Lymphoma Immunophenotyping: A New Era in Paraffin-Section Immunohistochemistry

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Summary: Recent advances in immunohistochemistry have made it possible to investigate lymphomas for the expression of a wide range of antigens in fixed tissues. Epitope retrieval, sensitive detection methods, and the availability of new monoclonal antibodies have all contributed to one’s ability to perform detailed immunophenotyping that previously could only be done in cryostat sections or by flow cytometry. Current lymphoma classifications make use of characteristic immunophenotypic profiles that aid in the reproducible diagnosis and subclassification of these neoplasms. The following is a review of the current state of immunophenotyping for lymphoid neoplasms in fixed tissues. Key Words: Immunohistochemistry—Lymphoma—Non-Hodgkin lymphoma—Hodgkin lymphoma—Heat-induced epitope retrieval—Monoclonal antibody

The diagnosis and subclassification of lymphomas has undergone a gradual transformation over the last 20 years from a purely morphologically based system (1) to a system that makes use of the knowledge gained in immunology and molecular biology over this period. The most recent classification system, the revised European and American Lymphoma Classification of Lymphoid Neoplasms (REAL classification)(2) and the closely related World Health Organization (WHO) classification (3) detail the common immunophenotypic and molecular genetic features. These features are used in everyday practice by pathologists in community practice as well as large academic medical centers.

Although standard hematoxylin- and eosin-stained sections still provide the basis for all lymphoma diagnosis, immunophenotyping (IP) is by far the most common ancillary technique used today. Modern hematopathology relies heavily on immunophenotyping both for distinguishing benign from malignant processes as well as for accurate subclassification. In addition, paraffin section IP allows us to make diagnoses on small biopsies that would have previously been deemed inadequate for diagnosis. As we begin a new century, it is now possible to assess formalin-fixed paraffin-embedded tissues for a wider array of antigens than ever before. In addition to numerous new commercially available antibodies, the recent advances in heat-induced epitope retrieval (HIER) now allow one to obtain a detailed immunophenotype in routinely processed lymphomas that was only possible using flow cytometry or frozen section immunostaining. The following is a review of the major diagnostically useful antigens in lymphoma diagnosis for which paraffin-reactive antibodies are available. An attempt is made to list the antigen, briefly describe its lineage specificity, and expand on how it may be useful to the practicing pathologist in the diagnosis or subclassification of lymphomas. This review will be organized into antigens useful in the evaluation of B-cell lymphomas, T/NK-cell lymphomas, Hodgkin’s lymphoma, and finally miscellaneous antibodies.

Antigens Useful in Diagnosis of B-Cell Non-Hodgkin Lymphomas

B-cell NHL is the most common type of lymphoma and thus is encountered by the general pathologist with
some degree of regularity. Advances in the classification of these lymphomas, particularly the B-cell lymphomas of small lymphocytes, are reflected in the most recent classification systems (2,3). In conjunction with the morphologic features, immunophenotypic patterns for these lymphomas are useful with CD5, CD23, cyclinD1, and CD10 expression serving as major discriminators (4). All of these antigens can now be assessed in formalin-fixed, paraffin-embedded tissue. Table 1 lists the common phenotypes of the B-cell NHLs. Since kappa and lambda immunoglobulin (Ig) light chain expression is a marker of monoclonality, demonstration of monotypic light chain expression can be helpful in confirming a diagnosis of lymphoma.

**CD5**

CD5 is a 67 kD glycoprotein that appears to be involved in modulation of T- and B-cell receptor signalling (5,6). It is considered a pan-T cell marker and is present on postthymic T-cells as well as thymocytes. However, it is also present on a small subset of nonneoplastic B-cells (so-called B-1 lymphocytes) that may be increased in autoimmune disorders (7). Its diagnostic utility in B-cell lymphoproliferative disorders is due to CD5 expression on virtually all cases of small lymphocytic lymphomas/chronic lymphocytic leukemias, and the great majority of mantle cell lymphomas (8–10). It is generally absent in other B-cell lymphomas and lymphoproliferative disorders of small lymphocytes, including follicle center lymphoma, marginal zone lymphomas, lymphoplasmacytic lymphoma, and hairy cell leukemia (2). Thus, CD5 expression in B-cell lymphomas serves as an important immunophenotypic discriminator between CLL/SLL, MCL, and the other B-cell lymphomas (4,11).

CD5 (Figures 1, 2, and 3) antibodies immunoreactive in formalin-fixed paraffin-embedded tissue are now available. In particular, the clone 4C7 has been shown in several studies to be a useful antibody for paraffin section IHC (4,12,13). Similar results have been reported using the Leu-1 antibody (originally for frozen tissue) in paraffin-embedded tissue after antigen retrieval and tyramide-enhanced detection (14). 4C7 has been found useful in formalin-fixed tissues with less distinct staining in B5-fixed tissues. While of greatest utility in the differential of small B-cell lymphomas, CD5 expression can occur in large B-cell lymphomas either *denovo* or in the setting of CLL. The significance of CD5 expression in large B-cell lymphoma is uncertain (15); however, some studies have shown CD5 expression in large cell lymphoma to be a poor prognostic factor (16).

**CD10**

CD10 (common acute lymphoblastic leukemia antigen (CALLA)) is a 100kD cell-surface endopeptidase involved in modulating cell responses to peptide hormones (17,18). It is expressed in many cell types including some epithelial cells (liver canaliculi, renal tubules, enterocytes), as well as hematopoietic cells such as lymphoid cells and granulocytes (17,19). It is not lineage restricted, and among normal lymphoid cells, is expressed on precursor cells as well as in mature lymphocytes, particularly germinal center B-cells. Diagnostically, CD10 is useful in characterizing acute lymphoblastic lymphoma/leukemia of T- or B-cell type, follicle center lymphomas, Burkitt lymphoma, and subsets of diffuse large B-cell lymphoma (2). CD10 is expressed in at least 60% and up to 95% of follicle center lymphomas, depending on the series (4,20–22). The authors note occasional follicle center lymphoma (FCL) cases in their practice that express CD10 by IHC that were negative by flow cytometry. In the case of lymphoblastic lymphoma, CD10 is expressed in approximately 90% of B-ALL

<table>
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<tr>
<th>Lymphoma</th>
<th>Immunophenotypic Features</th>
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<tr>
<td>Small lymphocytic lymphoma/chronic lymphocytic leukemia</td>
<td>CD20+, CD5+, CD10−, CD23+, cyclin D1−, bcl-6−</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>CD20+, CD5+, CD10−, CD23+, cyclin D1+ bcl-6+</td>
</tr>
<tr>
<td>Marginal zone lymphoma</td>
<td>CD20+, CD5−, CD10−, cyclin D1+, bcl-6−</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>CD20+, CD5−, CD10+, bcl-6+, bcl-2+, cyclin D1−</td>
</tr>
<tr>
<td>Lymphoplasmacytoid lymphoma</td>
<td>CD20+, CD5+, CD10−, clg+</td>
</tr>
<tr>
<td>Plasmacytoma</td>
<td>CD20−, CD45RB−, clg+, CD79a+</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>CD20+, CD5−, CD10−, clg−, bcl-6+/−, MIB1−</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>CD20+, CD5−, CD10+ bcl-2, MIB1+</td>
</tr>
<tr>
<td>B-lymphoblastic lymphoma</td>
<td>CD20−/+, CD79a+, CD10−/−, TdT+</td>
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Clg, cytoplasmic Ig
**FIG. 1.** Example of SLL/CLL. (A) Hematoxylin and eosin stain showing an infiltrate of small lymphocytes with a proliferation center (right). (B) CD20, (C) CD5, (D) CD23. B-cells coexpress CD5 and CD23 and were negative for cyclin D1 (not shown).

**FIG. 2.** Pleomorphic type of blastoid mantle cell lymphoma. (A) Hematoxylin and eosin, (B) CD20, (C) CD5, (D) cyclin D1. The large B-cells coexpress CD20, CD5 and cyclin D1 (nuclear). The CD5 staining is less intense than the small T-cells present in the infiltrate. This case was originally diagnosed as diffuse large B-cell lymphoma.
and 30% of T-ALL. CD10 expression has been associated with a favorable prognosis, which may be due to its association with other favorable features including cytogenetics (23–26). It is also expressed in the great majority of Burkitt lymphomas (17,27). CD10 can also be seen in a subset of diffuse large B-cell lymphoma (16). The significance of this finding is uncertain but may identify some DLCLs that represent transformed follicle center lymphomas (28).

Until recently, CD10 expression was only assessed using flow cytometry or frozen section immunohistochemistry. Within the last 2 years, we and others have demonstrated the utility of a paraffin-reactive monoclonal antibody whose performance compares favorably to flow cytometry detection of CD10 (4,19,22). Immunoreactivity appears to be membrane and cytoplasmic and is superior in B5-fixed tissues although adequate staining is seen in formalin-fixed tissue.

CD20

CD20 is a 35 kd transmembrane protein involved in signal transduction. It appears to have calcium channel properties and is associated with tyrosine kinases (29,30). It is expressed normally on mature B-cells and a subset of immature B-cells. CD20 is also expressed on the great majority of mature B-cell lymphomas (90% to 100%) (31,32). The L-26 antibody is a commonly used antibody and shows strong membrane immunoreactivity in B-cell lymphomas (33,34). In the authors’ experience the antibody is among the most robust and reliable B-cell markers in diagnostic use. Thus, it is the favored “pan-B” cell antibody. Expression strongly favors a B-cell lineage. Only rare cases of T-cell lymphoma have been reported to express CD20 (35–37). Some cases of Hodgkin lymphomas (HL) express CD20 on Reed–Sternberg cells and their variants. Percentages vary considerably depending on the series (largely due to differences in methodology as well as interpretive criteria) (38–40). Strong expression in the majority of cells probably occurs in 10% to 20% of cases (39). However, since HL is now thought to be of B-cell origin (41), this is not considered aberrant expression.

Following the expression patterns in normal B-cell development, this antigen is not expressed on many B-
demonstrated by several groups (4,22,51 and its diagnostic utility, particularly for SLL, has been
and SLL) (4). CD5-positive lymphomas of small lymphocytes (MCL
dexpression is most useful in the differential diagnosis of
mas such as follicle center lymphoma (4). Thus, CD23
phomas has diagnostic relevance (51). CD23 is ex-
pressed in the majority of small lymphocytic lymphomas
including activated B-cells and its expression on lym-
finity receptor for IgE. It also has homology to calcium-
dependent animal lectins and interacts with other cell-
surface molecules such as CD21, CD11b, and CD11c,
suggesting that CD23 is also involved in cell-cell inter-
actions (50). It is expressed on a variety of cell types
including activated B-cells and its expression on lymph-
has diagnostic relevance (51). CD23 is ex-
pressed in the majority of small lymphocytic lymphomas
(SLL), whereas it is generally absent in mantle cell lym-
phoma. However, CD23 is expressed in other lympho-
mas such as follicle center lymphoma (4). Thus, CD23
expression is most useful in the differential diagnosis of
CD5-positive lymphomas of small lymphocytes (MCL
and SLL) (4).

CD23 expression can be evaluated in paraffin sections
and its diagnostic utility, particularly for SLL, has been
demonstrated by several groups (4,22,51–53) The great
majority of SLL cases (90%) appear to express CD23
(53). The level of expression may be variable and CD23
expression levels appear to be greater in the larger cells
seen in proliferation centers of SLL than in the small
lymphocytes (53,54). In contrast, the great majority of
MCLs (>95%) appear to lack CD23. However, several
cases of MCL have been noted in which CD23
expression varies in the same patient when immunophenotyp-
ing is performed on cells from different sites (e.g., lymph
node and bone marrow) using flow cytometry (55).
These differences have been seen even when sequential
biopsies were taken within 24 hours of each other with-
out interval therapy(personal observation). Thus, as with
any single marker, one must use caution and interpret the
overall immunophenotype in context of the morphologic
findings.

CD23 is also expressed on a subset of follicular den-
dritic cells, those in the light zone of the follicle center.
Thus, CD23 can be useful in demonstrating the follicular
dendritic network, although other markers such as CD21
and CD35 may also be used for this purpose.

CD43
CD43 (leukosialin, sialophorin) is a transmembrane
sialoglycoprotein that functions in cell-cell interactions
(56). Fixation resistant antibodies such as Leu22, MT-1,
and DFT-1 stain T-cells, myeloid cells, plasma cells, and
histiocytes in normal tissues with a membrane reactivity
(57–59). CD43 is expressed in the great majority of T-
cell lymphomas. For example, Said and colleagues found
90% of T-cell lymphomas expressed CD43 (58). How-
ever, it is also useful in the evaluation of small B-cell
lymphomas, since it is aberrantly expressed in approxi-
mately 40% of B-cell lymphomas (60,61). More specifi-
cally, it is expressed in the great majority of CD5+ B-cell
lymphomas (small lymphocytic lymphoma and mantle
cell lymphoma) (4,22,60,62). A lower percentage (20%)
of marginal zone lymphomas of MALT type may also be
positive (63). In a recent large series of cases, CD43
expression was found in a minority of large B-cell lym-
phomas, Burkitt-like lymphomas, and lymphoplasmacy-
tic lymphomas. CD43 is uncommonly seen in follicle
center lymphoma (62).

As can be seen, CD43 expression is not lineage spe-
cific. Lineage assignment should not be done solely on
the basis of CD43 expression alone, since nonlymphoid
malignancies such as granulocytic sarcomas and histio-
cytic neoplasms express CD43 (64).

CD45RA
CD45 is a differentially spliced molecule with mul-
tiple epitopes that demonstrates different degrees of lin-
eage specificity. CD45 restricted (CD45R) epitopes in-
clude CD45RA, CD45RB, and CD45RO (65,66).
CD45RA is preferentially expressed on B-cells and subsets
of T-cells. Using the MT2 antibody, CD45RA is
expressed on normal mantle cells and not on germinal
center cells or marginal zone cells (67). Clone specific
differences in reactivity exist and are probably related to
the carbohydrate component specificity of some of these
antibodies (65). The MT2 antibody recognizes mantle
cells and some T-cells and has been suggested to be
useful in the differential diagnosis of follicular hyperpla-
sia and follicle center lymphoma (68); however, the bcl-2
antibody performs better and is considered more useful
for this purpose (69). The 4KB5 antibody is a pan-B-cell
marker that can recognize many B-cell lymphoma types
(31,70). Using the KiB3 antibody, studies have reported
that splenic marginal zone lymphoma is negative, similar
to normal splenic marginal zone cells (65,71). Pawade, however, reported CD45RA expression in their series of splenic marginal zone lymphomas using the CD45RA antibody 4KB5 (72). Some B-cell lymphomas will lack CD45RA and some T-cell lymphomas will express it (73,74). Given this, and the excellent performance of anti-CD20 and CD79a antibodies, CD45RA antibodies are of secondary importance in routine diagnostic use as a pan-B-cell marker.

**CD79a**

CD79a(mb-1) is one member of the dimer that comprises CD79(mb-1/B29). The other member is CD79b(B29). These molecules are associated with surface Ig and are involved in intracellular signalling (75,76). CD79a is an excellent, lineage-specific marker of B-cells and is expressed at all stages of B-cell differentiation, from B-precursor to mature plasma cell. The JCB117 monoclonal antibody (MoAb) was shown to recognize 97% of B-cell lymphomas with a cytoplasmic pattern (77). Other recent studies also report a very high percentage of B-cell lymphomas of all types to express CD79a (22). Unlike antiCD20, antiCD79a also label most immature B-cell neoplasms (upto 100%) (43,78) and many plasma cell disorders (42,77). Thus, CD79a is the B-cell marker of choice when attempting to characterize a lymphoblastic lymphoma or acute lymphoblastic leukemia by paraffin section IHC.

The strong lineage specificity of CD79a is attested to by the proposal of the European Group for the Immunological Characterization of Leukemias to make CD79a expression worth two points in the flow cytometric scoring for lineage assignment of acute leukemias (79). Of note, one report suggests CD79a is expressed in a significant number of acute promyelocytic leukemias but was not seen in another series using the same clone (78,80). A recent abstract reported expression in some myeloid leukemias, including acute promyelocytic leukemia. This expression was variable and clone dependent, suggesting cross reactivity (81).

In addition, expression of CD79a has also been reported in 10% of T-lymphoblastic lymphomas in one series (82).

**Bcl-2**

The bcl-2 gene, located at chromosome 18q21, encodes a 25 kd inner mitochondrial membrane protein that plays a central role in protecting the cell from apoptosis or programmed cell death. Translocation of this gene with the immunoglobulin heavy chain gene at 14q32 is the most common translocation seen in follicle center lymphoma, occurring in approximately 70% to 95% of cases (83–85). This leads to overexpression of the bcl-2 protein in these lymphomas. Consequently, the majority of cases of FCL show expression of bcl-2 protein with the highest percentage of cases seen in grade I FCL and the lowest in FCL, grade III (86). Since reactive germinal center cells do not express bcl-2, immunostaining in paraffin section for this protein is most useful in the differential diagnosis of FCL versus follicular hyperplasia. Bcl-2 protein is expressed in normal T-cells as well as normal mantle B-cells. Since numerous T-cells can be present in FCL follicles, it is good practice to always perform B- and T-cell immunostains in addition to a bcl-2 stain in order to determine with certainty which cells are expressing bcl-2. Bcl-2 protein expression is not restricted to follicle center lymphomas and so expression of this protein alone must not be taken as evidence of a follicle center lymphoma. A recent study also points to the utility of bcl-2 immunostaining in reactive and neoplastic marginal zone proliferations (36).

It is worth noting here that primary cutaneous follicle center lymphomas may be an exception in that previous studies suggested that bcl-2 translocation and protein expression was uncommon in these lymphomas (87). However, a recent study using antigen retrieval and careful immunostaining for CD10 and bcl-2, and bcl-6 proteins suggests that at least some cases of true follicle center lymphoma primary in the skin are similar to their nodal counterpart (88). Another differential diagnostic situation in which bcl-2 protein expression is useful in Burkitt lymphoma versus Burkitt-like lymphoma. Expression of bcl-2 protein favors the latter (86).

Several studies have suggested that bcl-2 protein expression is of prognostic significance in large B-cell lymphoma, with bcl-2 expressing cases more likely to relapse than bcl-2 negative cases (89).

**Bcl-6**

The bcl-6 gene, located at chromosome 3q27, encodes a 79kd DNA binding protein and transcriptional repressor that appears to be involved in mediating growth suppression (90–92). It is also involved in germinal center development (93). Translocations involving this gene and immunoglobulin heavy and light chain genes are seen in approximately 25% to 30% of diffuse large B-cell lymphomas but no definite clinical significance has been ascribed to this (94–96). Mutations in the 5’ non-coding region of the gene is also common in some B-cell lymphomas as well as in germinal center cells (97–101). Normally, the bcl-6 protein is expressed in germinal center cells and some CD4+ T-cells (102–103). In non-Hodgkin lymphoma, bcl-6 protein expression does not require gene translocation and is expressed in a wide

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variety of lymphomas. It is, in fact, expressed in the majority of diffuse large B-cell lymphomas, almost all follicle center lymphomas, and in many lymphomas occurring in immunocompromised patients (104–106). In Hodgkin lymphoma, bcl-6 protein is expressed in the L&H cells of nodular lymphocyte predominant Hodgkin lymphoma and in a subset of classical Hodgkin lymphoma (107–108). In addition, some cases of anaplastic large cell lymphoma also express bcl-6 (109). From a diagnostic standpoint, it has been found most useful in distinguishing follicle center lymphomas (positive) from small B-cell lymphomas with a nodular architecture such as mantle cell lymphoma and marginal zone lymphomas (negative) (105).

Some studies suggest that bcl-6 translocation has prognostic significance in large cell lymphoma (110) while others find no difference (94).

Cyclin D1

Cyclin D1 is the product of the bcl-1 gene and is an important G1 cyclin involved in cell cycle progression (111). Located at chromosome 11q13, the cyclin D1 gene was found to be transcriptionally activated via rearrangement in a subset of parathyroid adenomas and lymphomas (111–113). In particular, the t(11;14)(q13;q32) involving bcl-1 and the immunoglobulin heavy chain gene is found in the great majority of mantle cell lymphomas. The percentage of cases with the translocation has varied depending on the method (113–119); however, recent studies using FISH methods spanning a large area of possible breakpoints demonstrates that the translocation is probably present in close to 100% of cases of MCL (120–121). This correlates with the fact that cyclin D1 expression has been seen in the majority of cases even if the translocation was not detectable (122).

Polyclonal and commercial monoclonal antibodies have been reported that are reactive in formalin and B-5—fixed tissues that identify the majority of cases (70% to 100%) of MCL, demonstrating a nuclear reactivity (4,123–128). Cyclin D1 is not expressed in normal lymphoid cells. It should be noted that these antibodies may be difficult to use in the clinical setting, requiring intensive antigen retrieval. Careful quality control of the performance of this antibody is needed to avoid a high false negative rate (129–130). Cyclin D1 expression is seen not only in typical MCL but also in the blastoid variants (131). With the rare exception of some cases of chronic lymphocytic leukemia, hairy cell leukemia, prolymphocytic leukemia, and plasma cell disorders, expression of cyclin D1 is specific for MCL (4,123,127, 128,132–134).

**Immunoglobulins (Igs)**

Monotypic Ig light chain expression is strong evidence of a B-cell lymphoma, although some lymphomas may lack surface Ig expression. Immunohistochemistry in paraffin sections can easily demonstrate cytoplasmic expression seen in plasma cell dyscrasias and some lymphomas with significant cytoplasmic Ig expression such as lymphoplasmacytic lymphoma, marginal zone lymphoma, and some cases of large B-cell lymphoma. Improved methods allow some laboratories to successfully demonstrate surface Ig expression in fixed tissues, a feat which was previously only possible in cryostat sections (135,136). Interpretation of these stains can be difficult and examination of serial sections with kappa and lambda stains in the same areas is suggested. A ratio of greater than 10:1 or less than 0.2 for kappa: lambda is required to be confident of light chain restriction. A note of caution in interpreting light chain staining is that some cells may take up Ig, thus leading the observer to mistakenly interpret a cell as positive for a light chain. In such cases, staining for J-chain will prove that Ig was synthesized in the cell since J-chain is not found in cells that have taken up Ig (137–138).

Ig heavy chains are, on occasion, diagnostically useful. For example, in the differential diagnosis between anaplastic plasmacytoma and immunoblastic lymphoma. Expression of IgA favors plasmacytoma, whereas IgM expression favors lymphoma (45). Recently, a distinction between splenic and MALT-type marginal zone lymphomas has been suggested that makes use, in part, of the IgD expression in the former type (139). In most cases of lymphoma, however, heavy chain assessment is not required. Figures 1 and 2 demonstrate IP in two cases of B cell lymphoma.

**T/NK-CELL NON-HODGKIN LYMPHOMAS**

T-cell lymphomas are relatively uncommon lymphomas. The incidence worldwide is 12% of all non-Hodgkin lymphomas, but in Asia it is higher (approximately 30%) (140–142). Their relative rarity compared to B-cell NHL is probably because T-cells lack the somatic hypermutation and class switching that occurs in B-cell maturation. In B-cells, these molecular processes allow for chromosomal translocations into Ig gene loci, with subsequent neoplastic transformation (143).

Immunophenotyping plays a definitive role in distinguishing reactive from malignant B-cell processes. T-cells in contrast, lack phenotypic markers of clonality; they usually outnumber B-cells in reactive processes; and, a predominance of either CD4+ or CD8+ T-cells is not definitive evidence of malignancy. The lack of a single specific marker expressed in all T-cells com-
pounds the issue further, necessitating the use of a combination of markers. CD3 (polyclonal) and CD45RO (UCHL-1) antibodies have a 79% and 94% sensitivity, respectively, in predicting T-cell phenotype; but used together, the sensitivity is 98% (144). Other “pan” T-cell markers may also be useful such as CD2 or CD7, both of which may be assessed in formalin-fixed, paraffin-embedded sections. Features that are suggestive of, but not diagnostic of malignancy, include aberrant phenotypes (coexpression or loss of both CD4 & CD8), expression of immature markers like CD1a by “mature” cells, and loss of one or more pan T-cell antigens (commonly CD7) (145). But there are exceptions since cortical thymocytes are CD1a+ and CD4 + 8+, γδ T-cells are usually CD4–8–, and loss of pan T-cell antigens such as CD7 can rarely be seen in reactive/inflammatory lesions (146). Thus, immunophenotyping of T-cell lymphomas is complex, with ALK expression being perhaps the only specific marker of malignancy. Table 2 lists common phenotypes of T-cell lymphomas.

**CD1a**

CD1a, a nonpolymorphic MHC class I related glycoprotein, is normally expressed in cortical thymocytes, Langerhans cells and interdigitating dendritic reticulum cells. For identifying neoplastic and normal Langerhans cells, it is as sensitive but more specific than S-100 (147,148).

CD1a expression in mature T-cells is aberrant and is evidence of a T-lymphoproliferative process, particularly a lymphoblastic lymphoma; but the possibility of normal thymocytes must be excluded. About 11% of PTCL (frozen section) (149) and 43% to 50% of T-ALL are positive, while AML and B-ALL are negative (147,150).

**CD3**

CD3 complex is the invariant, signal transducing component of αβ and γδ T cell receptors expressed in all T-cells. It consists of γ,δ and ε chains, and a largely intracytoplasmic homodimer of ξ chains. Although the exact stoichiometry is not known, it is thought that there are two ε chains and one each of δ and γ chains in the CD3 complex. During T-cell development, CD3 expression follows rearrangement of TCRβ chain, with cytoplasmic CD3 preceding expression of the membranous form.

Polyclonal and monoclonal antibodies recognizing CD3 ε are available. A recent review of monoclonal (PS1) and polyclonal antibodies shows comparable immunoreactivity in T-cell lymphomas (151). Our impressions of the monoclonal antibody PS1 are favorable, with strong immunoreactivity using EDTA antigen retrieval (unpublished observations). Polyclonal antibodies may suffer from batch-to-batch variation and background staining, though reactivity has been reported to be stronger than the monoclonal antibody (151).

CD3 expression is observed in 41% to 95% (polyclonal) (34,152,153) and 66% (monoclonal) (151) of T-cell lymphomas. In T-LBL/ALL, 38% (monoclonal) (154) to 100% (polyclonal) are CD3+ (150,155). Thus, some T-cell lymphomas will lack detectable CD3. Cytoplasmic and membranous staining is seen with polyclonal antibodies (144,156), while the staining is membranous with the monoclonal antibody (151). Membranous staining is said to be specific for T-cell lymphomas, as NK-cell lymphomas may show cytoplasmic staining (157,158). T-ALL/LBL, in keeping with an immature stage of development, can exhibit both membranous and cytoplasmic staining (150,154).

Reed–Sternberg cells have been reported to express CD3 with the polyclonal (7% to 11%) (159,160), but not monoclonal antibodies (151). A recent report with T-cell clonality data, however, suggests that a true T-cell origin of RS cells is very uncommon (159). B-cell lymphomas and L&H cells of lymphocyte predominant Hodgkin lymphoma do not express CD3 (160–162).

Thus, CD3 is a specific marker of T lineage. However, since cytoplasmic expression can be seen in NK-cells and NK-cell neoplasms, lineage assessment should be

### Table 2. Typical T-cell NHL Immunophenotypes

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<tr>
<th>Lymphoma</th>
<th>Immunophenotypic Features</th>
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<tr>
<td>Mycosis fungoides</td>
<td>CD3+, CD4+, CD8–, CD7–/+</td>
</tr>
<tr>
<td>Nasal T/NK lymphoma</td>
<td>CD2+, CD3–/+ (cytoplasmic), CD5–/–, CD56+, EBER+, TIA-1+, βF1–, TCRδ–/–</td>
</tr>
<tr>
<td>Angioimmunoblastic lymphoma</td>
<td>CD3+, CD4+, CD8–, βF1+, TCRδ–/–</td>
</tr>
<tr>
<td>Subcutaneous panniculitic</td>
<td>CD3+, CD4+, CD8+, TIA-1+, βF1+, TCRδ– (occ. cases +), CD56–/–, EBER–</td>
</tr>
<tr>
<td>Hepatosplenic lymphoma</td>
<td>CD3+, CD4–, CD8–, TCRδ–, TCRα–</td>
</tr>
<tr>
<td>Intestinal T-cell lymphoma</td>
<td>CD3+, CD4+, CD8+, TIA1+, EBER–, CD103+</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma</td>
<td>CD3–/+ (cytoplasmic), CD15–, CD30+, EMA–/+ (occ. cases +), CD56–/+ (occ. cases +), ALK1–, EBER–, TIA1–</td>
</tr>
<tr>
<td>Peripheral T-cell lymphoma, not otherwise specified</td>
<td>CD3–/+ (cytoplasmic), CD4–/–, CD5–/– (cytoplasmic), CD7–/+ (cytoplasmic), TIA1–</td>
</tr>
<tr>
<td>T-lymphoblastic lymphoma</td>
<td>CD3+, CD7+, CD4–/–, CD8–/–, TdT+</td>
</tr>
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done with attention to staining pattern and gene rearrangement studies, if required. Furthermore, CD3 negativity does not rule out a T-cell lineage since expression can be lost in T-cell lymphomas.

**CD4 & 8**

CD4, a coreceptor in MHC class II restricted, antigen-induced T-cell activation, is expressed in thymocytes, T-helper cells, monocytes, macrophages, and granulocytes. CD8 is a coreceptor in MHC class I restricted, antigen recognition, and is expressed in T-cytotoxic/suppressor cells and at a low level on NK-cells. Developing T-cells are initially CD4–8–; later they express both, and on maturation, they express either of these coreceptors. The staining pattern with CD4 and CD8 antibodies is cytoplasmic with membrane accentuation.

The majority of T-cell lymphomas are CD4+. γδ T-cell lymphomas are usually CD4–8–, or CD4–8+ in a minority (163). More ALCL (systemic and primary cutaneous) are CD4+ than CD8+ (164,165). Paraffin section staining correlates with frozen section and flow cytometry (166,167). Coexpression of CD4 and CD8 or loss of expression of both in mature T-cells suggests an aberrant phenotype and indirectly supports a diagnosis of T-cell lymphoma, although normal γδ-T-cells are “double negative.”

**CD5**

Assessment of CD5 expression in the diagnosis of B-cell NHL is discussed above. Among T-cell neoplasms, 75% to 100% of T-NHL, most T-LBL and some ALCL are CD5+ (12,14). When assessed with CD3 or other T-cell specific markers, CD5 is helpful in assigning lineage. Loss of CD5 expression in a T-cell process suggests a malignant process. NK-cells may be negative for CD5, and thus NK-cell malignancies should be considered when a weakly CD3+, CD5- “T-cell” neoplasm is encountered (168).

**CD7**

CD7, a membrane bound glycoprotein, is the earliest pan T-cell antigen to be expressed. It is thought to play a role in signal transduction and cell adhesion. It is expressed in 90% of T lymphoblastic lymphomas and most NK-cell neoplasms, but is deleted in many peripheral T-cell lymphomas (PTCL). In fact, it is the T-cell antigen most commonly deleted in T-cell lymphoma (61,149). Deletion of CD7 is commonly seen in mycosis fungoides (169). However, rare cases of reactive lesions have been reported to lack CD7 (146). Recently, a new paraffin reactive antibody has become available, showing strong membrane staining. Our experience suggests that it compares favorably to frozen section staining, giving strong staining and excellent morphologic correlation. In conjunction with PCR for TCR-γ gene rearrangement, it is a useful stain in the evaluation of suspected cases of MF (170).

**CD43**

CD43 is a glycosylated cell surface protein found on T-cells and histiocytes. It is a very sensitive, but not lineage specific marker expressed also on reactive lymphocytes, B-cells in infectious mononucleosis, various B-cell neoplasms and nonneoplastic and malignant myeloid cells. Its expression in B-cell lymphoma is addressed above. In T-cell neoplasms, 81% to 100% of PTCL and almost all T-lymphoblastic lymphomas/ALL are positive with a membrane staining (62,154,171). RS cells and variants are reactive in a minority of cases (56). Commonly used monoclonal antibody clones include Leu-22, MT-1, and DF-T1.

**CD45RO**

The T-cell restricted isoform of CD45, CD45RO is detected by the UCHL1 monoclonal antibody in fixed tissue. The A6 and UCHL1 antibodies stain mature T-cells in T-cell areas in lymphoid organs, thymocytes, mature myeloid cells, and some macrophages, with a membrane pattern; glandular epithelium may exhibit a diffuse cytoplasmic staining (172). CD45RO expression is replaced in 78% to 94% of PTCL (144,173), 63% to 78% of T-ALL (154,172), and all MF (172). Forty-two percent of CD45 negative ALCL (174) and 31% of RS cells (160) are CD45RO+. Though <10% of B NHL (especially diffuse subtypes) are immunoreactive (173,175), 27% of AIDS-related B NHL are positive (175). B-ALL are negative (154,172). Granulocytic sarcomas may also express CD45RO (176). Thus, CD45RO is a sensitive but not specific marker of T-cells.

**CD56/NCAM**

CD56 (neural cell adhesion molecule (NCAM)) is widely expressed in central and peripheral nervous systems, and thyroid follicular and renal proximal tubular epithelium (177). Rare lymphoid cells (single/2–3 cells) in normal and reactive lymphoid tissue, and nasal/nasopharyngeal mucosa exhibit membrane staining, while occasional plasma cells show cytoplasmic staining (178). A commonly used monoclonal antibody clone is 123C3. CD56 reactivity is useful in delineating the nasal/nasal type NK/T-cell lymphomas which are usually sCD3–, cCD3+, CD56+, and TCR gene germline. These are extremely aggressive tumors with a broad morphologic spectrum and strongly associ-
CD56 expression is not limited to nasal/nasal type NK/T-cell lymphomas and is seen in intestinal T-cell lymphomas (180), hepatosplenic γδ T-cell lymphomas (181–183), γδ subcutaneous panniculitis-like T-cell lymphoma (184), ALCL (185,186), rare B cell lymphomas (187), ALL, AML, neoplastic plasma cells (cytoplasmic), and CML blast crisis (178). Nonhematopoietic neoplasms that can express CD56 include rhabdomyosarcomas and other soft tissue tumors (178).

CD57

CD57 is an NK-cell marker, also expressed in a subset of GC T-cells (188). But expression by T-cells does not necessarily imply NK activity (189). Leu7, a monoclonal antibody against CD57, stains with a membrane pattern (188). Diagnostically, CD57 expression can be helpful in distinguishing LPHL from morphologic mimics such as T-cell rich B-cell lymphoma (TCRBCL) and cHL. CD57+ T-cells are increased in LPHL compared to both cHL and TCRBCL. Classically, the CD57+ T-cells form a rosette around the large L&H cells (189,190); however, in our experience, and that of others (191) this is not a sensitive marker of LPHL. In distinguishing PTGC from LPHL, Nguyen et al. found no appreciable differences in the overall numbers of CD57 positive cells, although in 3/16 LPHL cases, CD57 positive cells formed rings around some of the L&H cells (191). Though CD57 is an NK-cell marker, expression is found in only 8% of NK-cell neoplasms; some ALCL and T-LBL/ALL can also express CD57 (186,188).

ALK

ALK expression defines a distinct entity among ALCL, “ALKoma,” as a single disease with a broad morphologic spectrum (192). Both polyclonal (p80 (193), ALK11 (194)) and monoclonal (ALK1 (195) ALKc (196)) antibodies are available. The monoclonal antibodies, directed against the cytoplasmic portion of ALK protein, exhibit almost identical reactions (196).

ALK is not detected in normal and reactive hematolymphoid tissues, and nonhematopoietic tumors (with the exception of some neuroblastomas and inflammatory myofibroblastic tumors (198)) (197); a few neuronal cells in brain are weakly reactive (195,196). Among hematopoietic tumors, NHL other than ALCL, HL, and myeloid neoplasms are negative (195,199,200). An exception has been the reported expression in a diffuse large B-cell lymphoma (201). With rare exceptions, primary cutaneous ALCL are ALK negative (202,203).

The characteristic (2;5) translocation of ALCL juxtaposes the ALK (anaplastic lymphoma kinase) gene at 2p23 with the NPM (nucleophosmin) gene at 5q35, resulting in an 80kD chimeric NPM-ALK protein. The N terminus of NPM is linked to the entire cytoplasmic domain of ALK. In approximately 15% of ALK+ ALCLs, ALK gene fuses with genes other than NPM (204–209). The abnormally expressed protein products of these variant translocations may be detected with the monoclonal antibodies, but with different patterns. With the NPM-ALK translocation, expression is nuclear and cytoplasmic, while with the variants it is cytoplasmic alone or membrane. The NPM-ALK fusion protein heterodimerizes with wildtype NPM protein and is translocated to the nucleus (210). This does not occur when the fusion protein is other than NPM. This can be further assessed with antibodies to NPM (211). But the clinical features of these cases with “variant” translocations appear to be essentially the same as the classic ones (210).

βF1 and TCRδ1

Most normal T-cells express αβ-TCR, while a minority express the alternate γδ-TCR. Overall, both types of T-cells express a similar subset of pan T-cell markers. But in contrast to αβ T-cells, γδT-cells are predominantly CD4-8- and express cytotoxic molecules. βF1 and TCRδ-1 are monoclonal antibodies specific for the β chain of TCRγδ and δ chain of TCRγδ, respectively, and are useful in assessing lineage. Reactivity with the βF1 antibody is membranous (212) or cytoplasmic (213).

The majority of T-cell neoplasms express αβ-TCR. Thus, βF1 expression is seen in 66% to 76% of PTCL (212,213) and 75% to 80% of T-ALL/LBL (154,213). Expression may also be seen in RS cells of 13% of HL (212). About 10% of PTCL are TCRδ-1 positive, while approximately 20% express neither T-cell receptor αβ nor γδ (214) All B NHL are negative (212,213).

The significance of assigning T-cell lymphomas to αβ or γδ types is uncertain. The prototypic γδT-cell lymphoma is the hepatosplenic γδT-cell lymphoma (2); however, αβ types have been reported (215). γδ T-cell lymphomas have also been reported in sites other than the liver and spleen, including mucosa and skin (216,217). Thus, it appears that γδ T-cell lymphomas are a heterogeneous group, with behavior of T-cell lymphomas not clearly linked to the type of TCR expressed (163).

BNH9

BNH9 monoclonal antibody recognizes red blood cell-related antigens, and is expressed in endothelial cells, stellate reticulum cells, squamous cells, various glands in
the GI tract, bronchus, salivary gland, and pancreas (218). Staining is membranous, associated with a dot-like staining in the golgi region. Twenty-four percent to 51% of T/null cell ALCL express BNH9, along with 50% of various carcinomas, 3% to 6% of NHL other than ALCL, occasional L&H cells and rare RS cells (218,219). In both referenced papers, most BNH9+ cases were also EMA+. Thus, it maybe of use in differentiating CD30+, EMA+ HL-like ALCL from CD30+, EMA-RS cells in cHL (218). With the recognition that most cases of HL-like ALCL are more closely related to HL than ALCL (220–222), and the development of ALK antibodies, the utility of this antibody in routine diagnosis is marginal.

EMA

Antibodies against epithelial membrane antigen recognize the breast epithelial mucin complex. Numerous monoclonal antibodies are available and the E29 MoAb is commonly used. Primarily, an epithelial marker, EMA is also expressed on nonneoplastic plasma cells (223). Among lymphoid neoplasms, it has been used to distinguish LPHL from cHL. About 60% of L&H cells, but <3% of RS cells are EMA+ (223). It is also seen in the majority of ALCL, particularly the ALK+ subset (224,225). About 20% of T-cell lymphomas, 5% of B-cell lymphomas, and >85% of plasma cell neoplasms are EMA+ (223,226).

TIA/Granzyme/Perforin

TIA-1, perforin, and granzyme are cytolytic proteins present within the cytoplasmic granules of NK- and cytotoxic T-cells. Activated cytotoxic cells express all three proteins, while nonactivated cytotoxic cells express only TIA-1 (227). TIA-1, a 15kD protein related to the TNF receptor family, is expressed in occasional small lymphocytes in GC, mantle zones, and sinuses of normal and reactive lymphoid tissues; some myeloid precursors and mature granulocytes show a fine granular, diffuse staining. The characteristic staining pattern in cytotoxic cells is cytoplasmic and granular (227). Perforin (Pf) is a pore-forming protein similar to C9 component of complement, and Granzyme B (GrB) is a member of the serine protease family (185). A few GrB+ lymphocytes (mostly NK-cells) are present in the sinuses and medullary cords of nonneoplastic lymphoid tissues. TIA-1 expression is seen in all hepatosplenic lymphomas, intestinal T-cell lymphomas, most nasal/nasal-type NK/T-cell lymphomas (227), most subcutaneous panniculitis-like T-cell lymphoma (184,227,228), and the majority of ALCL (186,227,229). RS cells in a few cases of cHL express TIA-1 (227,229). CD4+ PTCL, B NHL, LPHL, and T-LBL/ALL are negative (227,229). Pf and GrB immunoreactivity is reported in 90% of CD8+ PTCL, all nasal lymphomas, 60% of ALCL, and <2% of CD4+PTCL (185). HL do not express Pf (229), while 14% to 18% are GrB+ (230,231). Though a cytotoxic phenotype is usually not associated with CD4+ T-cells, among ALCL (systemic and primary cutaneous), expression of cytotoxic molecules is seen in a greater proportion of CD4+ cases (165,229).

Thus, T-cell lymphomas with a cytotoxic phenotype include hepatosplenic T-cell lymphoma, nonhemopoietic γδ T-cell lymphoma, intestinal T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, nasal/nasal-type NK/T-cell lymphoma, and ALCL. But the significance of cytotoxic molecule expression in T-cell lymphomas is uncertain and will likely be the subject of future studies (163) An example of IP in a T-cell lymphoma is shown in Figure 3.

HODGKIN LYMPHOMAS (HL)

Most HL are now considered tumors of monoclonal B cells, with lymphocyte predominant Hodgkins lymphoma (LPHL) arising from GC B-cells (with ongoing mutations), and classical HL (cHL) from crippled GC B-cells (without ongoing somatic mutations) (232–235). They are two distinct entities with different clinical behavior and immunophenotype. The classic phenotype of RS cells and variants is CD45+, 30+, 15+, 20-. The majority of cHL are CD30+, 15+, 20- (83% of cases); 12% are CD30+, 15-, 20-, and about 5% are CD20+ (236). A subset of cHL cases express bcl-6 protein (108). T-cell markers are expressed in a few cases, with CD3 in 7% to 11% (159,160). The neoplastic cells of LPHL (L&H cells) in contrast are CD45+, 30-, 15-, 20+, EMA+ and express bcl-6 protein (108). The nodules, which are actually altered follicles, consist of polyclonal B-cells, CD57+ T-cells, and a prominent FDC network. These CD57+ T-cells may form a characteristic ring around the L&H cells (189).

Immunophenotyping can be very helpful in the distinction between cHL and LPHL. Up to 25% of LPHL by morphology express a cHL phenotype; and 12% of cHL by morphology show a LPHL phenotype (190).

CD15

Antibodies directed against CD15 (including Leu-M1) recognize X hapten, expressed in mature neutrophils, monocytes, and a subset of T-cells (237). There is variable expression in normal epithelia (238). Reed–Sternberg cells and variants characteristically express CD15 in 84% of cHL (226) with a membranous and paranuclear (Golgi) pattern. cHL lacking CD15 expres-
sion may have a worse prognosis (236). Though characteristic of RS cells, L&H cells in a variable number of LPHL express CD15 (190). Other neoplasms that are CD15+ include 5% of B NHL, 57% to 67% of T NHL, 10% of ALCL, and 2/3rd of AML and extramedullary myeloid tumors (176,237–240). Immature T-cell neoplasms are CD15-negative.

**CD30 (Ber-H2, Ki-1)**

A member of the TNF receptor family with pleiotropic effects, CD30 is an activation associated marker (241). It is normally expressed on activated lymphocytes and large lymphoid cells in tonsils, lymph nodes, and thymus. In lymph nodes, it is seen in a few large mononuclear cells with an evident nucleolus (immunoblasts), which are mainly grouped around B-cell follicles and at the edge of GCs (243). Lymphocytes infected with HIV, HTLV-1, or EBV, and occasional decidual cells are CD30+ (241,243). Ber-H2 monoclonal antibody is commonly used in fixed tissue. The characteristic staining pattern is membranous with a dot-like positivity in the Golgi region (241).

ALCL strongly expresses CD30. CD30 expression can also be seen in the cutaneous CD30+ lymphoproliferative disorders—lymphomatoid papulosis and primary cutaneous ALCL (241).

CD30 is an excellent marker for RS cells and variants, and is expressed in the great majority (98%) of cHL (236). It is also expressed by L&H cells in 8% to 22% of LPHL (190,244).

CD30 expression may be seen in approximately 30% of T-cell lymphomas (241,244,245), 15% to 20% of nonanaplastic B NHL (241), and 58% of primary effusion lymphomas (246). Immature lymphoid neoplasms and AML are CD30-negative (241,244). A few CD30+ cells are present in FCL, MCL, and SLL (244,247). In FCL, they are present at edges of neoplastic follicles and occasionally in perinodular and T-cell areas, resembling the distribution in reactive lymph nodes and tonsils (247). Among nonhematopoietic neoplasms, embryonal carcinoma and embryonal carcinoma components of mixed germ cell tumors (241,243) are consistently positive. Carcinomas of pancreas, salivary glands, nasopharynx (241), and mesotheliomas (243) can express CD30, with pancreatic and salivary tumors showing a cytoplasmic pattern. Thus, the expression of CD30 is by no means specific for HL or ALCL.

**CD45RB**

As discussed earlier, is not lineage specific but confirms a hematolymphoid origin. CD45RB immunoreactivity is seen in 97% of B-NHL, 90% of T-NHL, and 69% of B- & T-ALL (65,154), while RS cells and variants are negative (65).

**Fascin**

Fascin is a 55kD actin bundling protein. Normal cells with reactivity include dendritic cells and some endothelial, epithelial, and neural cells. Lymphocytes, myeloid cells, plasma cells, and histiocytes are negative. Strong diffuse cytoplasmic staining is seen in RS cells and variants, while L&H cells are negative. Weak reactivity is present in approximately 15% of non-Hodgkin’s lymphomas (248).

Fascin has been proposed as a useful marker for RS cells in difficult cases of HL. One should note, however, that fascin is expressed in a subset of ALCL (10/15 cases), albeit weakly as compared to RS cells. Similar results were reported in a recent series published in abstract form (249).

**MISCELLANEOUS**

**CD21 and CD35**

These antibodies recognize complement receptors, C3d and C3b, respectively. They are normally expressed in follicular dendritic cells (FDC), which are antigen presenting and processing cells present in GCs. Thus, they are useful in identifying neoplasms arising from FDCs (250). However, besides neoplasms of dendritic cell origin, these antibodies are useful in highlighting nodal architecture and the presence of true lymphoid follicles since the follicular dendritic network is demonstrated. Follicular lymphomas may show a tight, well-developed network, while mantle cell lymphoma, even in a nodular pattern, shows less well-defined or disrupted networks (251,252). AILD-like T-cell lymphoma is characterized by expanded FDC aggregates (252). These antibodies have been used to demonstrate the presence of follicles in cases of so-called follicular HL (251,253).

Nodules of LPHL exhibit a prominent concentric network of FDC. In cHL-nodular sclerosis type, FDC foci are found in the center of nodules, while there is disruption of the network in cHL-mixed cellularity type and cHL-lymphocyte depleted type (251,252).

In 16% of cHL, RS cells and variants are CD21+, while none of the L&H cells of LPHL express CD21 (254).

**CD99/MIC2**

As a cell surface glycoprotein product of the MIC2 gene, it is recognized by numerous monoclonal antibodies including RFB-1, 12E7, HBA71, and O13. The 12E7 antibody was developed against a human thymus leuke-
mia antigen and, as one might expect, expression can be seen in lymphoid cells. It is primarily used in the diagnosis of Ewing’s sarcoma and primitive neuroectodermal tumors, positively staining these cases (255).

CD99-positive normal cells include cortical thymocytes, pancreatic islet cells, Sertoli cells, granulosa cells of ovary, ependymal cells (256), T-cells in T-cell areas of lymphoid organs, and scattered lymphocytes in the skin (257). The staining pattern is membranous.

Among hematolymphoid neoplasms, reactivity is seen in the majority of lymphoblastic lymphomas (except Burkitt’s) and in a minority of other NHL (258). Reactivity in lymphoblastic lymphomas correlates with TdT expression (259). Thus, it is a sensitive but nonspecific marker and is said to be of use in distinguishing immature from mature lymphoid neoplasms (260). But using O13 antibody and HIER, Vartanian et al., report reactivity with most NHL, HL, and LBL (261).

**EBV LMP1**

Epstein-Barr virus (EBV) infection is associated with lymphomas and lymphoproliferative disorders in immuno-deficient patients. This includes those patients with acquired immunodeficiencies such as post-transplantation patients (post-transplantation lymphoproliferative disorders, PTLDs) and AIDS (262,263), as well as patients with primary immunodeficiencies (264). The latent membrane protein 1 (LMP-1) expression has been assessed in paraffin sections in many cases of lymphoproliferative disorders and is of interest because of its oncogenic properties (265). LMP-1 expression occurs in latently infected cells, although it is only expressed in cells demonstrating latency pattern 2 or 3. Thus, endemic Burkitt lymphoma, an EBV-associated lymphoma expressing latency pattern 1, does not typically show LMP-1 expression (266). Alternate methods such as EBER in situ hybridization, are superior for detecting latent infection compared to LMP-1 expression (267).

LMP-1 expression occurs in the majority of PTLDs (268) and LMP-1 expression has been related to NFkB activation (269). EBV-negative PTLDs are increasingly recognized, although the clinical significance of such a finding is uncertain (270). In HIV infected individuals, EBV has been found in approximately one-third of Burkitt-type lymphomas and in most other aggressive histology lymphomas (immunoblastic lymphoma) (263). It is also found in most CNS lymphoma as opposed to lymphomas at other sites (271). With respect to Hodgkin lymphoma, varying percentages of cases can be found to contain EBV. Factors that appear to influence whether EBV may be present include geographic region, socioeconomic circumstances, and age (265,272–275). Occasional T-cell lymphomas contain EBV and its presence has been associated with a poor outcome (276). One type, in particular, the nasal/nasal-type NK/T-cell lymphoma is strongly associated with EBV infection (157).

**Ki-67**

The Ki-67 antigen marks cells that are in the cell cycle (277). The Ki-67 antibody recognizes two nuclear proteins with the apparent molecular weights of 345 and 395 kD that are the product of a gene on chromosome 10q25 (278–280). The Mib-1 antibody recognizes the Ki-67 antigen in paraffin sections and is useful in assessing the proliferation index of tumors and thus, in the grading of lymphomas (281). As a prognostic marker in aggressive lymphomas, Ki-67 has proven to be useful with tumors showing greater than 80% labeling having a poor prognosis compared to those with less labeling (282). From a diagnostic standpoint Ki-67 labeling may be useful in the evaluation of Burkitt lymphoma versus other large cell lymphoma, since virtually 100% of cells should be in cycle in the former. Likewise, when evaluating a follicle center lymphoma, the neoplastic follicle should show a relatively low proliferation rate as compared to the high labeling in reactive follicles. These, however, are “soft” findings and other more reliable criteria exist (2).

**p53**

p53, the tumor suppressor gene, is involved in cell cycle regulation and is the most frequent genetic alteration in human malignancy. While probably not of primary importance in the pathogenesis of lymphoma, p53 may be important in lymphoma progression and transformation (283–285). Immunohistochemical assessment of p53 expression has been useful, since its overexpression has been correlated with p53 mutation. However, it has been shown in many tumor systems, including lymphoma, that p53 overexpression does not necessarily equate with gene mutation (286,287). Nevertheless, assessment of p53 expression by immunohistochemistry may have some diagnostic utility. For example, aggressive variants of mantle cell lymphoma are associated with p53 mutation and overexpression (288–290). From a prognostic viewpoint, p53 expression may have utility in predicting poor outcome in aggressive NHL (283,291).

One potential problem with p53 immunostaining is the dependence on technical factors such as antigen retrieval, interpretation, and clone selection for valid results (292–295). In order to minimize these problems, careful quality control with well-characterized positive and negative control samples should be done when performing this stain. Clones DO7 and Pab 1801 have been recommended for immunohistochemistry (293).
**CONCLUSIONS**

Modern classification of lymphoid neoplasms has progressed significantly over the past decade such that immunophenotyping is helpful and often essential in routine diagnostic practice. Paraffin-section immunohistochemistry has clearly become the method of choice, allowing excellent morphologic correlation to the immunophenotype. Technical advances in antigen retrieval techniques, sensitive detection systems, and rapid development of new antibodies have made this possible. As an aid, the antibodies and general conditions are listed which were found useful in the laboratory (Table 3). However, one must realize that there may be more than one way to produce acceptable staining in fixed tissues. Each laboratory must establish the performance of their immunohistochemical stains as part of good laboratory practice and in accordance with the recent analyte specific reagent ruling (302–304).

This review demonstrates the wide variety of antibodies that are most useful in the diagnosis and classification of lymphoid neoplasms. Properly controlled, these reagents are invaluable in improving the diagnostic accuracy of lymphoma diagnosis in all clinical settings—from the community hospital to the academic center to the commercial reference laboratory. In addition, these reagents have helped define specific entities and will continue to have a major impact on the diagnosis, understanding the biology, and defining prognosis of lymphoid malignancies (101,121,124–126).

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**TABLE 3. Example conditions for performing immunohistochemical stains**

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<thead>
<tr>
<th>Antibody/clone</th>
<th>Vendor</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
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<td>None</td>
</tr>
<tr>
<td>IgG polyclonal</td>
<td>Dako</td>
<td>1:100000</td>
<td>None</td>
</tr>
<tr>
<td>IgM polyclonal</td>
<td>Dako</td>
<td>1:25</td>
<td>None</td>
</tr>
<tr>
<td>Kappa polyclonal</td>
<td>Ventana</td>
<td>Predilute</td>
<td>Protease</td>
</tr>
<tr>
<td>Lambda polyclonal</td>
<td>Ventana</td>
<td>Predilute</td>
<td>Protease</td>
</tr>
<tr>
<td>Ki67 antigen MM1</td>
<td>Novocasta</td>
<td>1:10</td>
<td>MW, C</td>
</tr>
<tr>
<td>P53 D07</td>
<td>Dako</td>
<td>1:20</td>
<td>MW, C</td>
</tr>
<tr>
<td>TdT polyclonal</td>
<td>Supertech</td>
<td>1:10</td>
<td>MW, C</td>
</tr>
<tr>
<td>TIA-1 26gA10F5</td>
<td>Immunotech</td>
<td>1:80</td>
<td>MW, E</td>
</tr>
</tbody>
</table>

* Modeled after Cleveland Clinic Immunohistochemistry Laboratory procedures and selected references.

MW, microwave; C, citrate; E, EDTA; T, trilogy (Cell Marque, Texas); BD, Becton Dickinson.

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**TDT**

Terminal deoxynucleotidyl transfersase (TdT) is the polymerase responsible for addition of deoxynucleotides to the 3′-hydroxyl ends of DNA without a template. Its expression is a sensitive and fairly specific marker of precursor B- and T-cells. Thus, its presence in lymphoma is indicative of a precursor B- or T-lymphoblastic lymphoma. TdT is not, however, lineage specific. Antibodies exist which are suitable for use in paraffin-embedded tissue as well as tissue that has been decalcified (296–299). TdT is a nuclear enzyme and, therefore, a nuclear staining pattern should be seen.

TdT is expressed in over 80% of LBL and is absent in all other types of lymphoma (2). Several studies have demonstrated the utility of evaluating for TdT in the diagnosis of lymphoblastic lymphoma, with most studies showing the great majority (over 90%) of cases being immunoreactive (260,296,298–300). It is also well known that a minority (20%) of acute myeloid leukemias (AMLs) may express TdT (301). Not unexpectedly, TdT expression has been found in extramedullary myeloid cell tumors (297). Lack of other lymphoid associated antigens and expression of myeloid markers (such as CD43 only or myeloperoxidase) should make the diagnosis evident (64). It is also worth remembering that normal thymocytes express TdT. Therefore, when dealing with mediastinal biopsies for suspected lymphoma, one should always consider the possibility of a lymphocyte-rich thymoma since the thymocytes will phenotype similar to a T-lymphoblastic lymphoma.
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