Epstein-Barr Virus Associated B Cell Lymphoproliferative Disorders Following Bone Marrow Transplantation


B cell lymphoproliferative disorders (BLPD) developed in eight patients following bone marrow transplantation (BMT) for leukemia (five patients) or immunodeficiency (three patients). Recipients of T depleted marrow from a mismatched donor were at particularly high risk of this complication. Six of 28 (24%) recipients of mismatched T depleted bone marrow developed BLPD. In contrast, none of 47 matched T depleted transplants, one of ten (10%) who received non-depleted marrow from an unrelated donor, and only one of 424 matched non-depleted transplants were associated with BLPD. Epstein-Barr virus (EBV) specific serology and DNA hybridization studies demonstrating five to 50 copies of EBV genome/cell in involved tissues implicate this virus as an associated etiologic agent. Restriction fragment length polymorphism (RFLP) and cytogenetic analysis of involved tissue demonstrated donor origin (five of seven) or host origin (two of seven). Histologic appearance was similar to EBV-induced polymorphic B cell proliferations described following solid organ transplantation, or which occur de novo in primary immunodeficiency. Six of seven patients with adequate tissue available for study were found to have monoclonal proliferations by: in situ immunofluorescence (six of seven), and/or immunoglobulin gene rearrangement. (four of six). Cytogenetic analysis of involved tissues from four patients showed a normal karyotype, whereas two had multiple clonal chromosomal abnormalities. Seven patients died despite aggressive attempts at therapy with combinations of antiviral, immunologic, and chemotherapeutic agents.

ONE MARROW transplantation can be lifesaving for patients with otherwise fatal malignant and non-malignant diseases. Since most patients lack an HLA identical sibling for use as a donor, much effort has gone into finding alternative donor sources, such as partially mismatched family members or unrelated donors. Major obstacles to the use of such donors for BMT have been graft rejection and graft-vs-host disease (GVHD).1 Intensification of preparative regimens and improved methods of removing mature T cells from bone marrow have decreased the incidence of these complications.2-4 More recently, we have experienced an additional complication of mismatched BMT affecting primarily recipients of T depleted marrow: B cell lymphoproliferative disease (BLPD) associated with Epstein-Barr virus (EBV).5-6

EBV is a herpes group virus that is closely associated with the development of BLPD in patients with compromised immunologic function. The virus infects and immortalizes B lymphocytes in vitro7 and in vivo,8 resulting in polyclonal activation and proliferation. In the normal host, EBV-driven lymphoproliferation is primarily controlled by EBV-specific cytotoxic T cells9-11 with a lesser role being played by humor responses,12 antibody-dependent cellular cytotoxicity,13 natural killer cell activity,14 and possibly gamma interferon.15 In the immunodeficient host, the proliferation of EBV-infected B cells can go unchecked. This may result in death from organ failure secondary to infiltration by lymphoid cells, or proliferating cells may undergo evolution from a polyclonal reactive process to a monoclonal malignant lymphoma.16 BLPD has been documented in both primary immunodeficiency (severe combined immunodeficiency,17-20 ataxia telangiectasia,21 Wiskott-Aldrich syndrome,22 X-linked lymphoproliferative syndrome,23-24 and as a consequence of secondary immunodeficiency (iatrogenic, as in solid organ transplant recipients,25-29 or viral induced, as in the acquired immunodeficiency syndrome [AIDS].30). BLPD has been a surprisingly infrequent complication of matched allogeneic BMT despite intensive immunosuppression by preparative regimens before BMT, which include high-dose chemotherapy and irradiation, followed post-BMT by immunosuppressive agents used to prevent and/or treat GVHD. Reports of BLPD occurring after BMT have been sporadic and for the most part have occurred in the context of mismatching and/or T depletion11-13 or following in vivo anti-T cell immunotherapy with either antithymocyte globulin (ATG)34 or monoclonal antibody.35 This series, consisting of eight patients, represents the largest and most extensively studied group of post-BMT BLPD reported to date. In addition to clinical presentation, course, and treatment, we present data regarding serologic and genomic evidence of the association with EBV, as well as multiple studies characterizing these tumors, including histologic classification by light microscopy, determination of host v donor origin by restriction fragment length polymorphism and cytogenetic analysis, and also the assessment of clonality by immunophenotyping and immunoglobulin gene rearrangement studies.

From the Departments of Pediatrics, Laboratory Medicine and Pathology, and the Bone Marrow Transplantation Program, University of Minnesota Hospitals and Clinics, Minneapolis.

Submitted September 6, 1987; accepted December 16, 1987.

Supported in part by Grants No. PO1-CA-21737, U01-CA-44120, ROI-CA-25097, and T32-HL07143 from the National Institutes of Health (NIH), Minnesota Medical Foundation, and the Children’s Cancer Research Foundation of the University of Minnesota.

Bruce Blazar and James Greenberg are Fellows of the Leukemia Society of America.

Address reprint requests to Ralph S. Shapiro, MD, Box 214 Mayo, University Hospitals and Clinics, 420 Delaware St SE, Minneapolis, MN 55455.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1988 by Grune & Stratton, Inc.

0006-4971/88/7105-0005$3.00/0

Blood, Vol 71, No 5 (May), 1988; pp 1234-1243
PATIENTS AND METHODS

Between 1968 and 1986, 506 allogeneic bone marrow transplants were performed at the University of Minnesota: 424 allogeneic matched non-T depleted, 25 mismatched T depleted, 47 matched T depleted, and ten unrelated non-T depleted. Of these 506 patients, eight developed BLPD following BMT. All patients were advised of procedures and attendant risks in accordance with institutional guidelines and gave their informed consent.

Bone marrow transplantation. Characteristics of BMT for the eight patients who developed BLPD are summarized in Table 1. Five were transplanted for leukemia and three for lethal immunodeficiency. Their ages at time of BMT ranged from 8 months to 48 years. Six patients received marrow from related donors who were not fully matched, one from a phenotypically matched unrelated donor, and one from a matched sibling. Pretransplant conditioning included combined chemotherapy and total body irradiation in seven patients and chemotherapy alone in one.* Six received donor marrow that was depleted of mature T cells ex vivo before infusion by one of several methods previously described: soybean lectin agglutination and chemotherapy alone in one.* Six received donor marrow that combined chemotherapy and total body irradiation in seven patients one from a matched sibling. Pretransplant conditioning included cytosine arabinoside (3 g/m² twice daily for six days (no. 469); procarbazine (12.5 mg/kg) days -8, -6, -4, cytoxan (60 mg/kg) days -8, -6, -4, -3, -2, -1 followed by 750 cGy TBI day 0 (no. 309); procarbazine (12.5 mg/kg) days 8, 6, 4, cytoxan (60 mg/kg) days 8, 6, 4, -3 followed by 750 cGy TBI day 0 (no. 309); cytoxan (60 mg/kg) days 8, 6, 4, -3 followed by 1,320 cGy fractionated TBI (165 cGy twice daily) over four days (no. 541 and 596); 850 cGy TBI followed by cytoxan (50 mg/kg) days 8, 6, 4, -3, -2, -1 (no. 588 and 600).

*Conditioning regimens included: cytoxan (50 mg/kg) and ATG (15 mg/kg twice daily) days -4, -3, -2, -1 followed by 750 cGy total body irradiation (TBI) (no. 332); 850 cGy TBI followed by cytosine arabinoside (3 g/m² twice daily for six days (no. 469); procarbazine (12.5 mg/kg) days 8, 6, 4, cytoxan (60 mg/kg) days 8, 6, 4, -3, ATG (12.5 mg/kg) days 7, 5, 3 followed by 750 cGy TBI day 0 (no. 309); cytoxan (60 mg/kg) days 3, 2 (no. 190); cytoxan (60 mg/kg) days 7, 6 followed by 1,320 cGy fractionated TBI (165 cGy twice daily) over four days (no. 541 and 596); 850 cGy TBI followed by cytoxan (50 mg/kg) days 8, 6, 4, -3, -2, -1 (no. 588 and 600).

These patients were treated with prednisone (60 mg/m²/d). Patient no. 596 received five days of ATG 15 mg/kg twice daily after failing a course of steroids.

Histopathology. Seven patients were diagnosed as having an atypical lymphoproliferative disorder by premortem biopsy. One patient (no. 600) was diagnosed postmortem from autopsy specimens. Premortem diagnostic material was obtained from liver (four patients), lymph node (four), lung (two), brain mass (one), gall bladder (one), and appendix (one). Biopsy and autopsy specimens were fixed in B5, embedded in paraffin, and sections were stained with hematoxylin and eosin.

Classification of the lymphoproliferative lesions was based on the criteria developed for the polymorphic B cell processes that develop following renal transplantation. In brief, polymorphic diffuse B cell hyperplasia (PBCH) and polymorphic diffuse B cell lymphoma (PBCL) are both characterized by extensive invasion of blood vessels and other organ structures as well as by obliteration of the nodal architecture in lymph nodes. Both contain a mixture of B cells with plasmacytic differentiation (lymphoplasmacytoid, plasma cells, and immunoblasts), and small cells with angulated and cleaved nuclei, morphologically compatible with small cleaved follicular center cells. However, lesions termed PBCH show a striking plasmacytic component with small cleaved cells, no atypia in the large cells, and no necrosis. PBCL is characterized by frequent atypical and sometimes multinucleated large cells and extensive coagulative necrosis, with an irregular "geographic" pattern.

In addition to PBCH and PBCL, other categories were used to distinguish lesions that did not fit the criteria for either PBCH or PBCL. The term, atypical lymphoid hyperplasia (ALH), indicates lesions characterized by involvement of the paracortex of lymph nodes or interstitial tissue of extranodal organs by collections of small lymphocytes, many with irregular nuclear contours, some transformed lymphocytes or immunoblasts, lymphoplasmacytoid cells, and mature as well as immature plasma cells. These lesions are nonspecific and only appear "atypical" because of the relative abundance of immunoblasts and the irregular nuclei of the small cells. They do not show any morphologic features of invasiveness or obliteration of normal structures. The term, atypical PBCH (APBCH), was used to describe a few lesions that had features intermediate between PBCH and PBCL, with intense plasmacytic differentiation and lack of necrosis, characteristic of PBCH, but distinct nuclear atypia, and at times multinucleation of the large cells. Finally, some lesions were composed of a spectrum of plasmacytoid cells, with abundant and atypical B cells, which fit the criteria established by Lukes and Collins and Lennert, for B immunoblastic sarcoma. It was the absence of small cleaved cells that differentiated these tumors from PBCL.

EBV ASSOCIATED BLPD FOLLOWING BMT

Table 1. Characteristics of BMT for Eight Patients Who Developed BLPD

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age at BM/Sex</th>
<th>Pre-BMT Diagnosis</th>
<th>Mismatched Donor</th>
<th>T Depleted Donor Marrow</th>
<th>Post-BMT</th>
<th>Engraftment</th>
<th>GvHD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>588</td>
<td>12 yr/M</td>
<td>CML</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Donor</td>
<td>II</td>
</tr>
<tr>
<td>469</td>
<td>14 mo/F</td>
<td>ANLL</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Donor</td>
<td>—</td>
</tr>
<tr>
<td>309</td>
<td>8 mo/M</td>
<td>SCID</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Donor</td>
<td>III</td>
</tr>
<tr>
<td>600</td>
<td>48 yr/M</td>
<td>CML</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Donor</td>
<td>IV</td>
</tr>
<tr>
<td>596</td>
<td>30 yr/F</td>
<td>CML</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Host</td>
<td>—</td>
</tr>
<tr>
<td>541</td>
<td>36 yr/M</td>
<td>CML</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Donor</td>
<td>—</td>
</tr>
<tr>
<td>332</td>
<td>7.5 yr/M</td>
<td>WAS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Donor</td>
<td>—</td>
</tr>
<tr>
<td>190</td>
<td>13 mo/F</td>
<td>SCID</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Donor</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: UPN, unique patient number; CML, chronic myelogenous leukemia; ANLL, acute nonlymphocytic leukemia; SCID, severe combined immunodeficiency; WAS, Wiskott-Aldrich syndrome; D, donor; H, host.

*Overall grade by Seattle criteria.
†Unrelated matched.
Immunophenotyping. The tissues were phenotyped in frozen sections and/or in suspensions of viable cells by direct and indirect immunofluorescence as previously described.\(^4^4\) Phenotyping included murine monoclonal antibodies against the following leukocyte cluster specificities: CD1, CD8 (Ortho Diagnostics, Raritan, NJ); CD4, CD11, CD19, CD22, HLA-DR (Ia) (Becton Dickinson Co, Mountain View, CA); CD3, CD13, CD14, CD15, CD16, CD20 (Coulter Immunology, Hialeah, FL); CD2, CD5, CD7 (Daniel Valler, PhD, University of Minnesota); CD9, CD10, CD21, CD24 (Tucker LeBien, PhD, University of Minnesota). Frozen sections were routinely double-stained to determine the number and the distribution of cells that carried the same light and heavy immunoglobulin (Ig) chain, and to define whether the cells positive for a monotypic surface or cytoplasmic Ig were also reactive with each monovalent antibody applied. To meet the definition of monotypic B cell proliferation 75% or more Ig-positive cells had to carry the same heavy and light chain in double stained section. In one patient, only paraffin embedded material was available. This was studied by immunoperoxidase techniques as described by Sternberger et al.\(^4^5\)

Cyto genetic analysis. Cytogenetic studies were done according to previously described methods.\(^4^6\) Briefly, direct preparations and 24-hour unstimulated cultures were set up in each case. When sufficient material was received, 24-hour methotrexate-synchronized cultures were also set up. Chromosome spreads were G-banded using the Wright's technique of Sanchez et al.\(^4^7\) Twenty to 30 metaphase spreads were analyzed. QFQ and COB banding were also done to detect polymorphic differences between same sex donor and recipient pairs.

DNA studies: RFLP, EBV genomes, and Ig gene rearrangement. High molecular weight DNA was extracted from peripheral blood mononuclear cells, bone marrow, or minced tumor tissues by overnight digestion with proteinase K and sodium dodecyl sulfate (SDS) followed by phenol/chloroform extraction and ethanol precipitation. DNA was digested to completion by incubation with frequenting bands (RFLPs). Methods, including hybridization conditions of radiolabeled probes, stringency of washes, and densitometric scanning of autoradiographs have been previously described.\(^4^8\) Donor-specific and/or recipient-specific polymorphisms were detected in each case. Sex mismatched donor-recipient pairs were studied with the probes pDP34 (gift of Dr David Page, Whitehead Institute, Cambridge, MA) with or without additional screening using 13.1.25 (gift of Dr Michael Litt, University of Oregon, Portland). Same sex donor recipient pairs were studied using the probes D1JS2 (gift of Dr Webster Cavenee, University of Cincinnati), D1JS1 (gift of Dr Ray White, University of Utah and the Howard Hughes Medical Center, Salt Lake City), and/or pBL4-52 (gift of Dr Carmen Sponzo, Montreal, Canada).

The presence of EBV-specific DNA sequences in DNA extracted from involved tissue was determined by probing DNA blots using radiolabeled BamHI-A,E, or H fragments of the EBV genome (gift of Dr Elliot Kieff, University of Chicago). The presence or absence of a clonal rearrangement of the immunoglobulin heavy chain gene was detected by probing DNA blots from involved tissues using either the heavy chain constant region probe C\(\mu\) or the joining region probe JH (gifts of Dr Stanley J. Korsmeyer, NIH, Bethesda, MD).

RESULTS

Clinical course of BLPD. The clinical course of the eight patients who developed BLPD is summarized in Table 2. Time from transplant to onset of symptoms related to BLPD ranged from 30 days to 49 months (median, 72 days). The most common presenting clinical features included fever, jaundice, and lymphadenopathy. More than half the patients (6 of 8) developed symptoms related to the EBV infection within the first 3 months after BMT. The clinical course of the eight patients who developed BLPD is summarized in Table 2. Two patients developed symptoms related to BLPD within 72 days after BMT, while the other six had symptoms of BLPD ranging from 2 to 49 months after the transplant. The clinical course of the patients who developed BLPD is summarized in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Clinical Course of BLPD Following BMT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UPN</strong></td>
</tr>
<tr>
<td><strong>Onset of symptoms (days post-BMT)</strong></td>
</tr>
<tr>
<td><strong>Presentation</strong></td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>Anorexia</td>
</tr>
<tr>
<td>Abdominal pain</td>
</tr>
<tr>
<td>Hepatitis</td>
</tr>
<tr>
<td>Lethargy</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
</tr>
<tr>
<td>CNS symptoms</td>
</tr>
<tr>
<td>Pharyngitis</td>
</tr>
<tr>
<td><strong>Therapy</strong></td>
</tr>
<tr>
<td><strong>Outcome (survival, days post-onset)</strong></td>
</tr>
<tr>
<td><strong>Cause of death</strong></td>
</tr>
</tbody>
</table>

Abbreviations: Acy, acyclovir; Ig, intravenous immunoglobulin (400 mg/kg/wk); St, steroids; IF, alpha interferon; Ly, pheresised irradiated lymphocytes; d, donor; di, donor irradiated; pi, non-donor parent irradiated; ALG, antilymphocyte globulin; COP, cyclophosphamide, vincristine, prednisone.
(eight), anorexia (eight), hepatitis (seven), and abdominal pain (seven). Other commonly associated findings included lethargy (six), lymphadenopathy (five), CNS symptoms including behavioral changes, confusion and irritability (four), and pharyngitis (three). Seven of seven patients studied were seronegative for human immunodeficiency virus (HIV) at the time of diagnosis of BLPD.

Based on presentation and tempo of disease, patients could be divided into three groups. In the first group were patients no. 588, 469, 309, and 600 who presented early post-BMT (30 to 72 days) and had rapidly progressive symptoms related to widely disseminated lymphoid infiltration of vital organs. All four were recipients of mismatched T depleted BMT and three had received high-dose ATG and prednisone between day -4 and day +28 post-BMT in an attempt to facilitate engraftment. These patients received various combinations of treatments for BLPD, as listed in Table 2 and although two had transient symptomatic improvement, there were no persistent objective responses. These patients rapidly developed multiple system organ failure: hepatic dysfunction, progressive ascites, renal failure, and ultimately interstitial pneumonitis. Time from onset of symptoms to death ranged from 11 to 45 days. Autopsy confirmed the presence of diffuse infiltration of virtually all organs examined by activated B cells.

A second group, patients no. 596 and 541, presented at 72 and 89 days post-BMT, respectively. Onset of symptoms was more insidious, and they had a slower pace of disease with primarily nodal involvement. Both received non-T depleted marrows and received standard GVHD prophylaxis with methotrexate, prednisone, and ATG. Acute grade III to IV GVHD developed in each requiring treatment. Patient no. 596 failed initial steroid treatment, and so received a five-day course of high-dose ATG (15 mg/kg twice daily), while patient no. 541 had initial improvement with steroids but acquired a primary cytomegalovirus (CMV) infection. Both patients developed fever, pharyngitis, and tender cervical adenopathy. Multiple enlarged nodes seen on computed tomography (CT) scanning led to the suspicion of BLPD and biopsy confirmation. Alpha interferon (2,000,000 IU/m2/d) was added to acyclovir therapy in these patients. One patient (no. 596) had complete resolution of BLPD and is now off therapy more than 10 months post-BMT. The other (no. 541) had no further progression of BLPD by CT scan while on therapy; however, fatal interstitial pneumonitis with CMV developed and was confirmed at autopsy. The lymphoproliferative process in this patient was primarily nodal with some direct extension into perinodal tissues of the lung, pancreas, greater omentum, and mesentry.

A third group consisting of the remaining two patients, no. 332 and 190, presented at a much later date (16 and 49 months post-BMT). Both patients were transplanted for immunodeficiency. One patient rejected the transplanted marrow and had autologous marrow recovery. The other maintained a graft of maternal T cells but had persistent host B cells. Several months before the diagnosis of BLPD each developed a febrile illness and had serologic, as well as clinical evidence of an acute EBV infection. Fever and symptoms improved with institution of acyclovir therapy. Patient no. 332 developed intermittent abdominal pain and bizarre behavioral changes that persisted for several months, at which time fevers redeveloped and the patient had a generalized seizure. CT scan revealed bilateral basal ganglia masses and a CT-guided needle biopsy confirmed the diagnosis of BLPD. After diagnosis no further therapy was given. Autopsy showed bilateral masses in the patient’s basal ganglia, and in addition, isolated involvement of one mediastinal and one mesenteric lymph node. Other organs and lymph nodes were uninvolved. Patient no. 190 experienced apparent resolution of acute infectious mononucleosis, but 3 months later redeveloped fever, cough, and lymphadenopathy. Progressively enlarging lymph nodes required the placement of a tracheostomy for airway management. Despite multiple therapeutic agents, ascites, hepatic dysfunction, and pulmonary interstitial disease with respiratory distress progressed. Hemophils influenza sepsis and status epilepticus developed as terminal events. Autopsy revealed extensive diffuse BLPD involving both lymph nodes and extranodal tissues.

At the onset of symptoms that led to the diagnosis of BLPD, six patients were receiving prophylactic acyclovir: 15 mg/kg orally three times daily (no. 332); 10 mg/kg intravenously (IV) three times daily (no. 190); or 5 mg/kg IV three times daily (no. 588, 596, and 541). On suspicion of diagnosis of EBV associated BLPD, acyclovir was increased or added by IV administration at doses that achieved and maintained serum levels of 15 to 20 μg/mL. In addition, multiple other therapeutic modalities were instituted (Table 2).

Association with EBV. Serologic data regarding prior exposure of donors and recipients to EBV are shown in Table 3. Five patients had positive IgG antibody titers to EBV VCA before BMT, indicative of past infection. Patient no. 332 was receiving IV immunoglobulin when titers were measured; however, elevated titers to the early antigen at the onset of symptoms suggested either an ongoing or reactivated infection. One patient was seropositive pre-BMT and received marrow from a seronegative donor. Three patients had negative titers pre-BMT and two of these (no. 469 and 309) received marrow from donors who were seropositive. At the onset of BLPD both were found to have elevated IgG titers to VCA without IgM antibodies or antibodies to EBNA. The third seronegative patient received marrow from

<table>
<thead>
<tr>
<th>UPN</th>
<th>Pre-BMT Recipient</th>
<th>Donor</th>
<th>Maximum Post-BMT EBV Genome Copies/Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>588</td>
<td>640 &lt;10</td>
<td>320</td>
<td>640 Donor 5-50</td>
</tr>
<tr>
<td>469</td>
<td>640 &lt;10</td>
<td>320</td>
<td>160 Donor 5-20</td>
</tr>
<tr>
<td>309</td>
<td>640 &lt;10</td>
<td>320</td>
<td>320 ND ND</td>
</tr>
<tr>
<td>600</td>
<td>640 &lt;10</td>
<td>320</td>
<td>ND Donor 20</td>
</tr>
<tr>
<td>596</td>
<td>640 &lt;10</td>
<td>320</td>
<td>160 Donor 5-20</td>
</tr>
<tr>
<td>541</td>
<td>640 &lt;10</td>
<td>320</td>
<td>320 Donor 5-20</td>
</tr>
<tr>
<td>332</td>
<td>640 &lt;10</td>
<td>ND</td>
<td>640 Host 20-50</td>
</tr>
<tr>
<td>190</td>
<td>640 &lt;10</td>
<td>ND</td>
<td>160 Host 20-50</td>
</tr>
</tbody>
</table>

*IgG (VCA)—immunoglobulin G antibody titer to Epstein-Barr viral capsid antigen (reported as reciprocal).
a seronegative donor. This patient developed symptoms of acute infectious mononucleosis 3 1/2 years after BMT and had an increase of both IgG and IgM titers to EBV VCA indicative of a newly acquired primary infection.

Multiple copies of EBV genome per cell were detected in all seven cases where DNA extracted from involved tissue was available for analysis (see Table 3). Copy numbers varied from tissue to tissue within the same patient ranging from five to >50 genomes per cell.

Characterization of BLPD. Determination of host or donor origin of tumor specimens was made by RFLP (Fig 1) and/or cytogenetic analysis, and results are found in Table 3. Patients in groups one and two had BLPD of donor origin, while both group three patients had host-derived lymphoproliferative processes.

Tumor characteristics are summarized in Table 4. Several patients manifested more than one morphologic type of BLPD in consecutive specimens or in tissues from different sites biopsied at the same time. There was an excellent correlation (89%) between the morphologic classification of the lesions and clonality as determined by immunologic or genomic studies. Of six specimens classified as ALH or PBCH, five were immunologically polyclonal and only one, from an extranodal site, monoclonal by immunologic as well as genomic criteria. Of 12 specimens classified as PBCL or ISB, 11 were shown to be monoclonal. One classified as PBCL was not monoclonal by immunophenotyping and was germ line by gene rearrangement studies.

Nine of 15 specimens studied by immunofluorescence showed immunologic evidence of a monotypic (monoclonal) B cell proliferation by our criteria. In contrast to large cell malignant lymphomas occurring in the non-transplant setting, this monoclonal proliferation was mixed in several tissues with a large population of polyclonal plasmacytoid and immunoblastic B cells. For some patients in whom more than one tissue was examined, differences were found between lesions from different sites. Patient no. 541 had evidence of two different clonal proliferations at separate sites. Another patient (no. 588) had tissues with many cells devoid of surface or cytoplasmic Ig. These tissues, where Ig-negative cells carrying B cell surface antigens prevailed, were considered consistent with a monotypic proliferation. Indeed, genomic studies confirmed the presence of a population of cells with a clonal rearrangement of immunoglobulin heavy chain genes in these specimens.

Monoclonal antibodies detected B cell surface antigens on cells from all involved tissues. In every case the cells were HLA-DR positive. Associated B cell markers varied from tissue to tissue within each patient, as well as between patients. The most frequent markers were CD22 (six of seven), CD19 (four of seven), CD20 (four of seven), and CD21 (three of seven). Interestingly, the only patient whose tissues lacked the CD22 antigen was no. 596 in whom no clonal population of transformed B cells could be detected by immunofluorescence or genotyping. Genotypic analysis was performed on samples from six patients. A clonal rearrangement of the immunoglobulin heavy chain gene was detected in tumor tissues from four. Of the two cases with only germ line bands detected in tumor samples, one had immunophenotypic evidence of monoclonality on the same specimen.

Cytogenetics. The results of cytogenetic analysis performed on involved tissues from seven patients are shown in Table 4. Four had normal karyotypes in 20 of 20 metaphases examined. Two patients (no. 332 and 190) were found to have clonal chromosomal abnormalities in multiple specimens. Both had normal bone marrow and/or peripheral blood cytogenetic studies performed after BMT before development of BLPD. No metaphases were found for evaluation in specimens from patient no. 600.

DISCUSSION

Overwhelming BLPD is a well-known complication of immunodeficiency, be it primary (inherited) or secondary (acquired). Many of these processes, when adequately examined, have been found to be associated with EBV. In this study, we present eight recipients of BMT who developed BLPD similarly associated with EBV.

Three clinical groups were defined: patients in group 1...
Table 4. Characteristics of B Cell Lymphoproliferative Processes Occurring After BMT

<table>
<thead>
<tr>
<th>UPN</th>
<th>Post-BMT Day</th>
<th>Site</th>
<th>Morphology</th>
<th>Morphology</th>
<th>Immunophenotype</th>
<th>Ig Gene Rearrangement</th>
<th>Cytogenetic Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>588</td>
<td>36</td>
<td>Liver</td>
<td>ALH</td>
<td>X</td>
<td>Polytropic</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>36</td>
<td>Cervical LN</td>
<td>PBCH</td>
<td>X</td>
<td>Polytropic</td>
<td>Clonal</td>
<td>46,XX</td>
</tr>
<tr>
<td>43</td>
<td></td>
<td>Hilir LN</td>
<td>PBCH</td>
<td>X</td>
<td>Monotypic</td>
<td>Clonal</td>
<td>46,XX</td>
</tr>
<tr>
<td>469</td>
<td>79</td>
<td>Liver</td>
<td>PBCH</td>
<td>X</td>
<td>Monotypic</td>
<td>Clonal</td>
<td>46,XY</td>
</tr>
<tr>
<td>86</td>
<td></td>
<td>Peripheral blood*</td>
<td>X</td>
<td></td>
<td></td>
<td>Clonal</td>
<td>46,XY</td>
</tr>
<tr>
<td>86</td>
<td></td>
<td>Spleen*</td>
<td>X</td>
<td></td>
<td></td>
<td>Clonal</td>
<td>46,XY</td>
</tr>
<tr>
<td>309</td>
<td>89</td>
<td>Appendix</td>
<td>X</td>
<td></td>
<td></td>
<td>Polytropic</td>
<td></td>
</tr>
<tr>
<td>89</td>
<td></td>
<td>Liver</td>
<td>X</td>
<td></td>
<td></td>
<td>Polytropic</td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>78</td>
<td>LN*</td>
<td>X</td>
<td></td>
<td></td>
<td>Clonal</td>
<td>No metaphases</td>
</tr>
<tr>
<td>78</td>
<td></td>
<td>LN*</td>
<td>X</td>
<td></td>
<td></td>
<td>Clonal</td>
<td>No metaphases</td>
</tr>
<tr>
<td>596</td>
<td>77</td>
<td>Auxiliary LN</td>
<td>X</td>
<td>Polytropic</td>
<td>Germ line</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>541</td>
<td>104</td>
<td>Pulmonary LN</td>
<td>X</td>
<td>Monotypic</td>
<td>Germ line</td>
<td>46,XY</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td></td>
<td>Cervical LN</td>
<td>X</td>
<td>Monotypic</td>
<td>Germ line</td>
<td>46,XY</td>
<td></td>
</tr>
<tr>
<td>332</td>
<td>549</td>
<td>Brain</td>
<td>X</td>
<td></td>
<td></td>
<td>Abnormal clone*</td>
<td></td>
</tr>
<tr>
<td>593</td>
<td></td>
<td>Pericardial LN</td>
<td>X</td>
<td>Monotypic</td>
<td></td>
<td>Abnormal clone*</td>
<td></td>
</tr>
<tr>
<td>190</td>
<td>1501</td>
<td>Lung nodule</td>
<td>X</td>
<td>Monotypic</td>
<td></td>
<td>Abnormal clones†</td>
<td></td>
</tr>
<tr>
<td>1526</td>
<td></td>
<td>Cervical LN</td>
<td>X</td>
<td>Monotypic</td>
<td></td>
<td>Abnormal clones†</td>
<td></td>
</tr>
<tr>
<td>1562</td>
<td></td>
<td>Spleen*</td>
<td>X</td>
<td>Monotypic</td>
<td></td>
<td>Abnormal clones†</td>
<td></td>
</tr>
<tr>
<td>1562</td>
<td></td>
<td>LN*</td>
<td>X</td>
<td></td>
<td></td>
<td>No metaphases</td>
<td></td>
</tr>
<tr>
<td>1562</td>
<td></td>
<td>Kidney*</td>
<td>X</td>
<td></td>
<td></td>
<td>No metaphases</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: LN, lymph node; ALH, atypical lymphoid hyperplasia; PBCH, polymorphic B cell hyperplasia; APBCH, polymorphic B cell hyperplasia with atypia but no necrosis; PBCL, polymorphic B cell lymphoma; ISB, immunoblastic sarcoma; *, autopsy specimen.

*46,XY,del(7)(q22),t(3;6)(q25;q23),t(13;18)(q14;q23),t(14;21)(q24;q22) (20/20 metaphases).
†46,XX (12/23 metaphases); 49,XX, +9, +10, +11 (5/23 metaphases); 50,XX, +9, +9, +10, +11 (11/23 metaphases); 92,XXXX (3/23 metaphases).
‡46,XX (9/22 metaphases); 47,XX, +9 (6/22 metaphases); 47,XX, +11 (3/22 metaphases); 47,XX, +15 (3/22 metaphases); 48,XX, +9, +15 (1/22 metaphases).

developed BLPD early after BMT, had rapidly progressive and diffuse disease; group 2 patients had insidious onset of symptoms with primarily nodal involvement; and group 3 were two patients transplanted for underlying immunodeficiencies who developed BLPD long after their BMT, occurring several months after an acute EBV infection. In this last group, given the long interval between BMT and the development of BLPD it is uncertain what, if any influence, BMT had on its development.

Tissue from organs involved with BLPD were histopathologically similar to those described in patients after renal transplantation, and basically fell into two main categories: PBCH and PBCL. Several patients manifested more than one morphologic type of BLPD in consecutive specimens or in samples from different sites taken at the same time. Generally, there was good correlation between morphology and clonality; however, we found instances where morphology and clonality were discordant. It might be noted that finding monoclonality in a lymphoproliferative disorder may represent extreme clonal expansion and is not necessarily indicative of neoplastic transformation. To summarize our pathological correlations, some of the BLPD clearly were hyperplastic in that they were immunologically polyclonal and had reactive morphologic features and normal karyotypes. Others were monoclonal, neoplastic in morphology, and had abnormal karyotypes, leaving little doubt as to their malignant nature. Less definite were processes from patients in group 1 and 2, which showed malignant morphologic features, monoclonality by immunologic and genomic studies, but normal cytogenetic profiles. Our findings support the hypothesis that BLPD represents a spectrum of disease where a reactive polyclonal process precedes the development of a monoclonal process. The rate of evolution differs at separate sites or within a specific site, explaining the heterogeneity of morphology between and within specimens from individual patients.
Predisposing factors to the development of BLPD remain to be clarified. EBV serology and DNA hybridization studies in our patients suggest that this virus plays a major role in the etiology of BLPD. Since BLPD is rare in standard matched BMT, additional factors appear to be involved in our patients. Possible contributing factors include: histoincompatibility between donor and recipient, ex vivo T cell depletion of bone marrow, and in vivo anti-T cell therapy.

Our experience suggests that mismatching plays a major contributing role. Six of 25 (24%) recipients of mismatched T depleted bone marrow and one of ten (10%) who received unrelated non-depleted marrow developed BLPD. This incidence is in sharp contrast to that observed in matched related patients: none of 47 matched T depleted transplants and only one of 424 matched non-depleted transplants. Two factors associated with the development of BLPD in the setting of mismatched BMT may be chronic antigenic stimulation and activation of endogenous viruses.

In support of antigenic stimulation, Metcalf reported that repeated injections of antigen into mice results in an increased incidence of reticulum cell sarcomas and plasma cell tumors. Other studies show that lymphomas develop only in the setting of major histocompatibility complex (MHC) incompatibility in a murine model of lymphomagenesis involving a parental graft into F1 hybrid. It is hypothesized that in the mouse, GVHD and/or antigenic stimulation of B cells leads to expression of endogenous viruses that in turn activate cellular oncogenes leading to malignant transformation. In humans, endogenous DNA viruses of the herpes group are commonly reactivated from latency during immunostimulation or immunosuppression. A classic example of chronic antigenic stimulation (endemic malaria) interacting with endogenous virus (EBV) to produce a B cell malignancy in humans is Burkitt’s lymphoma. The recent discovery of the new herpes-like human B lymphotropic virus (HBLV) in patients with angioimmunoblastic lymphadenopathy and immunoblastic lymphoma raises the additional possibility that interactions between this or other viruses and EBV lead to transformation of B cells during the period of most profound immunoregulatory dysfunction following bone marrow transplantation.

Although mismatching appears to be a major risk factor, depletion of T cells from donor bone marrow and the use of in vivo anti-T cell immunotherapy may be other factors that increase the risk of BLPD. At the University of Wisconsin in Madison, eight of 76 recipients of T depleted bone marrow developed BLPD compared with none of 37 matched non-T depleted patients. Two patients who developed BLPD were HLA identical with their donors (Dr Paul Sondel, personal communication, November 1986). Martin et al reported the development of BLPD in two patients following in vivo treatment of steroid-resistant GVHD with the murine monoclonal antibody 64.1, which reacts with the p19 antigen on human T cells. While the use of ATG as GVHD prophylaxis has not been associated with an increased risk of BLPD, the doses used in previous trials were relatively low (7 mg/kg every other day for a total of six doses). Iwatsuki et al reported the development of lymphoproliferative processes in three of seven renal transplant recipients who received more than 15 doses (15 mg/kg) of ATG compared with none of eight who received less than 15 doses. Our experience also supports the contention that high-dose ATG (15 mg/kg twice daily) may contribute to the development of BLPD in that the symptomatic presentation in four patients was temporally related to administration of high-dose ATG.

The nature of the specific immunologic defect or defects that can lead to the development of BLPD is uncertain; however, the degree of immunosuppression is apparently important. In organ transplant recipients, the incidence of BLPD is related to the degree of immunosuppression, and numerous reports indicate that reduction or cessation of immunosuppressive drugs may lead to a regression of tumor in some patients. Regression of EBV-transformed B cells in vitro has been shown to depend on the presence of viral specific cytotoxic T cells. Renal allograft recipients treated with cyclosporine A (CsA) have been shown to lack EBV-specific cytotoxic T cell function, and reduction of the dose of CsA to <10 mg/kg/d leads to functional recovery of these cells. Gaston et al showed that patients treated with azathioprine and prednisone also lack this specific T cell function. In the matched allogeneic BMT setting, EBV-specific cytotoxicity has been shown to recover early following BMT. Recovery of this T cell function following T depleted and/or mismatched BMT has not been examined, nor has the influence of ATG or anti-T cell monoclonal antibody on this aspect of immunity been systematically studied. It is therefore possible that the absence and/or deficiency of the specific anti-EBV cytotoxic T cell predisposes marrow recipients to EBV-induced BLPD.

Prophylaxis against BLPD with the anti-viral agent acyclovir has been reported to be of benefit; however, in our experience it did not appear to be effective, since six patients developed BLPD while receiving this drug. Results of therapy, including antiviral, immunologic, and chemotherapy are disappointing, although some encouragement can be derived from the results obtained in two patients with the use of alpha interferon. The rapid resolution of BLPD in patient no. 596 following institution of alpha interferon must be interpreted with caution. Although biopsy specimens were fully consistent by morphology with a malignant lymphoma, they showed no evidence of monoclonality by immunophenotyping or gene rearrangement studies, and had a normal karyotype. In addition, it is not clear whether the response was a direct effect of alpha interferon or resulted from recovery of specific cytotoxic T cell function as the effects of recent ATG administration diminished.

In summary, EBV can lead to fatal BLPD in recipients of BMT. Characterization and classification of these lesions is complicated by the existence of a spectrum of disease ranging from a polyclonal to monoclonal processes. Factors that contribute to the risk of BLPD in transplant recipients include a mismatched marrow graft, ex-vivo depletion of T cells from donor marrow, and, possibly, in vivo anti-T cell therapy. How these factors interact in the BMT setting to allow BLPD to develop remains to be determined. Potential mechanisms include chronic alloantigenic stimulation, activation of endogenous viruses, and/or inhibition of autologous EBV-specific T cell immunity. Prophylaxis of high-risk
patients with the antiviral agent acyclovir is not of clear benefit since six patients developed BLPD while receiving this drug. While multiple avenues of therapeutic intervention have had little success, alpha interferon deserves further investigation as it may have been beneficial in two patients.

REFERENCES


31. Shearer WT, Ritz J, Finegold MJ, Guerra IC, Rosenblatt HM, Lewis DE, Pollack MS, Tuber LH, Sumaya CV, Grumet FC, Cleary ML, Warkne R, Sklar J: Epstein-Barr virus-associated B-cell proliferations of diverse clonal origins after bone marrow transplan-
55. Gleichmann E, Melief CJM, Gleichmann H: Lymphomagenesis and autoimmunization caused by reactions of T lymphocytes to incompatible structures of the major histocompatibility complex: A concept of pathogenesis. Recent Results Cancer Res 64:292, 1978
67. Crawford DH, Edwards JMB: Immunity to Epstein-Barr
Epstein-Barr virus associated B cell lymphoproliferative disorders following bone marrow transplantation

RS Shapiro, K McClain, G Frizzera, KJ Gajl-Peczalska, JH Kersey, BR Blazar, DC Arthur, DF Patton, JS Greenberg and B Burke