Posttransplantation Lymphoproliferative Disorders in Bone Marrow Transplant Recipients Are Aggressive Diseases With a High Incidence of Adverse Histologic and Immunobiologic Features

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Posttransplantation lymphoproliferative disorders (PT-LPDs) occurring in T-cell depleted (TCD) allogeneic bone marrow transplant recipients seem to be different from those that arise in solid organ recipients in their early development, the high incidence of extensive dissemination at presentation, and their aggressive course and high fatality rate. We report a series of 10 patients with PT-LPDs after TCD allogeneic bone marrow transplant. We studied the correlation between the morphology of the lesions; their clonality based on immunoglobulin (Ig) heavy chain gene rearrangement analysis and immunohistochemistry; their proliferative activity as measured by immunoperoxidase staining for the proliferating cell nuclear antigen (PCNA) and the presence of p53 gene product overexpression. Histologically, our cases corresponded to the two morphologic categories of polymorphic B-cell lymphoma (PBCL, seven cases) and malignant lymphoma immunoblastic (ML-IB, three cases). Ig light-chain staining showed monoclonality in a minority of the cases, whereas Ig gene rearrangement analysis by polymerase chain reaction revealed B-cell clonality in three of seven cases of PBCL and in all three cases of ML-IB. The Epstein-Barr virus (EBV) genome, the expression of EBV latent membrane protein or both were found in all 10 specimens. High proliferative activity (PCNA ≥66%) was found in all cases, with a mean PCNA value of 56% in PBCL and 84% in ML-IB. Five specimens were p53+ (two of seven PBCL and three of three ML-IB). Two of four PBCL cases resolved with the administration of donor leukocytes. All of the remaining patients died of the PT-LPD within a short time from admission. Our results show that the PT-LPDs after TCD bone marrow transplantation are characterized by a high frequency of high-grade histologic subtypes, frequent monoclonality, high proliferative activity, frequent overexpression of p53 gene product, and poor prognosis. These characteristics observed in only a minority of cases of PT-LPDs occurring after solid organ transplantation may account for the less aggressive clinical behavior observed in those diseases. (Key words: Posttransplantation lymphoproliferative disorders; Bone marrow transplantation; Immunohistochemistry; Proliferating cell nuclear antigen; p53; Cytogenetics) Am J Clin Pathol 1997;107:419–429.

Posttransplantation lymphoproliferative disorders (PT-LPDs) are a well-documented complication in organ transplant recipients receiving immunosuppressive therapies.[1,2] The incidence of this complication varies depending on the type of organ transplantation and immunosuppressive regimen administered. Among the solid organ transplant recipients, the highest incidence (>10%) has been reported in heart[3] and combined heart-lung transplants.[4] In the setting of allogeneic bone marrow transplantation (BMT), the overall reported incidence of PT-LPDs varies from 0.6% to 7.4%.[5–7] An even higher incidence (16%–24%) has been observed in patients who have undergone BMT and are receiving T-cell-depleted (TCD) marrows from unrelated or mismatched donors.[5,7]

The clinical behavior of PT-LPDs after solid organ transplantation is extremely variable, and accurate prognostication is still imperfect. Recent clinicopathologic studies, however, suggest that a combination of morphologic, immunophenotypic, and molecular genetic data can be effectively used to identify factors

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that determine disease progression and eventual clinical outcome for the patients.[8,9] Much less data are available in cases of PT-LPDs after BMT, which seems to be different from those that arise in solid organ recipients because of their high incidence of extensive dissemination at presentation, rapid clinical course, and high mortality rate.[5–7,10] This study is focused on the clinical characteristics, histologic and immunohistologic features including the assessment of the proliferative fraction, and the genotypic status of 10 cases of PT-LPDs occurring among the 245 patients who received allogeneic BMT between January 1985, and January 1995, at Indiana University Medical Center, Indianapolis, Ind.

MATERIALS AND METHODS

Patients

Between January 1985, and January 1995, 245 allogeneic BMTs were performed at the Indiana University Medical Center. Of these, 110 were TCD transplants by the soybean lectin agglutination/sheep red blood cell method[11] or by depletion of CD5 and CD8 cells using the CELLector T-634 device (AIS, Menlo Park, Calif). Patients who received TCD were conditioned with hyperfractionated total body irradiation and chemotherapy (cyclophosphamide alone or cyclophosphamide with etoposide or thiotepa). Rejection prophylaxis consisted of antithymocyte globulin (ATG) and methylprednisolone. Of the TCD BMTs, 50.9% (56/110) used unrelated donors, of which 64% were serologically matched for human leukocyte antigens (6/6 match) and 36% were disparate at one DR locus (5/6 match). All patients were serologically negative for the human immunodeficiency virus (HIV) at the time of the BMT.

Morphology and Immunohistochemistry

Eight patients were given the diagnosis of PT-LPD based on premortem biopsy specimens, and two diagnoses were determined from autopsy specimens. Histologic material was obtained from lymph nodes in six patients, and one each from lung, liver, stomach, and skin. All specimens were fixed in B5 or 10% formaldehyde and embedded in paraffin, and sections were stained with hematoxylin and eosin.

All specimens were reviewed independently by two of us (A. O. and R. S. N.) without knowledge of the clinical data or any of the additional tissue studies. Classification of the PT-LPDs was based on the criteria of Frizzera et al[12,13] that were originally developed for the PT-LPDs observed in renal transplant recipients.[13] In brief, polymorphic B-cell hyperplasia (PBCH) and polymorphic B cell lymphoma (PBCL) are both characterized by an invasive growth pattern with loss of the normal organ architecture and a polymorphic lymphoid cell infiltrate composed of a mixture of B cells with plasmacytic differentiation (lymphoplasmacytoid cells, plasma cells, and immunoblasts) and follicular center cells. However, PBCL shows less prominent plasmacytoid differentiation and displays significant cytologic atypia with atypical and sometimes multinucleated immunoblasts and large areas of coagulative necrosis. PBCH and PBCL have been recently grouped together into a new category of polymorphic PT-LPDs.[9] Lesions composed of a monomorphic collection of cytologically malignant cells are classified as immunoblastic malignant lymphoma (ML-IB).

Paraffin sections for immunohistochemistry were available for all cases. Sections were stained with the panel of antibodies listed in Table 1. Primary antibodies were stained with a biotin-conjugated goat antimouse or antirabbit antibody followed by a peroxidase-conjugated streptavidin and 3,3'-diaminobenzidine tetrahydrochloride (DAB) development, according to standard methods. Paraffin sections were pretreated with microwave oven heating when using the antibody anti-human wild and mutated p53 protein (p53/Pab1801), as previously described.[14] A case was considered p53 positive when the protein was expressed by at least 5% of the lymphoid cells. Immunohistochemical results for the anti–proliferating cell nuclear antigen (PCNA/PC10) stain were expressed as a percentage of positively-stained lymphoid cells.[15]

Polymerase Chain Reaction Analysis for IgH Chain and EBV-DNA

All 10 samples were analyzed under identical conditions. A 15 μm thick paraffin section was cut from each paraffin block. The section was then placed on a No. 30 circular glass fiber filter that was lying on a pad of paper towels, and xylene was dripped onto the filter to remove the paraffin. This tissue-filter unit was soaked in 0.5 mL of xylene at room temperature for 5 minutes. After removing the xylene, the tissue-filter unit was washed twice with 100% ethanol. The ethanol was then removed under vacuum, and the tissue-filter unit was digested with proteinase K (500 μg/mL) in 100 μL of proteinase K buffer (50 mmol/L KCl, 15 mmol/L Tris-HCl [pH

TABLE 1. ANTIBODIES USED FOR IMMUNOHISTOCHEMISTRY*

Antibody	*Clone*	*Dilution*	*Main Reactivity*
CD45RB	PD7/26*	1:150	Leukocytes
CD30	BerH2*	1:2	HRS, ALCL, plasma cells
Kappa	p*	1:2500	Plasma cells
Lambda	p*	1:3500	Plasma cells
CD20	L-26*	1:150	B-cells
CD21	1F8*	1:10	DRCs, B-cells
CD3	p*	1:20	T-cells
CD45RO	UCHL-1*	1:100	T cells and some B-MLs
PCNA	PC10*	1:50	Proliferating cells
p53	Pab1801†	1:8	p53 gene product
EBV-LMP	CS1,4*	1:5	EBV LMP

HRS = Hodgkin's Reed-Sternberg cells; ALCL = anaplastic large cell lymphoma; p = polyclonal antibody; DRCs = dendritic reticulum cells; MLs = malignant lymphomas; PCNA = proliferating cell nuclear antigen; EBV-LMP = Epstein-Barr virus latent membrane protein.
*From Dako, Carpinteria, Calif.
†From Oncogene Science, Manhasset, NY.

8.3, 0.5% Nonidet P-40]) overnight at 37°C. After incubation, the mixture was extracted with phenol and chloroform. The DNA in aqueous phase was precipitated with ethanol that was then removed by vacuum drying, and the DNA was then dissolved in 50 mL of TE (10 mmol/L of Tris-HCl [pH 8.0]; 1 mmol/L EDTA). Ten microliters of this solution was used for the polymerase chain reaction (PCR) analysis. PCR was performed as previously published[16] using Thermus aquaticus (Taq) polymerase, 150 µg DNA, and 35 cycles of amplification involving 1 minute each of sequential denaturation at 95°C, annealing at 55°C, and extension at 72°C. Control tubes containing no DNA or a known DNA sample containing a rearranged immunoglobulin (Ig) gene were set up with each experiment.[16] A portion of the B-globin gene was amplified with primers JBP1 and PC04 as amplifiability controls. A size marker (OX174/HaeIII; New England Biolabs, Beverly, Mass) was used with each run to allow base pair (bp) determination of PCR products. Oligonucleotide primer sequences were: for the third framework portion of the V region, 5'ACA CGG C(C/T)(G/C) TGT ATT ACT GT 3' (termed Fr3A)[17]; for the J region 5'TGA GGA GAC GGT GAC C 3' (termed LJH) or 5'GTG ACC AGG GTN CCT TGG CCC CAG 3' (termed VLJH).[17] Fr3A is based on a consensus sequence for codons 89 through 95 from 17 human V regions, while LJH and VLJH are based on consensus sequences for codons 109 through 113 and 103 through 110 from the 6 J regions.[17] The primers are expected to generate a fragment of approximately 100 to 120 bases in length. The amplified material was electrophoresed in 2% agarose gel and was visualized and photographed by ethidium bromide staining and UV illumination. Specimens showing one or two discrete product band(s) migrated between the 72-bp and the 118-bp size markers were considered positive for IgH gene rearrangement. Molecular analysis for the Epstein-Barr virus (EBV) genome was performed by PCR by using consensus primers for the iterated Bam H1 W sequence of EBV-DNA, according to published methods.[18] A product band of 110 bp was considered positive for the presence of the EBV genome.[18]

Cytogenetics

Cytogenetic analysis was performed with Giemsa-banding according to standard techniques. Cytogenetic results were only available in four PT-LPD cases.

RESULTS

Clinical Findings

During the 10 years studied, there were no cases of PT-LPDs in the 135 patients treated with unmanipulated marrow, while PT-LPD developed in 10 of 110 patients receiving grafts depleted of T cells (Table 2). The incidence was significantly higher in patients undergoing TCD BMT from unrelated donors compared with those using sibling donors, 16% and 2% respectively (P=.0163, Fisher exact test).

TABLE 2. CHARACTERISTICS OF PATIENTS WITH POSTTRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS

Patient No./Age, y/Sex	*Diagnosis*	*Match*	*T-Depletion Method*	*GVHD†*
229/41/F*	CML	6/6	CD5/CD8	0
237/51/M	CML	6/6	CD5/CD8	0
251/37/M	CML	6/6	CD5/CD8	2
264/33/M	MDS	6/6	SBA/sRBC	2
271/25/F	ALL	5/6	SBA/sRBC	0
295/47/M	AML	5/6	SBA/sRBC	1
303/24/F	AML/ALL	6/6	SBA/sRBC	0
314/31/F	CML	6/6	SBA/sRBC	1
316/30/F	CML	6/6	SBA/sRBC	1
339/30/F	CML	6/6	SBA/sRBC	1

GVHD = graft-*vs*-host disease; Staging according to the Seattle criteria. CML = chronic myeloid leukemia; MDS = myelodysplastic syndrome; SBA/sRBC = soybean lectin agglutination/sheep red blood cell method; ALL = acute lymphoid leukemia; AML = acute myeloid leukemia.
*The donor was related to patient 229; all other donors were unrelated to the graft recipients.
†Most severe stage before the diagnosis of posttransplantation lymphoproliferative disorder.

TABLE 3. CLINICAL COURSE OF PT-LPD AFTER BMT

	Patient No.									
	229	**237**	**251**	**264**	**271**	**295**	**303**	**314**	**316**	**339**
Onset, d post-BMT	105	98	57	161	163	273	166	84	98	63
Manifestation										
Fever	+	+	+	+	+	+	+	+	+	+
Nausea	−	−	+	+	−	+	−	−	+	+
Anorexia	−	−	−	−	−	−	+	−	−	+
Weight loss	−	−	−	−	−	−	+	−	−	+
Rash	+	+	+	−	+	−	−	+	+	−
Abdominal pain	+	−	−	−	−	+	−	−	+	−
Hepatosplenomegaly	−	−	−	−	+	+	+	−	−	−
Gastrointestinal bleeding	−	−	−	−	−	+	−	−	+	−
Lethargy	−	−	−	−	−	+	−	−	−	+
Lymphadenopathy	+	+	−	−	+	+	+	−	−	+
Central nervous system symptoms	+	−	+	−	−	−	+	−	+	−
Cough	−	−	−	−	−	−	+	+	−	−
Site diagnosed clinically	LN, Lv	LN		Lu	LN	LN	LN, NpM, Spl		GI, skin	LN
Site(s) found at autopsy		Ln, Lv	Adr, Br, Bm, GI, Ht, Kd, Lv, Lu, Ms, Pn, Pthy, skin, Thy, Ts			Adr, GI, Kd, LN, Lv, Pn, Spl		Gb, GI, Kd, Lv, Lu, Pn	Bm, Br, GI, GU, Ht, LN, Lv, Lu, Pn, Pr, skin	LN
Histology	PBCL	PBCL	ML-IB	ML-IB	PBCL	PBCL	PBCL	PBCL	ML-IB	PBCL
Therapy	None	CTX	None	None	IFN, IVIg	VCR, DLI	VCR, Acy, DLI	None	DLI	DLI
Survival, d after diagnosis	9	22	0	2	8	5	1008	0	12	70
Cause of death	PT-LPD, MOF, sepsis	PT-LPD, bleeding	PT-LPD, fungal infection	PT-LPD, MOS, ARDS	PT-LPD, hepatic failure	PT-LPD, ARDS	Alive	PT-LPD, Asp	PT-LPD, MOF, IP	ARDS, Asp

PT-LPD = posttransplantation lymphoproliferative disorder; BMT = bone marrow transplantation; LN = lymph nodes; Lv = liver; Lu = lung; NpM = nasopharyngeal mass; Spl = spleen; GI = gastrointestinal tract; Adr = adrenal glands; Br = brain; Bm = bone marrow; Ht = heart; Kd = kidney; Ms = skeletal muscle; Pn = pancreas; Pthy = parathyroid gland; Thy = thyroid gland; Ts = testis; Gb = gallbladder; GU = genitourinary system; Pr = prostate gland; PBCL = polymorphic B-cell lymphoma; ML-IB = immunoblastic malignant lymphoma; CTX = cyclophosphamide; IFN = interferon; IVIg = intravenous immunoglobulin; VCR = vincristine; DLI = donor leukocyte infusion; Acy = acyclovir; MOF = multiorgan failure; ARDS = adult respiratory distress syndrome; Asp = aspergillosis infection; IP = interstitial pneumonitis.

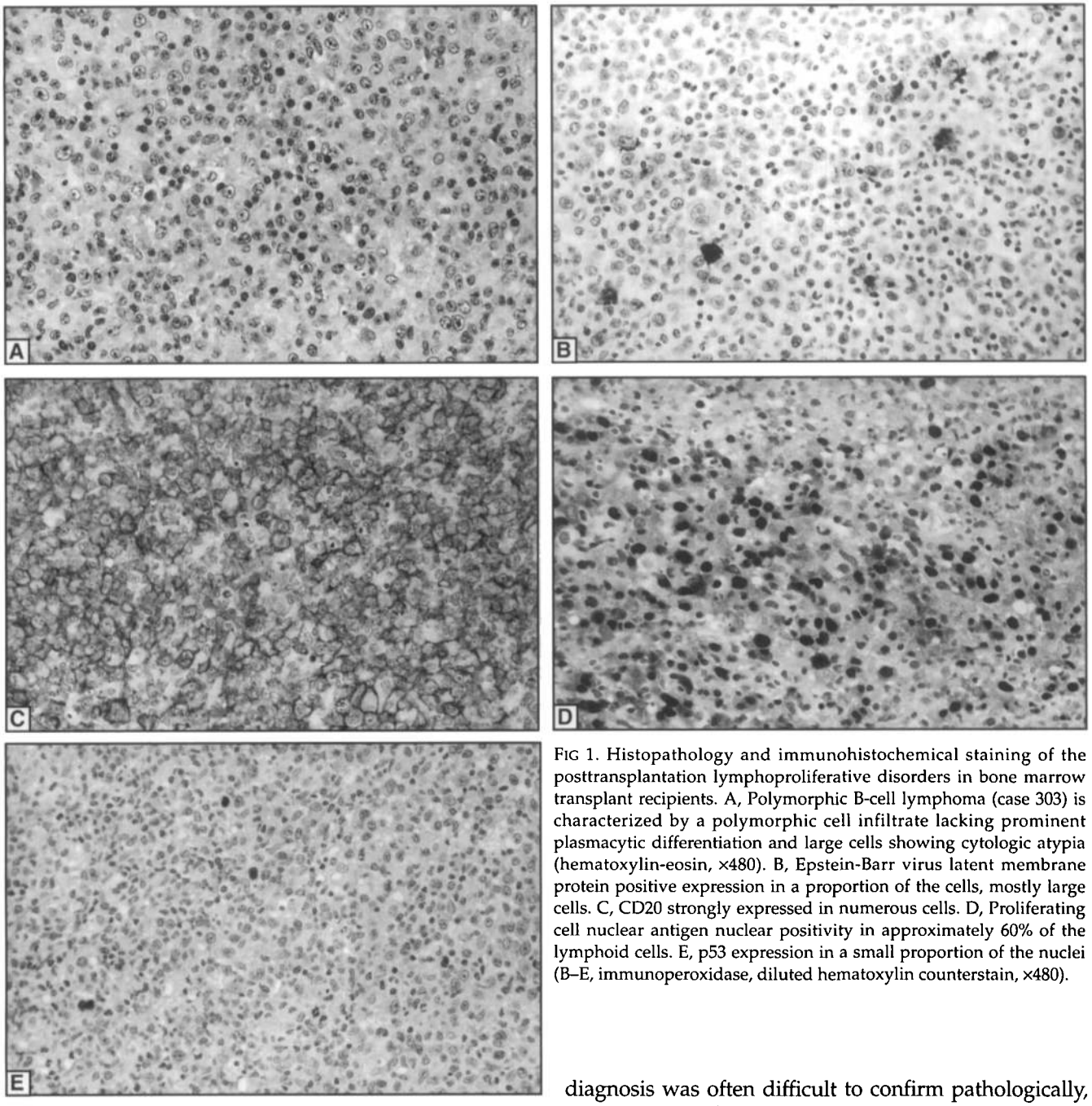

FIG 1. Histopathology and immunohistochemical staining of the posttransplantation lymphoproliferative disorders in bone marrow transplant recipients. A, Polymorphic B-cell lymphoma (case 303) is characterized by a polymorphic cell infiltrate lacking prominent plasmacytic differentiation and large cells showing cytologic atypia (hematoxylin-eosin, ×480). B, Epstein-Barr virus latent membrane protein positive expression in a proportion of the cells, mostly large cells. C, CD20 strongly expressed in numerous cells. D, Proliferating cell nuclear antigen nuclear positivity in approximately 60% of the lymphoid cells. E, p53 expression in a small proportion of the nuclei (B–E, immunoperoxidase, diluted hematoxylin counterstain, ×480).

The clinical course of patients in whom PT-LPD developed are listed in Table 3. Common presenting clinical features included fever (100%), lymphadenopathy (60%), rash (60%), central nervous system symptoms (40%), and hepatosplenomegaly (30%). Most patients had manifestations of a fever unresponsive to antibiotics and symptoms encompassing a variety of potential causes in this patient population. Even when clinical suspicion was high, the diagnosis was often difficult to confirm pathologically, and two cases of PT-LPD in our patients were diagnosed at autopsy. Interestingly, the three patients with ML-IB constituted three of four patients who did not have clinically or radiographically significant lymphadenopathy.

ML-IB was associated with diffuse internal organ involvement and presented the most difficulty in establishing a histologic diagnosis until late in the disease. Infusion of donor leukocytes, as described by Papadopoulos et al[19] and Hromas et al[20] proved the only successful treatment for patients with PT-LPDs, eliminating the disease in two of four patients.

Pathologic Findings

Biopsy sites and histologic characteristics are summarized in Table 4 and Figures 1 and 2. One patient (No. 229) underwent two consecutive biopsies. The histologic appearance of the original and the subsequent biopsy specimens was similar. Autopsy results available in five patients (three cases diagnosed while the patient was alive) confirmed the presence of widespread infiltration of extranodal sites, especially the liver and lung, as well as generalized involvement of lymph nodes. In patient 251 the postmortem histologic analysis revealed a polymorphic lymphoid infiltration

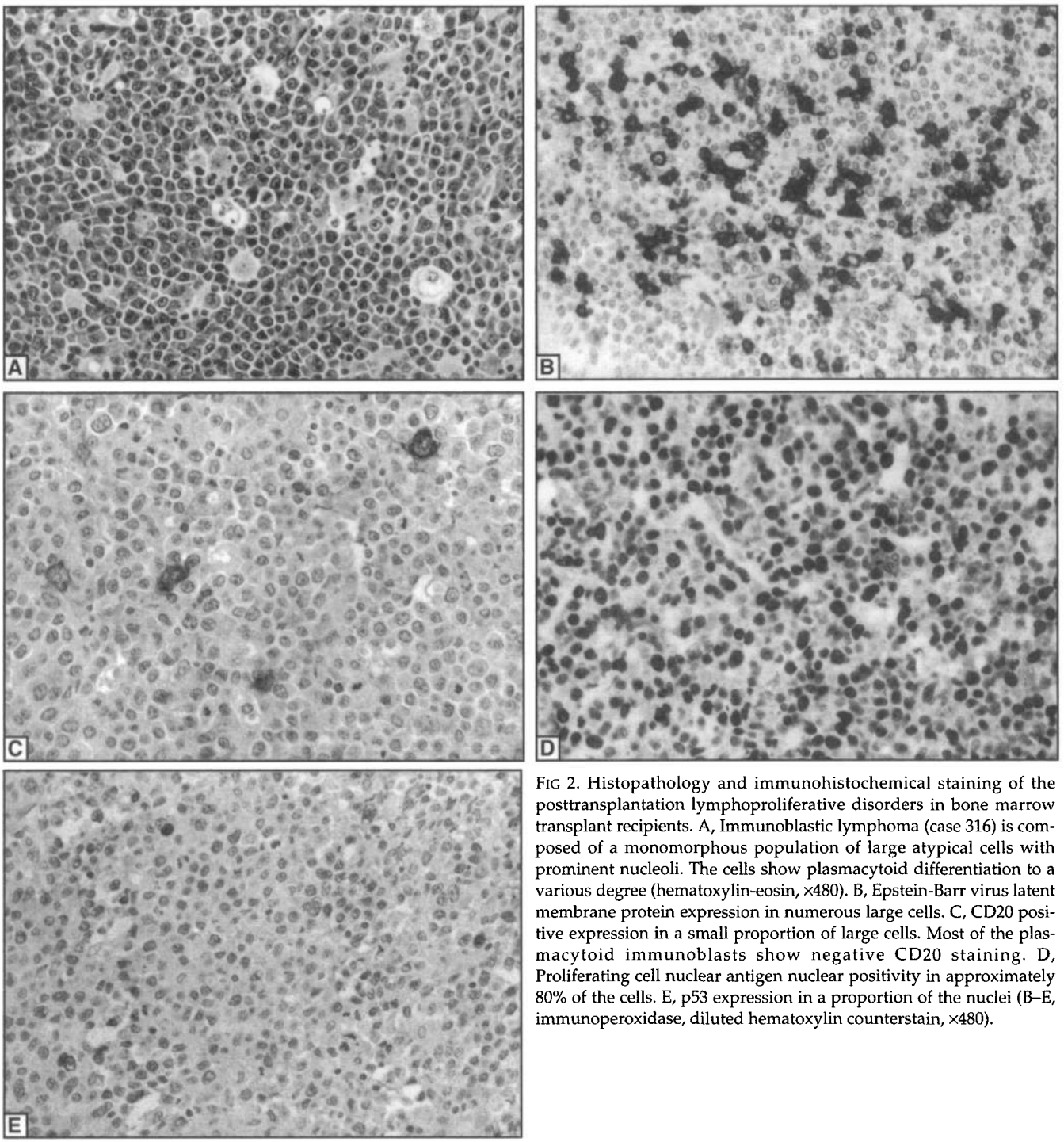

FIG 2. Histopathology and immunohistochemical staining of the posttransplantation lymphoproliferative disorders in bone marrow transplant recipients. A, Immunoblastic lymphoma (case 316) is composed of a monomorphous population of large atypical cells with prominent nucleoli. The cells show plasmacytoid differentiation to a various degree (hematoxylin-eosin, ×480). B, Epstein-Barr virus latent membrane protein expression in numerous large cells. C, CD20 positive expression in a small proportion of large cells. Most of the plasmacytoid immunoblasts show negative CD20 staining. D, Proliferating cell nuclear antigen nuclear positivity in approximately 80% of the cells. E, p53 expression in a proportion of the nuclei (B–E, immunoperoxidase, diluted hematoxylin counterstain, ×480).

consistent with PBCH, whereas the original skin biopsy specimen showed a morphologic appearance consistent with ML-IB.

B-cell clonality was determined by the demonstration of light chain class (κ or λ) restriction by immunohistochemistry, IgH gene rearrangement by PCR analysis, or both (Fig 3, A). Of seven specimens classified as PBCL, four were monoclonal by immunologic or genomic criteria or both. All three specimens classified as ML-IB were shown to be monoclonal, one by immunophenotypic analysis and IgH gene PCR, the other two cases only by the latter technique. In the patient 229 in whom biopsy specimens were obtained from two separate sites, similar results were observed in both specimens.

Immunohistologic analysis detected positive expression of the B-cell antigen CD20 in all PT-LPD cases (Fig 1, C and 2, C). In each case, a variable number of immunoblasts, large atypical cells, or both were positively stained with CD30. In eight of 10 cases, a small proportion of cells, mostly large, showed CD21 expression. CD45RO reactivity was confined to reactive small lymphocytes, with only rare large cells staining. PCNA immunostaining showed a high proliferative activity in all cases, with a mean PCNA value of 56% in PBCL (Fig 1, D) and 84% in ML-IB (Fig 2, D). The staining was particularly intense in the nuclei of large noncleaved cells and immunoblasts. Immunohistochemical analysis for p53 expression was positive in five of 10 specimens, two of seven PBCL (Fig 1, E) and three of three ML-IB (Fig 2, E). The percentage of cells showing p53 reactivity varied from 5% to 70% positivity. The p53-positive cells corresponded morphologically to the large strongly PCNA-positive cells previously described. Statistical analysis confirmed this finding by showing a positive correlation (r=.86) by linear regression analysis between the percentage of $p53^+$ and $PCNA^+$ cells. The not quite significant P value (.06) observed, can be easily explained on the basis of the limited number of cases analyzed.

EBV Detection

EBV-DNA sequences were detected by the PCR method in nine of 10 cases (Fig 3, B). Because PCR analysis for EBV provides no morphologic information about EBV harboring cells, sections were also immunostained with anti-EBV latent membrane protein (LMP) antibody.[21] LMP expression could be demonstrated in a variable proportion of the cells in every case (Fig 1, B and 2, B). Positive cells included large atypical cells, immunoblasts, and lymphoplasmacytoid lymphocytes.

Cytogenetics

Cytogenetic results were available for only four patients (Table 4). Two patients had normal karyotypes, and two showed clonal chromosomal abnormalities:

Patient 295: 46,XX,add(6)(p23)[10]/46,XX[15]/nonclonal(XX)[5]

Patient 339: 47,X,del(X)(q24),t(1;1)(p36.3;q21),+2,add(4)(q21), add(7)(p22),del(9)(q22q3?3),inv(12)(p13q24.1), add(15)(q26), ?t(16;16)(q13;q24)[2]/47,idem,del(6)(q2?1q2?3)[6]/46,XX[2].

TABLE 4. IMMUNOHISTOCHEMISTRY, MOLECULAR, AND CYTOGENETIC RESULTS IN 10 CASES OF PT-LPD AFTER BMT

Patient No.	*Histologic Class*	*PCNA, %*	*p53*	*IgK/L*	*PCR IgH*	*EBV LMP*	*PCR EBV*	*Cytogenetic Analysis*
229	PBCL	66	–	poly	–	+	+	nd
237	PBCL	40	–	poly	–	+	+	nd
251	ML-IB	85	+	poly	+	+	+	nd
264	ML-IB	88	+	poly	+	+	+	nd
271	PBCL	59	–	mono	+	+	+	46,XX
295	PBCL	70	+	mono	+	+	+	abn
303	PBCL	60	+	mono	–	+	+	46,XX
314	PBCL	ns	–	poly	–	+	+	nd
316	ML-IB	80	+	mono	+	+	–	nd
339	PBCL	42	+	mono	+	+	+	abn

PT-LPD = posttransplantation lymphoproliferative disorder; BMT = bone marrow transplantation; PCNA = proliferating cell nuclear antigen; IgK/L = immunoglobulin light chain κ on λ; poly = polyclonal; mono = monoclonal; PCR = polymerase chain reaction; IgH = immunoglobulin heavy chain; EBV = Epstein-Barr virus; LMP = latent membrane protein; PBCL = polymorphic B-cell lymphoma; nd = not done; ML-IB = malignant lymphoma immunoblastic; abn = abnormal karyotype; ns = no staining (inadequate staining).

DISCUSSION

In this study, we describe 10 patients in whom EBV-associated PT-LPDs developed after a TCD BMT. All but one of the 10 patients had received a marrow graft from an unrelated donor. Previous reports have indicated that unrelated and mismatched donors for BMT and TCD, as well as additional therapies to prevent or treat graft-vs-host disease, such as the use of high-dose ATG, anti–T cell monoclonal antibodies in vivo, and cyclosporine, are associated with an increased risk of developing PT-LPDs.[5–7,10] Our results confirm the low overall incidence of PT-LPDs in patients who have undergone BMT and the higher risk of PT-LPDs in cases with TCD and unrelated donors. Similar to other series of PT-LPD in BMT recipients, and in contrast with the majority of PT-LPDs in solid organ recipients, the disease arose in our patients at a very short interval (median 101 days) from the transplantation. It is interesting that this period corresponds closely with the interval of 3 to 6 months during which EBV-specific cytotoxic T lymphocytes are absent from the peripheral blood of BMT recipients.[22,23] As in previously published series, all our patients presented with disseminated involvement, and the mortality rate was high.

PT-LPDs are rarely karyotyped, and there are only a few cytogenetic studies in the literature.[7,10,13,24-26] Previously published cases have shown variable chromosomal abnormalities. One patient recently described by Waller et al[26] had break-point abnormalities at 1p36, 7p22, and 12q24 similar to our patient 339. Another patient (No. 295) in our study showed an add(6)(p23), an abnormality previously unreported in PT-LPDs. The significance of the abnormalities found in these two patients are unknown. However, the good therapeutic response observed in patient 339 despite the complex cytogenetic abnormalities argues against a prognostic value for cytogenetics in this group of disorders.

In the past, the effort to treat or prevent PT-LPDs in BMT recipients with conventional multiagent chemotherapy has been largely ineffective. According to the literature, reduction of therapeutic immunosuppression is the most reliable intervention in treating PT-LPDs after solid organ transplantation.[27–30] High-dose acyclovir has also been found effective in some cases,[23] but not in others.[5,7] Several other therapeutic approaches, including the use of interferon-α,[7,31] intravenous gamma globulins, and chemotherapy[32] have been proposed as more effective treatments. Our experience with these treatments, although limited, has been disappointing. Two of four patients treated with infusions of donor leukocytes[19,20] experienced complete remission; one patient, alive at more than 2 years, seems clinically cured. With this approach, donor leukocytes are infused to provide adoptive immunotherapy against the EBV.[19] Preliminary data have shown that this treatment may induce complete remission in EBV-associated PT-LPDs[19] as well as in overwhelming adenoviral infections in TCD BMT recipients.[20]

Pathologic Findings

Most PT-LPDs after solid organ transplantation have a polymorphic lymphoid cellular composition

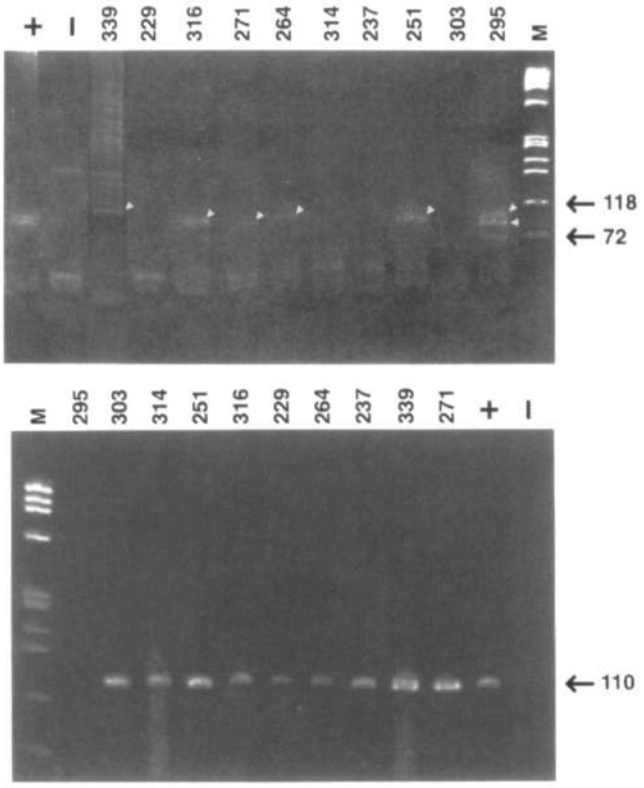

FIG 3. A, Polymerase chain reaction (PCR) amplification of V-D-J segments from 10 cases of posttransplantation lymphoproliferative disorders in bone marrow transplant recipients. The positive results (cases 251, 264, 271, 295, 316, and 339) are indicated (arrowheads) by one or two discrete bands between 72 and 118 base pairs (bp). Lane M contains marker DNA; lane (+) is DNA from a B-leukemia cell line, which acted as a positive control; and lane (–) is a negative control in which DNA had been omitted from the amplification reaction. B, PCR amplification for Epstein-Barr virus (EBV) DNA. The positive results (in nine of 10 cases) are indicated by the presence of a product band at 110 bp. Lane M contains marker DNA; lane (+) is DNA from a Raji EBV$^+$ cell line, which acted as a positive control; and lane (–) is a negative control in which DNA had been omitted from the amplification reaction.

that distinguishes them from the various types of malignant lymphoma that are observed in the immunologically competent patient. The most frequently used classification of PT-LPD is that of Frizzera et al[13] who proposed the separation of polymorphic PT-LPDs into the two histologic entities of PBCH and PBCL according to the degree of plasmacytic differentiation, the presence or absence of cytologic atypia, and the type and degree of necrosis. More recently, Knowles et al[9] proposed that PBCH and PBCL should be grouped into one category designated as polymorphic PT-LPD because of their extensively overlapping clinicopathologic characteristics. Reactive plasmacytic hyperplasia has also been considered an entity within the spectrum of PT-LPDs.[12,13] Rare cases resembling immunoblastic lymphoma have also been reported in several studies.[5–7,9,13]

While the morphology of the PT-LPDs occurring after solid organ transplantation has been extensively described,[12] few studies have described in any detail the histologic features of PT-LPDs in BMT recipients.[5–7] The results of these studies show that although a variable proportion of the cases of PT-LPD after BMT are histopathologically similar to the polymorphic PT-LPDs described in patients after solid organ transplantation, a relatively high proportion (25%–55%) of BMT-associated cases of PT-LPD exhibited the more aggressive histologic features of immunoblastic lymphoma.[5–7] In our series, PBCL and ML-IB accounted for 70% and 30% of cases, respectively. In addition, none of our PT-LPD cases exhibited the morphology of PBCH or plasmacytic hyperplasia, whereas these two less aggressive subtypes represent a sizable proportion of PT-LPD cases observed after solid organ transplantation.

As found in other studies of BMT-associated PT-LPDs, EBV association and B-cell derivation were demonstrated in all of our cases. B-cell clonality was investigated by applying a recently developed method for detecting rearrangements of the Ig heavy chain (IgH) gene based on DNA amplification using the PCR and consensus primers for the V and J regions of the IgH gene.[17] With this technique we uncovered IgH gene clonality in a proportion of cases previously classified as polyclonal on the basis of immunoperoxidase Ig light chain analysis. By PCR analysis, clonality was observed in all three cases classified as immunoblastic lymphoma and in three of seven polymorphic PT-LPDs. A morphologic diagnosis of ML-IB was therefore predictive of the monoclonality of the lesion, while polymorphic PT-LPDs seemed heterogeneous in this regard. The high number of IgH gene–negative results in our PBCL cases may be due to the relatively poor sensitivity of the PCR analysis in detecting B-cell rearrangement compared with the analysis by Southern blot hybridization.[33] Southern blot hybridization could not be performed in our cases because of the lack of suitable fresh frozen material.

In this study, we extended the characterization of the PT-LPD cases by analyzing their proliferative activity using PCNA immunohistochemistry. Several studies have shown a strong correlation between PCNA immunohistochemical analysis and other well-established techniques used to assess cell proliferation in tissue sections[15,34] and its value in evaluating the histologic grade of malignant lymphomas.[15] High proliferative activity was observed in most of our cases but with highest values in the ML-IB group. These results, although obtained in a small series, suggest that a high proliferative fraction is associated with the aggressive histologic subtype of immunoblastic lymphoma. A larger database is needed to assess the importance of this technique as an independent prognostic indicator.

In this study, we have also identified p53 overexpression in a significant proportion of BMT-associated PT-LPDs. Overexpression of the p53 gene can be demonstrated by immunohistochemistry in a wide range of human malignancies,[35,36] including high-grade lymphomas.[37] p53 protein detection by immunohistochemistry has been shown to be restricted to tissues in which the protein half-life is prolonged enough to allow sufficient material to be detected.[35,36] In most but not all cases, p53 overexpression is caused by a missense point mutation of the p53 gene, which results in the translation of a mutant p53 protein immunohistochemically detectable because of a prolonged half-life. Occurrence of p53 gene mutation, a common event in human cancers,[38–40] has also been demonstrated in cases of high-grade malignant lymphoma,[41–43] and, although infrequently, in cases of high-grade PT-LPDs.[9]

The association between p53 protein overexpression and gene mutation has led to the proposal that p53 detection may be synonymous with p53 mutation. Nevertheless, the findings of recent molecular studies, in which no mutation was found in several cases of high-grade malignant lymphoma,[44,45] have suggested that p53 accumulation in lymphoma may occur by a posttranslational mechanism independent of gene mutation. Because monoclonal antibody PAb1801, used in our study as well as other antibodies which can be used to detect p53 protein in fixed material, does not discriminate between mutant and wild-type

p53 protein, p53 staining in some of our cases may correspond to the accumulation of wild-type p53 protein, contrary to the popular notion about the meaning of p53 protein detection. Recent findings have suggested a role for p53 in cell cycle regulation with a high level of protein expression in highly proliferating cells.[44,46] Such high accumulation of the protein could therefore be viewed as a protective mechanism intended to halt the cell cycle of highly abnormal lymphoid cells and cause them to enter apoptosis.[47,48] Whatever the reason for p53 overexpression, positive staining was observed in all immunoblastic lymphomas and in two of the seven PBCLs included in the present series. A relation between growth fraction as assessed by PCNA immunohistochemistry and p53 expression was also noted in this series. Similar findings have been observed in high-grade malignant lymphoma[44] and have not been associated with polymorphic lymphoproliferative disorders observed in solid organ transplant recipients.

Our study of 10 PT-LPDs after BMT demonstrated a high frequency of high-grade histologic subtypes, including immunoblastic lymphoma, frequent clonality, high proliferative activity, and frequent overexpression of p53 gene product. Similar findings are observed in a minority of cases of PT-LPD occurring after solid organ transplantation and may account for the more aggressive clinical behavior observed in the subgroup of patients with PT-LPD after BMT. The complete remission observed after donor leukocyte infusion in two of our four patients raises the possibility that this type of adoptive immunotherapy may represent the most effective treatment available for this type of disorder.

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