REVIEW

The Clinical Relevance of Lymphocyte Marker Studies in the Diagnosis of Lymphoma

Nancy L. Harris

Department of Pathology, Harvard Medical School, and the Immunopathology Unit
Department of Pathology, Massachusetts General Hospital, Boston, MA, USA

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Abstract

Immunologic analysis has greatly improved diagnostic accuracy in several clinically important areas, however, the ultimate goal of a lymphoma classification based on immunophenotype has not been reached and may not be forthcoming. In the evaluation of a patient suspected of having lymphoma, lymphocyte marker studies may be essential for a correct diagnosis, helpful but not essential, or entirely noncontributory, depending on the specific clinical setting and histological differential diagnosis. This review will cover the basic principles and technical aspects of marker studies, and will explore several problems in differential diagnosis to clarify the role of marker studies in determining patient management.

Key words: lymphoma, immunophenotyping, cluster of differentiation

In the past decade, immunologic marker studies have made a major contribution to the diagnosis and classification of lymphoproliferative disorders. Although the ultimate goal of a lymphoma classification based on immunophenotype has not been reached and may not be forthcoming, immunologic analysis has greatly improved diagnostic accuracy in several clinically important areas. Because of the rapidity with which the field of lymphoma immunophenotyping has advanced, many physicians, both pathologists and clinicians, may be uncertain as to the precise role of these studies in

Reprinted request to: Dr. Nancy L. Harris, Department of Pathology, Massachusetts General Hospital, Boston, MA 02115, USA
the care of individual patients. Both physicians caring for patients suspected of having lymphoma and pathologists faced with making the diagnosis on biopsy specimens must be aware of these issues, since often the manner in which the initial diagnostic biopsy is handled is crucial in determining whether or not a definite diagnosis will be made. In the evaluation of a patient suspected of having lymphoma, lymphocyte marker studies may be essential for a correct diagnosis, helpful but not essential, or entirely noncontributory, depending on the specific clinical setting and histological differential diagnosis. This review will cover the basic principles and technical aspects of marker studies, and will explore several problems in differential diagnosis, to clarify the role of marker studies in determining patient management.

Principles of immunophenotyping

Lymphocyte Differentiation

In many respects, lymphoid neoplasms resemble their normal counterparts, the T and B cells of the immune system. For this reason, some understanding of normal T and B cell differentiation is essential to an understanding of the lymphomas. At various stages of differentiation, lymphoid cells undergo changes in both morphology and surface antigen expression, which are characteristic for each stage. These features can be used to identify and characterize both normal and neoplastic lymphoid cells in tissue sections or cell suspensions. The constellation of antigens expressed at a given time by a cell is called its immunophenotype.

The nomenclature for both the morphologic stages and the surface antigens can be confusing, since different authorities use different morphologic terms for both normal and neoplastic lymphoid cells, and a variety of monoclonal antibodies with different names detect the same antigens. Morphologic terms used by four major lymphoma classifications are summarized in Table 1. International workshops have recently developed a standardized nomenclature for many of the antigens detected by more than one monoclonal antibody. These clusters of differentiation (CD) are summarized in Table 2. Not all of the antigens listed are currently useful in the diagnosis of lymphomas: some are not restricted to a particular cell type, while others cannot distinguish between neoplastic cells and infiltrating benign lymphocytes. In the discussion of practical application of marker studies, only those antibodies that are useful in clinically important differential diagnoses will be emphasized; the others are included because they are of interest for the understanding of lymphocyte differentiation and the subclassification of lymphomas in ways that do not yet have clinical relevance.

There are two distinct phases of development within both the T and B lymphoid cell lines: antigen-independent and antigen-dependent (Figs. 1 and 2). Antigen-independent proliferation and differentiation occur in the primary lymphoid organs (thymus
Table 1  Nomenclature of Neoplastic Lymphoid Cells

<table>
<thead>
<tr>
<th>RAPPAPORT</th>
<th>LUKEs AND COLLINS WORKING FORMULATION</th>
<th>KIEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated lymphocyte</td>
<td>Small lymphocyte (T or B)</td>
<td>Lymphocyte (T or B)</td>
</tr>
<tr>
<td>Plasmacytoid lymphocyte</td>
<td>Plasmacytoid lymphocyte</td>
<td>Immunocyte (B)</td>
</tr>
<tr>
<td>Poorly differentiated lymphocyte</td>
<td>Small Cleaved FCC*</td>
<td>Centrocyte (B)</td>
</tr>
<tr>
<td>Histiocyte</td>
<td>Large cleaved FCC (B)</td>
<td>Centrocyte (B)</td>
</tr>
<tr>
<td>Histiocyte</td>
<td>Large noncleaved FCC (B)</td>
<td>Centroblast (B)</td>
</tr>
<tr>
<td>Histiocyte</td>
<td>Immunoblast (B or T)</td>
<td>Immunoblast (B or T)</td>
</tr>
<tr>
<td>Undifferentiated cell</td>
<td>Small noncleaved FCC (B)</td>
<td>Lymphoblast (B)</td>
</tr>
<tr>
<td>Lymphoblast</td>
<td>Convoluted lymphocyte (T)</td>
<td>Lymphoblast, convoluted (T)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lymphoblast, unclassified</td>
</tr>
</tbody>
</table>

* FCC = Follicular center cell. This modifier is omitted in the Working Formulation terminology, as is the lineage designation (T or B).

Table 2  Selected Monoclonal Antibodies Useful in the Diagnosis of Lymphoid Neoplasia

<table>
<thead>
<tr>
<th>CD</th>
<th>EXAMPLES OF ANTIBODIES</th>
<th>SPECTRUM OF REACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1</td>
<td>T6, OKT6, Leu6</td>
<td>Cortical thymocyte/Langerhan's cell</td>
</tr>
<tr>
<td>CD2</td>
<td>T11, OKT11, Leu5</td>
<td>Pan T (E-rosette receptor)</td>
</tr>
<tr>
<td>CD3</td>
<td>T3, OKT3, Leu4</td>
<td>Pan T</td>
</tr>
<tr>
<td>CD4</td>
<td>T4, OKT4, Leu3</td>
<td>T Helper (MHC II cytotoxic)</td>
</tr>
<tr>
<td>CD5</td>
<td>OKT1, Leu1</td>
<td>Pan T, rare B, B cell lymphoma</td>
</tr>
<tr>
<td>CD7</td>
<td>Leu9, 3A1</td>
<td>Pan T</td>
</tr>
<tr>
<td>CD8</td>
<td>T8, OKT8, Leu2</td>
<td>T Suppressor (MHC I cytotoxic)</td>
</tr>
<tr>
<td>CD10</td>
<td>J5</td>
<td>CALLA: B&amp;T precursor, GC</td>
</tr>
<tr>
<td>CD15</td>
<td>Leu M1</td>
<td>Granulocyte, monocyte, RS cell</td>
</tr>
<tr>
<td>CD19</td>
<td>B4, Leu12</td>
<td>Pan B (not plasma cell)</td>
</tr>
<tr>
<td>CD20</td>
<td>B1</td>
<td>Pan B (                )</td>
</tr>
<tr>
<td>CD21</td>
<td>B2</td>
<td>B subset, Follicular dendritic cell</td>
</tr>
<tr>
<td>CD22</td>
<td>Leu14, To15</td>
<td>Pan B (not plasma cell)</td>
</tr>
<tr>
<td>CD30</td>
<td>Kil</td>
<td>Activated T, B; RS cell</td>
</tr>
<tr>
<td>CD45</td>
<td>LCA, T29/33</td>
<td>Leukocyte common antigen (T&amp;B&amp;M)</td>
</tr>
<tr>
<td></td>
<td>Immunoglobulin</td>
<td>Pan B</td>
</tr>
<tr>
<td></td>
<td>TdT (terminal deoxynucleotidyl transferase)</td>
<td>B&amp;T precursor, cortical thymocyte</td>
</tr>
<tr>
<td></td>
<td>HLA-DR</td>
<td>Pan B, activated T</td>
</tr>
</tbody>
</table>

and bursa-equivalent), and do not require exposure to antigen for their initiation. These stages occur during fetal and neonatal development, as well as during adult life, and take the lymphocyte from a stem cell to a mature, so-called “virgin” T or B cell,
T CELL DIFFERENTIATION

Fig. 1 Hypothetical scheme of T cell differentiation. Antigen-independent stages are on the left; antigen-dependent stages on the right. The anatomical sites and immunophenotypes of putative normal T cell stages are summarized below. The neoplasms thought to correspond to each stage are indicated at the bottom of the chart. (reprinted with permission, from “Lymphoma 1987: an interim approach to diagnosis and classification” in: Pathology Annual, Rosen, P. P. ed., Appleton-Century-Crofts, East Norwalk, CT. 1987.)

which is capable of responding to antigen, but has not yet been exposed to it. On encountering an antigen that fits its surface receptor, the mature T or B cell undergoes “blast transformation” into a large, proliferating cell whose progeny differentiate into an expanded population of antigen-specific effector cells and memory cells. The suffix, “-blast” is used for the proliferating cells of both phases, with “lymphoblast” referring to the proliferating cell of the antigen-independent phase, and “immunoblast” referring to the proliferating cell of the antigen-dependent phase. This biphasic life cycle dis-
Fig. 2 Hypothetical scheme of B cell differentiation. Antigen-independent stages are on the left; antigen-dependent stages on the right. The anatomical sites and immunophenotypes of putative stages of normal B cell differentiation are summarized below. Note that antigen-dependent B cell differentiation has two possible pathways, corresponding to the early (immunoblastic) reaction and the late (germinal center) reaction. The neoplasms thought to correspond to each stage are indicated at the bottom of the chart. (reprinted with permission, from “Lymphoma 1987: an interim approach to diagnosis and classification” in: Pathology Annual, Rosen, P. P. ed., Appleton-Century-Crofts, East Norwalk, CT. 1987.)

Figures and text display:
- Lymphoma Marker Studies
- B cell differentiation
- Antigen-independent stages
- Antigen-dependent stages
- Normal cell differentiation
- Location
- Normal cell
- B-precursor
- Pre B
- Early B
- Normal B
- "Virgin"
- Centromblast
- Centrocye
- B-immunoblast
- Plasma cell
- Normal cell divisions
- Bone marrow
- Differing lymphoid cells from other cell types
- Myeloid, erythroid, epithelial cells
- Understanding lymphoid neoplasms
- Pathology Annual
- Rosen P. P.
- Appleton-Century-Crofts
- East Norwalk, CT
- 1987
Table 3 Major Categories of Lymphoproliferative Diseases Recognizable by Morphology and Immunologic Markers

<table>
<thead>
<tr>
<th>NAME</th>
<th>IMMUNOPHENOTYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. B CELL NEOPLASMS</strong></td>
<td></td>
</tr>
<tr>
<td>1. B-precursor lymphoblastic lymphoma/leukemia</td>
<td>TdT+ CALLA+ Cmu+ CD19+ CD20+/-</td>
</tr>
<tr>
<td>2. Burkitt’s and Burkitt-like (“non-Burkitt”) lymphoma</td>
<td>S IgM+ CALLA+ pan B antigens +</td>
</tr>
<tr>
<td>3. Small lymphocytic lymphoma/B-chronic lymphocytic leukemia</td>
<td>S IgM+D+ pan B antigens + CD5+</td>
</tr>
<tr>
<td>4. Small lymphocytic lymphoma, plasmacytoid (immunocytoma)</td>
<td>S IgM+Clg+ pan B antigens +</td>
</tr>
<tr>
<td>5. Centrocytic lymphoma (intermediate lymphocytic)</td>
<td>S IgM+D+ pan B antigens + CD5+</td>
</tr>
<tr>
<td>6. Follicular lymphoma (centroblastic/centrocytic)</td>
<td>S Ig+ (M, G, A) pan B antigens + CD21+ CALLA+</td>
</tr>
<tr>
<td>7. Large cell lymphoma (centroblastic, immunoblastic)</td>
<td>S Ig+ (M, G, A) pan B antigens + (immunoblastic may lose CD20)</td>
</tr>
<tr>
<td>8. Hairy cell leukemia</td>
<td>S Ig+ (M, D, G, A) pan B antigens + Leu M5+</td>
</tr>
<tr>
<td>9. Plasmacytoma, myeloma</td>
<td>Clg+ (G, A) pan B antigens − CD45−/+ , HLA−DR−/+</td>
</tr>
<tr>
<td><strong>II. T CELL NEOPLASMS</strong></td>
<td></td>
</tr>
<tr>
<td>1. T lymphoblastic lymphoma/leukemia</td>
<td>TdT+ CD1+ CD2+ CD7+ CD4+ CD8+</td>
</tr>
<tr>
<td>2. T chronic lymphocytic leukemia</td>
<td>CD2+ CD3+ CD5+ CD7+ (variable), most CD4+</td>
</tr>
<tr>
<td>3. Large cell immunoblastic lymphoma</td>
<td>CD2+ CD3+ CD5+ CD7+ (variable), CD4 or CD8</td>
</tr>
<tr>
<td>4. Adult T cell lymphoma/leukemia (endemic)</td>
<td>CD2+ CD3+ CD5+ CD7+ CD4+ HLA−DR+ (HTLV 1+)</td>
</tr>
<tr>
<td>5. Nonendemic pleomorphic T cell lymphoma</td>
<td>variable; antigen loss common</td>
</tr>
<tr>
<td>6. Mycosis fungoides/Sezary’s syndrome</td>
<td>CD2+ CD3+ CD5+ CD3+ CD7− (HTLV 1−)</td>
</tr>
<tr>
<td>7. T8 lymphoproliferative disease</td>
<td>CD2+ CD3+ CD8+ CD7+ HLA−DR+</td>
</tr>
<tr>
<td><strong>III. NEOPLASMS OF UNCERTAIN LINEAGE</strong></td>
<td></td>
</tr>
<tr>
<td>1. Hodgkin’s disease</td>
<td>lymphocytes; mature T, CD4 predominant, plus polyclonal B RS cells CD15+ CD30+ CD45− HLA−DR+</td>
</tr>
<tr>
<td>2. Angiocentric lymphoma</td>
<td>CD2+ CD3+ CD4+ (T cell receptor gene rearranged)</td>
</tr>
</tbody>
</table>
Table 4 Procedure for Lymph Node Biopsy Processing
("Lymphoma Workup")

1. Send the entire specimen fresh in saline to frozen section laboratory.
2. Freeze a representative piece in cryostat embedding medium, and cut a diagnostic frozen section to get an impression of the differential diagnosis.
3. If the frozen section shows malignant tumor, fix a portion for electron microscopy.
4. SAVE the frozen tissue in an airtight container at −70°C for immunohistologic studies.
5. If the specimen is small and no more tissue is available for permanent sections, cut 10–20 additional unstained frozen sections and SAVE the slides frozen for immunohistologic studies.
6. Fix remaining tissue for processing into paraffin for routine histological examination.
7. If non-Hodgkin's lymphoma is suspected on the basis of the frozen section, order immunohistochemical stains immediately; otherwise wait for permanent sections, to see whether immunophenotyping will be required.

At each of these stages of differentiation, lymphoid cells have characteristic immunophenotypes,10–12 as well as characteristic morphologic features. The diagrams in figures 1 and 2 illustrate the general morphologic appearance (size and shape) of each stage in the T and B cell systems, and the accompanying text indicates the name and location of each stage, following by its immunophenotype. (For a more complete review, see ref. 1).

Immunological Phenotypes of Lymphomas

Lymphoid neoplasms are usually composed of a relatively homogeneous population of cells, all of which resemble some type of cell found in non-neoplastic lymphoid tissues. The neoplasm can thus be classified according to the normal cell it most resembles, in a manner analogous to the classification of tumors of other tissues. The similarity between neoplastic and normal lymphoid cells may be morphologic, immunologic, functional, or any combination of the three. In practice, a combination of morphologic and immunologic features are used in the diagnosis and subclassification of these neoplasms.1–4

Although the current understanding of both the immune system and the lymphomas is inadequate to permit this to be done in all the cases, the normal counterpart of many lymphomas can now be postulated with reasonable certainty.1,9 The term normal counterpart does not mean cell of origin: it is possible to determine the stage of differentiation of many lymphomas, but this does not mean that neoplastic transformation occurred in a cell that had reached that stage. It may be that neoplastic transformation occurs in a primitive cell, and that the level of differentiation achieved by the tumor, depends on factors such as specific oncogene translocation. The immunological features of the lymphomas that can be recognized at present, arranged in order of their putative stage of differentiation, are summarized in Table 3 and illustrated in Figs. 1 and 2.
Technical Factors

Immunophenotyping can be done either on viable cell suspensions or on fresh-frozen tissue sections, using either polyclonal or, more commonly, monoclonal antibodies. Each technique has advantages and disadvantages, but in general similar information can be gathered from both methods, and the choice depends on the particular expertise available at a given institution. Cell suspension analysis is most suited to cells that naturally occur in suspension, particularly peripheral blood cells, while tissue section analysis is preferable for many biopsy specimens, particularly small or fibrotic specimens, such as those obtained from extranodal sites, or tissues only partially involved by tumor.2,13

Although limited immunophenotyping can be done on formalin-fixed, paraffin-embedded tissue sections, of the sort used for routine histologic diagnosis, most lymphocyte-associated antigens detected by currently available monoclonal antibodies do not survive this processing. Although many laboratories are working to develop antibodies that can reliably detect lineage-specific antigens in paraffin sections, for the present fresh tissue remains essential. This means that both surgeons and pathologists must plan ahead, to ensure that the diagnostic specimen is handled appropriately at the time of the biopsy procedure.

Whenever a biopsy is performed on a lesion for which lymphoma is in the clinical differential diagnosis, fresh tissue should be obtained for immunophenotypic studies. In practice, this includes all diagnostic lymph node biopsies, biopsies of suspected tumors in other sites, for which the cell type is not clinically evident, and fluids from patients known or suspected to have lymphoma. The procedure we use for handling such cases is outlined in Table 4. Performing and interpreting immunophenotyping studies requires experience, and is best done in high volume laboratories. In small laboratories, frozen tissue can be obtained, and if immunohistologic studies are deemed necessary, the frozen tissue can then be sent on dry ice to a reference laboratory for staining.

Situations in which immunophenotyping is clinically useful (Table 5)

Lymphoma vs non-lymphoid tumor

The distinction between lymphoma and nonlymphoid tumors is of great clinical importance, since the treatment and prognosis will in most cases be vastly different depending on the diagnosis. This is an extremely common problem; lymphomas can have a wide variety of morphologic appearances, and seem to have replaced syphilis as the “great imitator” of other diseases. The list of tumors that can be mimicked by lymphomas includes nasopharyngeal carcinoma (lymphoepithelioma), small cell carcinomas of the lung and other sites, other poorly differentiated or undifferentiated carcinomas, small round cell tumors of childhood (neuroblastoma, Wilm’s tumor, rhabdo-
myosarcoma, Ewing's tumor), glioblastoma, thymoma, seminoma, melanoma, and even spindle cell sarcoma. The single most important factor in making the correct diagnosis in these cases is a high index of suspicion: recognizing that lymphoma is in the differential diagnosis. Frequently the correct diagnosis, even if suspected, cannot be established with certainty without immunophenotypic confirmation. In the distinction between lymphoma and non-lymphoid tumor, markers may be essential for diagnosis: tissue should be prepared for immunophenotypic studies on every biopsy of a poorly differentiated malignant tumor.

The most useful marker in this differential diagnosis is the leukocyte common antigen (CD45), since it is expressed by the majority of lymphomas of either T or B cell type. Immunoglobulin light chains and pan-B and pan-T cell antibodies should also be used, since CD45 may be absent from some lymphomas. If frozen tissue is not available, paraffin-section immunophenotyping has a high likelihood of being useful in this differential diagnosis. The leukocyte common antigen, which is present on most, but not all lymphomas, is preserved in paraffin sections, as are many epithelial cell markers such as cytokeratin and epithelial membrane antigen. Thus, in contrast to most of the other differential diagnoses to be discussed, fresh tissue may not be required in all cases. Finally, electron microscopic examination may yield a correct diagnosis in many of these cases, if immunophenotyping is unsuccessful.

The use of immunohistologic studies has identified as lymphomas many tumors that would have been otherwise classified as undifferentiated carcinomas or sarcomas. As a result, the pathologist's index of suspicion that a poorly-differentiated neoplasm may be lymphoma is increased, and the spectrum of morphologic and clinical manifestations of large cell lymphoma is better understood than it was a decade ago. The impact on patient care is particularly important, since many patients who might have been treated palliatively for widespread tumor presumed to be carcinoma, may be cured, or at least enjoy prolonged remission, if treated appropriately for high grade lymphoma.
Neoplastic versus reactive lymphoid infiltrates

As with the distinction between lymphoid and nonlymphoid tumors, it is essential for clinical management to distinguish between benign and malignant lymphoid infiltrates. In contrast to the former differential, in which a number of antigens are useful, and in which other techniques such as electron microscopy may be diagnostic, there is only one antigen that is reliable in distinguishing between reactive and neoplastic lymphoid infiltrates: immunoglobulin. The usefulness of immunoglobulin in this differential diagnosis lies in the phenomenon of light chain restriction. Each B cell can make only one light chain; in normal (polyclonal) populations, about 2/3 of the B cells make kappa, and 1/3 lambda. In neoplasms, in which all cells are derived from a single precursor cell (monoclonal), all of the B cells will carry the same light chain, either kappa or lambda. In practice, an excess of one light chain (monotypic or monoclonal immunoglobulin) is taken as evidence of B cell neoplasia.

There are two major types of lymphoid proliferation in which the distinction between reactive lymphoid hyperplasia and B cell lymphoma may be difficult or impossible on histologic grounds alone: reactive follicular hyperplasia vs. follicular lymphoma, and small lymphocytic (well-differentiated lymphocytic) lymphoma vs. benign inflammatory infiltrates. The histologic diagnosis of follicular lymphoma is not usually difficult, but there are occasional cases in which even the most experienced hematopathologist is happy to have immunohistologic confirmation of the diagnosis, in the form of monotypic staining for immunoglobulin light chains.16,17

A more difficult problem for the pathologist is the differential diagnosis of extranodal infiltrates of small lymphocytes that lack significant cytologic atypia. These infiltrates occur commonly in the orbit, lung, gastrointestinal tract, and skin.18,19 In lymph nodes or bone marrow, the diagnosis of lymphoma of small lymphocytic type can be made, even in the absence of cytologic atypia, by virtue of the fact that a neoplastic infiltrate does not respect normal architectural boundaries: it fills sinuses, extends beyond the lymph node capsule, or replaces normal marrow elements. In extranodal sites, these architectural landmarks are lacking, and a diagnosis of small lymphocytic lymphoma is difficult or impossible.

Prior to the development of techniques for detecting immunoglobulin light chains in tissue sections, a diagnosis of extranodal lymphoma was made almost exclusively on lesions that had sufficient cytologic atypia to be recognized as malignant on histologic grounds.20 Infiltrates of normal-appearing lymphocytes were called "pseudolymphomas." Immunophenotyping studies have now shown that many of the lesions that had been considered to be histologically benign are in fact clonal B cell proliferations with immunoglobulin light chain restriction.18,19 These infiltrates are now recognized as low-grade B cell neoplasms, of small lymphocytic or plasmacytoid lymphocytic type.

Translation of this observation into clinical practice must be done with caution.
Since in the past, lymphomas were only diagnosed in these sites when the cells "looked" malignant, they were usually of intermediate or high clinical grade and required aggressive therapy. The concept of extranodal lymphoma must now be expanded to include those of so-called well differentiated or small lymphocytic type, which are of low-grade malignancy. For the most part, the infiltrates now being called B cell lymphoma of small lymphocytic or lymphoplasmacytic type are the same lesions that were called pseudolymphoma five years ago. Thus, it is likely that they will not require therapy as aggressive as that for high grade extranodal lymphomas. The ability to distinguish these monoclonal infiltrates of small B lymphocytes from truly non-neoplastic lesions offers the opportunity to devise more effective treatment strategies for these low-grade extranodal lymphomas.

Other than immunoglobulin light chain restriction, there are at present few if any immunologic markers that will permit a definite distinction between reactive and neoplastic lymphoid proliferations. There is as yet no marker for monoclonality in the T cell system, analogous to the immunoglobulin light chain, although it is possible that panels of antibodies against various configurations of the T cell receptor molecule may eventually be useful in this regard. Histologically recognizable malignant lymphomas do often have anomalous phenotypes—that is, phenotypes not recognized on normal T or B cells—of which immunoglobulin light chain restriction is only one example. These anomalous phenotypes include absence of immunoglobulin in cells expressing pan B antigens, coexpression of CD5 or CD10 and B lineage antigens, and failure of a T cell population to express one or more T lineage antigens. Theoretically, expression of one of these anomalous phenotypes could permit distinction between a benign infiltrate and lymphoma. In practice, this is difficult for two reasons. First, it is possible that some of these anomalous phenotypes may be expressed by uncommon populations of normal cells, and therefore may not be reliable indicators of neoplasia. Second, the results of immunostaining can be difficult to interpret when the infiltrate contains an admixture of atypical cells and normal-appearing cells, which is often the case in lesions that cannot confidently be classified as benign or malignant on routine sections alone. This latter factor is a particular problem in the distinction of benign from malignant T cell infiltrates, since benign T cells of both helper and suppressor phenotypes may be very numerous in both, and detection of a small population of cells that lacks a particular antigen can be very difficult.

Subclassification of lymphomas

The importance of the immunological subclassification of lymphomas is less generally accepted than the importance of distinguishing between benign and malignant lymphoid infiltrates and between lymphoid and nonlymphoid neoplasms. For the most part, the clinically relevant subclassification of lymphomas still rests primarily on
morphologic features. However, there are certain situations in which the lineage of a lymphoma may not be apparent morphologically, and may be clinically significant.

1. Hodgkin's disease (HD) vs. non-Hodgkin's lymphoma. The diagnosis of HD is usually straightforward on routinely-fixed and stained sections. However, several subtypes of HD may occasionally be confused with non-Hodgkin's lymphoma. Distinction between HD and B cell neoplasia is usually straightforward using antibody to immunoglobulin, since B cell lymphomas usually express immunoglobulin with restricted light chain expression. Distinction between T cell lymphoma and HD remains difficult, since the background lymphocytes in HD are predominantly T cells of the CD4 (helper) phenotype, and the majority of T cell lymphomas also express the CD4 phenotype.

As yet, there are no specific markers for Reed-Sternberg cells. Expression of two antigens, CD15 and CD30, is characteristic of Reed-Sternberg cells, as is lack of CD45. However this phenotype is not specific for Reed-Sternberg cells, and may not be found in all cases of Hodgkin's disease. Thus, the characteristic staining pattern is helpful to support the diagnosis of HD, but is neither necessary nor sufficient for the diagnosis. At present, the diagnosis of HD is still made almost exclusively on morphological criteria.

2. Lymphoblastic neoplasms of T vs B precursor types. Lymphoblastic lymphomas and acute lymphoblastic leukemias may be composed of primitive cells of either T or B lineage. The natural history and response to treatment of the tumor may differ depending on its lineage, so that in this diagnosis it is important to know the phenotype. There are no reliable morphologic criteria for distinguishing T from B precursor lymphoblastic neoplasia, but the immunophenotypes are distinctive. In the diagnosis of lymphoblastic lymphoma/leukemia, markers are essential for diagnosis and fresh tissue must be obtained in all the cases.

3. Diffuse mixed small and large cell lymphomas of B vs T cell types. This category in the Working Formulation is acknowledged to be heterogeneous, and contains lymphoplasmacytoid lymphomas, follicular center cell lymphomas, and probably the majority of non-lymphoblastic, non-mycosis fungoides T cell lymphomas (so-called "peripheral" T cell lymphomas). While the B cell lymphomas in this category may behave in a relatively indolent fashion, there is some evidence that T cell tumors may be more aggressive. An important member of this group is the virus-associated Adult T Cell Lymphoma/Leukemia. This rare but aggressive malignancy does not have pathognomonic histologic features, and can only be diagnosed by a combination of marker studies and testing for antibodies to the human T cell leukemia virus. Thus, immunophenotyping studies should be performed in diffuse lymphomas that fall into the mixed small and large cell category of the Working Formulation.

4. Subclassification within the T or B cell lineage. Immunophenotyping as a way
of subclassifying neoplasms within a given lineage is still in its developmental stages. Currently its most important use is in the distinction between lymphoblastic lymphomas of either T or B-precursor type and more differentiated tumors of the lineage, chiefly Burkitt or Burkitt-like lymphomas and large cell lymphomas in children. Immunophenotypes characteristic of some other subtypes of T or B cell lymphomas have been described as panels of antibodies have been applied to large series of cases.\textsuperscript{1,3,4,17,28,29} However, differences in classification schemes and antibody panels, as well as the small numbers of cases in any one study impair the usefulness of many of these observations. Finally, it remains to be shown that subclassification by immunophenotype is as accurate or more accurate than morphology in predicting natural history and response to treatment. This is a field whose potential has not been fully explored, and which will doubtless have more impact on diagnosis as more experience is gained.

Advances in the treatment of lymphomas will also influence the usefulness of marker studies. The current Working Formulation for non-Hodgkin’s lymphomas\textsuperscript{23} subclassifies lymphomas according to their response to currently available treatment modalities. Clearly, if new modalities are discovered, this scheme may become irrelevant. If treatment with monoclonal antibodies becomes widespread, the immunophenotype of a lymphoma will become its most important feature.

**Conclusion**

The correct diagnosis and subclassification of the lymphomas is of enormous clinical importance. These are tumors that affect individuals of all ages. They can be highly aggressive and fatal, or they can be indolent. Effective therapy exists for many of the more aggressive tumors, but the therapy is toxic and, occasionally, life-threatening, and should not be given unnecessarily. The treatment for lymphomas differs from that for nonlymphoid tumors in many cases, and also differs for the different subtypes of lymphoma.\textsuperscript{30} All these factors combine to make the diagnosis and subclassification of lymphomas much more important than it was only two decades ago.

The diagnosis of lymphoma on routinely processed histologic sections is one of the most difficult and poorly reproducible areas of histopathology. With the judicious application of immunophenotyping, diagnostic accuracy can be greatly improved. At present, immunophenotyping should be done in most cases of non-Hodgkin’s lymphoma and in virtually all undifferentiated malignant tumors, to provide confirmation of the diagnosis.

The impact of immunophenotyping studies on diagnosis is threefold. First, in an individual case, a definite diagnosis can often be made which would have been impossible, incorrect, or only presumptive, if only routine histologic sections had been available. Second, the pathologist’s “eye” is improved for future cases: if, knowing the
results of the immunohistologic stains, routine sections are reexamined, the histologic
criteria for making the diagnosis may be refined. Third, our understanding of the
morphologic and clinical spectrum of the lymphomas is enhanced.

The contribution of marker studies to the diagnosis of lymphoma is well established.
Their potential contribution to subclassification has not been fully explored. The
classification of the non-Hodgkin's lymphomas remains an area of controversy. Under-
standing the relationship of lymphoid neoplasms to their normal counterparts in the
immune system, through a combination of immunophenotype and morphologic features,
should help to predict their natural history and response to treatment. Large, coopera-
tive studies are needed, combining careful morphologic subclassification, large panels
of monoclonal antibodies, and clinical follow-up information, to determine the contri-
bution of immunophenotyping studies to the clinically relevant subclassification of this
group of diseases.

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