

Histogenetic Phenotypes of B Cells in Posttransplant Lymphoproliferative Disorders by Immunohistochemical Analysis Correlate With Transplant Type

Solid Organ vs Hematopoietic Stem Cell Transplantation

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Abstract

We immunohistochemically defined the histogenesis of posttransplantation lymphoproliferative disorders (PTLDs; B-cell phenotype) occurring after allogeneic T cell–depleted hematopoietic stem cell transplantation (HSCT; n = 15) or solid organ transplantation (SOT; n = 11) to determine whether transplantation type or morphologic subtype of PTLD affected the histogenetic subtype. Immunohistochemical stains using histogenetic markers for germinal center (GC) B cells, late GC and post-GC B cells, and post-GC B cells were performed on paraffin-embedded samples. Morphologically, 14 cases were polymorphic; 12 were monomorphic. Histogenetic marker expression was as follows: 1 monomorphic case (4%), GC phenotype expressing bcl-6 and CD10; 17 cases (65%; polymorphic, 9; monomorphic, 8), late GC–early post-GC phenotype expressing MUM1/IRF4; 8 cases (31%; polymorphic, 5; monomorphic, 3), post-GC phenotype expressing MUM1/IRF4 and CD138 but not bcl-6. PTLD cases after HSCT more frequently were post-GC phenotype than after SOT (7/15 vs 1/11, respectively; P = .040) and were independent of morphologic subclassification. Results suggest that most PTLDs are late GC–early post-GC phenotype with a minor group of post-GC phenotype and rare cases of GC phenotype. Findings also suggest a correlation between histogenetic phenotype of B-cell PTLD and type of transplantation.

B-cell posttransplantation lymphoproliferative disorder (PTLD) represents a complex group of lymphoproliferative disorders covering a spectrum from Epstein-Barr virus (EBV)-driven polyclonal proliferations resembling infectious mononucleosis to monomorphic proliferations that are indistinguishable morphologically from diffuse large B-cell lymphoma. The World Health Organization (WHO) classification divides PTLDs into 4 major categories: (1) early (reactive) lesions, (2) polymorphic PTLD, (3) monomorphic PTLD, and (4) Hodgkin lymphoma or Hodgkin lymphoma–like PTLD.¹

The majority of PTLD cases are related to the significant immune suppression secondary to regimens used to prevent or treat graft rejection (solid organ transplantation [SOT]) or graft-vs-host disease (hematopoietic stem cell transplantation [HSCT]) that allows unchecked EBV-driven lymphoproliferation.¹⁻⁶ This relationship is supported further by the observation that patients receiving T cell–depleted HSCTs have a significantly higher risk of developing PTLD than recipients of T cell–replete HSCTs.⁷ Similarly, patients who receive stronger immunosuppressive regimens after SOT, such as after heart-lung transplantation, are at higher risk for PTLD than those receiving less aggressive immunosuppressive regimens.⁸⁻¹⁰

Although PTLDs after SOT (PTLD-SOT) or HSCT (PTLD-HSCT) are indistinguishable morphologically, significant differences in clinical course, particularly prognosis, are observed.⁷⁻¹⁵ For example, the interval for development of PTLD is much shorter in HSCT recipients than in SOT recipients, with most PTLD-HSCT cases appearing during the first 6 months after transplantation, although PTLD-SOT might arise months to years after transplantation.^{7,10,11} The lymphoreticular system (lymph nodes, liver, and spleen) frequently is involved in patients with PTLD-HSCT, whereas PTLD-SOT

most commonly is extranodal and frequently involves the allograft.^{8,12} PTLD-HSCT usually is manifested as disseminated disease with early and rapid organ dysfunction resulting in a fulminant clinical course with a high mortality rate, whereas PTLD-SOT varies from localized to disseminated disease.¹³ The 5-year survival in PTLD-HSCT is dismal in most series (8% to 12.5%),^{7-9,13} although studies have shown that survival may be improved by anti-B-cell monoclonal antibody treatment.^{12,14} Reduction of immunosuppression is the first step in treating PTLD-SOT, with complete responses in 31% to 83% of cases. This approach is ineffective in PTLD-HSCT.^{15,16}

The mechanism leading to the clinical differences between PTLD-HSCT and PTLD-SOT is not well understood. It has been hypothesized that PTLDs following SOT vs HSCT are different biologically. It has been shown that PTLD-SOT is predominantly of host origin; in contrast, PTLD-HSCT usually is of donor origin.¹⁷⁻¹⁹ It may be further hypothesized that the B cell of origin (ie, germinal center [GC] vs post-GC) also might differ between these entities, leading to differences in clinical behavior and outcome as observed in diffuse large B-cell lymphoma (DLBCL). By using complementary DNA (cDNA) microarray technology, it has been shown that patients with DLBCL with neoplastic cells of GC B-cell origin have a better prognosis than those with neoplastic cells of non-GC B-cell origin.^{20,21} The results of cDNA microarray studies recently have been validated further with immunohistochemical analysis by using GC-B-cell markers and activation markers.^{22,23}

The histogenesis of PTLD has not been well studied, despite the recent development of well-defined immunohistochemical markers that correlate with histogenesis of B cells, including CD10, bcl-6, MUM1/IRF4, and CD138.²⁴⁻⁴¹ CD10 and bcl-6 are well known markers for GC B cells and are expressed highly in follicular lymphomas of GC B-cell origin. MUM1/IRF4 expression begins only at the centrocyte (late GC B-cell) stage. This suggests that MUM1/IRF4 expression by GC B cells occurs in a final step of B-cell differentiation in the GC. When B cells exit the GC, they maintain their MUM1/IRF4 expression. As an additional step toward differentiation into plasma cells and memory cells, they acquire expression of CD138.³¹⁻³⁷

The purpose of the present study was to determine whether the histogenetic phenotypes of PTLD-SOT and PTLD-HSCT could be defined using the aforementioned histogenetic markers by immunohistochemical analysis of paraffin-embedded tissue sections and to correlate histogenetic phenotypes with the current WHO classification of PTLD.

Materials and Methods

Cases

We retrospectively studied 26 cases of B-cell PTLD (15 after T cell-depleted allogeneic HSCT; 11 after SOT) in material

from the Department of Pathology, Medical College of Wisconsin, Milwaukee, archived from September 1994 to July 2002. T-cell depletion was performed using the protocol reported previously.⁴² Cases of T-cell and Hodgkin PTLD were excluded. The morphologic diagnosis of PTLD was reviewed and confirmed. The cases were classified further as polymorphic or monomorphic according to the WHO classification.¹

Immunohistochemical Analysis

Immunohistochemical stains were performed on formalin-fixed, paraffin-embedded tissue samples with the method described previously.^{43,44} Briefly, paraffin sections were mounted on Fisherbrand/Plus Superfrost Precleaned slides (Cardinal Health, McGaw Park, IL), heated at 60°C for 30 minutes, deparaffinized, and rehydrated. The stains for EBV latent membrane protein (LMP; clone CS1-4, DAKO, Carpinteria, CA), CD20 (clone L26, DAKO), and histogenetic markers, including CD10 (clone 56C6, Novocastra, Newcastle upon Tyne, England), bcl-6 (polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA), MUM1/IRF4 (polyclonal, Santa Cruz Biotechnology), and CD138 (clone MI-15, DAKO), were performed with antigen-retrieval techniques. The CD20 (L-26) stain was performed using the section consecutive to that stained for histogenetic markers to help in evaluating the expression of the histogenetic markers in B cells.

Nonspecific protein binding sites were blocked by serum for 5 minutes, and the primary antibody was added at different dilutions (CD138, 1:30; MUM1/IRF4, 1:200; bcl-6, 1:300; CD10, 1:50; EBV-LMP, 1:50; CD20, 1:400). All slides were stained using an automated immunostainer (DAKO). The slides were incubated for 15 minutes in labeled streptavidin-biotin for linking and labeled subsequently with diaminobenzidine chromogen. Appropriate positive and negative control samples were used in all cases.

In Situ Hybridization for EBV-Encoded RNA

Twelve cases (EBV-LMP+, 3; EBV-LMP-, 9) were studied by in situ hybridization for EBV-encoded RNA (EBER). This test was performed at the Mayo Clinic Reference Laboratory, Rochester, MN, and the Department of Pathology, University of Utah, Salt Lake City, using established methods. Briefly, the test was performed with the automatic Ventana Benchmark (Ventana Medical Systems, Tucson, AZ) and the Inform EBER probe (Ventana Medical Systems) with an iView blue counterstain (Ventana Medical Systems). Tissues were cut onto silene-coated slides and loaded using the in situ hybridization module.

Immunoglobulin Heavy Chain Gene Rearrangement

The immunoglobulin heavy chain gene rearrangement study was performed in 19 cases to evaluate clonality. DNA was extracted from the tissue blocks used for immunohistochemical

stains as described. Polymerase chain reaction was performed using the *IgH* Gene Rearrangement/B-Cell Clonality Assay (Invivoscribe Technology, San Diego, CA) using ABI fluorescence detection by the ABI Prism 377 DNA sequencer (ABI, Foster City, CA).

Interpretation of Expression of Histogenetic Markers

Expression of CD10, bcl-6, MUM1/IRF4, and CD138 was reviewed systematically in a semiquantitative manner independently by 2 pathologists (L.N.-T. and C.-C.C.) without the knowledge of transplantation type or clinical outcome of the patients. Each case was evaluated to estimate the percentages of CD20+ B cells that stained positively for CD10, bcl-6, MUM1/IRF4, and CD138. Samples were stratified semiquantitatively into 4 groups: negative, fewer than 20% of B cells positive; +, 20% to 50% of B cells positive; ++, 51% to 80% of B cells positive; and +++, more than 80% of B cells positive. This stratification provided the best intraobserver consistency and interobserver agreement. In only 2 cases was the semiquantitative stratification into the 4 groups different between the 2 observers. These 2 cases were rereviewed, and a consensus was reached for these cases. Mature plasma cells positive for CD138 and MUM1/IRF4 were not included in the count because the expression of these markers normally is acquired during the differentiation toward plasma cells. The plasma cells further served as positive internal control cells for MUM1/IRF4 and CD138.

Results

The clinical and pathologic data for the cases are summarized in **Table 1** and **Table 2**. The vast majority of PTLD cases were EBV-related (24/26 [92%] were LMP1 and/or

EBER positive; Table 2). During the study period (September 1994 to July 2002), 3.6% of adults and 4.5% of children who underwent T cell-depleted allogeneic HSCT developed PTLD. The interval between transplantation and development of PTLD was significantly shorter in the HSCT population (mean ± SD, 6.4 ± 13.5 months in HSCT recipients vs 53.3 ± 51.0 months in SOT recipients; *P* = .002; Student *t* test).

Morphologically, 14 cases were polymorphic PTLD according to the WHO classification (Table 2). These cases showed destructive polymorphic lymphocytic infiltrates composed of small to intermediate-sized lymphocytes, large transformed lymphocytes, immunoblasts, and plasma cells **Image 1A**. The remaining 12 cases were monomorphic PTLD, 1 Burkitt-like and 11 DLBCL cases. The case of Burkitt-like PTLD revealed a diffuse lymphocytic infiltrate with a “starry-sky” pattern. The neoplastic cells were monotonous medium-sized lymphocytes with round nuclei, multiple nucleoli, and scant to moderate basophilic cytoplasm **Image 1B**. The neoplastic cells expressed CD10 and MIB-1 (>95%) and were negative for bcl-2. The 11 cases of DLBCL showed a monotonous proliferation of large atypical lymphocytes with morphologic characteristics of predominantly centroblasts **Image 1C** or immunoblasts **Image 1D**. Areas of necrosis were present in all polymorphic and monomorphic cases **Image 1E**.

Evaluation of the B-cell staining pattern for each histogenetic marker and the resulting histogenetic phenotype in each case is summarized in Table 2. Only 1 case (4%) showed nuclear bcl-6 expression (++) **Image 1F** and membranous CD10 expression (+++). Of the 26 cases, 24 (92%) expressed nuclear (strong) and cytoplasmic (weak) staining of MUM1/IRF4 (++ in 12 cases and +++ in the remaining 12 cases) **Image 1G**. CD138 was expressed in a membranous (strong) and cytoplasmic (weak) manner in 8 cases (31%; + in

Table 1
Clinical Information for Patients With Posttransplantation Lymphoproliferative Disorder After Hematopoietic Stem Cell or Solid Organ Transplantation

	Type of Transplantation	
	Hematopoietic Stem Cell (n = 15)*	Solid Organ (n = 11)†
Sex (M/F)	10/5	5/6
Median age (y)	Adults, 43; children, 12	32
Type of transplant‡	NRPM, 6; RPM, 2; Hap, 3; RM, 4	Kidney, 8; liver, 2; heart, 1
Diagnosis‡	AML, 4; ALL, 3; CML, 5; MDS, 1; NHL, 1; AA, 1	Renal transplantation: HTN, 2; DM, 2; MPGN, 1; Alport syndrome, 1; HUS, 1; unknown, 1 Liver: autoimmune hepatitis, 1; cryptogenic cirrhosis, 1 Heart: CHF, 1
Conditioning regimen	Cyclophosphamide, cytarabine, TBI, steroids	—

AA, aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CHF, congestive heart failure; CML, chronic myeloid leukemia; DM, diabetes mellitus; Hap, haploidentical; HTN, hypertension; HUS, hemolytic-uremic syndrome; MDS, myelodysplastic syndrome; MPGN, membranoproliferative glomerulonephritis; NHL, non-Hodgkin lymphoma; NRPM, nonrelated partial match; RM, related matched; RPM, related partial match; TBI, total body irradiation.

* Includes 8 adult and 7 pediatric cases. The histogenetic phenotypes and clinical outcomes of the pediatric cases have been studied and reported by Abed N, Casper JT, Camitta BM, et al. Evaluation of histogenesis of B-lymphocytes in pediatric EBV-related post-transplant lymphoproliferative disorders. *Bone Marrow Transplant*. 2004;33:321-327.

† All adult patients.

‡ Data are given as number of patients.

Table 2
Morphologic Findings, EBV Status, Histogenetic Marker Expression, and Clinical Outcome in HSCT and Solid Organ Transplantation Recipients

Case No.	Biopsy Site	Morphologic Findings	LMP	EBER	Clonality	Histogenetic Phenotype	CD10	bcl-6	MUM1/IRF4	CD138	PTLD Interval (mo)	Treatment for PTLD	Outcome After PTLD
HSCT Recipients													
Adults													
1	Node	PM	+	+	Clonal	Late GC–early post-GC	–	–	+++	–	2	Rituximab	Died, PTLD, <i>Aspergillus</i> infection, 1 mo
2	Node	Mono, CB	+	ND	Clonal	Late GC–early post-GC	–	–	+++	–	2	Chemo; DLI	AWOD, 18 mo
3	GI	PM	–	+	ND	Late GC–early post-GC	–	–	++	–	2	Rituximab	AWOD, 5 mo
4	Lung	Mono, IB	–	+	No amplifiable DNA	Post-GC	–	–	++	+++	5	Surgery	Died, ANLL/GVHD, 2 mo
5	Node	Mono, IB	+	+	Clonal	Post-GC	–	–	++	+++	6	DLI	AWOD, 73 mo
6	Lung	PM	–	+	Clonal	Post-GC	–	–	+++	++	55	DC CSA	Died, PTLD/GVHD, 3 mo
7	Node	PM	+	ND	Clonal	Post-GC	–	–	+++	+++	2	Rituximab, DLI	Died, PTLD/bacteremia, 4 mo
8	Node	PM	+	+	No amplifiable DNA	Post-GC	–	–	–	+++	5	DC CSA	Died, <i>Aspergillus</i> infection, 5 mo
Children*													
1	Lung	PM	+	ND	ND	Late GC–early post-GC	–	–	++	–	3.2	Rituximab, DLI	Died, PTLD/ <i>Pseudomonas</i> infection, 3 mo
2	Liver	PM	+	ND	Clonal	Late GC–early post-GC	–	–	++	–	1.6	DC CSA	Died, PTLD/ <i>Aspergillus</i> infection, 4 mo
3	Tonsil	PM	+	ND	Clonal	Late GC–early post-GC	–	–	++	–	2.4	DC CSA	AWOD, 80 mo
4	Tonsil	Mono, CB	–	+	Clonal	Late GC–early post-GC	–	–	+++	–	3.1	DC CSA	Died, PTLD, 9 mo
5	Tonsil	Mono, IB	+	ND	Clonal	Late GC–early post-GC	–	–	++	–	2.8	DC CSA	AWOD, 93 mo
6	Node	Mono, IB	+	ND	Polyclonal	Post-GC	–	–	+++	+	1.7	Rituximab	Died, <i>Aspergillus</i> infection, 17 d
7	Node	PM	+	ND	Clonal	Post-GC	–	–	++	++	2.5	DC CSA	AWOD, 121 mo
Solid organ recipients													
1	GI	Mono, Burkitt	–	+	Clonal	GC	+++	++	–	–	72	Chemo	Died, liver failure, 3 mo
2	GU	Mono, CB	–	–	Clonal	Late GC–early post-GC	–	–	+++	–	132	Cyclophosphamide	Died, PTLD, 2mo
3	Brain	Mono, CB	–	+	Clonal	Late GC–early post-GC	–	–	++	–	10	Unknown	Died, RF, 24 mo
4	Tonsil	PM	+	ND	Polyclonal	Late GC–early post-GC	–	–	+++	–	0.7	Rituximab	AWOD, 14 mo
5	Tonsil	Mono, IB	–	+	No amplifiable DNA	Late GC–early post-GC	–	–	+++	–	72	Unknown	Died, MI, 1 mo
6	Node	Mono, CB	+	ND	ND	Late GC–early post-GC	–	–	++	–	3	Rituximab	AWOD, 10 mo
7	Lung	PM	+	ND	Clonal	Late GC–early post-GC	–	–	+++	–	28	Rituximab	AWOD, 15 mo
8	Node	PM	+	ND	ND	Late GC–early post-GC	–	–	+++	–	17	Rituximab	AWOD, 13 mo
9	GI	Mono, CB	–	ND	ND	Late GC–early post-GC	–	–	+++	–	144	None	Died, PTLD, 1 mo
10	GI	PM	–	+	ND	Late GC–early post-GC	–	–	++	–	24	Rituximab	Alive with PTLD, 8 mo
11	Spleen	PM	+	ND	ND	Post-GC	–	–	++	++	84	Rituximab	AWOD, 8 mo

ANLL, acute nonlymphocytic leukemia; AWOD, alive without disease; CB, centroblastic variant; Chemo, multidrug chemotherapy; CSA, cyclosporin; DC, discontinue; DLI, donor lymphocyte infusion; EBER, EBV-encoded RNA; EBV, Epstein-Barr virus; GC, germinal center; GI, gastrointestinal; GU, genitourinary; GVHD, graft-vs-host disease; HSCT, hematopoietic stem cell transplantation; IB, immunoblastic variant; LMP, EBV-latent membrane protein; MI, myocardial infarction; Mono, monomorphic; ND, not done; PM, polymorphic; PTLD, posttransplantation lymphoproliferative disorder; RF, renal failure; +, 20%-50% of B cells positive; ++, 51%-80% of B cells positive; +++, >80% of B cells positive; –, negative (fewer than 20% of B cells positive).

* The histogenetic phenotypes and clinical outcomes of the pediatric cases have been studied and reported by Abed N, Casper JT, Camitta BM, et al. Evaluation of histogenesis of B-lymphocytes in pediatric EBV-related post-transplant lymphoproliferative disorders. *Bone Marrow Transplant*. 2004;33:321-327.

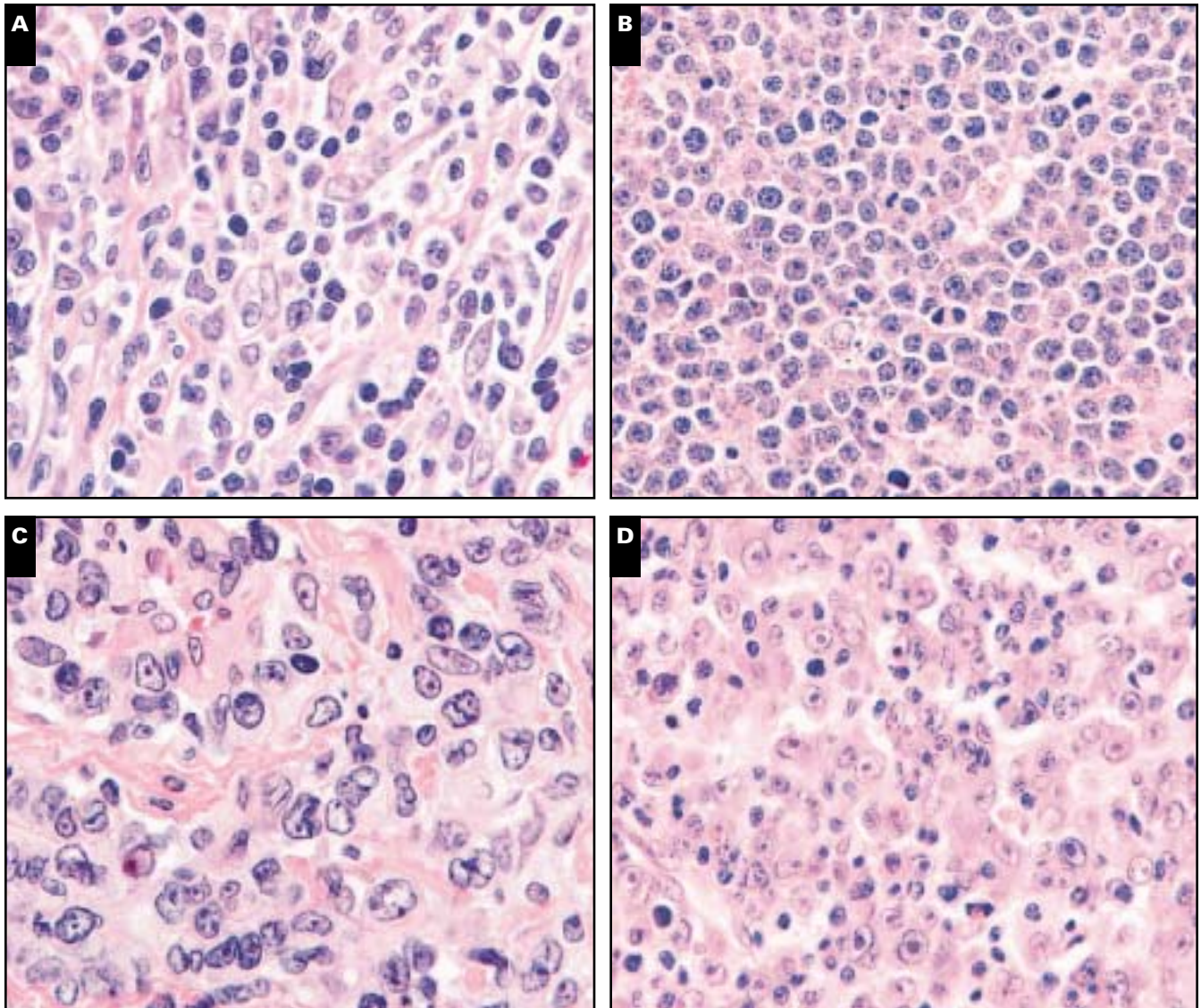


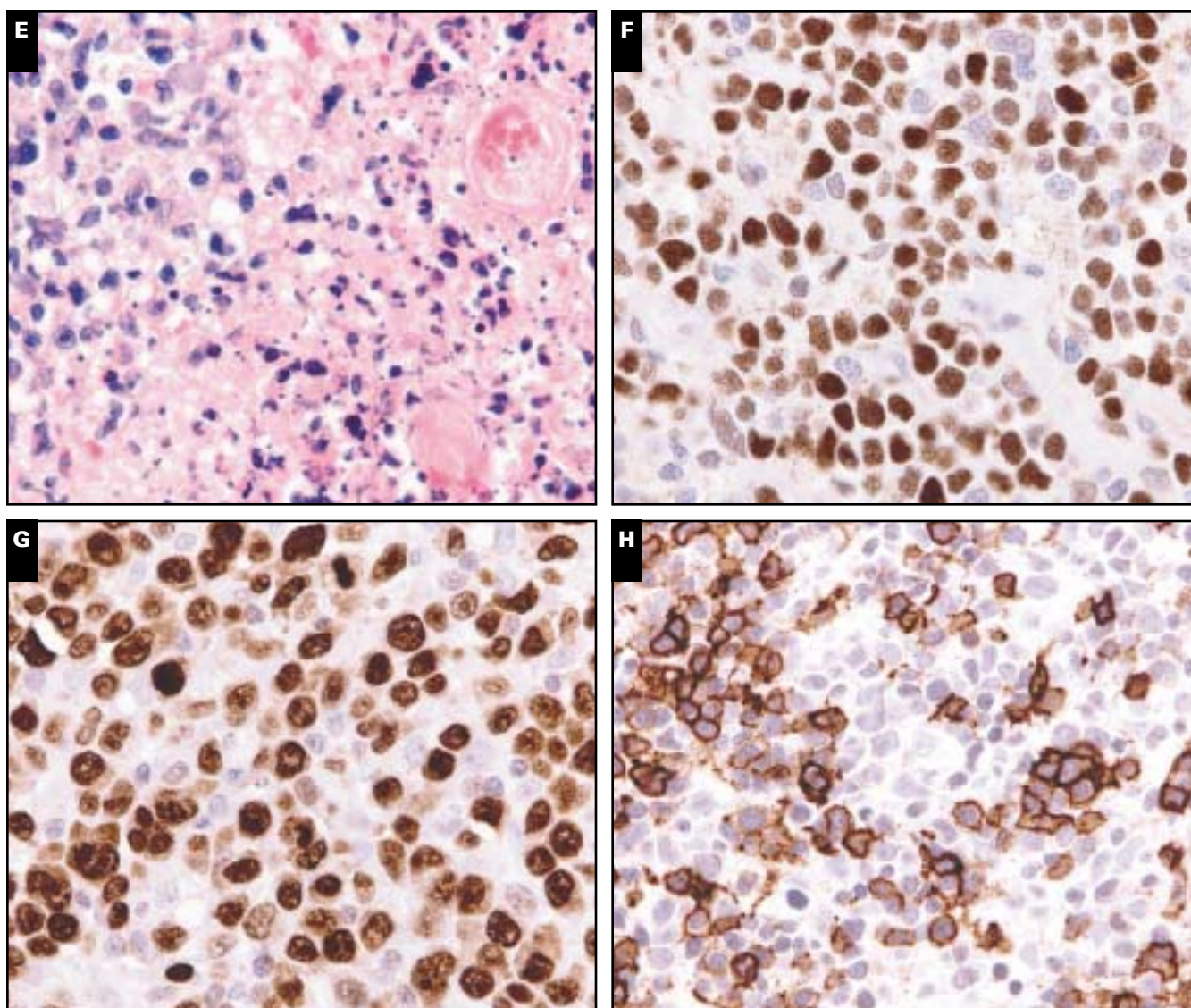
Image 1 Morphologic variants and expression of each marker in posttransplantation lymphoproliferative disorder (PTLD). **A**, Polymorphic PTLD (H&E, $\times 400$). **B**, Monomorphic PTLD, Burkitt-like lymphoma (H&E, $\times 400$). **C**, Monomorphic PTLD, diffuse large B-cell lymphoma (DLBCL), centroblastic variant (H&E, $\times 400$). **D**, Monomorphic PTLD, DLBCL, immunoblastic variant (H&E, $\times 400$).

1 case, ++ in 3 cases, and +++ in 4 cases) **Image 1H**. Further analysis of the expression profile of these histogenetic markers showed that 17 cases (65%) had a late GC–early post-GC phenotype (defined by expression of only MUM1/IRF4), 8 (31%) had a post-GC phenotype (defined by expression of MUM1/IRF4 and CD138 but not bcl-6 and CD10), and 1 (4%) had a GC phenotype (defined by expression of bcl-6 and CD10).

The histogenetic phenotypes of B lymphocytes in the PTLD did not correlate with the clonality of the lesion (Table 2) or with the WHO classification (polymorphic vs monomorphic) **Table 3**. The percentage of monoclonality in cases of late GC–early post-GC B-cell phenotype was similar to that in cases of post-GC B-cell phenotype (9/10

[90%] vs 4/5 [80%], respectively). Of 17 cases of late GC–early post-GC B-cell phenotype (expressing only MUM1/IRF4), 9 were polymorphic PTLD, and the remaining 8 were monomorphic PTLD. Similarly, 5 of 8 cases of post-GC B-cell phenotype (expressing MUM1/IRF4 and CD138) were polymorphic PTLD, and the remaining 3 cases were monomorphic PTLD. Of note, among the monomorphic group, the centroblastic variant was more frequently of the late GC–early post GC phenotype than the immunoblastic variant (6/6 vs 2/5, respectively; $P < .03$; χ^2 ; Table 3), which was more likely to be of the post-GC phenotype.

The type of transplantation did seem to correlate with the histogenetic subtype. Cases of PTLD-HSCT were more



E, Necrosis is a common finding in PTLD (present in all cases studied) (H&E, $\times 400$). **F**, Expression of bcl-6 showing nuclear staining pattern in the case illustrated in **B** (immunohistochemical stain, $\times 400$). **G**, Expression of MUM1/IRF4 showing a nuclear-cytoplasmic staining pattern in a case with PTLD, monomorphic, DLBCL, centroblastic variant (immunohistochemical stain, $\times 400$). **H**, Expression of CD138 showing a cytoplasmic-membranous staining pattern in a case of PTLD, monomorphic, DLBCL, immunoblastic variant (immunohistochemical stain, $\times 400$).

frequently of the post-GC phenotype than those associated with PTLD-SOT (7/15 vs 1/11, respectively; $P = .040$; χ^2 ; Table 3). In contrast, the morphologic WHO classification (polymorphic vs monomorphic) did not correlate with the type of transplantation (9 polymorphic and 6 monomorphic in HSCT vs 5 polymorphic and 6 monomorphic in SOT; $P = .540$; χ^2 ; Table 2). However, there was no significant correlation between the histogenetic phenotypes and the overall survival or disease-free survival among the cases of PTLD-HSCT. Similarly, we did not observe significant correlation between the histogenetic phenotypes and site of involvement (nodal vs extranodal; Table 2).

Discussion

By using a combination of specific immunophenotypic markers, including CD10, bcl-6, MUM1/IRF4, and CD138, that are associated with B-cell differentiation, we have shown that B-cell lineage PTLD can be classified into 3 histogenetic groups. The major type of PTLD (17/26 [65%]) is of the late GC–early post-GC phenotype expressing MUM1/IRF4 but not bcl-6, CD10, or CD138, with a second group (8/26 [31%]) of the post-GC phenotype expressing MUM1/IRF4 and CD138 but not bcl-6 or CD10. A third group (1/26 [4%]) was of the GC phenotype, expressing bcl-6 and CD10, and was

Table 3
Histogenetic Phenotype vs Morphologic Features and Transplantation Type

Histogenetic Phenotype	Polymorphic	Monomorphic	Transplantation Type	
			Hematopoietic Stem Cell (n = 15)	Solid Organ (n = 11)
GC (CD10+, bcl-6+, MUM1/IRF4-, CD138-)	0	1	0	1
Late GC-early post-GC (CD10-, bcl-6-, MUM1/IRF4+, CD138-)	9	8 (IB, 2; CB, 6)	8	9
Post-GC (CD10-, bcl-6-, MUM1/IRF4+, CD138+)	5	3 (IB, 3)	7	1

CB, centroblastic variant; GC, germinal center; IB, immunoblastic variant.

encountered very infrequently. In addition, our results suggest that the histogenesis of B cells in PTLD lesions differs between cases following SOT and those following HSCT. SOT is highly associated with late GC-early post-GC derivation and very infrequently expresses a post-GC phenotype (only 1 of 11 cases studied).

Our results suggest that the histogenesis of PTLD is comparable to that of HIV-associated lymphoma.⁴¹ This is not unexpected when considering that both entities are associated with immunosuppression and immunodeficiency. Carbone et al⁴¹ reported that expression of bcl-6, MUM1/IRF4, and CD138 segregates HIV-associated lymphomas into 3 major histogenetic patterns with the following profiles: (1) bcl-6+/MUM1/IRF4-/CD138- pattern, (2) bcl-6-/MUM1/IRF4+/CD138- pattern, and (3) bcl-6-/MUM1/IRF4+/CD138+ pattern, corresponding to the GC, late GC-early post-GC, and post-GC B-cell phenotypes, respectively. All 3 patterns also were observed in the PTLD cases in the present study. However, the most common pattern in our study was the late GC-early post-GC phenotype. This is in contrast with HIV- and AIDS-associated lymphoma, in which this pattern is rare and the GC pattern and post-GC pattern predominate.⁴¹

Furthermore, our findings are in partial agreement with a recent study by Paessler et al.⁴⁵ They reported that B-cell PTLD could be divided into 2 broad categories corresponding to the early (CD10-/bcl-6-/SHP-1+/CD138-) and late (CD10-/bcl-6-/SHP-1+/CD138+) post-GC B-cell origin. The former was observed in about 60% of cases and the latter in about 40% of cases. It would be of great interest to study the MUM1/IRF4 expression in the former group to determine whether any of these cases might correspond to the late GC-early post-GC origin defined in the present study. Our findings, however, disagree somewhat with the findings of Capello et al.⁴⁶ They reported that their PTLD cases were composed of GC (22.7%), late GC-early post-GC (41%), and post-GC (30%) phenotypes. The reason for this discrepancy is not completely certain. We note that the study by Capello et al⁴⁶ included a significant portion (40%) of EBV- PTLD cases that might arise owing to a different pathophysiologic mechanism from EBV-driven lymphoproliferative disorders. Furthermore, the details defining the expression of each histogenetic marker

tested were not reported by Capello et al,⁴⁶ making comparisons between the 2 studies difficult.

Our finding that a significant difference exists in histogenetic phenotypes between PTLD-HSCT and PTLD-SOT provides additional evidence to support the notion that the biology of PTLD-HSCT and PTLD-SOT is different. PTLD-SOT is rarely of the post-GC B-cell phenotype; in contrast, the PTLD-HSCT is more heterogeneous in histogenetic phenotypes (8/15 [53%] with the late GC-early post-GC B-cell phenotype and 7/15 [47%] with the post-GC B-cell phenotype). This relationship has not been reported previously. Recent studies using cDNA microarray and immunohistochemical analysis have shown that in DLBCL cases, patients with a non-GC B-cell genetic expression pattern have a significantly worse prognosis than those with a GC B-cell genetic expression profile.²⁰⁻²³ However, in this limited series, we were not able to demonstrate a definite association between prognosis and histogenetic phenotype among the cases of PTLD-HSCT. Further studies are warranted to determine whether the histogenetic phenotypes of PTLD influence prognosis. In addition, all of the allogeneic recipients in our study received T cell-depleted HSCTs. Further investigation is required to determine whether PTLDs in recipients of T cell-replete HSCT show a similar pattern of histogenetic distribution.

We were able to determine the histogenetic subtype of PTLD using immunohistochemical analysis with a panel of specific histogenetic markers on paraffin-embedded tissue samples. Furthermore, our results demonstrated that there was no correlation between the histogenetic phenotype and the morphologic subtype of PTLD (polymorphic vs monomorphic). The lack of such correlation also was observed by Paessler et al.⁴⁵ Although histogenesis also can be evaluated by immunoglobulin heavy chain mutation status,^{47,48} this type of evaluation is too labor intensive to be applicable for clinical use. Furthermore, study of the immunoglobulin heavy chain mutation status requires frozen tissue samples, which frequently are unavailable in clinical practice. Histogenetic phenotypes using immunohistochemical analysis might be considered as an additional factor for classification of PTLD in the future to further determine whether these phenotypes correlate with the clinical course of PTLD.

Our results indicate that most PTLDs are derived from late GC–early post-GC B cells. A second minor group is derived from post-GC B cells, and rare cases are derived from GC B cells. No specific histogenetic staining pattern was associated with the morphologic subtype of PTLD (monomorphic vs polymorphic). In addition, PTLD-HSCT more frequently is of post-GC B-cell origin than that of PTLD-SOT. Further studies with a larger sample are warranted to confirm our observations and to evaluate the prognostic significance of the histogenetic phenotype of PTLD.

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