Cytochemical and Immunological Aspects of the Acute Lymphocytic Leukemias

(As Related to FAB Classification)

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ABSTRACT

The FAB classification of acute leukemias was proposed by a cooperative French-American-British group as an easily applied classification useful for uniform assessment of large clinical therapeutic trials. Although the classification is based on morphological criteria, cytochemical, biochemical, immunological and cytogenetic studies supplement the morphologic criteria for accurate identification of the leukemic blasts.

The FAB criteria are shown by these supplementary studies to be deficient for classifying the acute lymphocytic leukemias (ALL) since various subgroups of ALL appear in more than one FAB group. Precise definitions of clinical-pathological entities within the framework of the FAB classification are desirable.

Introduction

As with so many other accomplishments, the first classification of leukemia has been credited to Virchow.34 This classification was based primarily on the anatomic distribution of the major tumor mass, splenic or lymphoid. The subsequent development of the Romanowsky stains provided the tools to define more precisely types of leukemias on cytologic grounds. In the absence of effective therapy, the earlier classifications, based entirely on morphological criteria, were useful primarily in distinguishing between chronic and acute leukemias. Patients with chronic leukemias, i.e., those characterized by cells resembling mature normal cells, had a natural life span of several years, whereas patients with acute leukemias, i.e., those characterized by cells resembling primitive cells, generally had a life span of only several months.

With the advent of chemotherapy and the first successful attempts to achieve remissions in 1948,10 this situation changed entirely. Since the favorable response to the early chemotherapeutic
agents was not at all uniform in patients with acute leukemia, it became necessary to achieve a clinically useful classification, one which would be predictive regarding response to chemotherapy. Most of the remissions with the early chemotherapeutic agents were achieved in children rather than adults, especially in those children with acute lymphocytic leukemia or acute leukemias in which the cells were so undifferentiated in appearance that they were called “stem cell” leukemias. As more drugs became available and prolonged remissions in other types of leukemias were achieved in adults as well as children, the urgent need for precise identification of the leukemic cell type became apparent. At the same time, it became apparent that classifications based entirely on morphological criteria obtained from Romanowsky-stained smears were not satisfactory because they were overly dependent on subjective interpretation. The introduction of various cytochemical stains, described in the preceding papers, greatly aided in assigning undifferentiated appearing acute leukemias into myelogenous, monocytic or “probable” lymphoid categories. The reason for the term “probable” lymphoid leukemia is that this diagnosis is frequently made on the basis of the lack of, rather than the presence of, specific morphological characteristics and cytochemical reactions. This category, therefore, may include leukemias of pre-lymphoid or pre-myeloid origin.

The classification of acute leukemias which is presently being discussed, FAB classification, was proposed by a cooperative French-American-British group as an easily applied classification useful for uniform assessment of large clinical therapeutic trials. Although the classification is based on morphological criteria, the need for cytochemical and immunological studies is emphasized by the FAB group, and such studies are performed at most large participating centers. According to the FAB classification, acute lymphocytic leukemias (ALL) are divided into three groups: L₁, in which small lymphoblasts predominate; L₂, in which large, heterogeneous lymphoblasts comprise more than 10 percent of the blasts; and L₃, in which large, uniform lymphoblasts with deep blue cytoplasm, resembling Burkitt cells, predominate.

Recent advances in immunological methodology, permitting the subclassification of the lymphocytic malignancies into T, B or null cell types, further confirm that the FAB classification of acute lymphocytic leukemias based on morphology alone is not sufficient.

**Diagnostic Procedures in the Evaluation of Acute Lymphocytic Leukemias**

The following methods are employed for characterizing the blast cells of acute leukemias: (1) Morphologic examination (light and electron microscopy), (2) Immunologic examination, (3) Cytochemical examination (light and electron microscopy), (4) Biochemical examination and (5) Cytogenetic examination. In this report, only those methods especially useful for the evaluation of acute lymphocytic leukemias will be presented.

**Morphologic Examination**

*Light microscopy*: Smears of blood and bone marrow are stained with a Romanowsky dye and examined with the light microscope.

L₁. Blasts are small, up to twice the diameter of a small lymphocyte. Cytoplasm is scanty, making the nuclear-cytoplasmic ratio high. The nuclear shape is usually regular and the chromatin pattern is fine. Nucleoli are inconspicuous. This is the predominant type in children.

L₂. The population of blasts is more heterogeneous. The larger cells have more cytoplasm. The nuclear shape is irregular and the chromatin pattern varies
from fine to coarse. Nucleoli, which vary in size and number, are usually present. This is more commonly seen in adults. Both L1 and L2 may have occasional azurophilic cytoplasmic granules.

L3. The blasts are large (up to 30 μ), of uniform size, with a deeply blue staining cytoplasm and clear cytoplasmic vacuoles. The nucleus is round and regular. The chromatin pattern is fine. One or more prominent nucleoli are seen. This leukemia is rare.

Electron microscopy: L1 and L2 lymphoblasts have few features distinguishing them from very young myeloid cells without cell-specific organelles. Myeloperoxidase-positive granules, seen at the electron microscopic level, help to rule out the lymphoid origin of such primitive cells. The blasts of the T cell leukemias may contain lysosomal granules, which have been shown to contain acid phosphatase,1,8,22 near the Golgi region.

The L3 blast has a more distinctive picture. The cytoplasm contains numerous polysomes and large cytoplasmic fat vacuoles. The numerous polysomes are responsible for the deep blue cytoplasmic staining at the light microscopic level.

IMMUNOLOGIC EXAMINATION

Surface immunoglobulin markers: Surface or membrane immunoglobulins, secreted by the cell, are presently the most reliable markers for B lymphocytes. Leukemic cell suspensions are reacted with mono-specific fluorescent antisera to the various heavy and light chains of human immunoglobulins.24 Surface immunoglobulins under the usual test conditions appear as distinct, bright fluorescent dots on the cell membrane. Such surface immunoglobulins are found primarily on L3 cells and are usually IgM, kappa, thus distinguishing this as B cell ALL. L3 cells usually do not have IgD, which is frequently associated with IgM on the surface of other B cell malignancies.6 The L3 ALL is the Burkitt type11 usually seen in children and young adults and probably is the leukemic phase of the non-African Burkitt's lymphoma. Few L2 and occasional L1 type leukemias may be of B cell type. These usually represent either blastic transformation of chronic lymphocytic leukemia2 or the leukemic phase of poorly differentiated lymphocytic lymphoma (lymphosarcoma cell leukemia).13

It is important to ascertain that the surface immunoglobulins are secreted by the cell and not derived from an extrinsic source such as the patient's serum and attached to the cell via the receptor for the Fc portion of immunoglobulin. This receptor is found on myeloid cells and monocytes as well as on lymphocytes. Overnight incubation in serum-free medium, occasionally requiring previous removal by trypsinization, obviates this problem.

Cytoplasmic immunoglobulin: The recently described pre-B cell leukemias display intracytoplasmic μ chains but no detectable intracytoplasmic light chains or surface immunoglobulins.25 The blasts are thought to resemble the pre-B cells found in fetal liver.12 These cells are the likely early precursors of surface Ig-bearing B cells. In the adult, pre-B cells are normally found in the bone marrow.28 In one series of patients, 20 percent of non-T, non-B ALL were found to be of this type.4

The cytoplasmic immunoglobulin (μ) is demonstrated in alcohol-fixed smears with fluorescein conjugated monospecific antisera.4 The fluorescence is very faint. Immunoelectron microscopic studies have shown that the μ chains are located at the level of free polysomes rather than inside endoplasmic reticulum.28 Pre-B cell leukemias fall into L1 and L2 categories.4

E-rosette formation: E-rosette formation by lymphocytes is the most commonly used test to identify T lympho-
cytes. Leukemic cell suspensions are incubated with unsensitized sheep red cells. In T cell leukemia, the sheep red cells attach to the surface of the blasts, forming rosettes which are counted in a hemacytometer chamber. The E-rosettes formed by T lymphoblasts have the unusual property of remaining stable at 37°C; whereas the E-rosettes formed by normal human peripheral T cells dissociate at this temperature. Normal thymocytes, however, like T lymphoblasts, also form stable E-rosettes at 37°C. This property of T lymphoblasts and the fact that complement receptors have been found on such blasts, as well as on thymocytes, have given rise to speculation that T cell ALL may arise from thymocytes rather than from peripheral T cells. However, in our experience with a large number of various lymphoid disorders, the present authors have found large populations of such stable E-rosette-forming T cells in the lymph nodes of both malignant and non-malignant disorders, suggesting that peripheral T cells responding to antigenic stimulation may acquire the property of forming stable E-rosettes at 37°C.

More than 20 percent of ALL cases are of T cell type. The incidence varies in different series depending on availability of anti-T serum for testing. Approximately 20 percent of T-ALL do not form E-rosettes but do react with anti-T cell sera. T lymphocytes can be subdivided into several functional groups, such as helper, suppressor and effector T cells. The methodology available for the identification of such subgroups is complex and as yet not clinically applicable. So far, one case of T-ALL with suppressor cell properties has been identified.

The advantages of being able to identify subgroups of leukemic cells are great. The availability of large volumes of a homogeneous cell population facilitates in-depth investigation to define the properties of such cells. It also allows the production of specific antisera against the leukemic cell populations. Such antisera may provide the fastest and most accurate means of identifying the leukemic cell type. Automated equipment, such as the fluorescence-activated cell sorters, provides the means for accurate and rapid identification of cells that have reacted with these specific antisera.

Receptors for complement and the Fc portion of immunoglobulin: These markers do not appear clinically useful at this time. Although they are found on B and null lymphocytes, they are also found on monocytes, granulocytes and their precursors. Thus, their detection is diagnostically non-specific. Under somewhat different test conditions, receptors for the Fc portion of IgM and IgG are also found on T lymphocytes. T cells with receptors for IgM were originally thought to be helper, and those with receptors for IgG, suppressor T cells. However, these demarcations have not been proven to be so clear cut. T cell ALL blasts express receptors for the Fc portion of IgG and/or IgM.

Antisera: Antisera to leukemic populations may prove to be our best diagnostic aid in the future. The most useful antisera for diagnostic purposes at this time are those prepared against various human T cells, including leukemic blasts, and against leukemic blasts with neither T nor B cell characteristics. Leukemias composed of such blasts have been termed null-ALL.

The diagnosis of null-ALL, which previously had been based on negative findings, can now be based on positive reactions with anti-null cell sera. Most (about 75 percent) of the ALL are of null cell type. In children, they fall primarily into the L1 category; in adults, into L2 category; in adults, into L2. Antisera to B lymphocytes are less useful. The antibody seems to be directed against the Ia antigen which is also found on monocytes, granulocytes and all early
myeloid and erythroid precursors. Null-ALL blasts and pre-B cells also react with these antisera.6

Cytochemistry

One of the diagnostic features of acute lymphoblastic leukemia is that the blasts are negative for several cytochemical reactions specific for cells of the granulocytic and monocytic series. In some laboratories, two non-specific cytochemical reactions are considered useful in identifying and differentiating lymphoblasts from myeloblasts and monoblasts. Neither of these stains is specific for the lymphocytic series, and it is the pattern of staining that is considered of diagnostic significance.

The first of these is the periodic acid Schiff (PAS) reaction30 which detects a variety of products such as glycogen, mucopolysaccharides, mucoproteins, glycoproteins and glycolipids. Since several of these chemicals are present in all blood cells, this cytochemical reaction is obviously of limited value. Some observers, however, consider that coarse granules or block-like aggregates of PAS positive material in the cytoplasm of a blast suggest a lymphoid origin. In our experience, this has not been a helpful stain. We and others have found such positive reactions in monocytic leukemia.13 Conversely, the blasts in T-ALL have usually been PAS negative.

The other popular cytochemical test, the acid phosphatase reaction, is helpful primarily in distinguishing the T-ALL from the other ALL.13 It has been found that acid phosphatase granules are concentrated in the Golgi region of the cell in T lymphocytes,8 whereas they appear as more dispersed granules in other lymphocytic subgroups as well as in other leukocytes, erythroblasts and platelets.

Various other enzymes, such as acid α-naphthyl acetate esterase,37,33 β-glucuronidase and various other acid hydrolases are being investigated in normal lymphocyte subgroups as well as in lymphoid malignancies.26,27 Their clinical usefulness at this time is limited.

Biochemistry

Terminal deoxynucleotidyl transferase (TdT) is an enzyme which catalyzes the polymerization of deoxyribonucleotides on the 3' OH end of polydeoxyribonucleotides. Until recently it was regarded primarily as a rather specific enzyme of thymic cells.9,20 Its detection in a child with acute lymphoblastic leukemia initially suggested that this enzyme was found in leukemic lymphoblasts derived from the thymic cell population.21 However, recently this enzyme has been found in null as well as T-ALL, in lymphoblastic lymphoma, in chronic myelogenous leukemia in blast crisis18 and in pre-B cell ALL.4 Occasionally, it has also been found in acute myeloblastic leukemia.18

The significance of finding this enzyme in the various lymphoid and myeloid malignancies is at present not entirely clear. Normally, immature thymic lymphocytes and bone marrow lymphoid cells which may have prothymocyte characteristics contain TdT. Mature peripheral T and B lymphocytes and the B cell precursors in the avian bursa of Fabricius lack this enzyme.19 However, TdT may be present in early B-cell precursors in the mouse.31

Further studies are obviously necessary to assess the significance of the presence of TdT in various malignancies. However, one example of the clinical usefulness of the assay is its capacity to predict the response to therapy in the blastic phase of chronic myelogenous leukemia (CML). CML patients with high levels of TdT respond better to the chemotherapeutic agents vincristine and prednisone (agents effective in lymphoblastic leukemia) than do patients with low levels.19
Cytogenetics

A Philadelphia (Ph1) chromosome, previously thought to be limited to non-lymphoid cells of the Ph1-positive type of chronic granulocytic leukemia, was recently found in several patients with null-ALL. Since blast cells in some Ph1-positive granulocytic leukemias in blast transformation were shown to react with anti-null ALL serum and also to contain TdT, it is possible that such leukemias represent malignant transformations of a pluripotent, i.e., pre-lymphoid and pre-myeloid stem cell.

Summary

The FAB classification of acute leukemias was proposed in an effort to achieve an easily applied and consistent classification of acute leukemias that would allow the accurate placement and later assessment of cases in national therapeutic trials. Although the classification is based primarily on morphological criteria, the proper identification of leukemic cells is supplemented by and frequently dependent on additional studies including cytochemical, biochemical, immunological and cytogenetic studies.

The correlation of the FAB classification with the results of all these diagnostic approaches has proven to be difficult in the case of the acute lymphocytic leukemias. Two of the three FAB morphologic types of ALL, L1 and L2, are heterogeneous in that each contains leukemias which vary immunologically, chemically and biologically. It may become necessary later to subdivide ALL into several subdivisions based more on immunological and/or chemical findings rather than exclusively on morphological criteria. This type of subdivision should result in more precisely defined clinical-pathological entities for which specific drug therapies can be developed and more accurately evaluated. Understand-

References


