:80</td>
<td>+</td>
<td>1:320</td>
</tr>
<tr>
<td>1,847</td>
<td>1:320</td>
<td>+</td>
<td>1:640 nonspecific</td>
</tr>
<tr>
<td>2,481</td>
<td>1:40</td>
<td>-</td>
<td>1:20 nonspecific</td>
</tr>
<tr>
<td>2,594</td>
<td>1:80</td>
<td>+</td>
<td>1:40</td>
</tr>
<tr>
<td>2,762</td>
<td>&lt;1:10</td>
<td>-</td>
<td>&lt;1:10</td>
</tr>
<tr>
<td>2,764</td>
<td>1:40</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>2,779</td>
<td>1:10</td>
<td>+</td>
<td>1:10</td>
</tr>
<tr>
<td>2,784</td>
<td>1:320</td>
<td>+</td>
<td>1:20</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 Category*</th>
<th>Total 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>24.0</td>
<td>0.0002</td>
</tr>
<tr>
<td>GVHD II-V†</td>
<td>723</td>
<td>1.19</td>
<td>0.0503</td>
</tr>
<tr>
<td>GVHD 0-†</td>
<td>1308</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>HLA mismatched,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GVHD II-V†</td>
<td>232</td>
<td>2.16</td>
<td>0.040</td>
</tr>
<tr>
<td>GVHD 0-†</td>
<td>1173</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>HLA mismatched‡</td>
<td>386</td>
<td>1.41</td>
<td>0.1721</td>
</tr>
<tr>
<td>HLA matched‡</td>
<td>1868</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>T cell depletion</td>
<td>64</td>
<td>6.16</td>
<td>0.0342</td>
</tr>
</tbody>
</table>

*The 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. 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 monotonity, decreased plasmacytoid differentiation, greater numbers of atypical immunoblasts, and increased nuclear atypia. This entire spectrum of lesions has been classified by the Working Formulation 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 lymphohadenoapathy 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. 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. 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. 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. Infective replicating virus can be recovered from the saliva of all patients with infectious mononucleosis, in approximately 20% of healthy seropositive persons, and in 50% of seropositive patients receiving immunosuppressive drugs. Epithelial cells in the nasopharynx represent the principal reservoir of replicating virus. 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, 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. 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. The association of EBV-LPS with HLA-disparity and GVHD may reflect an impairment in immune function that increases susceptibility to viral infection.
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 al.46 emphasized reversal of lymphomas after cardiac transplantation by reduction or discontinuation of immunosuppression. Acyclovir has been proposed for both prophylactic use and does not inhibit in vitro EBV transformation even when used at high concentrations.47-49 Acyclovir does not affect the cellular shedding of EBV.47-49 Clinical studies, acyclovir has been shown to decrease oropharyngeal infection as found in the nasopharynx.47-48 In clinical trials, acyclovir has been used at high concentrations.47

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 HVS 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.

ACKNOWLEDGMENT
We thank Dr Lawrence Corey and Rose Mary Obrigewitch for performing serologic assays; Gary Schoch for computer retrieval; Drs Ray White, A.J. Jeffreys, Elliott Kieff, Philip Leder, and Roger M. Pielmutter for supplying DNA probes; Charles Mahan and Clyde Cook for technical assistance; and Regina Warmoth for excellent secretarial assistance.

REFERENCES
17. Schneider DR, Taylor CR, Parker JW, Cramer AC, Meyer PR, Lukes RJ: Immunoblastic sarcoma of T- and B-cell types:
Morphologic description and comparison. Hum Pathol 16:885, 1985


19. Levy N, Nelson J, Meyer P, Lukes RJ, Parker JW: Reactive lymphoid hyperplasia with single class (monoclonal), surface immuno-


21. Palmier RD, Chen HY, and Brinster RL: Differential regu-


28. Reedman BM, Klein G: Cellular localization of an Epstein-


34. Halterman RH, Graves RG, Fucillo DN, Leventhal BG: Immunocompetence following allogeneic bone marrow transplanta-


39. Chang RS, Lewis JP, Abildgaard CF: Prevalence of orophar-


43. Filipovich AH, Krawczak CL, Kersey JH, McGlave P, Ramsay NK, Goldman A, Goldstein G: Graft-versus-host disease prophylaxis with anti-T-1 cell monoclonal antibody OKT3, predni-


46. Starzl TE, Porter KA, Iwatsuki S, Rosenthal JT, Shaw BW, Atchinson RW, Nalesnik MA, Ho M, Griffith BP, Hakala TR, Hardesty RL, Jaffe R: Reversibility of lymphomas and lymphoprolifera-
tive lesions developing under cyclosporin-steroid therapy. Lancet 1:583, 1984


52. Cleary ML, Warnke R, Sklar J: Monoclonality of lympho-


Epstein-Barr virus lymphoproliferation after bone marrow transplantation

MM Zutter, PJ Martin, GE Sale, HM Shulman, L Fisher, ED Thomas and DM Durnam