Electron microscopic and cytochemical studies of peroxidase-negative acute nonlymphoblastic leukemia

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Received: October 17, 2000 / Accepted: January 5, 2001

Abstract Cytochemical and ultrastructural studies of 42 patients with acute nonlymphoblastic leukemia (ANLL) lacking myeloperoxidase as detected by light microscopy were performed. These ANLL patients were classified into minimally differentiated acute myeloid leukemia (AML-M0) (26 cases), mixed-lineage leukemia (9 cases), and acute undifferentiated leukemia (AUL) (7 cases). Ultrastructural morphology revealed blasts from AML-M0 as very immature myeloid cells, blasts from mixed-lineage leukemia as having partially lymphoid cell features, and blasts from AUL as undifferentiated blasts with myeloid nature to some extent. Ultrastructurally, myeloperoxidase activity was positive in 81% of AML-M0 patients and less positive in the mixed-lineage leukemia and AUL patients. The DNA in the nuclei of the AML-M0 blasts was dispersed, as was observed in the immature myeloid cells. Some of the blasts from the mixed-lineage leukemia patients showed condensation of DNA, resembling blasts from acute lymphoblastic leukemia (ALL). The distribution of periodate-reactive glycoconjugates revealed that glycogen particles were absent or, if present, very scanty in AML-M0. Most of the blasts from the AUL patients lacked glycogen particles, and the blasts from those with mixed-lineage leukemia contained clustered glycogen particles, as was observed in the blasts from all patients. These findings disclosed that three subtypes of immature ANLL showed different cytochemical and ultrastructural features in accordance with their immunophenotypical classification.

Key words Minimally differentiated acute myeloid leukemia (AML-M0) · Acute undifferentiated leukemia (AUL) · Mixed-lineage leukemia · Myeloperoxidase · Electron microscopy

Introduction

The diagnosis of acute myeloid leukemia (AML) is based on morphological characteristics and myeloperoxidase activity detected by light microscopy, and a myeloperoxidase activity level of less than 3% is considered to be indicative of acute lymphoblastic leukemia (ALL). However, there have been reports of several cases who presented with myeloid markers without myeloperoxidase activity by light microscopy. Minimally differentiated acute myeloid leukemia was thus recognized as a distinct entity, and the French-American-British (FAB) Co-operative group proposed guidelines for the recognition of this form of leukemia, named AML-M0. The factors that distinguish AML-M0 from other types of leukemia are less than 3% myeloperoxidase activity by light microscopy, and positive myeloid markers such as CD13 or CD33, and negative lymphoid markers. AML-M0 was recently approved as a distinctive clinical entity. According to the foregoing criteria, not all cases of peroxidase-negative immature acute nonlymphoblastic leukemia (ANLL) are classified as AML-M0; some are undifferentiated leukemia (AUL) in which myeloid and lymphoid markers are negative, and some are mixed-lineage leukemia with myeloid and lymphoid markers.

Myeloperoxidase is a well-known myeloid marker, and several reports have described it as more useful than CD13 or CD33 for the detection of myeloid leukemia. However, the positivity differed according to the methods used in these studies. Myeloperoxidase examination by electron microscopy is reported to be the most sensitive, followed by immunological examination of myeloperoxidase, and the least sensitive method is light microscopy. Recent study showed that immunological examination distinguished most cases of immature AML. Because immature ANLL...
includes several subtypes of leukemia, a multiparameter analysis is necessary to clarify the nature of immature ANLL. In the present study, we examined blasts by several electron microscopy techniques, including an evaluation of myeloperoxidase activity. DNA staining of osmium ammine B discloses the distribution of DNA in the nucleus. We previously demonstrated different distribution patterns of DNA between AML and ALL blasts with this technique; we suspected that the technique could also reveal some information on immature ANLL. Periodic-acid-Schiff (PAS) reaction, the reaction with glycoconjugates, is fundamental cytochemistry for distinguishing the types of leukemia cells, and we therefore used the periodate-thiocarbohydrazide-silver proteinate (PA-TCH-SP) technique, staining for the glycoconjugates on the electron microscopic level, for more sensitive observation. In the present study, we examined blast cells from 42 patients with peroxidase-negative immature ANLL by these electron microscopic techniques to evaluate the characteristics of the blast cells.

**Materials and methods**

**Patients**

The subjects of this study were 42 patients with ANLL lacking myeloperoxidase activity by light microscopic observation. AML-M0 was diagnosed according to the criteria of the FAB co-operative group. AML-M0 was defined as CD13- or CD33-positive leukemia without T- or B-lineage markers; 26 patients were in this category. The remaining 16 cases of myeloperoxidase-negative immature ANLL leukemia were classified into two types by immunophenotyping: acute undifferentiated leukemia (AUL) showing no definite surface markers, and immature leukemia of mixed lineage (mixed-lineage leukemia) showing both myeloid and lymphoid surface markers. We have diagnosed AUL when immunological markers disclosed one of the earlier molecules, HLA-DR, CD34, or CD7, together with the lack of myeloid markers as CD13, CD33, and T- and B-lineage markers such as CD10, CD19, CD22, CD24, CD3, and CD5. Seven patients were diagnosed as AUL. Mixed-lineage leukemia was diagnosed when the blasts had the myeloid markers CD13 or CD33 and one of the T- or B-lineage markers. Nine patients were diagnosed as having this type of leukemia.

The immunological markers and characteristics of the patients are shown in Tables 1 and 2. In the AML-M0 group, markers not specific to myeloid cells or lymphocytes were occasionally positive. HLA-DR was positive in all patients examined, and CD7 was positive in 9 of 16 patients examined; CD34 was positive in 7 of the 8 adult patients, and all 3 pediatric patients examined were CD34 negative. CD14 was negative in most of the AML-M0 patients. In the mixed-lineage leukemia group, the myeloid markers CD13 or CD33 were positive in all cases, and the B-lineage markers

**Table 1. Immunological markers and characteristics of patients with minimally differentiated acute myeloid leukemia (AML-M0)**

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MP0, myeloperoxidase; MEM, membranous reactivity; GR, granular reactivity; GR&MEM, membranous-granular reactivity

Positive blasts: less than 10%, (−); 10%–20%, (±); 20%–40%, (+); 40%–70%, (++); more than 70%, (+++)