Clinical Significance of Low Levels of Myeloperoxidase Positivity in Childhood Acute Nonlymphoblastic Leukemia

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The clinical significance of a low percentage of myeloperoxidase-positive blast cells in childhood acute nonlymphoblastic leukemia was determined. Of 155 consecutive cases studied by cytochemical staining methods, 14 were characterized by 4% to 15% (median 6%) myeloperoxidase-positive blasts. All 14 cases showed reactivity to Sudan black B stain, and 7 had Auer rods. The morphological subtypes of leukemia were M1 (8 cases), M2 (3), M4 (1), and M5 (2). Immunological marker studies disclosed the lymphoid-associated T11 antigen on cells from 8 of the 11 cases tested. Other lymphoid-related findings in these 8 cases included the T3 antigen and E rosette formation in 1 case each. Among cases that were prospectively studied for the expression of lymphoid-associated markers, 6 of 8 with low levels of myeloperoxidase positivity compared with only 1 of 44 with higher levels (>15%) possessed such features (P < 0.001). We conclude that low levels of myeloperoxidase reactivity distinguish cases of acute leukemia in which the blast cells coexpress lymphoid (T11 antigen) and myeloid markers.

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CLASSIFICATION of the acute leukemias by the French-American-British (FAB) system requires analysis of the cytochemical features of the malignant blast cells. Although reactivity to Sudan black B (SBB) stain can no longer be considered a lineage-specific marker, myeloperoxidase (MPO) positivity is still regarded to be diagnostic of acute nonlymphoblastic leukemia (ANLL). Investigators in the FAB cooperative group selected ≥3% MPO positivity as a criterion for the diagnosis of ANLL. Although the percentage of blasts containing MPO activity ranges widely, from 3% to 100%, most cases of myelocytic, promyelocytic, or myelomonocytic leukemia are characterized by high levels of the enzyme. Whether cases with marginal MPO positivity are sufficiently different from more typical cases to warrant reclassification has not been established. The results reported here indicate that low levels of blast cell MPO activity distinguish a subgroup of children with ANLL whose blasts are more likely to express lymphoid-associated antigens.

MATERIALS AND METHODS

Patients. From April 1980 to April 1986, 155 consecutive children with newly diagnosed ANLL were enrolled in two successive clinical trials at St Jude Children's Research Hospital. Informed consent was obtained for all patients, and the investigations were approved by the institution's clinical trials committee.

Morphological and cytochemical studies. Bone marrow smears were stained by standard techniques, including Wright's-Giemsa, MPO, SBB, periodic acid-Schiff reagent (PAS), naphthol AS-D chloroacetate esterase (CAE) and α-naphthyl butyrate esterase (ANB). Blast cell morphology was classified according to FAB criteria. By convention, cytochemical staining was considered positive if >3% of the blasts reacted. Blast cell MPO positivity, based on analysis of 200 cells per sample, was reviewed and confirmed independently by two of us (F.G.B. and D.L.B.). There was good agreement of results between the two readers, with differences generally <2%. In no instance did the discrepancy lead to a different interpretation of MPO positivity.

Immunophenotyping. Leukemic blast cells from marrow aspirates were separated on a density gradient, and only samples containing >85% blasts were assayed. They were studied for spontaneous rosette formation with use of sheep erythrocytes incubated at 37°C. Cell-surface antigens were detected with a standard indirect immunofluorescence assay using monoclonal antibodies representative of the cluster of differentiation (CD) groups identified at the First and Second International Workshops on Human Leukocyte Differentiation Antigens. The monoclonal antibodies to myeloid-associated antigens included anti-My-1 (X-Hapten, CD-15), anti-MCS.1 (CDw-13, 150), anti-Mol (CD-11), and anti-SJ-D1 (CDw-13, 150). The blasts were also tested with monoclonal antibodies to lymphoid-associated antigens, including J5 (common acute lymphoblastic leukemia antigen, CALLA, CD-10), T11 (CD-2), T101 (CD-5), and T3 (CD-3). Two observers independently counted 200 cells in each assay; the results were judged positive if ≥40% of the blast cells had surface fluorescence. Terminal deoxynucleotidyl transferase (TdT) was detected by an indirect immunofluorescence assay.

RESULTS

MPO activity was demonstrated in 3% to 100% of the blast cells from 37 of 43 cases with an M1 FAB type, 47 of 47 with M2, 9 of 9 with M3, 29 of 29 with M4, and 4 of 25 with M5. The diagnosis of ANLL in the six M1 cases with negative MPO reactivity was based on the presence of Auer rods, ultrastructural myeloperoxidase or myeloid markers as defined by monoclonal antibodies; none of them expressed any lymphoid markers. The two cases of M7 leukemia lacked evidence of the enzyme. Of the 126 cases that showed MPO activity, 14 were characterized by 4% to 15% (median 6%) MPO-positive blast cells in bone marrow samples (Table 1);
the remaining cases had >25% blast cells with MPO positivity, most >80%. None of the cases had MPO positivity between 16% and 25%. Blast cells from each of these 14 cases with low levels of MPO positivity met standard FAB criteria for ANLL. All cases were positive for SBB, and seven contained Auer rods. Their FAB designations were M1 in 8 cases, M2 in 3, M4 in 1, and M5 in 2.

Blast cells from 12 of the 14 patients (excluding nos. 8 and 10) were examined for lymphoid- and myeloid-associated differentiation markers. All but three cases (nos. 3, 5, and 6) were defined by reactivity to one or more myeloid-associated monoclonal antibodies (data not shown). Studies with lymphoid-associated monoclonal antibodies, although not disclosing a consistent pattern of binding, indicated unequivocal lymphoid involvement in eight of the 12 cases tested (nos. 1, 2, 4, 6, 7, and 12-14, Table 1). Evidence of lymphoid involvement consisted of reactivity with antibodies to the T11 (CD-2) antigen in all eight cases, coupled with the expression of T3 (CD-3) or formation of heat-stable E rosettes in one case each. Blast cells from an additional patient (no. 5), which were not tested for T11, were positive for CALLA. TdT activity was detected in all but one of the cases that demonstrated T11 and in two others that were devoid of more definitive lymphoid markers.

The relationship between MPO reactivity and the expression of lymphoid-associated antigens was determined in the 56 consecutive patients who had been prospectively studied for these features since August 1983 and had adequate leukemic cell samples. Among these cases, eight (including cases 1, 3, 4, 6, 7, and 11 through 13) were characterized by 4% to 15% MPO-positive blasts, and the remainder were characterized by negative MPO activity (four cases) or >25% MPO positivity (44 cases). Six of eight cases with low MPO reactivity had blast cells with lymphoid-associated markers, contrasted with only one of those with >25% MPO positivity (p < 0.001). The patient with >25% MPO also expressed T11 antigen. None of the four cases with negative MPO activity expressed lymphoid-associated antigens.

The clinical features of patients whose leukemic cells expressed lymphoid-associated markers did not clearly discriminate between these cases and typical ANLL. Ages ranged from 6 to 18 years (median 14 years), presenting leukocyte counts from 2 to 171 x 10^9/L (median 16.2 x 10^9/L), platelet counts from 4 to 312 x 10^9/L (median 120 x 10^9/L) and hemoglobin levels from 4.1 to 14 g/dL (median 10.4 g/dL). All patients had lymphadenopathy but only three (nos. 1, 2, and 13) had hepatosplenomegaly. Initial CNS leukemia was evident in patient 4, and patient 2 had an anterior mediastinal mass.

**DISCUSSION**

Bennett and associates considered 3% as the lower limit for MPO positivity in the FAB system of leukemia cell classification. Results of the present study suggest that a higher level of positivity is required to distinguish "typical" cases of ANLL from those with equivocal features. We demonstrated that a criterion of 4% to 15% MPO positivity yields a high percentage of cases with lymphoid-associated differentiation markers, primarily the T11 antigen. Although identified on normal, activated B cells, T11 has not been detected on normal myeloid cells. Additional evidence of lymphoid involvement was provided by T3 positivity and E rosette formation in one case each. Although TdT activity and/or CALLA were also expressed in some of our cases, these markers can no longer be considered lymphoid-specific markers. TdT activity has been demonstrated in 5% to 10% of ANLL cases that were unequivocally myeloid in origin. CALLA has been identified on renal tubular and glomerular cells, granulocytes, fibroblasts, fetal small intestine epithelial cells, mammary epithelium, and myeloma cell lines (see ref. 18 for review). Nonetheless, we think it noteworthy that the majority of our cases with low MPO positivity expressed TdT activity, a far greater proportion than has been reported in ANLL.

The immunologic marker findings in cases with low MPO activity are most compatible with the newly recognized entity of acute mixed-lineage leukemia, in which the leukemic cells coexpress markers generally thought to be
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restricted to a single lineage.\(^{18-23}\) None of the cases in this study had cytogenetic evidence for biclonal disease, whereas evidence for simultaneous expression of lymphoid and myeloid markers was found in all four cases tested (data not shown). The diagnosis of acute mixed-lineage leukemia is best exemplified by case 2. The presence of an anterior mediastinal mass and the expression of T11 and T3 antigens in 83% and 87% of this child's blast cells would be considered by most investigators as diagnostic of T cell acute lymphoblastic leukemia. However, there was also strong evidence for myeloid differentiation, including 4% myeloperoxidase positivity, the presence of Auer rods, and the expression of myeloid antigens in blast cells [84% reacted with Mol (CD11) and 35% with MCS-1 (CDw-15) monoclonal antibodies.

Consistent with our data, the reported patterns of monoclonal antibody binding in cases of acute mixed-lineage leukemia have generally included high percentages of T11 positivity with variable levels of other lymphoid markers.\(^{21}\) These leukemias either may arise from multipotential progenitors with capacity of lymphoid or myeloid differentiation\(^{16,22}\) or had aberrant expression during malignant proliferation.\(^{19}\) The clinical importance of acute mixed-lineage leukemia lies in the suggestion that such patients may not respond to therapy conventionally prescribed for myeloid leukemia, but require treatment directed to the lymphoid pathway.\(^{22-23}\) None of the cases in this study had cytogenetic evidence for biclonal disease, whereas evidence for simultaneous expression of lymphoid and myeloid markers was found in all four cases tested (data not shown). The diagnosis of acute mixed-lineage leukemia is best exemplified by case 2. The presence of an anterior mediastinal mass and the expression of T11 and T3 antigens in 83% and 87% of this child's blast cells would be considered by most investigators as diagnostic of T cell acute lymphoblastic leukemia. However, there was also strong evidence for myeloid differentiation, including 4% myeloperoxidase positivity, the presence of Auer rods, and the expression of myeloid antigens in blast cells [84% reacted with Mol (CD11) and 35% with MCS-1 (CDw-15) monoclonal antibodies.

MPO, a major 135,000-dalton protein, is localized to the azurophil granules in the myeloid series of hematopoietic cells. MPO synthesis has been suggested to occur at the promyelocytic stage of differentiation and ceases as myeloid cells differentiate to myelocytes, metamyelocytes, granulocytes, and macrophages.\(^{24}\) Abundant MPO activity is typical of progranulocytic leukemia; the activity is decreased in myeloid variants of ANLL and low to absent in monocytic subtypes.\(^{25}\) Thus, the lack of lymphoid markers on blast cells in the single case of M5 leukemia that was tested is not surprising. If acute mixed-lineage leukemia originates in a pluripotent stem cell with the ability to differentiate in either the lymphoid or myeloid pathway,\(^{19,22}\) and if the developmental stage of such transformed cells is more primitive than is usually seen in cases of ANLL, one might expect to find low MPO activity as a characteristic feature of this form of the disease.

Currently, the diagnosis of acute mixed-lineage leukemia is based on an extensive battery of cytochemical and immunological tests.\(^{21}\) We believe that low MPO positivity in cases of ANLL should be regarded as a potential indicator of this clinical entity. Our interpretation assumes additional significance in view of the variability of clinical and laboratory features among such patients.

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