# HUMAN LEUKEMIAS:

## Cytochemical and Ultrastructural Techniques in Diagnosis and Research

Aaron Polliack (editor)

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## *Cytochemical and Ultrastructural Techniques in Diagnosis and Research*

*Edited by*

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# PREFACE

In recent years excellent progress has been made in the development of improved cytochemical and ultrastructural techniques for electron microscopy in hematology and in particular in leukemia and lymphoma. Subtypes of these disorders, which are essentially classified according to cell phenotype and the degree of maturation, can be well defined today using a combination of immunological methods, light microscopy, cytochemistry, and electron microscopy. Ultrastructural cytochemistry is now utilized routinely in leukemia in most major centers today and can also be performed using scanning electron microscopy (SEM), as described in this book. Immuno electron microscopy used with or without simultaneous ultrastructural cytochemistry can also be employed in the accurate classification of undifferentiated or unclassified leukemias, and studies of this nature are illustrated in this book. Undoubtedly some cases of so-called unclassified leukemia will be diagnosed as early myeloblast leukemia, on the basis of positive ultrastructural myeloperoxidase staining or as megakaryoblastic in nature if positive platelet peroxidase activity is present on transmission electron microscopy (TEM). A few cases of so-called acute lymphoblastic leukemia (ALL) may also be reclassified after myelo- and platelet peroxidase staining is performed using TEM.

In some cases surface architecture of the leukemic cells as seen by scanning electron microscopy may contribute to the diagnosis. The techniques for performing immuno SEM and SEM cytochemistry are now available and may be utilized more in the study of these disorders in the future. Recently freeze fracture techniques have also been employed in the study of normal and leukemic lymphocytes and much new information concerning leukemic cell membranes may be obtained from the use of this technique in the future.

The purpose of this book is to demonstrate the utility of these techniques in defining leukemic cell types and to illustrate the potential of some of the newer ultrastructural methods, including transmission and scanning immuno electron microscopy, SEM cytochemistry, and freeze fracture techniques, in the study of leukemia. Advances in cytochemical techniques and their use in the definition of nonlymphoid cells and in the classification of lymphoid subtypes are clearly demonstrated in this book. The importance of the detection of platelet and myeloperoxidase activity and nonspecific esterases on the ultrastructural level, in acute unclassified leukemia and blastic crisis of chronic granulocytic leukemia (CGL) is clearly outlined. Ultrastructural characteristics of the various types of ALL and in particular T-type leukemias and prolymphocytic leukemias are vividly illustrated in the chapters on ALL, and the contribution of ultrastructure in the accurate definition of therapy-related panmyelosis, secondary leukemia, and blastic crisis of CGL is clearly stated.

Some of the chapters dealing with ALL contain overlapping information, which is unavoidable when different authors contribute on related subjects in an edited book. However, on the whole, these chapters are complementary, adding different dimensions and personal perspectives to

the morphology of these disorders. Of particular interest is the use of combined ultrastructural cytochemistry and immuno TEM as described by Breton-Gorius and Catovsky and co-workers. Scanning EM vividly illustrates in three dimensional views, the spectrum of surface features seen in the different leukemic cells while immuno SEM and SEM cytochemistry show again how combined ultrastructural approaches can be effectively used to define different cell types. Ultrastructural aspects of cultured leukemic cells and some observations on virus production and differentiation in vitro are also reviewed in this book. This also demonstrates how TEM can contribute to the understanding of cell differentiation and etiology in these disorders.

In general it is hoped that this volume will focus more attention on the importance of ultrastructure in the definition of the leukemic cell phenotype and highlight the fact that much practical information can be obtained from these methods, which will influence decision making for patients with these disorders.

# CONTENTS

# I. CYTOCHEMISTRY, LIGHT MICROSCOPY

# 1. CONTRIBUTION OF CYTOCHEMISTRY IN LEUKEMIA

Gerassimos A. Pangalis

The recognition of subgroups of leukemias by morphologic and cytochemical criteria has greatly contributed over the past decades in the differential diagnosis, classification, and prognosis of the leukemic proliferations [1–13]. In 1964 Hayhoe and his associates [1] in a combined morphologic and cytochemical study of acute leukemias were able to determine four different types of leukemias: acute myeloblastic, acute myelomonocytic, acute lymphoblastic and erythremic myelosis. A few years later Schmalzl and Braunsteiner [14] and Daniel et al. [15] separated the acute monocytic leukemias from the other acute leukemias using the sodium fluoride sensitive naphthol AS-D acetate esterase reaction (NASDA + NaF).

In 1976 Bennet et al. [16] proposed the French-American-British (FAB) classification of acute leukemias based on morphologic and certain cytochemical criteria (myeloperoxidase and NASDA-NaF when necessary). In the FAB classification six classes of myeloid leukemias ($M_1$–$M_6$) were recognized on the basis of cell maturation and differentiation (table 1–1). This classification of myeloid leukemias appears to have prognostic significance. In the same classification (FAB) three groups of lymphoblastic leukemias ($L_1$–$L_3$) have been proposed. These subgroups of lymphoblastic leukemias did not correlate with immunologic and cytochemical markers, however.

In recent years the simultaneous study of lymphocytic cell with morphologic, immunologic, and cytochemical methods resulted in the demonstration of significant qualitative and semiquantitative enzyme differences in normal [17–34] and leukemic [35–58] B- and T-lymphocytes. As a result of these studies, T-cell lymphocytic proliferations, acute or chronic, could be recognized on the basis of the pattern and degree of positivity of the acid phosphatase, $\alpha$-naphthyl acetate esterase and $\beta$-glucuronidase reactions [20, 21, 33, 36–39, 42–46, 48, 51–58].

In this chapter the practical aspects of certain cytochemical reactions are analyzed, on the basis of their contribution in the following: (1) the differential diagnosis and classification of acute leukemias (myeloperoxidase, Sudan black B, naphthol AS-D chloroacetate esterase, NASDA + NaF, and periodic acid-Schiff); (2) the recognition of leukemic lymphocytic cell subpopulations according to their immunologic phenotype (acid phosphatase, $\alpha$-naphthyl acetate esterase and $\beta$-glucuronidase), and (3) the diagnosis and differential diagnosis of certain chronic hematopoietic disorders (neutrophil al-

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TABLE 1–1. Cytochemical markers for the differential diagnosis and classification of acute leukemia

| Type of acute leukemia | | Reactions | | | | |
|---|---|---|---|---|---|---|
| | | | Esterases | | | |
| Traditional terms | FAB classes | Myeloperoxidase and/or Sudan black B | NCA | NASDA | NASDA + NaF | PAS |
| Myeloblastic | $M_1$ | +/+ + + | ± | + | + | −/+ +ϕ |
| Myeloblastic with granulocytic differentiation | $M_2$ | +/+ + + | ± | + | + | −/+ +ϕ |
| Promyelocytic | $M_3$ | + + + | + + | + | + | + +ϕ |
| Myelomonocytic | $M_4$ | +/+ + + | + + | + + | + | +/+ +ϕ |
| Monocytic/ monoblastic | $M_5$ | −/+ + | − | + + + | ± | −/+ +ϕ |
| Erythroleukemia* | $M_6$ | − | − | − | − | −/+ + + |
| Lymphoblastic | $L_1$, $L_2$, $L_3$ | − | − | ± | ± | −/+ + +ϕ |

Note: FAB = French American British; NCA = naphthol AS-D chloroacetate; NASDA = naphthol AS-D acetate; NaF = sodium fluoride; PAS = periodic acid-Schiff; − = negative, ± = faintly positive; + = weakly positive; + + = moderately positive; + + + = strongly positive; ϕ = for the pattern of positivity—see text.
*Results are referred to erythroblastic component.

kaline phosphatase and acid phosphatase resistant to tartrate).

## *Cytochemical Reactions for the Differential Diagnosis and Classification of Acute Leukemias*

Within the group of acute leukemias, the most common problem is the differential diagnosis of acute lymphoblastic leukemia (ALL), especially the $L_2$ class of the FAB classification, from the acute myeloblastic leukemia (AML) without apparent morphologic evidence of granulocytic differentiation ($M_1$ class of the FAB classification) [16]. It is also of interest to distinguish the myelomonocytic leukemia ($M_4$ class), from the hypergranular promyelocytic ($M_3$) class of acute myelocytic leukemia and the monocytic variant ($M_5$ class) of the FAB classification. Finally it is important to confirm the monoblastic nature of the $M_5$ acute monoblastic leukemia variant of the FAB classification, which is likely to be confused with acute myeloblastic or acute immunoblastic leukemia.

For the differential diagnosis and classification of the leukemias just named, the application of the following cytochemical reactions may be necessary (see tables 1–1 and 1–2): myeloperoxidase, Sudan black B (SBB), periodic acid-Schiff (PAS), esterase with naphthol AS-D chloroacetate as substrate, esterase with naphthol AS-D acetate as substrate (NASDA), and esterase with naphthol ASD acetate as substrate and inhibition with sodium fluoride (NASDA + NaF).

*Myeloperoxidase Reaction.* Myeloperoxidase appears during the early stages of differentiation of the granulocytic series (from myeloblast to promyelocyte) and is localized in the lysosomes or primary granules. Neutrophils and eosinophils are myeloperoxidase strongly positive, whereas basophils are myeloperoxidase negative. This enzyme is also present in the cells of the monocytic series. The cytochemical

TABLE 1–2. Summary of the contribution of cytochemical markers in the differential diagnosis and classification of leukemic proliferations

| Cytochemical reaction | For the discrimination of |
|---|---|
| Myeloperoxidase<br>Sudan black B<br>NASD chloroacetate esterase<br>PAS | Acute myeloblastic<br>from<br>Acute lymphoblastic leukemia |
| NASD acetate esterase<br>NASD acetate esterase inhibited by sodium fluoride | Acute monocytic and myelomonocytic leukemias<br>from<br>Acute myeloblastic, lymphoblastic, and immunoblastic leukemias |
| Acid phosphatase<br>α-Naphthyl acetate esterase<br>β-Glucuronidase | T from null and B acute or chronic lymphocytic leukemias |
| Neutrophil alkaline phosphatase | Chronic myelogenous leukemia<br>from<br>Other chronic myeloproliferative disorders |
| Acid phosphatase resistant to tartrate | Cells of hairy cell leukemia<br>from<br>Other lymphoproliferative disorders |

Note: NASD = naphthol AS-D; PAS = periodic acid-Schiff.

[59] demonstration of myeloperoxidase activity in the blast cells of acute leukemias without apparent morphologic evidence of myelocytic differentiation, such as the $M_1$ class of the FAB classification, determine the myeloblastic nature of this proliferation [16] (figures 1C* and 2C). Thus demonstration of myeloperoxidase activity in this type of leukemia is very important, since the $M_1$ class may be confused with the acute lymphocytic leukemia ($L_1$ and especially $L_2$ of the FAB classification), which is by definition myeloperoxidase negative [1, 4, 16]. Auer rods, which are considered to be abnormal lysosomes, when present are myeloperoxidase positive [1]. The myeloperoxidase reaction is highly specific for the granulocytic and monocytic series. According to certain investigators more than 5% myeloperoxidase positive blast cells are required in order to classify a case of acute leukemia as myeloblastic [1, 3, 4, 12]. The percentage of positive blast cells may range from 5 to 100%, with faint to very strong positivity [1, 3]. This varying percentage of myeloperoxidase positive blast cells is probably due to maturation arrest of the abnormal leukemic clone at different stages of differentiation. In certain cases the enzyme may be localized in the paranuclear space, the endoplasmic reticulum, and in the Golgi apparatus, as has been shown by electron microscopy cytochemistry [60]. Such observations explain the finding of myeloperoxidase positivity in blast cells without other morphologic evidence of granulocytic differentiation [3]. Myeloperoxidase positivity may also be found in the acute monocytic leukemias ($M_5$ class of the FAB classification) [15]. Deficiency of this en-

*The letter *C* after a figure number denotes a color figure. All color figures appear in the color plate section in the middle of the book.

zyme has been demonstrated in the granulocytic series of patients with preleukemic states [61].

*Sudan Black B Reaction.* Although the exact nature of the SBB stain is not known, its cellular distribution is similar to that of myeloperoxidase [1, 3], in both normal and leukemic cells [1]. Therefore the SBB reaction may be used to discriminate the less differentiated type of myeloblastic leukemia ($M_1$ class of the FAB classification) from the acute lymphoblastic leukemia (figures 1C and 3C). The SBB stain is not as specific as the myeloperoxidase reaction, although it appears to be more sensitive [1, 3, 11]. Its sensitivity is characterized by the fact that the percentage of SBB positive cells in acute myeloblastic leukemia may occasionally be higher than the percentage of myeloperoxidase positive cells [1, 13]. In rare cases of myeloblastic leukemia the myeloperoxidase reaction by light microscopy may be entirely negative while the SBB stain is positive [1]. As in the myeloperoxidase reaction, more than 5% SBB positive blast cells are required in order to classify a case of acute leukemia as myeloblastic [3]. Since the SBB stain is not an enzymatic reaction, blood and bone marrow smears may be used even if the slides have been stored at room temperature for several weeks.

*Naphthol ASD Chloroacetate Esterase Reaction (62).* The specificity of NASD chloroacetate esterase in the myelocytic series is similar to that of myeloperoxidase [3, 4]. The reaction, however, is less sensitive than the myeloperoxidase reaction and the SBB stain [12]. This is probably due to the fact that this enzyme appears in the granulocytic series later than the myeloperoxidase. Therefore this reaction is not of help in the differential diagnosis of the $M_1$ class of the FAB classification from the acute lymphoblastic leukemia. It is of great significance though, for the differential diagnosis of granulocytic sarcoma (chloroma) from histiocytic lymphoma on tissue sections, since this reaction can be applied in formalin fixed and paraffin embedded material [63]. The cells of the monocytic series are NAS-D chloroacetate esterase negative [4]. Therefore this reaction may also be used simultaneously with the NASDA esterase, for the differential classification of the hypergranular myelocytic leukemia ($M_3$), the myelomonocytic leukemia ($M_4$) and the monocytic variant of the $M_5$ class of acute leukemia in the FAB classification (table 1–1).

*Naphthol ASD Acetate Esterase Reaction.* This esterase reaction [14, 64, 65] demonstrates various degrees of cytochemical activity in most normal [63, 66] and leukemic cells [4, 8, 14, 15, 67]. The cells of the monocytic series, however, are characterized by a strong NASDA positivity, which is extensively inhibited by sodium fluoride (NaF) [4, 8, 9, 14, 15, 67]. No evidence for NASDA inhibition by NaF exists in other normal or leukemic cells. Therefore this is a reaction of choice for the differential diagnosis of acute monocytic or monoblastic leukemia ($M_5$ class of the FAB classification) from other types of acute leukemias (figures 4C–6C). The NASDA reaction occasionally is weaker in acute monocytic or monoblastic leukemia, but it always decreases significantly after the addition of NaF to the incubation media [12]. Rare cases of monoblastic leukemia may be entirely negative for the NASDA reaction [4].

*Periodic Acid-Schiff (PAS) Stain.* The PAS reaction [1] has been extensively used for the differential diagnosis of acute lymphoblastic from acute myeloblastic leukemias [1, 4]. The presence of a granular