Lymphocyte Differentiation: An Essential Basis for the Comprehension of Lymphoid Neoplasia

ORIGINS OF OUR CURRENT CONCEPTS OF MALIGNANT LYMPHOMA

"Da steh ich nun, ich armer Tor, Und bin so klug als wie zuvor."
—Faust, Part I

Our modern concepts of lymphoid neoplasia are usually considered to have had their beginnings in an initially little noticed paper by Thomas Hodgkin published in 1832 (1). In the same year Goethe’s Faust was finally completed—a fitting coincidence, because the frustrations experienced by Faust in his search after the innermost secrets of nature have frequently been felt by pathologists and physicians alike in their endeavors to comprehend malignant lymphomas (2). The depth of understanding that can be achieved is, of course, a function of the available means of investigation and the prevailing doctrines of the era. In Hodgkin’s time, for example, the concept of disease as disordered physiology had only recently superseded the ancient ideas that had held sway for over 2,000 years—due largely to the enlightening influence of the brilliant French school of the late 18th and early 19th centuries. Pathologic histology was unknown, and of necessity the earliest reports of what we now know as lymphoid neoplasia were confined to descriptions of gross pathology and clinical features. There could have been no concept of the nature of these diseases, excepting that they appeared to arise de novo in lymph nodes and spleen, were of grave consequence, and could be distinguished from lymphadenopathy occurring as part of syphilis, tuberculosis, or adjacent inflammation.

But revolutionary new ideas were to take firm hold in the course of the 19th century. By the 1830’s the microscope had become an effective instrument, leading to much more detailed descriptions of cells and to Schwann’s theory, published in 1839, that the cells were the elementary units from which all animals are composed (3). Virchow (4) played a prominent role in promoting the importance of cellular disturbances as the basis for many pathologic processes, although his concept of “cellular pathology” was seriously challenged as the microbiologic discoveries of Pasteur and Koch in the 1870’s and 1880’s led, on the part of some of their protagonists, notably Klebs, to theories that all diseases are initiated by external causes.

In 1845, independent descriptions by Virchow (5), Craigie (6), and Bennett (7) of patients with very high numbers of circulating white blood cells (by no means the first such descriptions) captured the imagination of the new breed of pathophysiologists. These descriptions, coupled with Hodgkin’s earlier observations and subsequent descriptions by other physicians of patients with generalized lymphadenopathy and frequently splenomegaly but without an increase in circulating white blood cells (8-11), provided a basis for the subdivision of diseases presenting as primary, generalized, lymph node swelling. In Germany, such diseases could now be separated into “leukemia” and the overtly similar aleukemic form, “pseudoleukemia” (12), whereas the equivalent terms “leukocytchemia” and “Hodgkin’s disease” (also called “lymphadenoma”) were used in Great Britain (13).

Localized lymph node swellings (the general term for which was “lymphoma”) not due to scrofula, typhoid, or carcinoma, i.e., of primary lymphatic origin, had also been known for some time (10, 14). In his classical work “Die Krankhaften Geschwuelste” (15), Virchow, while admitting the existence of primary lymph node sarcomas, mentioned the difficulty in separating them from “simple hyperplasia” in which the histologic appearance resembled that of a normal lymph node. Interestingly, Virchow (15) also commented on the similarity of the cell type of most mediastinal tumors to that of normal lymph node cells and stated that it seemed appropriate to classify such neoplasms with the lymphatic tumors. Much later (in 1893), Kundrat (16) attempted to separate a syndrome of multiple lymphosarcomas (“lymphosarcomatosis”) from pseudoleukemia. He emphasized the frequency of progression from one lymph node to another: a behavioral characteristic subsequently to influence considerably the radiotherapy of Hodgkin’s disease. Echoing Virchow’s earlier obser-

ABBREVIATIONS USED: ALL = acute lymphoblastic leukemia; CLL = chronic lymphocytic leukemia.

1 Pediatric Oncology Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Bethesda, Md. 20205.

2 Received December 31, 1980.

Editor’s note: Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.
vations (15), Kundrat (16) suggested that lymphosarcoma and lymphosarcomatosis were more closely related to pseudoleukemia and leukemia than to other types of sarcoma. Although Kundrat's paper stimulated considerable interest and has often resulted in his being credited, inappropriately, with the original description of lymphosarcoma, his clinicopathologic "definition" of lymphosarcomatosis clearly included cases of Hodgkin's disease, as subsequently pointed out by Ghon and Roman (17).

From our modern perspective, classification on the basis of clinicopathologic features alone would seem hazardous indeed, but in spite of Virchow's lead, histologic examination of tissue (most often at autopsy rather than biopsy) appears initially to have been performed out of curiosity rather than as part of a studied attempt to evaluate the role of histologic examination of tissue in the subdivision of lymphoid neoplasia. By the end of the 19th century, however, such histologic peregrinations had revealed to many the mixed cellular infiltrates, including giant cells, which could be seen in a fraction of the patients with "pseudoleukemia" or "Hodgkin's disease" (terms which were, at the time, synonymous) (9, 18-20). Sternberg (19) and Reed (20) have received most of the credit for these observations, possibly because of their more detailed descriptions at a time when the value of histologic observation was becoming increasingly accepted. This appearance, suggestive to many of a chronic granulomatous process (hence "lymphogranuloma"), could be clearly separated from the remaining cases of pseudoleukemia in which histologic examination revealed "pure hyperplasia," a finding which permitted, as recorded by Ghon and Roman (17) in 1916, a three-part classification: pseudoleukemia, lymphosarcomatosis, and lymphogranuloma.

Although histology was at last recognized as a diagnostic tool, the distinction between pseudoleukemia and lymphosarcomatosis was still based on clinical features, and a uniform approach to classification was lacking. Hence, when Roulet (21) in 1930 described primary "retothelsarkom" of lymph nodes (reticulum cell sarcoma) as a histologic entity, he provided, if not a precise definition, at least the remaining element of a crude taxonomic concept of the histology of malignant lymphomas which has persisted until quite recently: lymphosarcoma, Hodgkin's disease, and reticulum cell sarcoma. The term "lymphosarcoma," originally applied to invasive, local lymphatic tumors, had gradually taken on a rather different histologic significance, indicating a neoplastic process in which the constituent cells resembled lymphocytes to a greater or lesser degree (although some authors continued to use the term very loosely). In earlier years, many of the more differentiated appearances would have been considered indicative of "hyperplasia" rather than sarcoma (4, 10, 15). Hodgkin's disease had for some time been defined histologically, the variety of constituent cells being characteristic, whereas reticulum cell sarcoma was a term used for all remaining entities—including those in which the cells resembled phagocytic cells (macrophages or histiocytes).

In spite of the greater attention to histology, the older clinical and gross pathologic concepts were not immediately superseded and remained important to the establishment of a diagnosis until 1942, when Gall and Mallory (22), recognizing the poor correlation between clinical features and the histologic appearance, proposed a purely histologic classification in which lymphosarcoma and reticulum cell sarcoma were each subdivided into more and less differentiated categories (table 1). They also recognized as a separate entity the follicular lymphoma described originally as a hyperplastic process by Brill et al. (23) and Symmers (24). The Gall and Mallory classification scheme provided the framework for subsequent classifications in which histomorphology alone was utilized as a basis for subdivision. The best known of these subsequent classifications are the schemes of Rappaport (25), the British National Lymphoma Investigation (26), and Dorfman (27).

Not until the 1960's, when understanding of the immune system suddenly entered its current phase of rapid growth, was a new basis for comprehending lymphomas provided. Cooper and his colleagues (28) may have been the first to attempt to apply this newer immunologic knowledge to the understanding of lymph neoplasia, and soon histologists, notably Lukes and Collins (29) in the United States and Gerard-Marchant et al. (30) in Europe, proposed classification schemes that incorporated immunologic concepts. These schemes were still, however, histologic classifications, i.e., entirely dependent on morphology without benefit of immunologic studies. Subsequently, the results of immunologic characterization (of cell surface immunoglobulin and other receptors) have been correlated with the histologic diagnoses (31-33), but until now no scheme based purely on immunologic characteristics has been proposed.

### Table 1.—Histologic classification of malignant lymphomas proposed by Gall and Mallory (22) in 1942

<table>
<thead>
<tr>
<th>Terms in general usage</th>
<th>Gall and Mallory classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphosarcoma</td>
<td>Lymphoeytic lymphoma</td>
</tr>
<tr>
<td></td>
<td>Lymphoblastic lymphoma</td>
</tr>
<tr>
<td>Reticulum cell sarcoma</td>
<td>Stem cell lymphoma</td>
</tr>
<tr>
<td></td>
<td>Plasmacytic lymphoma</td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>Hodgkin's lymphoma</td>
</tr>
<tr>
<td></td>
<td>Hodgkin's sarcoma</td>
</tr>
</tbody>
</table>

RAPPAPORT NON-LUKES: LIMITATIONS OF HISTOLOGIC CLASSIFICATION

The lack, until quite recently, of a comprehension of the functional aspects of the normal lymphoid system has meant that attempts to understand and classify...
lymphoid neoplasms have been limited to purely descriptive approaches, taking account of the anatomic location(s) of the tumor, its natural history, and more recently its microscopic appearance. The inadequacies of these parameters and the consequent repeated attempts to devise better systems have resulted in the present multiplicity of classification schemes. This superabundance of nomenclature, coupled with the repeated redefinition of older terms and a general reluctance to renounce previous notions, has ensured that the study of malignant lymphomas has for long been hampered by a difficulty in communication and an aura of confusion. Even now we suffer from the survival of terms dating from the prehistologic era. In lymphoid neoplasia among the pediatric age group, for example, therapeutic decisions are still often determined by an arbitrary (and debated) definition of "leukemia" on the basis of degree of involvement of the bone marrow and other clinical findings (both subject to temporal variation). Currently, a major cause for confusion (and contention) in the classification of malignant lymphomas is the juxtaposition of histologic classifications on the basis of purely descriptive morphology with those that take account of the advances in immunology that have occurred in the last 20 years (table 2).

In the United States, "pre-immunological" classification of 1956 by Rappaport et al. (35), modified in 1966 (25), is still the most widely used. It differs from the prototype scheme of Gall and Mallory (22) mainly in its rejection of the concept of a true follicular lymphoma. Although the follicular appearance had for long been assumed to reflect the origin of these tumors from the germinal follicles of lymph nodes and lymphoid tissue, Rappaport disputed this assumption and suggested that an architectural variant was involved (hence might better be called "nodular") and that any cell type could occur either in a diffuse or in a "nodular" pattern. This idea stemmed from Rappaport's observation that the nodular pattern was present even when the spread occurred outside lymphoid tissue. It was also fueled from the belief that the large cells, morphologically resembling histiocytes, which were frequently seen in nodular lymphomas, were truly of histiocytic origin. Since both "histiocytic" and lymphocytic cell types and tumors composed of mixtures of these cells could occur in a "follicular" pattern, it seemed unlikely that this appearance represented a

<table>
<thead>
<tr>
<th>Table 2.—Comparison of terminology in three major histologic classifications and the recently devised &quot;working formulation&quot;a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification</td>
</tr>
<tr>
<td>Lymphomas histologically similar or identical to Burkitt's lymphoma</td>
</tr>
<tr>
<td>Undifferentiated lymphoma, Burkitt's and non-Burkitt's types</td>
</tr>
<tr>
<td>Lymphoma of thymic origin</td>
</tr>
<tr>
<td>Lymphoblastic lymphoma</td>
</tr>
<tr>
<td>Lymphomas of germinal follicle cell origin</td>
</tr>
<tr>
<td>Poorly differentiated lymphocytic lymphoma c</td>
</tr>
<tr>
<td>Mixed lymphocytic–histiocytic lymphoma c</td>
</tr>
<tr>
<td>Histiocytic lymphoma c</td>
</tr>
<tr>
<td>Lymphomas of &quot;transformed&quot; lymphocytes</td>
</tr>
<tr>
<td>Histiocytic lymphoma</td>
</tr>
<tr>
<td>Lymphocytes resembling mature lymphocytes</td>
</tr>
<tr>
<td>Lymphocytic, well differentiated</td>
</tr>
<tr>
<td>Lymphocytic with plasmacytoid features</td>
</tr>
</tbody>
</table>

a Only the major subdivisions are included here. ML = malignant lymphoma.
b This classification was first proposed by Gerard-Marchant et al. (30) and has become known as the "Kiel classification" after the University in the Federal Republic of Germany in which the histologic examination was performed.
c May be follicular (nodular in Rappaport's classification) or diffuse.
single category of lymphoma. Subsequently, it has been well shown that tumors of presumptive histiocytic origin are most often the neoplastic counterparts of large lymphoid cells (29, 33), and the work of Kojima et al. (36, 37), Lennert (38), and Lukes and Collins (39) has clearly established the presence of such cells in normal lymphoid germinal centers. Thus the concept of a true follicular lymphoma, or perhaps a group of tumors derived from germinal follicle cells, has been revived. On the basis of cellular morphology (29, 40) as well as the long-recognized topographic or temporal transition from follicular to diffuse patterns (22, 41, 42), there seems little doubt that these same follicle center cells can give rise to "diffuse" lymphomas. The original classification of Rappaport et al. in 1956 (35) has also been modified to incorporate recently described histologically distinctive tumors with characteristic clinical features, particularly Burkitt's lymphoma (43) and lymphoblastic lymphoma (44, 45).

In the early 1960's two immunologic observations were made that have had far-reaching significance both to basic immunologists and to the fields of clinical immunology and oncology. The first was the realization that the immune system is composed of two compartments (B and T) (46, 47) and the second, that mature lymphocytes are not end-stage cells but can be "transformed," i.e., from activated into effector cells (48). The descriptions of B- and T-dependent areas of lymph nodes and spleen and the occasionally well-demonstrated preferential involvement of these areas by lymphomas that also expressed some of the markers of either T- or B-lymphocytes (31, 32) were consistent with data obtained from animal models showing that removal of either the thymus or the bursa of Fabricius could prevent T-lymphomas in mice and B-lymphomas in chickens, respectively (49, 50), and, therefore, that lymphomas could arise from one or other of the two main compartments of the immune system. Further, the resemblance of some lymphomas to lymphocytes transformed in vitro with mitogens into blast cells suggested that some lymphomas arise in vivo from activated T- or B-lymphocytes, the site at which this occurred being characteristic—i.e., in the germinal follicles in the case of B-cells and, less obviously, the paracortical and periarterial (T-cell) regions of lymph nodes and spleen in the case of T-cells (29, 31).

These immunologic concepts led to the development of new classification schemes for malignant lymphomas, notably those of Lukes and Collins (29) and Gerard-Marchant et al. (30)—the so-called Kiel classification—both of which emphasize a major subdivision into B- and T-cell types. Unfortunately, the terminology in each of the schemes is quite different (table 2). Lukes and Collins (29) have proposed descriptive terms for tumors believed to be the neoplastic counterparts of the cells of germinal follicles—i.e., large and small follicular center cells of cleaved and noncleaved types. The authors of the Kiel classification, however, have introduced the terms "centrocye" and "centroblast" for the small and large follicular center cells (30, 39). Some confusion has arisen in attempting to define individual entities, since follicular lymphomas and diffuse lymphomas derived from follicle cells contain various mixtures of both cell types (small and large or centrocyte and centroblast). Divisions between supposedly separate entities based purely on morphology must, therefore, depend on somewhat arbitrary designations, which differ in each classification scheme as to the requisite proportions of such cells for a particular diagnosis. Thus the "small cleaved" follicular center cell lymphoma of Lukes and Collins (29) corresponds more closely to the Kiel "centrocytic/centroblastic" lymphoma than to the "centrocytic" lymphoma. Similarly, there is no clear agreement as to the dividing line between diffuse, "large follicular center cell" lymphomas (or "centroblastic" lymphoma) and "immunoblastic" lymphomas [a term now used in several classifications (29, 30, 51)] of B-cell origin. Normal immunoblasts (i.e., activated lymphocytes) occur in the medullary cords rather than the germinal follicles so that there is a theoretical basis for attempting such a separation. Both large follicular center cell lymphomas and immunoblastic lymphomas, however, would be included in Rappaport's "histiocytic" lymphoma category. All classifications are in good agreement with regard to the well-differentiated lymphomas, including "lymphocytic" and "lymphoplasmacytoid" lymphomas. The term lymphoplasmacytoid is applied to tumors consisting of variable mixtures of lymphocytic and plasmacytic cells in which the cytoplasm contains large amounts of immunoglobulin and frequently secretes this in the form of a monoclonal paraprotein. The terminal stage of B-cell differentiation—the plasma cell—is represented in the neoplastic spectrum by myeloma.

A further potential cause for confusion resulting from the profusion of classification schemes is the application of identical terms to quite different tumors. European schemes, for example, tend to use the term "poorly differentiated lymphocytic" as essentially synonymous with "lymphoblastic" and include both T- and B-cell tumors in this group, e.g., mediastinal lymphoblastic lymphoma of T-cells and Burkitt's lymphoma (26, 30). In Rappaport's classification, however, poorly differentiated lymphocytic lymphomas are usually nodular and correspond most closely to the Kiel centrocytic/centroblastic lymphoma or the small cleaved follicular center cell lymphoma of Lukes. In the Rappaport scheme, "lymphoblastic" lymphoma was recently distinguished from the remainder of the diffuse, poorly differentiated group, being now recognized as a separate tumor occurring predominantly in the mediastinum (45)—one of several types of lymphoblastic lymphoma in the Kiel scheme. In the Lukes classification, this lymphoma is referred to as a lymphoma of convoluted lymphocytes (31, 44). Finally, some entities in each scheme do not obviously correspond to any of those in other schemes, e.g., Lennert's T-zone lymphoma (34). If such are truly separate entities, they appear to be presently included in larger
composite groups in those schemes in which they are not currently recognized.

Some of these difficulties will hopefully be removed by the utilization of a "working formulation" recently agreed upon after a survey of histologic material from 1,175 patients seen at four different institutions, in which the authors (52) of the major histologic classifications currently in use took part. The formulation utilizes terms from several of the schemes and, although not necessarily easier to apply and certainly not circumventing the major problem of reproducibility of histologic diagnosis, its originators hope that it will at least permit communication between centers using different classifications—i.e., that it will provide a means of "translating" from one scheme to another (see Table 2). While this determined effort is laudable, the practical difficulties of histologic diagnosis remain. Paramount among these are the variation in tissue preparation within, and particularly between, centers and the resultant artifacts that may arise during the processes of fixation, sectioning, and staining. Frequently, pathologists must make informed guesses as to the most representative slide or part of a slide, and although within a center a reasonable degree of uniformity can be obtained, reproducibility between centers is seldom greater than 60% (53).

The subjective nature of histology clearly limits its usefulness, and although of proved value to the clinician because of its correlation with prognosis, histology alone can never provide more than a crude basis for understanding the origins and nature of lymphomas, for lymphomas are disorders of cell behavior. As indicated by the above discussion, single histologic categories have often been shown to include lymphomas that differ markedly when examined at an immunologic level. Although this finding has sometimes led to the recognition of previously overlooked histologic differences and thus to new histologic subdivisions, modern methods of examination are clearly able to go beyond pure morphology, as is well illustrated by ALL. ALL has now been clearly subdivided according to the phenotypic characteristics of the cells into several distinct entities (text-fig. 1) with different clinical behavior and response to therapy (54, 55). Further, although cytoplastic variants have been described, these variants correspond poorly to the functional categories (56). Finally, phenotypic analysis of ALL cells has led to the identification of the normal counterpart cells of ALL and to a much greater comprehension of the nature of the disease and its relationship to various other lymphoid and hematopoietic neoplasms (52, 54, 57).

It has been frequently agreed that the most important test of a classification scheme is its value to the practicing clinician, and great emphasis has been placed on the correlation between histologic diagnosis and prognosis. However, whereas the practical value of such correlations cannot be denied, their demonstration does not represent a goal achieved. Progress in diagnosis and treatment is likely to be made much more readily if a serious attempt is made to understand the nature of the disease. Even if initial attempts to define individual lymphomas more precisely and to better understand the origins of these tumors are not immediately translated into improved patient management, it is essential that we move beyond purely descriptive histology and study lymphomas with all of the tools currently available—not simply as fixed tissue, but as living cells originating from the immune system.

OMNIS CELLULA E CELLULA

The concept that cells arise from other cells rather than in some way coalescing from an acellular matrix (the "blastema" theory) was probably first proposed in 1825 (59). It was not generally accepted, however, until the latter part of the 19th century. When proposing his cell theory in 1839, Schwann (3), for example, still adhered to the blastema theory. Our understanding of stem cells and the process of differentiation has been arrived at much more recently and constitutes a confirmation and extension of the original revolutionary concepts pertaining to the critical significance of cells and their origins. Similarly, our newfound knowledge of the immune system as well as attempts to utilize this knowledge in the classification of malignant lymphomas is not contrary to the original concepts of cellular pathology, based of necessity on simple microscopic observation of cell morphology, but provides us with more powerful and more discriminating tools with which to considerably extend and enhance our understanding of the cellular disorders that give rise to malignant lymphoid neoplasms. We now have the ability to separate cell types on the basis of functional differences or phenotypic characteristics—both potentially more sensitive markers of cell type than light microscopy alone. The value of these immunologic or biochemical discriminants is also considerably enhanced by their objective nature. Light microscopy is essentially a subjective tool, but immunologic, immunochemo, or biochemical analysis of cells provides information that can be numerically expressed and which may sometimes be of a binary nature, i.e., either a given protein is present or it is absent. Whereas such precise probes are of inestimable value to the study of differentiation of normal cells, whether or not they enhance our comprehension of lymphoid neoplasia is dependent on the degree of resemblance of the neoplastic cells to the normal cells from which they were derived.

It is becoming increasingly apparent that neoplastic cells are not necessarily bizarre monstrosities that bear little, if any, resemblance to the normal cells from which they originated. Rather, the degree of resemblance of neoplastic cells to normal cells is quite remarkable. Neoplastic phenotypes that appear to be aberrant may be consistent with those of rare normal cells, although abnormalities resulting from the neoplastic state cannot be excluded at present. A situation
of this kind has recently been described in CLL, where even surface immunoglobulin-bearing cells express an antigen also present on a subset of T-cells (60, 61). This is not a novel situation, for Ia-like antigens are present on both B-cells and activated T-cells.

In mature cell types, specialized function is often reflected morphologically; e.g., the necessity of immunoglobulin-producing cells to accumulate large quantities of rough endoplasmic reticulum is responsible for many of the characteristic features of plasma cells. In precursor cells, however, not only may the degree of commitment to a particular differentiation pathway be reflected predominantly at a macromolecular rather than a morphologic level, but also many precursor cell types may be present in such small numbers and frequently (as for example in the bone marrow) so admixed with numerous other cell types that they have hitherto remained unrecognized. Only now are the features of specific precursor cells being described, e.g., at the level of rearrangements in gene segments, messenger RNA species, or the synthesis and cellular location of particular proteins (54, 57, 62). Significantly, much of this information is derived from the study of neoplastic cells, which may provide purified populations of cells expressing many of the characteristics of specific normal precursor cells. Further development and application of these approaches to cell characterization are essential if we are to relate certain neoplasms to an origin from committed stem cells or precursor cells.

A considerable body of evidence has been accumulated that lymphoid neoplasms are clonal in origin; e.g., B-cell neoplasms express surface immunoglobulin or secrete immunoglobulin of a single light-chain type and idiotype (63-66). Data from the study of glucose-6-phosphate dehydrogenase isotypes for many tumors have led to similar conclusions (67). Both morphologic and immunologic characterizations also indicate that most lymphoid neoplasms are composed of a predominant cell type that can be shown, in cases where there is sufficient information, to closely resemble both phenotypically and functionally a normal cell located at a specific point in the differentiation sequences, e.g., ALL, T-cell mediastinal lymphoma, Sézary syndrome, and myeloma (52). Thus lymphoid neoplasia can be comprehended and classified in a rational fashion according to the differentiation pathways of normal lymphocytes.
MALIGNANT LYMPHOMAS AS DISORDERS OF LYMPHOID DIFFERENTIATION

The words “tumor” and “neoplasm” focus attention on abnormal proliferation as the underlying pathologic process in malignant disease. Yet viewed from the perspective of differentiation, it can be seen that changes in the rate of proliferation (i.e., compared to the normal counterpart cell) are not necessary to the development of a lymphoma from a stem cell or precursor cell and that the accumulation of abnormal numbers of immature cells of a specific type can sometimes be explained simply in terms of a failure of differentiation (text-fig. 2). Stem cells must have many attributes. Among the most characteristic of these attributes are the capacity for self-renewal, the ability to respond to a demand for more progeny cells, and, of course, the tendency to differentiate. There is little doubt that both positive and negative feedback controls govern the proliferation of stem cells (i.e., the fraction of cells in a proliferative phase), and the direction of differentiation of multipotential cells must also be influenced by external factors.

It is important to recognize that cell loss from any given stage (or compartment) in the differentiation sequence can result only from either maturation (whence the cells, by definition, enter the succeeding compartment) or cell death. Accumulation of precursor cells at any stage of differentiation, whether or not such cells are capable of self-renewal, will occur if either a) the fraction of stem cells in a proliferative phase is increased (which will tend to produce only a small increase in the size of early compartments because of continued cell loss by differentiation) or b) the tendency to differentiation is reduced or completely abrogated. In the second circumstance, the rate of proliferation will influence the rate of accumulation of cells, but since stem cells even under normal circumstances may have quite short cycle times, accumulation may be rapid though proliferation kinetics are unaltered.

Where the fraction of proliferating cells in a compartment is increased, but differentiation proceeds normally (e.g., as a result of the expansion of a single clone), all subsequent differentiation compartments will be increased in size. A clonal expansion of this type will result in compartments in which size relationship to each other is maintained, but since considerable amplification occurs in the course of the differentiation pathway, the absolute size increase will be much greater in larger (usually more differentiated) compartments, with the result that the clinical manifestations of such a disorder will be mainly or entirely perceived in the largest compartment. Myeloma appears to be a neoplasm of this type, because it has been shown that a fraction of the pre-B-cells in the bone marrow and a proportion of circulating lymphocytes in all immunologic heavy-chain classes express the same idiotype as the paraprotein, i.e., belong to the same cell clone (68). There is also good evidence that chronic granulocytic leukemia is a disorder of a pluripotent stem cell, because all the hematopoietic cell series and some lymphoid cells, among them B-lymphocytes, express a single glucose-6-phosphate dehydrogenase isoenzyme in female heterozygotes (69), whereas in some patients in blast crisis the blast cells possess the phenotype of pre-B-cells (70).

In these kinds of stem cell disorders in which the predominant expression is in more mature compartments, the possibility of additional disorders beyond the stem cell stage cannot be excluded. In fact the morphologic variation that can be seen at different

![Text-Figure 2](https://academic.oup.com/jnci/article-abstract/67/3/507/949858)
sites or as a function of time (so-called transformation) in lymphomas of follicle center cells suggests that in this case, and probably in other lymphoid neoplasms, the cellular expression of the tumor is influenced either by external factors or by newly expressed abnormalities in the genetic control of the tumor phenotype. Where changes are subtler, methods more precise than morphological, such as detailed phenotypic analysis, may be required to detect them. Such "instability" in the cellular expression of the malignant clone is probably also responsible for blastic transformation of tumors normally expressed as mature cells, e.g., chronic leukemias. Such occurrences are most simply explained as a sudden differentiation failure in a neoplastic clone, although a barrier to differentiation in one direction may not exclude at least partial maturation in another, as may occur in the pre-B-blast crisis of chronic myeloid leukemia (70). In some cases the nature of the blastic transformation can provide information concerning the cellular origin of the neoplastic proliferation. For example, the description of rare cases of conversion to ALL in some cases of CLL (71) and to histiocytic lymphoma (Richter's syndrome) (72) in others is consistent with the possibility that there may be at least two major types of CLL of B-cell type—one in which the normal counterpart cell is an immunocompetent but virgin lymphocyte (earlier differentiation arrest leading to the expression of the malignant clone as ALL) and another in which the neoplastic counterpart is a memory cell (in which differentiation arrest leads to immunoblastic-histiocytic lymphoma) (see text-fig. 3).

Transformation of follicular lymphomas is from a small cell to a large cell variety, with continued expression of the same heavy and light chain immunoglobulin classes. If this also represents differentiation failure, either the sequence of differentiation, small to large cell, as suggested by Lukes and co-workers (29, 31) and depicted in text-figure 3, is incorrect or prior to transformation large cells are rapidly lost from the tumor presumably by differentiation into lymphocytes. Failure of the last step would result in the progressive accumulation of large cells. An alternative explanation is that in small cell follicular lymphomas there is actually a complete or partial block to secondary differentiation beyond the small cell stage, which is lifted when transformation from a small cell to a large cell tumor occurs.

Prior to therapy the cellular expression of a malignant clone may be influenced to a degree by normal regulatory mechanisms, including, in the case of B-cell lymphomas, helper and suppressor T-cells—a possibility that is no less likely than hormonal dependency in neoplasms arising in hormonally regulated tissues. The particular balance of help and suppression may in turn be a consequence of environmental factors such as exposure to infectious agents. If this notion contains an element of truth, it may be at least partly responsible for the marked geographic variation in frequency of certain lymphomas, including Burkitt's lymphoma, Mediterranean lymphoma, subacute T-cell leukemia, nodular lymphomas, and myeloma (73, 74). The possible influence of the therapy of lymphomas on the subsequent cellular expression of the neoplasm should also be borne in mind, for a very wide range of compounds including dimethyl sulfoxide, bis-acetamides, phorbol esters, retinoids, nucleotides, and cytotoxic drugs has been shown to influence differentiation, although the mechanisms of action are for the most part unknown (75).

The concept of a lymphoid neoplasm as an expanded lymphoid clone expressed at a variable number of levels of differentiation has important implications:

Anatomic Location

Insofar that cells of the lymphoid series vary in location according to their stage of differentiation and to their function, the sites at which particular lymphomas predominantly occur are at least in part a function of the particular cell type that is predominantly expressed. Thus nodular lymphomas will mainly occur in lymph nodes or in any aggregation of secondary lymphoid tissue. CLL is a disease in which the predominant cell type normally migrates widely throughout tissues and blood. Tumors of T-cell precursors will most often occur in the bone marrow or thymus, whereas T-cell tumors expressed as the counterparts of later differentiation steps arise in lymph nodes and skin (e.g., mycosis fungoides and Sézary syndrome) and may also be frequently widespread (subacute T-cell leukemia) (76). Neoplasms of lymphoid stem cells arise in the bone marrow and always present as leukemia. One question that is continually debated, and which arises particularly with neoplasms of T-cell precursors, is the distinction between leukemia and lymphoma. Bone marrow and peripheral blood involvement can occur in several circumstances: 1) in a neoplasm arising in stem cells normally resident in bone marrow in which there is profound failure of differentiation (ALL); 2) in a neoplasm in which the predominantly expressed cell is one that normally migrates through blood and bone marrow; and 3) in tumors that arise at other locations, e.g., thymus, when either overspill spread occurs (i.e., the neoplasm outgrows the physical confines of its differentiation compartment), leading to secondary involvement of marrow and peripheral blood, or there is additional representation of the malignant clone at earlier or later differentiation steps, the cells of which normally reside in or pass through marrow. These three situations may be indistinguishable at a purely clinical level, but it appears probable that T-cell ALL is an example of the first situation and lymphoblastic lymphoma of the third. In some cases of ALL, the blast cells may be the neoplastic counterparts of normal "late thymocytes" destined to exit from the thymus as functionally competent T-cells (see below). Phenotypic evidence that should support or refute this contention is cur-
Primary Differentiation (antigen independent)

- Multipotential stem cell
- Pre-B cell
- B cell with Sig M

Secondary Differentiation (antigen dependent)

- CLL, lymphocytic lymphomas (?)
- Immunoblastic lymphoma
- Histiocytic lymphomas
- Immunocompetent 'virgin' lymphocyte
- Acute lymphoblastic leukemias
- Burkitt-like lymphomas (?)

Text-Figure 3.—B-lymphocyte differentiation sequences and the origins of B-cell lymphomas. Little is known of the primary sequence, i.e., the generation of antigen-responsive cells from stem cells, except the sequential acquisition of surface markers, as indicated. The secondary sequence is based primarily on the morphologic observations of Lukes and co-workers (29, 31) and Lennert (40), but the surface markers of both normal and (for the most part) neoplastic cells are as indicated. The anatomic location of the primary pathway is not known in its entirety, although cell types up until pre-B-cells certainly occur in the bone marrow. The cells of the secondary pathway are mostly found in germinal follicles of secondary lymphoid tissue, but immunoblastic and subsequent cell types are found in the medullary cords of lymph nodes and actually leave the node in efferent lymph. This "horizontal" arm represents B-cell activation in response to antigen, the final product being an antibody-producing cell or memory cell. Cell types are labeled to the left of the vertical arm and below the horizontal arm. Their neoplastic counterparts are indicated to the right of the vertical arm and above the horizontal arm. This text-fig. is modified from a fig. first published in the Annals of Internal Medicine and is reproduced here with permission of the Editor. c~ytoplasmic ~-chain; SIg = surface immunoglobulin; CR = complement receptor; SIg M = surface immunoglobulin M; FcR = Fc receptor.

Currently being collected in a number of centers, but in the interpretation of phenotypic data, e.g., with the use of various anti-T-cell or antithymocyte antibodies (58), it must be borne in mind that the biopsy site may be of critical importance (e.g., mediastinal mass vs. lymph node or marrow) and that clinical definitions of T-cell ALL versus lymphoblastic lymphoma may differ from center to center. Clearly, the concept of leukemia as a generic group of diseases makes no more sense than would the inclusion of a broad range of sarcomas and carcinomas under one descriptive term because of involvement of a specific tissue (e.g., lung). If the term "leukemia" is to be retained, subdivision into primary or secondary leukemia might be considered, according to whether the predominant cell type is the counterpart of a normal cell residing in the marrow (hematopoietic and lymphoid stem cell neoplasms) or secondary marrow involvement occurs in a disease in which the predominant cell corresponds to a normal lymphoid cell not normally present in the marrow. Such a distinction would, of course, depend on precise knowledge of the cell type, on the basis of phenotypic characteristics, and as such may ultimately completely obviate the term "leukemia."

Lymphomas in Different Age Groups

The spectrum of lymphoid neoplasia occurring in children and young people is quite different from that occurring in adults. Most lymphoid neoplasms in
children come under the category of lymphoblastic lymphoma in the Kiel classification (which includes ALL, T-cell mediastinal lymphoma, Burkitt's lymphoma, and Burkitt-like lymphomas). This group accounts for some 10% of all lymphomas in Lennert's series (34). In adults, however, there is a much broader range (though 70-80% are of B-cell origin), including well-differentiated lymphomas (lymphocytic, lymphoplasmacytoid, and myeloma), nodular lymphomas (which are extremely rare or absent in children), and large cell lymphomas including immunoblastic lymphoma (which are relatively uncommon in children although there is considerable variation between centers in this regard). Recent investigations using a range of antisera and monoclonal antibodies have established the phenotypic similarity of "common" ALL to a stem cell normally resident in the bone marrow and that of ALL and lymphoblastic lymphoma of T-cell type to pre-T-cells or thymocytes (52, 54, 57, 58) (text-fig. 1). The origin of B-cell lymphomas in childhood has not been established, but the invariable presence of IgM and usual absence of IgD, as well as the expression of the common ALL antigen on some cell lines derived from Burkitt's lymphoma, suggests an origin early in the differentiation pathway (77-79). It is probable, therefore, that lymphomas in children and young adults generally arise from lymphocyte precursor cells and not from immunocompetent cells. In adults, however, lymphomas are composed predominantly of either mature cell types, of cells of follicular center origin, or, if of T-cell origin, of cells mature enough to express functional activities such as help or suppression of immunoglobulin production (80). Tumors indistinguishable from those seen in childhood do arise, but they are rare. Thus most adult lymphomas are the neoplastic counterparts of immunocompetent cells, i.e., cells capable of actually taking part in an immune response.

The basic difference in the origins of childhood and adult lymphomas is clarified by an examination of the B-lymphocyte differentiation pathway. This pathway falls naturally into two parts. In the first part, the generation of immunocompetent cells from stem cells (a "vertical" process), which is antigen-independent, occurs mainly in primary lymphoid organs (marrow and thymus) and may thus be termed the "primary" pathway. The second part, although involving differentiation, e.g., of an immunoglobulin-secreting apparatus, is in fact a process of activation (usually by antigen) in which the immunocompetent cell organizes its programmed function of antibody production, i.e., a "horizontal" process (text-fig. 3). Lymphocyte activation occurs mainly in secondary lymphoid organs (spleen and lymph nodes) and may be called the "secondary" pathway. Similar considerations probably apply to the T-cell differentiation pathway, with the reservation that functionally active T-cells do not necessarily undergo profound morphologic changes as do B-lymphocytes. The separation of lymphoid differentiation into primary (vertical) and secondary (horizontal) pathways is of some significance to the study of lymphomas and their classification, for most childhood lymphomas appear to be neoplasms composed of cells in the primary pathway whereas lymphomas in adults are the neoplastic counterparts of cells in the secondary pathway.

**Pathophysiologic Effects of Functioning Lymphoid Cells**

As has been already implied, the resemblance of neoplastic lymphoid cells to their normal counterparts exists at functional as well as morphologic levels; tumors arising from the secondary differentiation pathways of B- and T-lymphocytes might therefore be expected to manifest some of the properties of cells taking part in an immune response. In the B-cell lymphomas, the production of immunoglobulin by tumor cells reaches a clinically obvious level only in the most differentiated tumors such as lymphoplasmacytoid lymphomas and myeloma. Very high levels of paraproteins may give rise to a number of clinical problems including the hyperviscosity syndrome and, when the protein is a cryoglobulin, peripheral vascular disease. It is rare that the antigen specificity of the immunoglobulin has recognized clinical implications. Lymphomas composed of functioning T-cells have been reviewed by Broder and Bunn (80) and a detailed description is not warranted here. Most reports concern tumors that help or suppress immunoglobulin production. Such tumors are neoplasms of relatively mature T-cells, the most studied being cutaneous T-cell lymphomas, particularly the Sézary syndrome, in which the cells frequently express polyclonal helper activity, although in a few cases suppressor activity has been described. In the subacute T-cell leukemia, particularly common in Japan, the cells may act as suppressors. A case of T-cell ALL in which the blast cells appeared to be prosuppressor cells capable of developing into actual suppressor cells under the influence of a separate T-cell population has also been described (80). For the most part, these neoplasms have few observed effects on the host that can be directly related to their functional activity. However, hypogammaglobulinemia, either general or confined to a specific immunoglobulin class, may occur with suppressor cell tumors, whereas high levels of IgA are frequently observed in patients with Sézary syndrome, and monoclonal serum immunoglobulin abnormalities of IgM, IgG, or IgA have also been documented. The abnormalities of immunoglobulins probably are a consequence of the helper activity of the tumor cells. Doubtless, many of the direct effects of functional T-cell tumors remain to be described, but effects on hematopoiesis and general or specific immunologic responsiveness might be expected.

Disturbances in the immunologic "equilibrium" may also result from the expansion of a particular cellular compartment, with its resultant potential influence on other cell populations. The complexities of these interactions remain to be elucidated, but the heightened
susceptibility to opportunistic infections of patients with malignant lymphomas is surely one of the consequences.

**Implications for Therapy**

Paradoxically, lymphomas that have for long been referred to as “unfavorable” or poor prognosis appear to be curable by modern chemotherapy \((81-84)\). However, few, if any, lymphomas in the “favorable” group are curable, even though the patients may live for several years \((84, 85)\). This situation may be the result of a difference in the number of stages of the differentiation pathway that are represented in these two classes of lymphomas. Where differentiation is prevented, only a small range of precursor cells, all rapidly dividing, may be represented in the lymphoma. Such tumors may (and do) respond well to chemotherapy. In tumors in which differentiation occurs, a wide range of cell types may be expressed in the malignant clone with a correspondingly wide range of proliferative rates and doubtless varying degrees of responsiveness to therapy. In follicle center cell lymphomas, this is morphologically obvious, whereas in myeloma it has been demonstrated, as previously mentioned, by use of anti-idiotypic. Even the complete destruction of the predominant cell type of the neoplasm could theoretically be followed by replenishment of this compartment from another compartment. Insofar that most adult lymphomas are of B-cell type and arise from the secondary differentiation pathway, it is quite conceivable, but unproven, that such replenishment might sometimes occur as it does under the circumstances of a normal immunologic reaction, i.e., by reactivation of a “memory” cell derived from the neoplastic clone. The presence in the peripheral blood of some patients with lymphomas of an excess of lymphocytes expressing the same light chain type as the tumor \((86, 87)\) is at least consistent with this possibility, although recrudescence of tumor via neoplastic memory cells will be difficult to confirm. The apparent curability of some large cell lymphomas that are of follicular center cell origin \((83, 84)\) may indicate that in such lymphomas the number of differentiation steps represented in the malignant clone is small and/or that reactivation of progeny cells cannot occur. In the presence of neoplastic memory cells, failure to reactivate them might be thought of as the neoplastic equivalent of immunologic tolerance—i.e., the situation resembles that of nonreactivation of normal, primed, lymphoid cells upon repeated antigen exposure.

In B-cell tumors the extent of the cellular expression of the malignant clone can be studied by use of anti-idiotypic directed against the variable region of the immunoglobulin expressed by the tumor cells (because of monoclonality, idiotypic is in essence tumor-specific), an approach which has been of demonstrable value in myeloma \((see \ above)\) \((66, 88)\). Highly specific (e.g., monoclonal) antibodies directed toward particular cell types or the immunoglobulin idotype of B-cell tumors also have a potential role in both imaging of tumor sites (by injecting radiolabeled antibody and scanning the patient for its location) \((89, 90)\) and treatment (either unmodified or by coupling the antibody to toxins or drugs) \((91-93)\). Neither of these approaches is new, but the combination of newer concepts of lymphoid neoplasia and improved immunologic technology renders them worthy of renewed study. When a neoplastic clone is expressed at several differentiation steps, one or more of which is refractory to chemotherapy (as may be the case in the neoplasms of activated B-cells), the potential of idiootype-directed therapy is particularly attractive, since cure of such lymphomas may be achieved only by elimination of the entire neoplastic clone. Therapy targeted to specific differentiation antigens by means of monoclonal antibodies is less attractive since normal cells may also be destroyed, but additional maneuvers such as allogeneic replacement of essential normal cell populations may circumvent this problem. The successful therapeutic use of monoclonal antibodies is of course dependent on solving a number of technical problems, including distribution of the antibody; blocking by soluble, circulating antigen, whether secreted by neoplastic cells or shed from their surfaces; the possible development of antibodies directed against the monoclonal antibody; and antigenic modulation of the malignant cells. In the future, with increased knowledge of the triggering mechanisms of lymphoid cells and the regulation of differentiation, modification of the cellular expression of the malignant clone or the prevention of reactivation of neoplastic lymphocytes may become feasible therapeutic approaches, whether primary or adjuncts to other modalities.

**THE FUTURE—MOLECULAR ONCOLOGY**

It has hopefully been demonstrated that a concept of lymphoid neoplasia on the basis of the normal differentiation pathways not only will aid comprehension of these tumors but also is of potentially profound significance to the design of treatment strategies. Further, many of the concepts discussed here may be applicable, with suitable modifications, to the understanding of some nonlymphoid tumors. The use of immunologic identification of the cells represented in the malignant clone will probably become increasingly refined and already provides, in many cases, much more objective diagnosis than descriptive histology alone. Even though no truly tumor-specific antigen has yet been defined in humans and such antigens may not in fact exist \((94)\), the characterization of lymphoid neoplasia in terms of the particular pattern of expression of “differentiation antigens” or phenotypic markers is likely to become increasingly important. The commitment of a cell to a specific differentiation pathway depends on restriction of gene expression or potential gene expression. In B-cells, commitment involves rearrangement of DNA sequences coding for the variable...
and constant regions of immunoglobulin chains, whereas "maturation" involves the regulation of transcription, posttranscriptional processing, and further regulation of translation and posttranslational processing. Similar processes will undoubtedly be demonstrated in other cell types. Thus nucleic acid probes may ultimately permit detailed examination of the state of commitment of a cell, whereas phenotypic analysis, utilizing hybridoma antibody technology and detailed protein mapping by two-dimensional gel electrophoresis, possibly coupled with chemical induction of "differentiation," may enable the precise point of a cell along the differentiation pathway to be identified. These approaches have particular importance to the study of relatively undifferentiated cells or where more standard methods provide inadequate or ambiguous information. For example, study of the rearrangement of immunoglobulin genes or mRNA in "common" ALL or so called null-cell lymphomas might reveal that some of these neoplasms are committed to B-cell differentiation, even though the earliest stage of maturation, i.e., the development of cytoplasmic μ-chains, has not been reached. Evidence for immunoglobulin gene rearrangement has in fact already been obtained in ALL (95). Much more needs to be learned, however, about gene rearrangements in different cell types before firm conclusions can be drawn. For example, it has recently been demonstrated that some T-cell lines contained mRNA for the constant region of the heavy μ-chain and also have demonstrable rearrangement of immunoglobulin genes—possibly a clue to the elusive T-cell receptor (96).

In the future, it is to be expected that the molecular derangements leading to disordered differentiation will be identified, although the cause of such derangement may not be apparent initially. Ultimately, etiologic diagnoses may supplement the cellular basis for classification of lymphoid neoplasia, but it would seem unlikely that reference to cellular identity will ever be dispensable, since the abnormal accumulation of specific cell types appears to be the very essence of neoplasia and, in the words of Raspail, "Omnis cellula e cellula."

REFERENCES

(6) Craigie D. Case of disease of the spleen, in which death took place in consequence of the presence of purulent matter in the blood. Edinburgh Med Surg J 1845; 64:400-413.
(7) Bennett JH. Case of hypertrophy of the spleen and liver, in which death took place from suppuration of the blood. Edinburgh Med Surg J 1845; 64:413-423.

JNCI, VOL. 67, NO. 3, SEPTEMBER 1981


(96) COREY S, ADAMS JM, KEMP DJ. Somatic rearrangements forming active immunoglobulin genes in B and T lymphoid cell lines. Proc Natl Acad Sci USA 1980; 77:4943-4947.