Surface Markers in Leukemias and Lymphomas

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Cell marker analysis has contributed to a better understanding of the biology and morphology of the lymphomas, and its application to the lymphomas has provided important insights into the working of normal lymphocytes. In the process, the immunologist, the pathologist, the clinician who deals with lymphomas, and the patient have all benefited. Continued study of cellular differentiation through analysis of the lymphoproliferative disorders should shed further light on this difficult but fascinating area of medicine. (Am J Pathol 90:451–460, 1978)

RATIONAL THERAPY, proper epidemiology, and meaningful immunobiology can be applied to or derived from consideration of lymphoproliferative diseases only if their classification can be rendered truly objective. The well-known difficulties encountered by the pathologist in the diagnosis of the lymphomas include a frequent lack of unanimity concerning classification both of a particular lesion and of a taxonomic scheme. The development, over the past decade, of markers of mononuclear cell differentiation, both in experimental animals and in humans, has permitted discrimination among morphologically similar cell types on objective grounds. Most of these new techniques have been developed by immunologists and geneticists concerned about basic questions of lymphocyte biology, but the enzymologist and the electron microscopist have also contributed to the development of cell markers applicable to the characterization of the lymphomas.[1]

On the basis of clinical and experimental data, the immune system of lymphocytes and their products has been divided into two components. The B cells, particularly after further differentiation into plasma cells, make immunoglobulins (Ig) and constitute the humoral immunity system. In avian species, although not in mammals, a thymus-like organ known as the bursa of Fabricius provides the environment for early differentiation of the B-cell line. The T cells, developing under the influence of the thymus, provide control for the B-cell response to antigens and act on their own in providing those functions traditionally included in cell-

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mediated immunity. These two lines of lymphoid differentiation have their counterparts in the development of experimental lymphoproliferative syndromes. In 1944, McEndy, Boon, and Furth first showed the thymic dependence of certain mouse leukemias, which are known to occur with high frequency in AKR strain mice and to arise within the thymus.[2] Peterson and his colleagues, in 1966, defined avian visceral lymphomatosis as a disease of bursa-dependent (B) lymphocytes, which begins in a single area within the bursa and later generalizes.[3] The newly developed markers capable of defining cells of the two types in humans have been widely applied in many laboratories. It has become clear that many human lymphoid neoplasms, as predicted, can be associated with one or the other mode of cellular differentiation and that certain morphologic or marker-defined entities can be localized within these developmental pathways according to similarities with normal stages of differentiation of the lymphoid system. Most, although not all, have been monoclonal expansions of a single cell type.

Despite the apparent simplicity of various schemes of classification based on cellular differentiation marker analysis, it seems to us that the approach must be regarded as still being at the descriptive level. Uncertainties exist concerning the significance of some of the markers in use; elucidation of these uncertainties may define more stages of differentiation than are currently appreciated. Through continued experience, however, these problems should be resolved. The best approach to cellular definition is that which takes into account as many independent criteria as possible. For this reason, multiple markers should be utilized in the characterization of the lymphomas.

In the present paper, we review not only our own experience but also that of many other laboratories in an attempt to synthesize what is known about lymphoreticular neoplasms. A more extensive bibliography has been compiled by Siegal and Good.[1] We do not attempt to put forth a new, improved classification scheme but to relate the lessons of the new marker systems to more conventional systems of taxonomy.

Functional Heterogeneity of Lymphocytes

The mononuclear cells comprising the lymphomas are usually round cells with the morphologic characteristics of lymphoid cells or histiocytes. Frankly monocytic–histiocytic cells can be identified relatively simply, as described below. The lymphoid cells, however, are functionally heterogeneous, as illustrated in Table 1. The variety of differentiation markers which discriminate among their lineages and stages of development are outlined in Table 2. The functional subset, the K cell, which is operation-

Table 1—Functional Categories of Cells Commonly Identified as Lymphocytes: Nonphagocytic Mononuclear Cells of Humans

	Approximate proportion among blood "lymphocytes" (%)
Precursors of plasma cells (B cells)	10
Thymus-processed lymphocytes (T cells)	80
Mononuclear cells involved in ADCC (K cells)	5
Promonocytes, monocytes	Variable (0-5)
Precursors of B cells	0.1
Precursors of T cells	0.1
Colony-forming cells	0.001
Others	??

ADCC = antibody-dependent cell-mediated cytotoxicity.

ally defined by its capacity to carry out antibody-dependent cytotoxic activity, falls within the cells of indeterminate type (Table 2) but is associated with no discrete marker of its own. Several detailed reviews of these markers have been published.[1,4-6]

Characterization of Lymphoreticular Neoplasia Employing Differentiation Markers

Certain forms of mononuclear cell neoplasia have been well characterized almost from the earliest use of the surface markers, although our knowledge even of these has expanded as more sophisticated techniques have been employed. The ordinary form of chronic lymphocytic leukemia (CLL), Waldentröm macroglobulinemia and multiple myeloma, with their variants (heavy chain diseases, CLL with monoclonal gammopathy) can be considered a spectrum of malignant expansions at various steps along the differentiation pathway of the B lymphocyte. They are all characterized by the manufacture of Ig. Gradations from one disease to another are readily understandable within this scheme. Other B-cell malignancies (see below) can also be included. A T-cell type of CLL has been described, occurring with a frequency perhaps one fiftieth that of CLL of B-cell type, although an extraordinary number of cases has been reported from certain islands off the coast of Japan. Cells of T-CLL form sheep rosettes at 4 C but not at 37 C and lack the enzyme terminal deoxynucleotidyl transferase (TdT) and consequently can be considered as representing postthymic T cells. Another form of T-cell neoplasm is the Sézary syndrome, involving cells which have markers similar to those of the more conventional T-CLL cells, although their cerebriform nuclei often render them identifiable as such. In certain cases, these cells can be further defined as belonging to the helper subset of T cells, providing "help" to

Table 2—Cellular Differentiation Markers Applicable to Lymphoreticular Neoplasia

Bone-Marrow-Derived (B) Lymphocytes:	
Their Precursors and Progeny	
Precursors	
Lymphoid stem cell	?B-cell alloantigens (Ia-like)
Pre-B lymphocyte	Cytoplasmic IgM
B lymphocytes	Surface IgM, IgD, (IgA, IgG)
	Mouse erythrocyte rosette
	Receptors for immune complexes
	IgG (as IC or as aggregates)
	C3d, C3b
	B-cell alloantigens (Ia-like)
	EBV receptors
Plasmacytoid B cells	Surface IgM
	Cytoplasmic Ig + others presumed
Plasma cells	Cytoplasmic Ig, J-chain
	Plasma cell antigens
Other characteristics	Synthesis and secretion of Ig
	Response to mitogens (T cells + PWM)
	Localization to B-cell areas of lymphoid tissue
	Tendency to aggregate
Thymus-Derived (T) Lymphocytes:	
Their Precursors and Progeny	
Precursors	
Lymphoid stem cell	? B-cell alloantigens (Ia-like)
	? TdT
Pre-T lymphocyte	TdT
Thymic T cells	TdT
	Sheep erythrocyte rosette at 37 C and 4 C
	Thymocyte heteroantigens
Peripheral T cells	Sheep erythrocyte rosette at 4C
	T cell autoantigens, heteroantigens
	Receptors for Ig
	Receptors for IgM ("T_{mu}")
	Receptors for IgG ("T_{gamma}")
	Rhesus monkey erythrocyte rosette*
	Hemagglutinin of *Helix pomatia**
	Acid phosphatase
	Adenosine deaminase (10 × B-cell levels)
Cells of Indeterminate Type	
Functions not clearly defined; heterogeneous	Receptors for IgG, often of high avidity
	Receptors for complement
	Sheep rosette formation under certain conditions
	Nonphagocytic
	Most negative for monocyte-associated enzymes (α-naphthylacetate esterase)
Monocytes	Receptors for IgG, often of high avidity
	Receptors for complement
	Phagocytic
	α-Naphthylacetate esterase positive (NaF sensitive)

* Expressed on some CLL B cells.

IC = immune complexes; EBV = Epstein-Barr virus; Ig = immunoglobulin; PWM = pokeweed mitogen; TdT = deoxynucleotidyl terminal transferase.

B-cell differentiation *in vitro*.[7] Burkitt lymphoma was the first lymphoid neoplasm on which surface Ig was noted, qualifying it as the original B-cell tumor. Interestingly, the expression of Epstein–Barr virus receptors on these cells can vary considerably. After initially having been considered a true monocytic neoplasm, leukemic reticuloendotheliosis (hairy cell leukemia) can now probably be classified within the B-cell line, although its morphology on scanning electron microscopy and tendency to combine with latex particles still suggest a derivation from the monocytes. A normal counterpart to these peculiar cells has not been definitely identified. Acute lymphoblastic leukemia appears to come in two forms. One type is associated with thymic lymphocytic (thymocytic) surface markers, forming sheep rosettes at 37 C as well as at 4 C; the other carries "B-cell alloantigens," or "Ia-like" antigens. These antigens characterize B cells and probably stem cells of both lymphoid and myeloid lines but are lacking on T-cell surfaces. Both types of ALL cells contain the enzyme TdT, which would appear to place them early in the T-cell lineage.[8-10]

Study of the solid-tissue non-Hodgkin lymphomas (NHL) using these techniques has been more difficult; therefore, their characterization has been delayed relative to those neoplasms appearing with greater frequency in a leukemic phase. Study of the NHL in leukemic phase has yielded considerable information about the nodal disease; combining this sort of information with that derived from direct study of lymph node lymphocytes in suspension and on frozen sections has led to our grouping the NHL into several categories on the basis of nodal architecture, cytology, cell surface, and other markers.[11,12]

Type I consists of small, round, uncleaved cells with scanty cytoplasm, which look like quiescent, small lymphocytes. Their nuclear chromatin is clumped and their nucleoli are inconspicuous. Most of these are associated with a CLL picture in the peripheral blood and a nodal architecture characterized as diffuse, well-differentiated lymphocytic lymphoma. The differentiation markers are characteristic of the CLL B cell: surface Ig is monoclonal (either κ or λ light chains), the heavy chain is usually IgM, with or without IgD. Other Ig classes are rarely represented. Unlike Type II cells, the staining by immunofluorescence of the Ig of these B cells is usually quite faint, and the amount of Ig, as revealed by other immunologic techniques, is generally scanty. These cells have a tendency to form spontaneous rosettes with mouse erythrocytes. In our series [11] 47% formed such rosettes, in contrast to the B cells of Type II (see below). The proportion of cells having receptors for complement does not differ from those of Type II. Markers characteristic of other cell types are usually

lacking. Type I B-cell neoplasia would thus be considered the nodal phase of CLL.

The Type II lymphoma B cell differs from that seen in Type I in several ways. These cells stain much more brightly for surface Ig and tend to make mouse rosettes only poorly: only approximately 7.5%, on the average, form these rosettes. Such cells appear in lymphomas characterized by diffuse or nodular architecture. They can be of the poorly differentiated, lymphocytic, histiocytic, or mixed types, as defined by Rappaport.[13] Both larger cell types (histiocytic) and smaller ones (lymphocytic), often with cleaved nuclear morphology, have the same surface marker configuration. These cells also lack the markers of monocytes and of T cells. Chiefly on the basis of size and appearance of "activation," we have grouped the smaller cell types with this marker display as Type II A and the larger ones as Type II B. It is these cells which were recognized by Aisenberg et al [14] as the brightly staining cells of chronic lymphosarcoma cell leukemia. The finding of two distinct types of B-cell lymphomas strongly suggests that two types of B cell exist as their counterparts among normal lymphocytes. The concept of follicular center cell neoplasia [15,16] fits best with our Type II cells, while the uncleaved, pregerminal center cell, fits best with that of Type I.

Certain rare, true histiocytic lymphomas of the monocyte–macrophage lineage are included in Type III. These cells have high-affinity receptors for the Fc portion of IgG, stain for nonspecific esterases and peroxidase, and produce lysozyme (muramidase). They usually exhibit some degree of phagocytosis when exposed to particulate markers such as latex or opsonized erythrocytes. These cells lack the definitive B-cell markers (mouse rosetting, production of Ig), but they do have complement receptors and carry the B-cell alloantigens (Ia-like) and bear surface Ig (IgG) which is adherent through the Fc receptor. They thus could be confused with B cells. The patients we studied presented with monocytic leukemia with lymphadenopathy.[11,12] The cells had the morphologic characteristics of monocytes rather than of activated lymphocytes. One must emphasize that most histiocytic lymphomas consist of cells of our Type II B rather than of Type III. Brouet et al [17] called attention to the infrequency of true histiocytic lymphomas (those composed of Type III cells).

Still another distinct lymphoid or stem-cell type is exemplified by medium-sized round cells with dispersed chromatin, round or convoluted nuclear morphology, and T-cell markers. On the basis of our own experience and the studies of Nathwani et al [18] and McCaffrey et al,[10] we have grouped these as Type IV. These cells carry the nuclear enzyme TdT, and form rosettes at 37 C and 4 C (attributes of normal human thymic

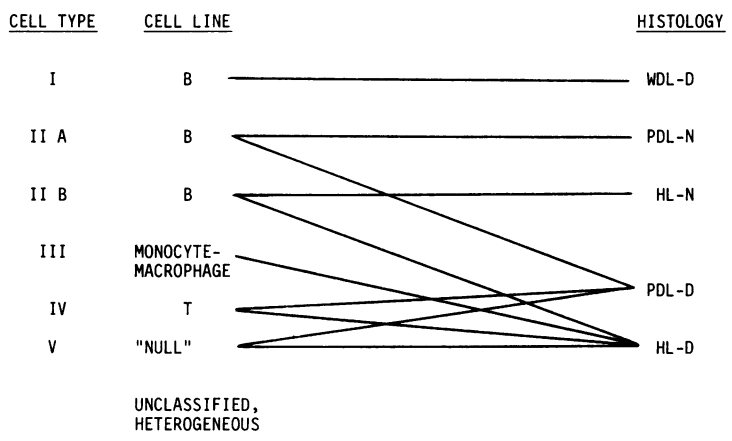
lymphocytes [thymocytes]). These lymphomas, included in Rappaport's 1966 classification [13] in the diffuse poorly differentiated lymphocytic type (DPDL), have been separated as a distinct morphologic type by Nathwani et al [18] on the basis of markers and other features. In our experience, such cells, lacking B-cell markers for the most part (complement receptors are occasionally present) are frequently found in the peripheral blood of young adults and older children with leukemic DPDL, despite the fact that most DPDL in adulthood is of B-cell type. This biologic tendency of T-cell DPDL to develop a leukemic phase is another feature of the cells of Type IV. The marker data and the fact that Type IV cells are frequently associated with a mediastinal tumor suggest that the disorder associated with these cells is a true thymic lymphoma, perhaps analogous to that observed by Furth and his colleagues [2] in high-leukemia–strain mice. Type IV cells are seen in approximately 25% of children with ALL.

Type V cells lack markers of mature T cells, although they generally seem to contain TdT. They would be expected, from the work of Fu et al,[9] to carry the Ia-like "B-cell alloantigens," which may indicate that such cells are counterparts of cells very early in the differentiation of hematopoietic cells. They lack other B-cell markers (rarely, they have complement receptors) and markers of monocytes. The presence of TdT would seem to have the same significance as the presence of Ia-like antigens, although it could indicate that differentiation along the T-cell line has begun. Cells of this type are occasionally seen in lymph nodes associated with the "null" type of childhood ALL, which accounts for approximately three fourths of the ALL cases. The histology of nodes consisting of Type V cells has been DPDL, in our experience, although perhaps they too (as with Type IV) should be included in the malignant lymphoma, lymphoblastic type (MLLB) of Nathwani et al.[18]

Rare cell marker constellations which do not seem to fit any of the above groupings are occasionally found. They may reflect the existence of normal lymphoid subsets which occur in low frequency, or the abberrant display of markers not usually expressed, by virtue of their neoplastic state. Cells bearing markers characteristic of both B and T cells, diclonal proliferations, apparently unrelated, and other sorts of neoplasms have been described.

Relationship Between Rappaport Classification (1966) and Cell Marker Analysis

Through the combined use of cellular morphology, nodal architecture, and the differentiation markers, it is possible to arrive at a description of several biologic cell types and to compare these types with the more traditional scheme of Rappaport's AFIP Fascicle on the lymphomas.[13] In



TEXT-FIGURE 1—Relationship of histologic group according to Rappaport classification (1966) to cell type defined by differentiation markers. (Modified from Filippa et al.[12])

general (Text-figure 1), the nodular lymphomas (histiocytic as well as lymphocytic) are B-cell neoplasms, although an occasional example of a nodular T-cell proliferation has been claimed.[1] Similarly, a morphologic definition as diffuse, well-differentiated lymphocytic lymphoma almost certainly warrants consideration as consisting of B cells (the node architecture associated with T-cell CLL has not been described). Confusion, however, arises when one considers the diffuse, poorly differentiated lymphocytic lymphomas or the diffuse, histiocytic lymphomas. Even here, cells with cleaved nuclear morphology are likely to be B cells,[16,19] if not invariably so. However, examples of virtually every mononuclear cell type have been seen among those other "lymphomas," as indicated in Text-figure 1. It would seem that in these cases, careful cell marker analysis becomes indispensable as a means of objectively identifying the type of proliferation.

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