Application of Immunohistochemistry in the Diagnosis of Non-Hodgkin and Hodgkin Lymphoma

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• ***Context.*—Beginning with the immunologic classifications of Lukes and Collins and Kiel and culminating in the Revised European-American Lymphoma and World Health Organization classifications, the diagnosis of lymphoid tumors relies heavily on the determination of cell lineage, maturation, and function, based on antigen expression in addition to morphology and clinical features. Technologic advances in immunology, antibody production, genetic analysis, cloning, and the identification of new genes and proteins by microarray and proteomics have provided pathologists with many antibodies to use in routine diagnosis.**

***Objective.*—To provide guidance to the practicing pathologist in the appropriate selection of an antibody panel for the diagnosis of lymphoma based on morphology and relevant clinical data and to avoid pitfalls in the interpretation of immunohistochemical data. Attention is given to some of the newer antibodies, particularly against transcription factors, that are diagnostically and prognostically useful.**

***Data Sources.*—The information presented in this article is based on review of the literature using the OVID database (Ovid MEDLINE 1950 to present with daily update) and 20 years of experience in diagnostic hematopathology.**

***Conclusions.*—Immunophenotyping is required for the diagnosis and classification of lymphoid malignancies. Many paraffin-reactive antibodies are available to the pathologist but most are not specific. To avoid diagnostic pitfalls, interpretation of marker studies must be based on a panel and knowledge of a particular antigen's expression in normal, reactive, and neoplastic conditions.**

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Immunohistochemistry (IHC) is an integral part of diagnostic hematopathology. Although in the early 1980s a routine panel included less than 10 antibodies, the current diagnostic armamentarium includes more than 50 antibodies. In addition, there are more than 300 antigens listed on the current cluster designation (CD) list including antigens expressed on nonleukocytes (stromal cells, endothelial cells, etc) (see http://www.hcdm.org/).[1] This explosion of antigens and reagents to detect them necessitates judicious use of carefully selected panels rather than a ''shotgun approach'' to diagnosis. In addition, it should always be remembered that no antigen is totally specific; therefore, immunostaining must be interpreted in the context of a panel to avoid errors in assignment of cell lineage or of an abnormal phenotype. The goals of this paper are to provide guidance to the practicing pathologist in the appropriate selection of an antibody panel based on morphology and relevant clinical data and to avoid pitfalls in the interpretation of immunohistochemical data. State of the art information regarding use of new antibodies particularly against transcription factors is given.[2] This article is not intended as a comprehensive review of each antibody, each lymphoma, or technical issues involved in IHC. The reader is referred to several reviews[3–6] for additional information.

LYMPHOMA DIAGNOSIS IN 2007

Lymphoma diagnosis is currently based on the World Health Organization (WHO) classification[7] and broadly divided into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Non-Hodgkin lymphoma is further subclassified based on the stage of maturation (immature vs mature) and cell of origin (B cell, T cell, or natural killer cell [NK cell]). Beginning with morphologic examination, a determination is made of the anatomic architectural alterations in the lymphoid compartment (ie, B-cell follicle [follicle center, mantle, or marginal zone] or T-cell regions [interfollicular or sinus areas]). If an abnormal population is present, determination of the cell size and nuclear characteristics (round, irregular, cleaved with condensed or dispersed or blastic chromatin, and the character of the nucleoli) is made. The associated reactive component may also be important, particularly in HL, in the classification of the process. At extranodal sites, the anatomic location, evidence of epithelial or vascular invasion, or presence of zonal necrosis may also be used in establishing the diagnosis. Immunohistochemistry is applied in 3 circumstances: to completely phenotype the abnormal population detected by morphology, to further characterize an abnormal population identified by flow cytometry, or to screen ostensibly ''reactive'' tissue to determine if a subtle abnormal population is present.

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A RECOMMENDED BASIC PANEL AND SOME IMMUNOHISTOCHEMICAL PEARLS

The beginning IHC panel includes antibodies against B-cell (CD20) and T-cell (CD3) antigens; κ and λ light chains (if numerous plasma cells are present); and CD45, CD15, and CD30 if large dysplastic cells are seen. Based on morphology, if there is alteration of B-cell areas, antibodies against CD5, CD10, CD23, CD43, B-cell lymphoma 2 protein (BCL-2), and B-cell lymphoma 6 protein (BCL-6; the prototype of the B-cell transcription factors) would also be useful initially to further characterize the process. If there is expansion of interfollicular areas and a T-cell lymphoma is a consideration, antibodies against CD2, CD4, CD5, CD7, and CD8 would identify the subset distribution and if there is abnormal loss of pan–T-cell antigens.

B-Cell Markers

CD20 is the most widely used pan–B-cell marker and is expressed from the naïve B cell until the final stages of B-cell development just prior to plasmacytic differentiation. Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) may be weakly positive[8] or occasionally negative for CD20 by IHC. This staining pattern parallels that seen by flow cytometry in which CD20 is often weakly expressed compared with normal B cells or other B-cell lymphomas. If an abnormal immature blast population is present or if the patient has received rituximab (anti-CD20 antibody) therapy, other antibodies to detect B-cell differentiation such as CD79a or transcription factor paired box gene 5 (Pax-5) should be included.[9] Pax-5 (also known as B-cell–specific activator protein) is expressed in the early stages of B-cell development (pro-B and pre-B cells) and in naïve and other mature B cells but not in plasma cells.[10,11] In control tissue, Pax-5 is expressed intensely in the mantle zone and has weak to moderate expression in germinal center B cells and in interfollicular B cells; monocytoid B cells and marginal zone B cells are mostly negative. T cells, plasma cells, and macrophages are Pax-5 negative. CLL/SLL and mantle cell lymphoma (MCL) have strong Pax-5 expression. A less intense staining pattern is seen in follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). Approximately half of marginal zone lymphomas (MZLs) are positive. Plasma cell myeloma (PCM) and peripheral T-cell lymphoma (PTCL) are Pax-5 negative.

The reader should be aware of a few caveats regarding the use of these B-cell markers. Very rare PTCLs have been reported to express CD20 and/or CD79a so, although not common, it is a potential pitfall.[12] CD79a has also been detected in 10% to 50% of precursor T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL)[11,13] making this marker somewhat less reliable as an indicator of B-cell lineage in blastic neoplasms. In addition, Pax-5 can be expressed in acute myeloid leukemia (AML), predominantly cases associated with t(8;21)(q22;q22) and in some precursor T-ALL/LBLs.[14,15] Pax-5 is expressed in approximately 70.6% to 93.5% of Merkel cell carcinomas and in approximately 70.6% to 73.3% of small cell carcinoma and rarely in breast, endometrial, and urothelial carcinomas.[16,17]

Follicular Dendritic Cell Markers

Immunostaining for CD21, CD23, or CD35 is useful to highlight follicular dendritic cell (FDC) meshwork if B-cell follicle structures are not readily apparent and to determine if there is colonization of follicle centers (MZL) or expanded FDC meshworks (angioimmunoblastic T-cell lymphoma [AILT]) (Figure 1, A through D).

Immunoglobulin Stains for Clonality Assessment

Staining for κ and λ light chains may help to identify the presence of an abnormal clonal population, particularly plasma cells. Immunohistochemistry is less sensitive than flow cytometry for the detection of immunoglobulin expression, as it does not detect surface immunoglobulin. Immunohistochemistry in fixed tissue relies on some degree of cytoplasmic immunoglobulin production. Plasma cells and immunoblasts strongly express cytoplasmic immunoglobulin, whereas only a small subset of other B-cell proliferations has detectable immunoglobulin expression by paraffin immunoperoxidase techniques. A word to the wise: With routine IHC, always examine the same areas in the tissue to compare κ and λ expression to confirm that any potential staining is specific and not an artifact, as these antibodies are polyclonal and have higher nonspecific reactivity than monoclonal antibodies. Colorimetric in situ hybridization is an alternate method used to detect immunoglobulin expression in paraffin-embedded material. Although more expensive and initially more difficult to examine cytologic detail, colorimetric in situ hybridization has increased sensitivity and is very useful when there is high nonspecific background staining using the polyclonal antibodies against κ and λ.[18]

T-Cell Markers

CD3 is the most commonly used pan–T-cell antigen and is normally expressed at the second stage of thymic differentiation and beyond. CD3 may be lost in some T-cell neoplasms, particularly anaplastic large cell lymphoma (ALCL).[19] Natural killer cells can also express the ϵ chain of CD3 detected by the commonly used paraffin-reactive anti-CD3 antibodies, so expression of cytoplasmic CD3 is not incontrovertible evidence of T-cell lineage. It should be noted, however, that surface CD3 as detected by flow cytometry is definitive evidence of T-cell lineage as is expression of the T-cell receptor (TCR) protein αβ (as detected by immunostaining with the antibody β-F1 by IHC). A paraffin-reactive antibody for the γδ TCR protein (present in approximately 5%–10% of normal T cells and in hepatosplenic lymphoma) is not currently available. T cells are composed of CD4 (helper T-cell) and CD8 (suppressor, cytotoxic T-cell) subsets. Antibodies to detect these antigens are included in the basic panel to determine if there is an abnormal T-cell distribution (normal CD4/CD8 ratio 1.5–3:1). Most reactive processes show a predominance of $CD4^+$ T cells. In the absence of human immunodeficiency virus (HIV) or other viral infection, a neoplastic T-cell or NK-cell process should be considered if a marked predominance of $CD8^+$ T cells is present, particularly at extranodal sites.

CD5 is a pan–T-cell antigen and is not expressed on NK cells. CD5, however, is not lineage specific. CD5 is present on only a very small subset of normal B cells but more importantly CD5 is expressed in CLL/SLL, in MCL, and less frequently in small subsets of other B-cell neoplasms. If CD5 is expressed in B-cell areas (usually weaker than in T cells), additional studies should be performed to determine the type of small B-cell neoplasm (see ''Small B-Cell Neoplasms''). If CD5 expression is lost in T-cell areas,

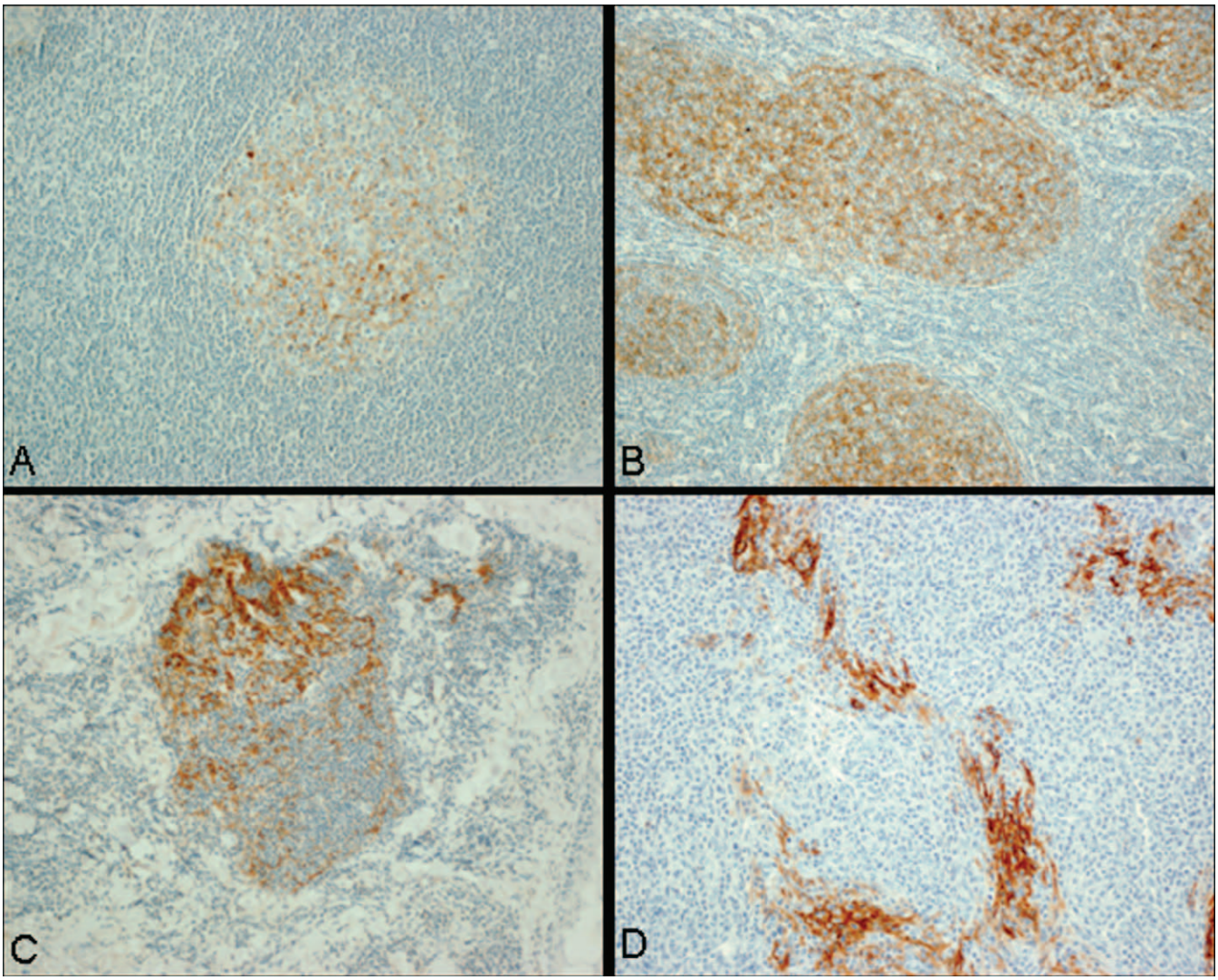

Figure 1. *Staining for follicular dendritic cell (FDC) meshwork demonstrates the presence and architectural features of B-cell follicles and is helpful in lymphoma diagnosis. A, Normal FDC meshwork in a reactive follicle in the tonsil. B, Preserved "tight" FDC meshwork in low-grade follicular lymphoma. C, Expanded and somewhat disrupted FDC meshwork in a follicle that is colonized by a marginal zone lymphoma. D, Markedly expanded and disrupted FDC meshwork extending beyond the follicle and surrounding vessels, a characteristic feature of angioimmunoblastic T-cell lymphoma (CD21 immunoperoxidase, original magnifications ×200).*

additional studies to evaluate for a T-cell or NK-cell lymphoma are indicated.

Antigens for Characterization of Large Cell Proliferations

Finally, if atypical large cells are present, antibodies against CD15, CD30, and CD45RB (leukocyte common antigen) should be added to the panel to rule out HL or ALCL (particularly with small cell variant morphology) or an epithelial neoplasm. One should remember that CD30 is an activation antigen and commonly expressed on large cells at the periphery of B-cell follicles and in the interfollicular areas. Also, reactive processes, such as infectious mononucleosis, can have numerous large atypical, dysplastic $CD30^+$ cells.[20,21] Immunostaining for anaplastic lymphoma kinase (ALK) protein (using the antibody ALK-1) and for CD15 is useful to evaluate $CD30^+$ large dysplastic cells. The lack of CD15 expression does not exclude classical HL (CHL) as approximately 25% to 40% of cases may be negative or have focal expression of this marker. CD30 and ALK-1 expression indicate an ALK-positive ALCL; absence of ALK can be seen in ALK-negative ALCL and other tests such as evaluation of Epstein-Barr virus (EBV) (more frequently positive in HL) or *TCR* gene rearrangement analysis (more frequently positive in ALCL) may be indicated. Expression of CD45RB rules out an epithelial tumor, but very weak or absent CD45RB can be seen in tumors with plasma cell differentiation, precursor lymphoid and myeloid tumors, and ALCL (in 20%–40% of cases), as well as in CHL. CD15 and rarely CD30 (particularly in embryonal carcinoma) are expressed in epithelial tumors.

Immunostaining of Necrotic Tissue

The presence of necrosis without an otherwise identifiable etiology (infection, vascular compromise, etc) is suspicious for a neoplastic process. Although necrosis is generally considered to cause nonspecific IHC staining, some hematopoietic markers may be reliable, particularly CD3, CD45RO, and CD43; variable preservation of CD20 and CD79a has been reported.[22–24] Although these markers

Table 1. Immunophenotype of Small B-Cell Neoplasms in Paraffin-Embedded Material*

Neoplasm	CD5	CD10	BCL-2	IgD	BCL-6	MUM-1	CD43	Cyclin D1	CD23	Annexin-A1
MCL	+	−	+	+	−	−	+	+	−/+	−
CLL/SLL	+	−	+	+	−	+/−†	+	−/+‡	+	−
FL	−	+	+	−/+	+	−§	−§	−	−/+	−
MALT	−	−	+	−	−	+/−	+/−	−	−	NT
MZL, nodal	−	−	+	+/−‖	−/+	+	+/−	−	−	−
MZL, splenic	−	−	+	+	−	+/−	−	−	−	−
LPL/WM	−	−/+	+	−	−	+¶	−/+	−	−/+	−
HCL	−	−/+	+	−	−	NT	NT	+	−/+	+

* IgD indicates immunoglobulin D; MCL, mantle cell lymphoma; +, more than 50%; −, less than 5%; −/+, 5% to 25%; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; +/−, more than 25% to 50%; FL, follicular lymphoma; MALT, mucosa-associated lymphoid tissue; NT, not tested in a large number of cases; MZL, marginal zone lymphoma; LPL/WM, lymphoplasmacytic lymphoma/Waldenström macroglobulinemia; and HCL, hairy cell leukemia.

† Positive in proliferation centers and variable in the small lymphocytes.

‡ Positive primarily in proliferation centers.

§ May be positive in FL, grade 3.

‖ Positive in nodal MZL of ''splenic type.''

¶ More positivity in the plasma cells rather than in the small lymphocytic component.

may be helpful, a lack of morphologic detail often prevents precise classification of the neoplastic process.

A WORD ABOUT EBV AND OTHER VIRUSES

Epstein-Barr virus is the etiologic agent of infectious mononucleosis and insignificant viral infections in early childhood. Consequently, many individuals have subclinical, lifelong latent infection by the virus. Detection of EBV is important in the diagnosis of infectious mononucleosis; lymphoproliferations in posttransplant or other immune deficient states; or neoplasms such as Burkitt lymphoma (BL), HL, NK-cell neoplasms (aggressive NK-cell leukemia and extranodal NK/T-cell lymphoma, nasal type), immunoblastic/plasmablastic tumors, and AILT. Immunohistochemistry detection of viral-encoded proteins, such as latent membrane protein (LMP) and Epstein-Barr nuclear antigen (EBNA-2), and in situ hybridization for EBV-encoded RNA (EBER) are used in determining the EBV latency state, which shows some correlation with diagnostic categories. Type I latency (EBER positive, LMP-1 negative, EBNA-2 negative) is typical of BL, type II latency (EBER positive, LMP-1 positive, EBNA-2 negative) is typical of HL, and type III latency (EBER positive, LMP-1 positive, EBNA-2 positive) is seen in many immunodeficiency-associated (including posttransplant) lymphoproliferative disorders.[25] Immunohistochemical detection of LMP is a specific but not a sensitive marker of EBV. Currently the best method for detection of EBV is in situ hybridization of EBER with IHC detection methods, and this is often sufficient for diagnosis without LMP or EBNA-2.[26]

Detection of human herpesvirus 8/Kaposi sarcoma–associated herpesvirus with the antibody against the latent nuclear antigen is used in the diagnosis of multicentric Castleman disease, plasmablastic lymphoma (PBL) arising in Castleman disease, and primary effusion lymphoma (PEL) and in the differential diagnosis of other PBLs (see ''PBLs or Lymphomas With Plasmacytic Differentiation'').

SMALL B-CELL NEOPLASMS

The Role of CD5 and CD10 in the Classification of Small B-Cell Neoplasms

Small B-cell neoplasms are stratified according to CD5 or CD10 expression (Table 1). Neoplasms that express CD5 and lack CD10 ($CD5^+CD10^-$) predominantly include MCL and CLL/SLL. CD5 expression has rarely been reported in other B-cell neoplasms. A small number of $CD5^+$ mucosa-associated lymphoid tissue (MALT) type MZL cases have been described.[27–29] Some $CD5^+$ MALT type MZL have involvement of the head and neck (particularly orbital) area, tumor cells in the bone marrow and peripheral blood, and a relapsing course.[27,28] Up to 15% of non-MALT MZL (splenic, nodal, and leukemic) have been reported to be $CD5^+$ using flow cytometry or IHC on frozen or fixed tissue.[30,31] Hairy cell leukemia (HCL) rarely expresses CD5.[32,33]

The lack of CD5 and expression of CD10 ($CD5^-CD10^+$) phenotype is seen in FL and in precursor B-cell acute lymphoblastic leukemia/lymphoma. Precursor B-cell acute lymphoblastic leukemia/lymphoma is usually excluded from this category by its blastic morphology and expression of terminal deoxynucleotidyl transferase (TdT) and/or CD34 (see ''Tumors With Blastic Morphology''). CD10 expression has been reported in approximately 10% of cases of HCL by IHC[34] and approximately 2% to 6% of MZLs.[30,35] Rare cases of blastoid variant of MCL may lack CD5 and express CD10.[36]

The lack of expression of CD5 and CD10 ($CD5^-CD10^-$) phenotype is most often present in MZL, lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, and HCL. A small group of FLs (5%–30%) and CLL/SLLs (less than 1% by flow cytometry but up to 25% in paraffin) are negative for CD5 and CD10.[8,37] A small but significant number (4%–25% of cases) of MCLs lack detectable CD5 expression using either flow cytometry or IHC.[8,38]

The neoplasms with dual expression of CD5 and CD10 ($CD5^+CD10^+$) are very uncommon representing less than 1% of B-cell neoplasms.[39] This immunophenotype has been described in approximately 1% of FLs (including rare cases with ''floral variant'' morphology),[40,41] 4% to 10% of MCLs by flow cytometry and IHC,[35,37,42] less than 1% of CLL/SLLs, and rarely in precursor B-cell acute lymphoblastic leukemia/lymphomas.

The Use of CD23 in Subcategorizing $CD5^+$ Small B-Cell Neoplasms

Most cases of MCL are clinically aggressive compared with other small B-cell neoplasms making recognition of MCL very important. Immunohistochemistry using CD23 is helpful in the differential diagnosis of the $CD5^+$ small B-cell group neoplasms CLL/SLL and MCL. CD23 is ex-

pressed in CLL/SLL (virtually all cases are positive by flow cytometry; however, the expression may be dim in up to 35% of cases, and 82%–95% of cases are positive by IHC).[8,7,42–46] Approximately 3% to 13% of MCLs can be CD23$^+$ by IHC with up to 45% (most having dim expression) by flow cytometry.[8,42,44,47,48] Morphologic clues to suggest MCL rather than CLL/SLL would include the lack of pseudofollicles (proliferation centers usually seen in CLL/SLL), small lymphocytes with hyperchromatic nuclei with more irregular nuclear profiles than typically seen in CLL/SLL, the presence of periodic acid-Schiff–positive thickened vessels and admixed histiocytes, and, in some but not all MCLs, a higher mitotic rate; cyclin D1 expression would confirm the diagnosis of MCL. CD23 is also expressed in approximately 16% to 33% of FLs by IHC.[42]

Is Cyclin D1 Expression Specific for MCL?

Several routine methods are available to confirm the histologic impression of MCL. The characteristic translocation, t(11;14)(q13;q32), can be detected by fluorescence in situ hybridization (FISH), Southern blot, reverse transcriptase–polymerase chain reaction, or polymerase chain reaction (PCR). Alternatively, the resultant overexpression of cyclin D1 can be demonstrated by IHC. Cytogenetic and Southern blot detection of the translocation are positive in only 40% to 70% of cases.[48–50] Detection of t(11;14)(q13;q32) by PCR is found in less than 50% of MCL cases using routine primer sets, as the breakpoints are widely distributed. Detection of cyclin D1 expression by IHC or demonstration of the t(11;14)(q13;q32) translocation by FISH are the preferred confirmatory tests of the diagnosis of MCL. Lack of cyclin D1 expression has been reported in approximately 10% to 25% of MCLs by IHC and in less than 10% of MCLs using FISH.[42,49–52] The lack of cyclin D1 expression by IHC may be attributed in part to technical factors, particularly the types of antibodies used to detect the antigen. Recent studies have demonstrated the efficacy of rabbit monoclonal antibodies that have increased sensitivity without significant loss of specificity.[53,54] A comparative study using multiple antibodies by Torlakovic et al[54] demonstrated the strongest staining with a rabbit monoclonal antibody SP-4 with 95% sensitivity. A polyclonal antibody CP236 had 100% sensitivity, but the staining was weaker. The authors recommend having both antibodies available and a second antibody run if the first is negative in cases in which there is a strong suspicion of MCL by morphology. Using the panel of 5 antibodies in their study, Torlakovic et al found that only 1 (3%) t(11;14)-positive case of 31 confirmed cases of MCL was a true false-negative by IHC.

Cyclin D1 is normally expressed in scattered large nuclei of histiocytes, endothelial cells, fibroblasts, and rare normal mantle cells (Figure 2, A). Cyclin D1 is expressed in MCL nuclei, and cytoplasmic expression alone is nonspecific (Figure 2, B). B5-fixation can be associated with equivocal results; therefore, formalin-fixed tissue is generally preferred; however, Torlakovic et al[54] did not find a significant difference. In cyclin D1–negative cases with the morphologic features and immunophenotype of MCL, immunostaining with a proliferation marker Ki-67 (MIB-1) in addition to FISH for the t(11;14) translocation would be useful in verifying such cases are not typical indolent small B-cell neoplasms. Some cyclin D1–negative MCLs overexpress other cyclins (cyclin D2 or D3), and rare cases show translocations juxtaposing the *CCND2* gene at 12p13 next to the κ light chain locus at 2p12.[55]

Although cyclin D1 expression in the appropriate morphologic context strongly supports MCL, other lymphoid neoplasms also overexpress cyclin D1. Cyclin D1 expression has been reported in 13% to 19% of CLL/SLLs (particularly in proliferation centers) (Figure 2, C),[37,56] 25% of PCMs, and 40% to 100% of HCLs (variable, usually weaker staining in a subpopulation of cells) (Figure 2, D).[56,57] A small number of cases (approximately 4%–15%) of splenic MZL are also reported to express cyclin D1 and the translocation t(11;14)(q13;q32).[31,58,59] In cases of suspected HCL with cyclin D1 expression, a new antibody, annexin A1, appears useful in making the correct diagnosis and in distinguishing HCL from splenic villous lymphoma.[60] Annexin A1 is also expressed in neutrophils requiring morphologic correlation, particularly in the bone marrow. Combined immunoreactivity for tartrate-resistant alkaline phosphatase staining and DBA.44 is seen in HCL; both antibodies show reactivity in other B-cell lymphomas (DBA.44 is frequently expressed in FL, and tartrate-resistant alkaline phosphatase is seen in 57% of MCLs, 41% of CLL/SLLs, and 54% of primary mediastinal large B-cell lymphomas), but only 3% of these tumors have expression of both.[61]

CD10 and BCL-6 Protein Expression in FL

CD10 and BCL-6 are expressed in normal and neoplastic follicle centers (Figure 3, A through D). CD10 is also expressed on granulocytes, precursor B and T cells, and T cells in AILT. In addition, CD10 is expressed in endometrial cells and in some epithelial neoplasms such as renal cell carcinoma and hepatocellular carcinoma. BCL-6 is expressed in a small number of T cells. BCL-6 is involved in germinal center function and is downregulated as the B cell undergoes apoptosis or as it exits the germinal center destined to become a memory B cell or a plasma cell. Nodal low-grade FL is characterized by its morphologic appearance with crowded, back-to-back neoplastic follicles, numerous cleaved cells, lack of zonation and tingible body macrophages, and loss of the mantle zones; in addition, follicles often invade the capsule and infiltrate the surrounding fat. The presence of CD10$^+$ lymphocytes and groups of BCL-6–positive small lymphocytes outside of follicles strongly supports a neoplastic proliferation (Figure 3, B and D).[62] A few isolated BCL-6–positive cells may normally be present in the interfollicular areas of lymph nodes (predominantly T cells) (Figure 3, C), but large numbers of interfollicular BCL-6–positive cells would be supportive of FL. Approximately 10% to 30% of nodal FLs lack expression of CD10 by IHC, and BCL-6 may be helpful in identifying a follicular origin.[8,62,63] However, rare cases of BCL-6–positive MCL (including the blastoid variant) have been described.[64] The presence of small numbers of BCL-6–positive large cells with numerous BCL-6–negative, BCL-2–positive small lymphocytes (other than can be accounted for by reactive T cells) in the follicle center may be a tip off to the presence of follicular colonization by MZL.

The diagnosis of FL in the bone marrow relies on distinction from reactive follicles and from other small B-cell neoplasms, particularly CLL/SLL and MCL. A paratrabecular location, increased numbers of CD20$^+$ B cells, expression of CD10 and BCL-2, and more well-developed follicular dendritic meshwork supports a diagnosis of

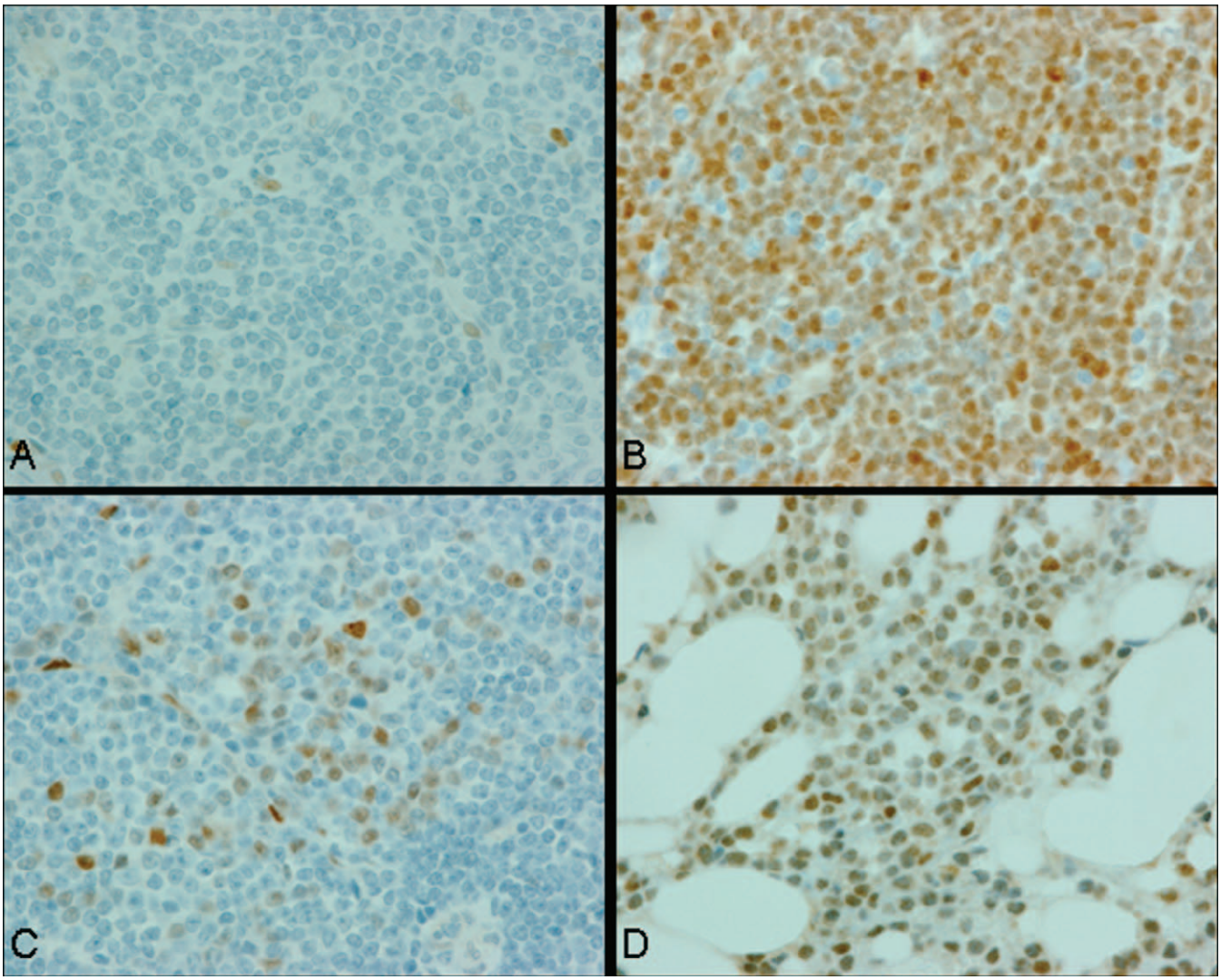

Figure 2. *Cyclin D1 expression is a defining feature of mantle cell lymphoma (MCL) but can be present in other small B-cell neoplasms. A, Cyclin D1 expression is present in scattered, reactive endothelial, histiocyte, and stromal cell nuclei in a benign tonsil. B, Strong nuclear expression of cyclin D1 is seen in MCL. C, Cyclin D1 is expressed in the nuclei of paraimmunoblasts and immunoblasts in the proliferation centers of chronic lymphocytic leukemia/small lymphocytic lymphoma. D, Cyclin D1 is also weakly expressed in the nuclei of hairy cell leukemia (SP4 cyclin D1 immunoperoxidase, original magnifications ×600 [A through C]; P2D11F11 cyclin D1 immunoperoxidase, original magnification ×600 [D]).*

FL.[5,65] The interpretation of small lymphoid aggregates may be difficult as CD10 can be expressed in bone marrow stromal cells and immature B cells. Mantle cell lymphoma (which also may be paratrabecular in location in addition to interstitial) should express CD5 and cyclin D1, and CLL/SLL should be $CD5^{+}CD23^{+}$.

The Use of BCL-2 Expression in the Evaluation of Small B-Cell Proliferation

BCL-2 is an antiapoptotic molecule normally expressed in pre-B cells, resting B cells including normal mantle zone lymphocytes, and certain types of proliferating B cells. BCL-2 is down-regulated in normal germinal center B cells (Figure 3, A). Virtually all of the small B-cell neoplasms express BCL-2.[66] Overall, approximately 85% to 90% of FLs are BCL-2 positive. BCL-2 expression varies with FL grade with almost 100% expression in grade 1 and approximately 75% in grade 3.[67] Immunostaining for BCL-2 is most useful in distinguishing reactive follicular hyperplasia from FL. B cells in reactive follicle centers lack BCL-2 expression, whereas the normal mantle zone and primary follicles are BCL-2 positive.[68] Immunostaining for BCL-2 must be interpreted in conjunction with staining for CD3 as T cells normally express BCL-2, and numerous T cells can be present in reactive follicles. Most BCL-2–negative FLs lack a t(14;18) translocation; however, some FLs have a mutation in the *BCL2* gene leading to a false-negative result on IHC (resulting from an alteration in the epitope recognized by the antibody Mab#100, Pharmingen, Franklin Lakes, NJ).[69]

Marginal zone lymphoma is also BCL-2 positive (approximately 83% of extranodal and 62% of nodal).[67] Care should be taken in using BCL-2 expression as evidence of a neoplasia in MZL, particularly in the spleen and abdominal lymph nodes where BCL-2 is expressed in reactive hyperplastic marginal zones.[70] Monocytoid B cells, as commonly seen in *Toxoplasma* lymphadenopathy, are morphologically distinct from marginal zone hyperplasia and are BCL-2 negative.[66]

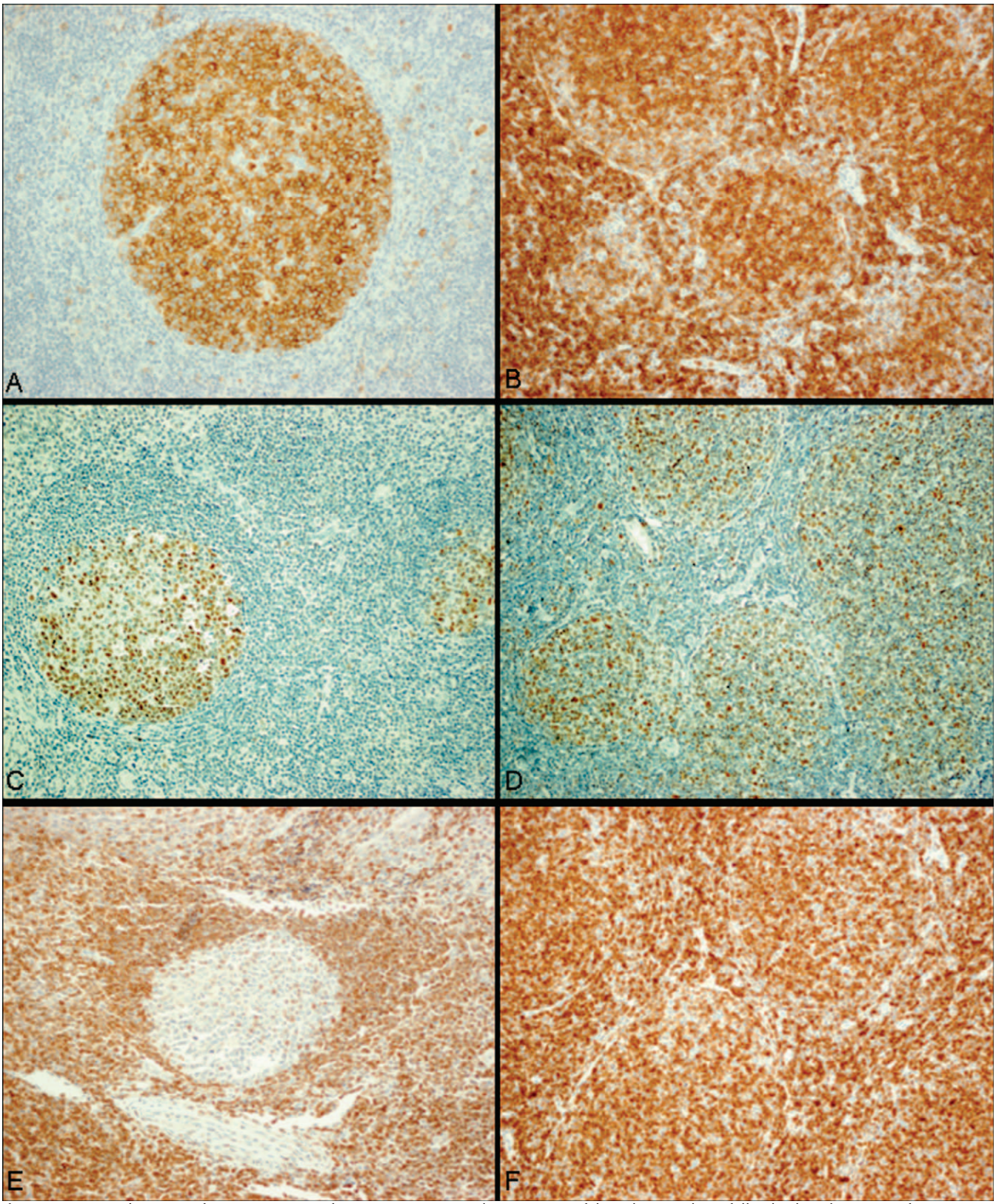

Figure 3. *A combination of immunostaining for CD10, BCL-6, and BCL-2 is useful in distinguishing follicular lymphoma (FL) from reactive lymphoid hyperplasia. This figure compares expression of these antigens in benign follicles from a reactive tonsil (A, C, E) and in neoplastic follicles in a low-grade FL (B, D, F). A and B, CD10 is expressed in germinal center cells in both reactive and neoplastic follicles; however, in FL (B) CD10 expression can be seen in cells invading the interfollicular area. C and D, BCL-6 is expressed predominantly in large cells in the reactive and neoplastic germinal centers with only rare positive large nuclei in the interfollicular area in the reactive process (C). BCL-6 is weakly expressed in FL (D) in the cleaved cells invading the interfollicular area. E, BCL-2 is expressed in T cells in reactive follicles and in the interfollicular area and in the normal mantle zone lymphocytes. F, In contrast, virtually all of the neoplastic cells in the follicle center in low-grade FL are BCL-2 positive as shown here (CD10 immunoperoxidase, original magnifications ×200 [A and B]; BCL-6 immunoperoxidase, original magnifications ×200 [C and D]; BCL-2 immunoperoxidase, original magnifications ×200 [E and F]).*

A Word About the Immunophenotype of Extranodal FL

Primary extranodal FL has more variable expression of CD10 and BCL-2 than nodal FL suggesting a different pathogenesis and making the diagnosis more difficult, as these cases often lack the t(14;18)(q32;q21) translocation. For example, in primary cutaneous follicle center lymphoma (PCFCL), expression of CD10 is more often absent, particularly in those cases with a diffuse growth pattern.[71] BCL-6 is usually present in PCFCL providing some evidence of germinal center origin, but the determination of malignancy is still problematic. BCL-2 expression is variable and, if present, is weaker than in reactive T cells; a higher incidence of BCL-2 expression is reported in cases from the United States and Canada than from Europe. Recent studies have shown cases of PCFCL with a follicular growth pattern have detectable t(14;18)(q32;q21) (41%) and t(3;14)(q27;q32) (7%)[72] and more expression of CD10 in the cases with translocations.[73] The lower detection of genetic abnormalities in earlier studies may relate to the methodology (PCR rather than FISH). At the present time, the diagnosis of PCFCL relies primarily on the morphologic features (increased number of cleaved cells, loss of tingible body macrophages, and mantle zones), presence of FDC meshwork, and the presence of a clonal population by immunoglobulin heavy chain gene rearrangement, particularly because most PCFCLs lack the t(14;18)(q32;q21) translocation.[72,73]

Other Antigens Useful in the Diagnosis of Small B-Cell Neoplasms

CD43.—CD43 is expressed in T cells, myeloid cells, plasma cells, and weakly in histiocytes. CD43 is usually negative in normal B cells, and CD43 expression in B cells is supportive of lymphoma. However, exceptions such as reactive lymphoplasmacytoid cells, B cells in infectious mononucleosis, and subpopulations of B cells of the terminal ileum[74] expressing CD43 have been described. CD43 expression has also been detected in benign lymphoepithelial lesions or expansion of monocytoid B-cell halos in the salivary gland.[75]

Immunohistochemistry for CD43 has some utility in categorizing small B-cell lymphomas when used in conjunction with other markers (Table 1). Approximately 20% to 50% of MALTs and approximately 40% to 50% of nodal MZLs express CD43[76,77]; splenic MZL is $CD43^-$.[78,79] Most (60%–95%) cases of MCL and CLL/SLL are $CD43^+$, whereas FL is very rarely (<5%–10%) $CD43^+$.[37,42,80,81] Therefore, the pathologist can anticipate CD43 expression in $CD5^+$ small B-cell lymphomas; conversely, CD43 expression in a $CD10^+$ nodular small B-cell tumor is unexpected and should prompt correlation with IHC markers for CD5 and cyclin D1 or FISH studies for cyclin D1 and a *BCL2* gene rearrangement t(14;18)(q32; q21) to determine if the tumor is a $CD10^+$ MCL or a $CD43^+$ FL. It should be noted that, as the histologic grade of FL increases, CD43 may be expressed. In one study by Lai et al[80] 6% of grade 3/3 FLs and 28% of DLBCLs expressed CD43.

FDC Meshwork and Proliferation Markers.—FL, MCL, and CLL/SLL may have a marginal zone growth pattern resembling MZL, particularly in extranodal sites such as the spleen.[82–86] In addition to CD5, CD10, CD43, and cyclin D1, immunostains for FDC meshwork and MIB-1 may be useful in this distinction. Immunostaining for CD21, CD23, or CD35 highlights FDC meshwork making it possible to delineate follicle structures and assess their morphology.[87] Reactive follicles have distinct, circumscribed meshwork and zonation is highlighted by a denser reaction in the light zone (Figure 1, A). Low-grade FL has a dense, expanded but tight, sharply defined FDC (Figure 1, B) that may merge between 2 follicles. Loss of FDC meshwork in FL is seen with transformation.[87,88] In MZL, the FDC meshwork may be disrupted by colonization of the follicle (Figure 1, C); expanded FDC meshworks can become confluent in gastric and salivary gland MALT.[87] Pseudofollicles (growth or proliferation centers) in CLL/SLL have delicate meshwork compared with entrapped reactive follicles[89]; the small tumor cells and larger tumor cells in proliferation centers express CD23. MCL has loosely arranged, ill-defined and expanded FDC meshworks resembling broken-up primary follicles.

Proliferation marker MIB-1 (Ki-67) is useful in differentiating FL from reactive follicular hyperplasia and from other small B-cell lymphomas.[90] Normal germinal centers have a high proliferation rate with some zonation (more proliferation in the dark zone); in contrast, a decreased proliferation rate within the germinal center is seen in low-grade FL and in follicles infiltrated by marginal zone cells (follicular colonization). Care should be taken in interpreting MIB-1 expression, however, as some grade 2 and grade 3 FLs have high proliferation rates similar to reactive follicles. In addition, tangential cuts through the mantle zone of the follicle will also have a lower amount of MIB-1 expression. MIB-1 staining in splenic MZL reveals a somewhat targetoid appearance (strong staining of the reactive follicle center cells and staining of tumor cells with prominent nucleoli in the marginal zone).[91]

Immunoglobulin D.—Immunoglobulin (Ig) D is expressed in normal and neoplastic mantle cells and in CLL/SLL. Immunoglobulin D is absent in most cells of the normal splenic marginal zone but is expressed in 30% to 40% of splenic MZLs particularly in those with an unmutated phenotype[79] and overall in approximately 30% of nodal MZLs (predominantly cases resembling splenic MZL, the so-called splenic type).[76,77] MALT type MZL is only rarely IgD positive; therefore, IgD IHC may be useful in small mucosal stomach biopsies in which IgD-positive lymphocytes are likely normal mantle cells or MCL. Therefore, staining for IgD in the context of clinical location and other markers can be useful in distinguishing mantle zone expansion versus MZL, particularly at MALT sites, and in determining if marginal zone expansion in the spleen is abnormal.

Clonal Plasma Cell Populations Are Seen in Several Small B-Cell Neoplasms

As emphasized in the WHO classification, clonal plasma cells and IgM paraproteins may be present in multiple small B-cell neoplasms.[92] A diagnosis of lymphoplasmacytic lymphoma/Waldenström macroglobulinemia is made only after exclusion of other small B-cell neoplasms. Clonal plasma cell populations have been detected in approximately 40% to 50% of MALT-type MZLs, in less than 5% of FLs and CLL/SLLs, and very rare cases of MCL.[93,94]

BURKITT LYMPHOMA

Burkitt lymphoma has a characteristic morphologic appearance (uniform population of small transformed lymphocytes with round nuclei) and a translocation involving *C-MYC* at 8q24 (with the immunoglobulin heavy chains

[14q32] or κ [2q11] or λ [22q11] light chains). Burkitt lymphoma typically has a high proliferation rate (approaching 100%) as detected by immunostaining for MIB-1 (Ki-67) and is $CD20^+$, $CD10^+$, BCL-6^+, $CD43^+$, BCL-2^-, TdT^-, MUM-1^-, and surface immunoglobulin positive. Some lymphomas with *C-MYC* abnormalities have atypical or ''Burkitt-like'' morphology with more variability in nuclear shape, some admixed large transformed lymphocytes, and less expression of CD43 (approximately 40%).[80] Virtually all endemic BLs, approximately 25% to 40% of BLs in immunodeficient patients, and less than 30% of sporadic BLs are EBV positive.

There is overlap of morphology, immunophenotype, genetic profile, and expression profile between BL and DLBCL. The majority of BLs adhere to the characteristic BL immunophenotype and genotype. Recently, a molecular profile has been identified in BL.[95,96] It appears that 3% to 8% of cases with DLBCL morphology have a molecular signature of BL.[95–97] The presence of *C-MYC* abnormalities often associated with a complex karyotype in 5% to 20% of DLBCL cases may indicate an aggressive course but not necessarily a BL molecular signature.[95–97] Of the small number of BL cases that express BCL-2 protein, some lack an identifiable *C-MYC* rearrangement and some have a *BCL2* rearrangement.[95–98] In the future, immunophenotypic markers will likely be developed that reflect the molecular signature of BL to further evaluate the diagnostic and prognostic utility of this ''molecular'' phenotype.

DIFFUSE LARGE B-CELL LYMPHOMA

Diffuse large B-cell lymphoma represents approximately 30% to 40% of NHLs in adults and is diagnosed based on the presence of a predominant population of large noncleaved or transformed lymphocytes expressing B-cell antigens CD20, CD79a, or Pax-5. B-cell transcription factors Oct-2 and Bob1 are expressed in DLBCL and are useful in distinguishing DLBCL from HL (see ''Hodgkin Lymphoma'').[99] The expression of the transcription factor multiple myeloma oncogene 1/interferon regulatory factor 4 (MUM-1/IRF4) is useful in the subclassification of DLBCL into prognostic groups. MUM-1 is a marker of late germinal center or post germinal center B cells. MUM-1 is normally expressed in the nuclei of plasma cells, a small percentage (approximately 5%) of B cells in the light zone (late germinal center B cells with little expression of BCL-6), and up to 5% of germinal center and activated, $CD30^+$ interfollicular T cells.[100] As MUM-1–positive B cells leave the germinal center, they lose BCL-6, maintain MUM-1, and acquire CD138 to become memory B cells or plasma cells. MUM-1 expression is present in grade 3 FL and in paraimmunoblasts and prolymphocytes in proliferation centers of CLL/SLL and predominantly in plasma cells in MZL and lymphoplasmacytic lymphoma.

Subclassification of DLBCL With Regard to B-Cell Differentiation Stage

Diffuse large B-cell lymphomas are biologically heterogeneous as reflected in their variable clinical outcome, immunophenotype, and cytogenetics and molecular genetics. Using molecular genetic and IHC data, progress has been made in the last 5 to 10 years in subclassifying DLBCL into clinically relevant groups based on tumor cell biology. Using gene expression profiling, 3 distinct groups (germinal center, activated B cell, and unclassified, type 3) of DLBCL have been identified and correlated with prognosis.[101,102] As a surrogate for the molecular analysis, expression of 3 antigens, CD10, BCL-6, and MUM-1, has been used to classify DLBCL as germinal center ($CD10^+$ or $CD10^-$, BCL-6^+, MUM-1^-) and non–germinal center or post germinal center or activated B-cell ($CD10^-$, BCL-$6^{+/-}$, MUM-1^+) origin.[103,104] Approximately 30% to 45% of DLBCLs have a germinal center phenotype, and 55% to 70% are non–germinal center with better survival in the germinal center type.

Predicting Prognosis in DLBCL Using Immunohistochemical Markers

Individual antigens related to apoptosis (BCL-2, survivin, cellular FLICE-inhibitory protein, X-linked mammalian inhibitor of apoptosis protein, etc), B-cell differentiation (CD10, BCL-6, Forkhead box protein P1, human germinal center–associated lymphoma, etc), adhesion molecules (intercellular adhesion molecule, sCD44, etc), and cell cycle regulators (p53, cyclin D2, D3, Ki-67) have also been evaluated for prognostic impact in DLBCL, and the results are controversial because of variability in patient age and stage, the cutoff for positive cases on IHC, genetic abnormalities such as the presence of a (14;18)(q32; q21), and lack of uniform treatment between various studies.[105] BCL-2 expression is the most extensively studied marker, and in most series is unfavorable (summarized in reference 105), particularly in cases with a non–germinal center phenotype.[103,105–107] The less adverse affect of BCL-2 in the germinal center phenotype likely relates to the mechanism of expression of BCL-2 in some cases through the 14;18 translocation or amplification at the *BCL2* locus rather than constitutive expression of NFκB activation. MUM-1 expression is associated with poor prognosis (that reflects the non–germinal center phenotype), and as a target of NFκB its expression likely is a reflection of activation of the NFκB pathway and other apoptosis-inhibiting genes.[106]

BCL-2 expression in primary cutaneous DLBCL deserves special comment. Traditionally, primary cutaneous DLBCL was divided into tumors on the leg and those located elsewhere. The distinction was made because of the worse prognosis of the former. In the new WHO classification of skin tumors,[108] the category of DLBCL, leg-type, is defined immunophenotypically by expression of strong positivity for BCL-2 and MUM-1, variable BCL-6, and lack CD10 and CD138. Recent studies have confirmed the utility of BCL-2 and MUM-1, and also transcription factor FOX-P1, expression in predicting a more aggressive course in primary cutaneous DLBCL.[109,110]

Currently, the IHC workup of DLBCL should include CD5, CD10, BCL-6, MUM-1, and BCL-2. The expression pattern of these markers is membranous (CD10), cytoplasmic (BCL-2), and nuclear (BCL-6 and MUM-1). In a recent study by Muris et al,[106] the optimal discriminative power of these antigens using log-rank analysis was 30% tumor cell positivity for CD10, BCL-6, and MUM-1 and 50% for BCL-2. If numerous plasmablasts or immunoblasts are present, CD138 should be included (see ''PBLs or Lymphomas With Plasmacytic Differentiation''). Most DLBCLs have a growth fraction of approximately 40% to 60%. In DLBCL with histologic evidence of a high growth fraction (numerous mitoses with or without tingible body macrophages and expression of Ki-67 in >80% of the tumor cells), FISH analysis to detect abnormalities of *C-MYC* (see ''Burkitt Lymphoma'' for further discussion of *MYC*

abnormalities in DLBCL) may be indicated. If there is a vaguely nodular growth pattern or CD10 is expressed, staining for FDC meshwork may be useful in ruling out transformation of DLBCL from FL. Approximately 5% to 10% of DLBCLs are $CD5^+$; most arise de novo but cyclin D1 and CD23 should be tested in these cases to rule out transformation from MCL or CLL/SLL, respectively.

Special Subtypes and Caveats in DLBCL

T-cell/histiocyte-rich large B-cell lymphoma (T/HRBCL) is recognized by randomly scattered single or small clusters of neoplastic $CD20^+$ B cells in a background of small cytotoxic ($CD8^+$, T-cell intracellular antigen 1 [$TIA\text{-}1^+$]) T cells and variable histiocytes.[111,112] Small B cells are rare, and if present, lymphocyte predominant HL should be considered. Distinguishing features are the predominance of $CD8^+$, $TIA\text{-}1^+$ T cells and lack of FDC meshwork in T/HRBCL versus the presence of $CD4^+$, $CD57^+$ T cells and presence of FDC meshwork in nodular lymphocyte predominant Hodgkin lymphoma (NLPHL).[112] Clonal cytoplasmic immunoglobulin light chain may be detectable by IHC in the large cells in T/HRBCL.

Mediastinal (thymic) large B-cell lymphoma (MLBCL) typically lacks CD5 and CD10 as well as surface and cytoplasmic immunoglobulin and HLA-DR by flow cytometry. MUM-1 is expressed indicating an activated germinal center or post germinal center B-cell origin. There is weak and variable expression of CD30. The clinical location and presence of large dysplastic cells with focal CD30 expression may suggest HL; however, the expression of CD45 (leukocyte common antigen) and lack of CD15 expression distinguishes MLBCL from HL.[113] Interestingly, on gene expression profiling MLBCL shows substantial overlap with CHL[114–116] and a new gene, *MAL*, has been identified that distinguishes MLBCL from other DLBCL.[117] At the present time, commercially available antibodies against *MAL* are only reactive in nonfixed cells or tissue. In addition, nuclear expression of reticuloendotheliosis viral oncogene homologue (a member of the NFκB family of transcription factors) and cytoplasmic expression of *TRAF1*, an NFκB target gene (seen in approximately 50% of MLBCL), is highly specific for this lymphoma.[118]

Intravascular large B-cell lymphoma is a subtle intravascular lymphoma that relies on IHC with CD20 for its detection.[119,120] Intravascular large B-cell lymphoma arises in patients that generally lack peripheral adenopathy and have protean clinical systems resulting from occlusion of small vessels in various organs (often the brain and skin). The majority of cases have a B-cell phenotype with small numbers of T-cell and rare NK-cell cases reported.[121,122] Approximately 20% to 40% of intravascular large B-cell lymphomas express CD5 or CD10.[123,124] The diagnosis is best made with careful attention to CD20 immunostaining looking for subtle distension of blood vessel lumina by large B cells.

Rare large B-cell lymphomas have a sinus growth pattern or an anaplastic or immunoblastic appearance. Staining for CD30, ALK-1, epithelial membrane antigen (EMA) (antibody MUC-1), CD56, and immunoglobulin light chains can be used to divide these tumors into 3 groups. An ALK-negative, $CD30^-$, EMA-negative, $CD56^{+/-}$ phenotype may be seen in a rare large B-cell lymphoma with microvillous features on ultrastructural examination.[125,126] An ALK^+, $CD30^-$, EMA^+, $CD56^-$, $CD57^{-/+}$, $CD4^+$, IgA^+ phenotype has been described in ALK^+ large B-cell lymphoma.[127,128] Finally, $CD30^+$ large B-cell lymphomas with variable cytology (anaplastic or nonanaplastic) and variable EMA expression are now included in the DLBCL category in the WHO classification.[129,130]

PBLS OR LYMPHOMAS WITH PLASMACYTIC DIFFERENTIATION

Plasmablastic tumors are composed of large cells with abundant, often eosinophilic, cytoplasm and immunoblastic (central prominent nucleolus), anaplastic, or plasmacytoid morphology.

The classification of tumors with plasmablastic morphology has become increasingly complex.[131,132] Important features to subclassify these neoplasms include the clinical site (oral cavity, body cavity, etc), morphologic spectrum (pure immunoblasts vs mixture of immunoblasts, plasmacytoid cells, and plasma cells), differential antigen expression (CD20, CD138, immunoglobulin, CD30, and CD56), and association with viruses (Table 2). Tumors with plasmablastic morphology typically occur in patients with an abnormal immune state (HIV positive, posttransplantation, or the elderly). These tumors often arise in the oral cavity[133] or other mucosal sites of the head and neck, or body cavity (PEL),[134] or in association with multicentric Castleman disease.[135] In addition, a Kaposi sarcoma-associated herpesvirus–positive solid lymphoma/ extracavitary PEL/human herpesvirus 8–associated DLBCL has been described, predominantly in HIV-positive patients and shows coexpression of EBV.[136,137] Atypical BL with plasmacytoid differentiation is seen in HIV-positive patients, representing approximately 20% of AIDS-related NHL.

Other tumors with plasmablastic morphology and less association with an immunocompromised state include PBL with plasmacytic differentiation defined by Colomo et al[131] as prominent immunoblasts or plasmablasts but with some admixed smaller cells with plasmacytic differentiation and by little or weak expression of CD20, DLBCL with prominent plasmablastic/secretory differentiation, pyothorax-associated lymphoma,[138] and PCM with a dysplastic, plasmablastic appearance. As mentioned earlier, a small subset of DLBCLs with plasmablastic/secretory features express ALK and EMA (ALK-positive DLBCL), contain IgA λ or κ, variably express CD4 with some heterogeneous expression of CD57, and lack CD30.[127,128]

Morphologically, the PBL of the oral cavity and the rare ALK-positive DLBCL are composed of a very monomorphic, sheetlike proliferation of immunoblasts. The other plasmablastic tumors tend to have a predominance of immunoblasts but show some cells with plasmacytic differentiation. The body cavity and extracavitary PELs have significant nuclear pleomorphism with plasmacytoid morphology in some cells. PBL with plasmacytic differentiation and DLBCL with secretory differentiation (immunoblasts and plasmacytoid cells) are distinguished by the presence of centroblasts in the latter.

Approximately 8 to 12 immunostains and EBER in situ hybridization are often necessary to distinguish B-cell neoplasms with plasmacytic differentiation (Table 2). CD138 and MUM-1, markers of post germinal center/terminal B-cell/plasmacytic differentiation, are useful in identifying the B-cell origin of these tumors that show variable or negative expression of CD20 and CD79a. Expression of Pax-5 has not been investigated in a significant number of cases to be informative at the present. Epstein-

Table 2. Immunophenotype of Lymphoid Neoplasms With Plasmablastic Features in Paraffin-Embedded Material*

Marker	PBL, Oral Type and Other†	PBL With PCD‡	PBL With Multicentric CD§	DLBCL With PCD‖	ALK+ DLBCL	PEL	PAL	Plasmablastic Plasma Cell Myeloma
CD20	−/+	−/+	+/−	+	−	−	+	−/+
CD79a	+/−	+	NT	NT	−	+	+	+/−
CD138	+	+	+	−/+	+	+	−/+	+
MUM-1	+	+	NT	NT	NT	+	+	+
Ig expression	+/−	+	+ (IgM/λ)	+	+ IgA	−	+	+
CD56	−/+	+/−	−	−	−	−	−	+
EBV	+	+	−	−	−	+	+	−/+ ¶
HIV	+	+/−	+	−	−	+	−	−
HHV-8/KSHV	−#	−**	+	−	NT	+	−	−
ALK	−	Rare +	−	−	+	−	−	−
EMA	+	+/−	NT	−	+	+	NT	+
CD30	+/−	−/+	−	−	−	+	+	−/+
CD10	+/−	NT	NT	+/−	NT	−	−	+/−

* PBL indicates plasmablastic lymphoma; PCD, plasmacytic/plasmablastic/immunoblastic "secretory" differentiation; CD, Castleman disease; DLBCL, diffuse large B-cell lymphoma; PEL, primary effusion lymphoma; PAL, pyothorax-associated lymphoma; −/+, 5% to 25%; +/−, more than 25% to 50%; +, more than 50%; −, less than 5%; NT, not tested in a large number of cases; Ig, immunoglobulin; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HHV-8/KSHV, human herpesvirus 8/Kaposi sarcoma–associated herpesvirus; and EMA, epithelial membrane antigen.

† Approximately 50% arise in sites other than the oral cavity; most are extranodal mucosal sites of the head area with a smaller number in lymph nodes.[131]

‡ Defined as predominantly plasmablasts with more mature plasma cells and some large lymphocytes but with little expression of CD20.[131]

§ Clinical presentation is in lymph nodes and spleen with frequent leukemic involvement.

‖ PBL with plasmacytic differentiation and DLBCL with PCD are distinguished by stronger expression of CD20 and the presence of more centroblasts in the latter.

¶ EBV infection in plasma cell neoplasms (PCM or plasmacytoma) may be related to anatomic location (head and neck) and the presence of EBV in the nasopharynx.[131]

Somewhat controversial; most series report as negative (see references).[202, 203]

** Rare positive cases recently described in HIV-negative patients[137] and in a small number of HIV-positive patients.[136]

Barr virus is strongly associated with PBL of the oral mucosa, PEL and extracavitary PEL/human herpesvirus 8–associated large B-cell lymphoma, and pyothorax-associated B-cell lymphoma. Cyclin D1, expressed in 20% to 40% of PCM cases, is also present in approximately 12% of plasmablastic tumors associated with PCM.[131] PBL with plasmacytic differentiation has EBV expression in approximately one third of patients. Lack of EBV is characteristic of most cases of DLBCL with plasmacytic/secretory differentiation (both ALK positive and ALK negative) and PCM. Lack of detection of cytoplasmic immunoglobulin expression and the presence of CD30 and human herpesvirus 8/Kaposi sarcoma–associated herpesvirus would favor a PEL. A subset of PCM has dysplastic or even plasmablastic morphology but is EBER negative, has bone involvement, often expresses CD56, and rarely expresses cyclin D1. Although both PEL and PBL associated with multicentric Castleman disease are human herpesvirus 8 positive, the tumors appear different as the former arises from late germinal center or post germinal center B cells and the latter from λ-restricted naïve B cells with unmutated immunoglobulin genes. Occasionally, T-cell antigens can be expressed in tumors with plasma cell/plasmablastic features and lead to confusion when a limited panel of markers is used, particularly in tumors that lack CD20.[127,138] Aberrant antigen expression may be attributed to the lack of *Pax-5* gene expression in cells with plasmacytic differentiation.

T- AND NK-CELL PROLIFERATIONS

T-cell and NK-cell neoplasms are difficult to diagnose because they are uncommon (representing 10%–15% of lymphoid tumors in the western hemisphere), often arise at extranodal sites, and have a somewhat reactive appearance because of a mixture of large and small lymphocytes with numerous inflammatory cells in some cases. There are no morphologic features that are diagnostic of T-cell differentiation comparable to plasma cells in the B-cell lineage. In addition, there are no specific routine immunologic markers of T-cell clonality like immunoglobulin light chain expression in B-cell neoplasms. Sophisticated TCR-V_β gene use analysis by flow cytometry with the detection of preferential use of one V_β gene family suggests a clonal population[139]; however, this is not widely used and is not currently possible by IHC in fixed tissue. If $CD3^+$ T cells are increased, staining for CD4, CD8, CD2, CD5, and CD7 is useful. The presence of a markedly skewed CD4/CD8 ratio (in the absence of HIV or other viral infection), loss of pan–T-cell antigens (CD2, CD3, CD5, CD7), lack of CD4 and CD8, or dual expression of CD4 and CD8 may indicate a T-cell neoplasm. Decreased CD7 expression can be seen in reactive conditions, particularly in the skin, and care should be taken in assuming a $CD7^-$ population is neoplastic; however, having said that, CD7 is often lost in mycosis fungoides (MF) and adult T-cell leukemia/lymphoma as well. It should be noted that normally expression of CD7 in paraffin is weaker than other T-cell antigens. Loss of CD3 or CD5 is more significant and supportive of a T-cell neoplasm or a NK-cell proliferation/neoplasm.

When to Suspect NK-Cell Malignancies and How to Determine NK-Cell Lineage

A NK-cell proliferation (extranodal NK/T-cell lymphoma, nasal type, and aggressive NK-cell leukemia involving extramedullary sites) should be considered at extranodal sites (particularly in nasal, mucosal/gastrointestinal sites, and skin lesions) if there is angioinvasion and angiode-

struction and zonal necrosis, if the lymphocytes contain cytoplasmic granules on Wright stain, if NK-cell associated antigen CD56 is expressed, or if EBV is present. As there are no specific markers of NK-cell lineage, the diagnosis of an NK-cell neoplasm is made after a T-cell lineage is excluded. NK cells and cytolytic T cells express cytolytic granule proteins (TIA-1, perforin, granzyme B) and NK-associated antigens CD56 or CD57 (Figure 4, A through F). NK cells lack CD5 and surface CD3 as detected by flow cytometry; it is very important to recognize that cytoplasmic expression of the CD3ε chain, as detected by routine IHC analysis, can be present in NK cells (Figure 4, A). Immunohistochemistry detection of the αβ TCR protein with the antibody β-F1 is definitive evidence of T-cell lineage and would exclude an NK-cell origin. However, if there is no staining with β-F1, a γδ T-cell origin has to be considered (such as that seen in hepatosplenic lymphoma or in cutaneous γδ T-cell lymphoma) or loss of the αβ TCR protein as seen in ALCL.[19] At the present time, no reliable paraffin-reactive antibody to detect the T-cell γδ receptor protein is available. Most γδ T-cell tumors and NK-cell tumors have a $CD4^{-}CD8^{-}$ or a $CD4^{-}CD8^{+}$ phenotype, and γδ T-cell tumors often lack CD5. *TCR* gene rearrangement by PCR analysis may be required to discriminate between a cytotoxic T-cell and a NK-cell proliferation. Even then, approximately 10% to 20% of T-cell neoplasms can lack a detectable *TCR* gene rearrangement.

Classification of PTCL According to T-Cell Subset (CD4 or CD8)

After an immature T-cell proliferation is excluded by the lack of blast morphology and lack of TdT or CD34 expression, the diagnosis of a T-cell malignancy begins with determination of the subset phenotype.

Tumors With a $CD4^{+}$ T-Cell Phenotype.—The majority of T-cell lymphomas (MF, adult T-cell leukemia/lymphoma, AILT, ALCL, and most PTCL, unspecified) have a $CD4^{+}$ phenotype. Differential morphologic features and antigen expression in these tumors are listed in Table 3.

Strong CD30 expression (membrane and focal cytoplasmic dotlike or Golgi-associated staining) in virtually every cell is a defining feature of ALCL. However, CD30 is an activation antigen and can be expressed in reactive conditions (infectious mononucleosis, drug reactions, bug bites, etc), and in AILT, transformed MF, a subset of DLBCL, HL, and nonlymphoid tumors such as embryonal carcinoma, pancreatic carcinoma, malignant melanoma, mesenchymal tumors, malignant mesothelioma, and AML.[140–145] In most NHLs other than ALCL, only a subset of large cells is $CD30^{+}$. In melanoma and AML, the immunoreactivity is more diffuse and cytoplasmic rather than the distinct membrane and Golgi expression seen in ALCL, HL, and embryonal carcinoma. Other antigens expressed in ALCL include EMA and the cytolytic granule protein TIA-1 and granzyme B. Epithelial membrane antigen is best detected with the E29/MUC1 antibody that detects all forms of EMA irrespective of its degree of glycosylation.[146]

ALK protein, most commonly activated by the translocation t(2;5)(p23; q35) or other cytogenetic abnormalities involving the *ALK* gene (2p23), is present in 60% to 80% of ALCLs with most cases in children and young adults. ALK expression may also be present in inflammatory myofibroblastic tumors (resulting from other ALK cytogenetic abnormalities) and in some tumors of neural origin.[147–149] ALK expression is typically nuclear and cytoplasmic (seen with the 2;5 translocation), but in approximately 20% of cases a diffuse cytoplasmic, punctate or membranous pattern can be seen because of involvement of translocation partners other than the *NPM* (nucleophosmin) gene located at 5q35.[148] Small cell variant and lymphohistiocytic ALCL variants may be difficult to diagnose as there are only a minor population of large $CD30^{+}$ cells; ALK-1 is expressed in more than 80% of these ALCLs with variant histology.[147] The presence of $CD30^{+}$ large cells preferentially distributed around vessels rather than randomly scattered is also characteristic of the small cell variant.[150] ALK-negative ALCL should only be diagnosed when there is a pleomorphic large cell infiltrate with strong membrane and Golgi-associated dotlike CD30 expression in virtually every cell. Bone marrow examination for staging of ALCL should include immunostaining for CD30 and ALK as tumor involvement is often subtle with only scattered tumor cells.[151,152] Although bone marrow involvement in ALCL is seen overall in only 10% to 25% of patients, the lesions are often subtle requiring immunostaining for CD30 and ALK-1 (if the tumor is ALK positive) for detection.[151–153] In the small cell variant, 50% or more of the patients may have marrow involvement.[150] It has been shown that bone marrow involvement is associated with a significantly lower survival.[151]

The morphologic diagnosis of AILT is supported by characteristic IHC findings.[154–159] A subset of the $CD4^{+}$ T cells expresses CD10, BCL-6, and chemokines CXCR3 and CXCL-13 (in a perivascular and perifollicular pattern). CXCL-13 is normally expressed in T cells and dendritic cells and histiocytes in the germinal centers and in single, evenly distributed histiocytes and small lymphocytes in the paracortex of reactive lymph nodes.[157] The presence of expanded FDC meshworks that wrap around arborizing vessels, as demonstrated by immunostaining for CD21, CD23, or CD35, is another characteristic feature of AILT (Figure 1, D). $CD20^{+}$ large B cells are present and can mimic a DLBCL (such as T/HRBCL) or HL; the large B cells are EBV (EBER) positive in approximately 80% to 95% of cases. The differential diagnosis of AILT includes T/HRBCL and HL. T/HRBCL has numerous admixed $CD8^{+}$ T cells, is EBV negative, and lacks the expansion of FDC meshwork. The background T cells in HL should have a normal cytologic appearance, express CD4, and lack expression of CD10, BCL-6, and CXCL13.

Mycosis fungoides is a clinicopathologic diagnosis. The presence of dysplastic cerebriform lymphocytes, particularly in the epidermis, along with Pautrier microabscesses is the most specific but least sensitive criteria.[160] Frequently, cerebriform lymphocytes surrounded by halos line up along the dermal-epidermal junction. Immunohistochemistry is useful in demonstrating a predominant population of $CD4^{+}$ T cells with variable loss of CD7 and CD5.[161] A few $CD30^{+}$ large cells are often present in MF and strong expression of CD30 is seen in approximately 20% of large cell transformation of MF.[162–164] The differential diagnosis of a $CD30^{+}$ large cell lymphoma should include transformed MF.

Tumors With a $CD8^{+}$ T-Cell Phenotype.—Neoplastic $CD8^{+}$ proliferations predominantly involve extranodal sites and may be T cell or NK cell in origin.[165] It should be remembered that a small subset of lymphomas that are characteristically $CD4^{+}$ (eg, MF, ALCL) can have a $CD8^{+}$ phenotype and otherwise typical clinical and morphologic

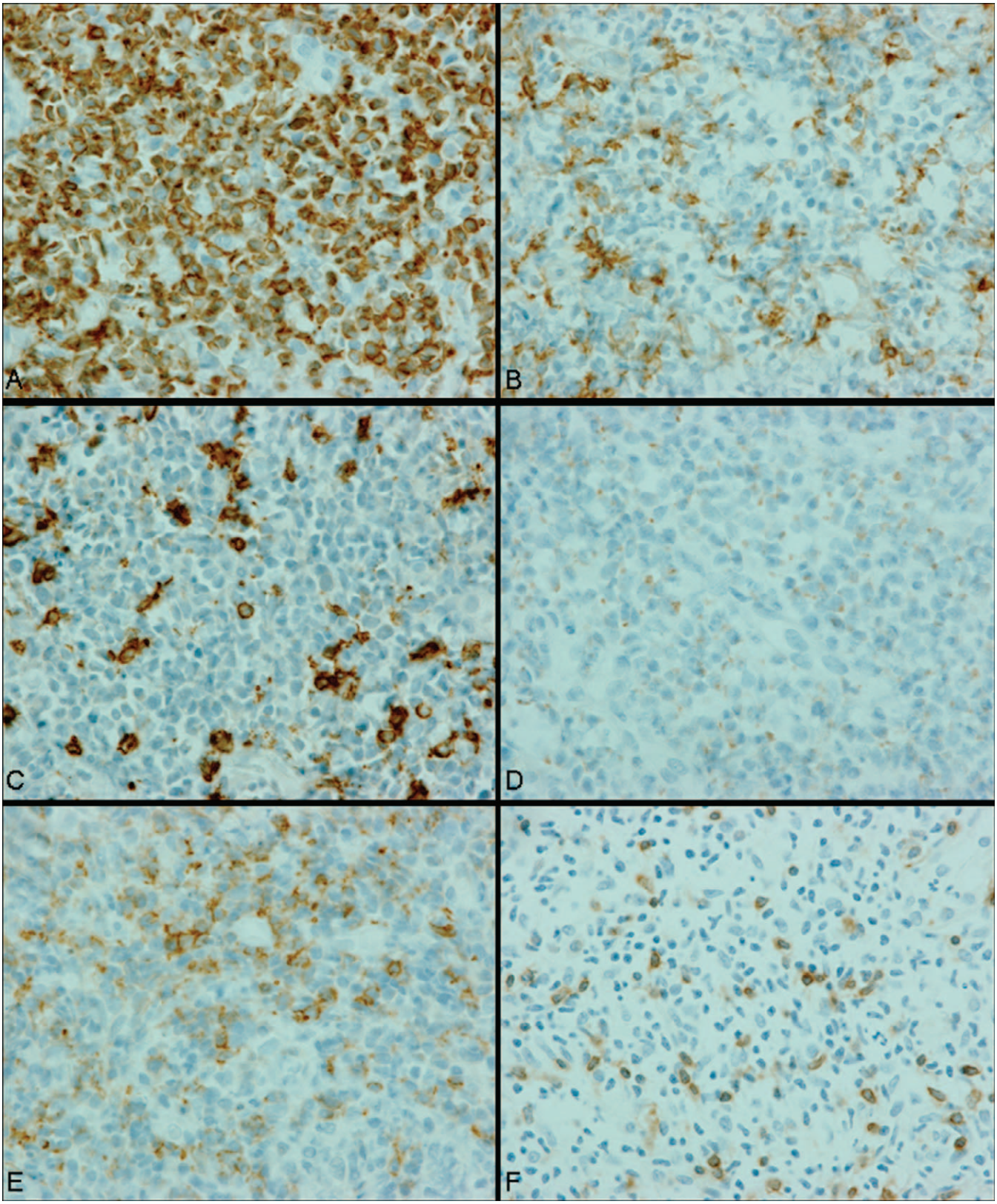

Figure 4. *Neoplasms of natural killer (NK)–cell origin have overlapping antigen expression with other tumors of cytotoxic lymphocyte origin (NK-like T cells), particularly γδ T-cell tumors (eg, hepatosplenic lymphoma, cutaneous γδ T-cell lymphoma, and subsets of other extranodal T-cell tumors). This figure illustrates the typical antigen profile of a nasal-type NK/T-cell lymphoma of true NK-cell origin diagnosed in the small intestine. A, Cytoplasmic CD3 (the ε chain) is nonspecific for lineage determination and can be in NK cells in fixed tissue, as shown here, as well as in T cells (original magnification ×600). B and C, The tumor cells lack CD4 and CD8, respectively (original magnifications ×600). D, The tumor cells express the cytolytic granule protein TIA-1 (original magnification ×600). E, Tumor cells express CD56 (original magnification ×600). F, Tumor cells lack expression of the αβ T-cell receptor protein (β-F1 immunoperoxidase, original magnification ×600). Absence of a clonal T-cell population on molecular studies excluded γδ T-cell neoplasm. The tumor cells were also Epstein-Barr virus–encoded RNA positive (not shown).*

Table 3. Morphologic and Immunophenotypic Markers Useful in Distinguishing CD4+ T-Cell Proliferations in Paraffin-Embedded Material*

Marker	MF	ATLL	AILT	ALCL
Cell cytology/morphology	Epidermotropic, dysplastic, cerebriform lymphocytes; Pautrier microabscesses	Folded "flower- like" nuclei; can be epidermotropic	Clear cells; immunoblasts; expanded FDC meshwork; arborizing HEV	Hallmark cells; sinus growth
CD10	−	−	+	−
BCL-6	−	NT	+	+/−
CXCL13	NT	−	+	−
CD21	−	−	Expanded FDC meshworks	−
CD30	Some large cells +†	−/+	Some scattered large cells +	++‡
TIA-1	Some large cells +†	−	−	+/−§
ALK-1	−	−	−	+ (60%–80%)‖
EMA	−	−	−	+/−§
EBER	−	−/+	+ large cells	−
HTLV-1 serology	−	+	−	−

* MF indicates mycosis fungoides; ATLL, adult T-cell leukemia/lymphoma; AILT, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; FDC, follicular dendritic cell; HEV, high endothelial venule; −, less than 5%; +, more than 50%; NT, not tested in a large number of cases; +/−, more than 25% to 50%; EMA, epithelial membrane antigen; EBER, Epstein-Barr virus–encoded RNA; −/+, 5% to 25%; and HTLV-1, human T-lymphotropic virus.

† More numerous after transformation to large cell lymphoma (>25% or masses of large cells with dispersed chromatin).

‡ Virtually every cell is $CD30^+$ with membrane and cytoplasmic "dotlike" Golgi-associated pattern.

§ More often in ALK-positive ALCL.

‖ More than 90% in pediatric ALCL; less frequent in adult series.

Table 4. Morphologic and Immunophenotypic Markers Useful in Distinguishing Extranodal Lymphomas With T-Cell or Natural Killer (NK)–Cell Phenotype in Paraffin-Embedded Material*

Marker	Subcutaneous Panniculitis-Like TCL	Cutaneous γδ TCL†	Hepatosplenic TCL	Nasal or Nasal-Type NK/TCL‡	Enteropathy-Type TCL
Distinctive morphologic features	Subcutaneous with a lacelike lobular pattern	Subcutaneous with some dermal and less often epidermal involvement	Sinus infiltration; sparing of the hepatic periportal area	Angiodestruction and invasion; zonal necrosis	Infiltration of the mucosa; evidence of celiac disease in the uninvolved epithelium
T-cell subset phenotype	$CD8^+$	$CD4^-CD8^- > CD4^-CD8^+$	$CD4^-CD8^- > CD4^-CD8^+$	$CD4^-CD8^+$ or $CD4^-CD8^-$	$CD8^+$
CD5	+	−	−/+	−	−
CD56	−	+	+	+	Subset +§
TIA-1	+	+	+	+	+
Granzyme B	+	+	−	+	+
EBER	−	Small subset +	−	+	−

* TCL indicates T-cell lymphoma; +, more than 50%; −, less than 5%; −/+, 5% to 25%; and EBER, Epstein-Barr virus–encoded RNA.

† Provisional category in the World Health Organization classification of skin tumors.

‡ May include some tumors with a γδ T-cell phenotype.

§ Subset with monomorphic small cell morphology.[204]

features. Immunostaining for CD56, CD57, TIA-1, granzyme B, the αβ TCR protein (as detected by β-F1), and EBER should be performed.[165] Many of these tumors have similar cytology and immunophenotype. Thus, the anatomic location and the architecture of the infiltrate become important in the classification. (See Table 4 for the characteristic morphologic features and antigen expression pattern of these neoplasms.)

Tumors With a $CD4^-CD8^-$ Phenotype.—A $CD4^-CD8^-$ phenotype may be seen in γδ T-cell lymphoma and in precursor T-ALL/LBL and rare, small subsets of other T-cell lymphomas (resulting from aberrant T-cell antigen loss). Patients with autoimmune lymphoproliferative syndrome have a reactive polyclonal expansion of double negative T cells. As mentioned earlier, there is no paraffin-reactive antibody that detects the γδ TCR protein. Absence of the αβ TCR protein, as demonstrated by lack of immunoreactivity with the antibody β-F1, is taken as indirect evidence of a possible γδ phenotype or a NK-cell neoplasm or aberrant antigen loss in an αβ T cell (most frequently seen in ALCL).[19] *TCR* gene rearrangement studies may be indicated to confirm a T-cell lineage. Both NK-cell and γδ T-cell malignancies express CD56 and the cytotoxic granule protein TIA-1. Hepatosplenic γδ T-cell lymphoma typically lacks granzyme B (ie, has a nonactivated cytotoxic T-cell phenotype), whereas most NK-cell tumors express both antigens. Precursor T-ALL/LBL typically expresses TdT and CD34 and may be $CD10^+$ (50%–70% of cases). In addition, some T-ALL/LBLs may express CD1a reflecting the second stage of thymic differentiation.

Potential Use of EBER In Situ Hybridization in Staging of Extranodal NK/T-Cell Lymphoma, Nasal Type

A recent study from Taiwan has emphasized the importance of EBER testing in staging bone marrow biopsies from patients with extranodal NK/T-cell lymphoma of nasal type.[166] Occult tumor involvement was detected in 43% of patients and was associated with a lower survival. The

Table 5. Immunophenotype of Tumors With Blast Morphology in Paraffin-Embedded Material*

Marker	AML	B-ALL/LBL	T-ALL/LBL	CD56+ Hematodermic Neoplasm†	MCL	FL
CD20	−	−/+	−	−	+	+
Pax-5	−/+‡	+	−	−	+	+
CD79a	−/+ or +/−	+	−/+ (10%)	−	+	+
CD2	−/+§	−	+	+/−	−	−
CD4	+/−	−	+/−	+	−	−
CD5	−	−	+	−	+	−
TdT	−/+	+	+	+/−	−	−
CD34	+	+	+	−	−	−
CD10	−	+	+/−	−	−	+
CD43	+	+	+	+	+	−
CD68	+/−	−	−	+ (focal dotlike)	−	−
CD15	−/+	−	−	−	−	−
CD56	−/+	−	−/+	+	−	−
CD123	−/+	−	−	+	−	−
TCL-1	−/+	+	−	+	+	+

* AML indicates acute myeloid leukemia; B-ALL/LBL, precursor B-cell acute lymphoblastic leukemia/lymphoma; T-ALL/LBL, precursor T-cell acute lymphoblastic leukemia/lymphoma; MCL, mantle cell lymphoma (blastoid variant); FL, follicular lymphoma (with blastic morphology); −, less than 5%; −/+, 5% to 25%; +, more than 50%; +/−, more than 25% to 50%; and TdT, terminal deoxynucleotidyl transferase.

† Designated as blastic natural killer–cell lymphoma in the World Health Organization classification; cell of origin is the plasmacytoid dendritic cell.

‡ Expressed in AML with the t(8;21) and other CD19+ AML.

§ CD2 may be expressed in microgranular acute promyelocytic leukemia (AML-M3v).

expression of EBER was an independent variable in predicting overall survival in patients with stage 1 and 2 disease. The bone marrow was interpreted as positive if EBER-positive nuclei were larger than those of admixed small lymphocytes and had irregular nuclear outlines. The median number of EBER-positive cells was 6.7 per high-power field (range, 1.2–17.0 EBER-positive cells per high-power field). However, it should be noted that 1 patient with cutaneous T-cell lymphoma and 1 with aplastic anemia in this study had 4.1 and 4.2 EBER-positive cells per high-power field in their bone marrow specimens as well.

TUMORS WITH BLASTIC MORPHOLOGY

Cells with blastic morphology (finely dispersed chromatin, scant cytoplasm, and often prominent nucleoli) are present in immature B-cell, T-cell, myeloid, and plasmacytoid dendritic cell neoplasms. In addition, some lymphomas (eg, follicular and mantle cell) also have ''blastic or blastoid variants.''[167,168] Blastic neoplasms are distinguished based on immunophenotype and may require a relatively large panel (Table 5). Blastic variants of MCL and FL are recognized by the expression of antigens typically seen in these lymphomas (see ''Small B-Cell Neoplasms''). Rare cases of immature B-cell neoplasms (lack of surface immunoglobulin and presence of TdT) arising in patients with FL have been described.[169]

Tumors of plasmacytoid dendritic cells (originally called blastic NK-cell lymphoma, now also known as CD4+CD56+ hematodermic neoplasm) are relatively uncommon, often present in the skin, and are recognized based on their expression of CD4, CD56, CD123 (IL-3 receptor α chain), and T-cell leukemia 1 and generally lack specific myeloid antigens (CD13, CD15, CD33).[170,171] T-cell leukemia 1 may be expressed in a small percentage of AMLs and is also expressed in B lymphoblastic lymphoma, T prolymphocytic leukemia, MCL, FL, CLL/SLL, BL, and approximately 60% of DLBCLs.[172] CD123 expression has been reported in chronic myelomonocytic leukemia and a subset of AMLs.[173,174] Acute myeloid leukemia, particularly with monocytic differentiation, can express CD4 and CD56[175] and should be distinguished from plasmacytoid dendritic cell tumors based on its strong expression of myeloid antigens such as CD15 and CD68; CD68 expression in plasmacytoid dendritic cell tumors is focal, granular, or punctate in the Golgi region of the cells.

Although CD79a is the B-cell marker of choice in evaluation lymphoblastic processes, it should be noted that CD79a is not entirely lineage specific and can be expressed in 10% of precursor T-ALL/LBL[13] and overall in approximately 10% of AML[9] with the highest incidence in acute promyelocytic leukemia. Immunoreactivity in AML appears variable according to the antibody clone and detection methods.

HODGKIN LYMPHOMA

Hodgkin lymphoma is diagnosed based on the morphology of the Reed-Sternberg (RS) cells, the character of the inflammatory background, and the immunophenotype of the RS cells. In the WHO classification HL is broadly divided into NLPHL with a CD45RB+ (leukocyte common antigen), CD20+, EMA+/−, EBER− phenotype and CHL with a CD45RB−, CD30+, CD15+, EBER+/− phenotype (Table 6). CHL is further subclassified into nodular sclerosing, mixed cellularity, or the rare (<5%) lymphocyte-rich and lymphocyte-depleted subtypes. CD30 expression is present in virtually all (and a small number of lymphocyte predominant HL) and CD15 expression is reported in 59% to 93% of CHL cases.[176,177] CD15 is not specific for RS cells and may be expressed in granulocytes, atypical cells in cytomegalovirus infection,[178] approximately 10% of ALCLs, rare PTCLs that express CD15 and CD30 (some of which have HL-like morphology),[179] AMLs, and up to 60% of carcinomas.

Distinguishing NLPHL and CHL

Overlap in morphologic features (areas of diffuse growth in NLPHL and nodular growth in classical lymphocyte-rich CHL) and antigen expression between NLPHL and CHL can cause confusion in the diagnosis. Approximately 25% to 40% of CHLs have some expression

Table 6. Immunophenotypic Markers to Distinguish Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL), Classic Hodgkin Lymphoma (CHL), and Other Large Cell Lymphomas in the Differential Diagnosis*

Marker	NLPHL	CHL	ALCL	T/HRBCL
CD20	+	−/+	−	+
CD79a	+/−	−/+	−	+/−
Pax-5	+	+ (weak)	−	+
MUM-1	−/+ weak or −	+	+, small number tested	NT
BCL-2	−/+	variable 7%–56%	− in ALK$^+$ ALCL; + in ALK$^-$ ALCL	+/−
BCL-6	+	+/− (weaker, fewer cells +)	+/−	+
CD15	−	+	−/+	−
CD30	−/+ (usually <10%)	+	+	−/+ (weak)
Clusterin	−	+/−, membranous	+, dotlike cytoplasmic	−/+ (usually <10%)
Fascin	−	+, strong cytoplasmic	+, weak cytoplasmic	NT
EMA	+/−	−/+	+/− (higher in ALK$^+$ ALCL)	+/−
ALK-1	−	−	+/−†	−

* ALCL indicates anaplastic large cell lymphoma; T/HRBCL, T-cell/histiocyte rich B-cell lymphoma; +, more than 50%; −/+, 5% to 25%; −, less than 5%; +/−, more than 25% to 50%; NT, not tested in a large number of cases; and EMA, epithelial membrane antigen.
† More than 90% in pediatric ALCL; less frequent expression in adult series.

of CD20, which usually shows variable intensity in a subset of RS cells.[180,181] If strong expression of CD20 is present in every large cell, a large B-cell lymphoma, such as a T/HRBCL, should be considered. Epithelial membrane antigen is detected in approximately 5% of CHLs.[177] Markers useful in distinguishing NLPHL and CHL include BCL-6, which is strongly expressed in NLPHL and weak in CHL. MUM-1 is present in most CHLs but only in a small number of NLPHLs in which the lymphocytic and histiocytic (L&H) RS cells are weakly positive.[100,182,183] Fascin, an actin bundling protein, is expressed in a strong, diffuse cytoplasmic pattern in RS cells of CHL and is negative in NLPHL.[184] CD57$^+$ T cells often ring the L&H cells in NLPHL, a feature not seen in most CHLs. Epstein-Barr virus is detected in the RS cells in 50% to 60% of CHLs, most often in the mixed cellularity subtype[185] and in less than 10% of NLPHLs.

Evaluation of the presence or absence of transcription factors is also useful in distinguishing CHL from NLPHL and NHL. Pax-5 is expressed in 90% to 98% of RS cells in CHL and 100% of RS cells in lymphocyte predominant HL.[11,186] In CHL the staining intensity varies from weak to moderate and is rarely strong compared with the normal reactive small lymphocytes. In lymphocyte predominant HL the staining is as strong as the small B cells in at least some of the tumor cells in approximately 60% of the cases.[186] A combination of Oct-2 and Bob1 staining can be helpful.[2] Oct-2 and Bob1 bind to an ATGCAAAT octamer motif that is present in immunoglobulin gene promoters and most enhancers. Oct-2 is normally highly expressed in germinal center B cells and to a lesser degree in normal mantle zones. Some monocytoid B cells and splenic marginal zones show strong Oct-2 signals. The nodal marginal zone expression is weaker. Bob1 shows strong nuclear and weak cytoplasmic expression in the germinal center cells and scattered rare mantle zone, marginal zone, and monocytoid B cells are positive. Both Oct-2 and Bob1 are present in plasma cells and weakly in interfollicular B cells and can stain thymocytes. In B-cell lymphomas, Oct-2 and Bob1 are expressed in FL, DLBCL, and BL; B-CLL, MALT-type MZL, and MCL are negative or have weak expression. Oct-2 can be expressed in CLL proliferation centers. In CHL, expression of Oct-2 and Bob1 is down-regulated consistent with the absence of immunoglobulin expression in RS cells. In CHL either Oct-2 or Bob1 can be expressed in approximately 25% to 50% of cases, whereas both transcription factors are positive in DLBCL and in NLPHL.[99,187,188] Lymphocyte-rich CHL can have expression of both Oct-2 and Bob1.[182] PU.1 is rarely expressed in CHL and is usually present in NLPHL; its variable expression in DLBCL and staining of histiocytes, which makes interpretation more difficult, has limited its general use.[189,190]

Distinguishing HL From NHL

Most cases of HL are readily distinguished from NHL based on the presence of a minor population of diagnostic RS cells, a characteristic predominant reactive background, and the typical immunophenotype of the RS cells. The presence of numerous RS cells or atypical morphologic or immunophenotypic features in some HL suggests the possibility of NHL. In a subset of NLPHL the presence of L&H cells outside of B-cell nodules in a background rich in T cells brings up the differential diagnosis of T/HRBCL.[112,191] Important distinguishing features include the presence of a partly nodular pattern with FDC meshwork and CD57$^+$CD4$^+$ T cells ringing the L&H cells in NLPHL, and the predominance of background cytotoxic CD8$^+$, TIA-1$^+$ T cells, lack of FDC meshwork, and scarcity of small B cells in T/HRBCL. In addition, clonal cytoplasmic immunoglobulin light chain can often be detected by routine IHC in the large tumor cells in T/HRBCL, and a clonal immunoglobulin heavy chain rearrangement would be present. Approximately 27% of NLPHL have IgD expression in L&H cells (which is expressed in only 5% of T/HRBCL), and in almost 70% of these cases the L&H cells were predominantly extrafollicular; clinically these cases were predominantly young males with a median age of 21 years.[192]

The syncytial variant of nodular sclerosing HL and the reticular variant of lymphocyte-depleted HL have numerous RS cells. If the tumor cells lack or show weak expression of CD15, then a CD30$^+$ NHL such as ALCL or a CD30$^+$ DLBCL should be considered. The latter would be diagnosed if there were expression of CD45RB (leukocyte common antigen), strong expression of CD20 in virtually every cell, and coexpression of Oct-2 and Bob1. Distinguishing ALCL, particularly with a null phenotype, would be more difficult. Some ALCLs have a nodular growth pattern and fibrosis, and a small subset can be CD15$^+$. The combination of clusterin, fascin and Pax-5 is useful in distinguishing ALCL and CHL. Clusterin, a ubiquitous

glycoprotein, is highly expressed in ALCL by DNA microarray analysis[193] and IHC.[194–196] Clusterin is distributed in a distinctive perinuclear, cytoplasmic dotlike pattern in approximately 90% to 100% of systemic ALCLs and in 40% to 50% of primarily cutaneous ALCLs.[195,196] Clusterin is also present in approximately 50% of CHLs, but the pattern is primarily membranous. Fascin is strongly expressed in the cytoplasm of RS cells but is also weakly expressed in approximately two thirds of ALCLs. Pax-5 is potentially useful in distinguishing HL from ALCL, as PTCL, including ALCL, is Pax-5 negative and CHL has weak expression in RS cells. A small number of ALK-positive ALCLs resemble nodular sclerosing HL,[197] but most ALCLs resembling nodular sclerosing HL lack ALK and other characteristic ALCL antigens such as CD4, TIA-1, and EMA. TIA-1 expression has been described in approximately 10% of HLs.[198]

When considering a diagnosis of HL, it is important to carefully examine the small lymphocytes in the reactive background to confirm they are cytologically normal and have the characteristic immunophenotype (ie, predominantly B cells particularly in the nodules of lymphocyte predominant HL and $CD4^+$ T cells in CHL). Some cases of AILT (with $CD30^+$, EBV^+ RS-like cells) and a very rare $CD15^+CD30^+$ PTCL can mimic HL,[179] but these lymphomas are distinguished by the presence of dysplastic small to medium-sized lymphocytes and a clonal *TCR* gene rearrangement. In AILT, expanded FDC meshworks are prominent, and the T cells often express CD10, BCL-6, and CXCL13.[154,155,157] It should be recognized that approximately 5% of typical CHL cases express T-cell antigens (CD2>CD4>CD3>CD5>CD8; CD7 expression has not been seen), but most of these cases have a clonal B-cell receptor gene rearrangement (by microdissection and immunoglobulin heavy chain gene analysis by PCR), and only very rare CHLs show a true T-cell genotype.[199–201]

Lastly, further difficulty in the diagnosis of HL may arise because some B-cell lymphomas have tumor cells with RS-like morphology (T/HRBCL, mediastinal large B-cell lymphoma, some PBLs, and rarely small B-cell lymphomas such as CLL/SLL) and/or express CD30 (MLBCL, a subset of DLBCLs, and PBL including pyothorax-associated B-cell lymphoma and PEL, Table 2). These lymphomas will be recognized by their previously described immunophenotype and the presence of a clonal immunoglobulin heavy chain rearrangement.

CONCLUSIONS

Immunophenotyping, although indispensable in the diagnosis and classification of hematopoietic and lymphoid neoplasms, has to be used cautiously with knowledge of the antibodies used. No antigen is totally lineage or lymphoma specific, and for this reason, immunostaining must be performed in the context of a panel. Each lymphoid neoplasm has a characteristic immunophenotype, but a potential pitfall is the small number of otherwise typical cases that can express phenotypic markers of other neoplasms or lack their characteristic markers. In addition, familiarity with the diagnostic criteria and differential diagnosis of each lymphoid tumor and ultimately correlation with morphology, ancillary molecular genetic and cytogenetic/FISH studies, and clinical history are essential to confirm the diagnostic impression.

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