Simultaneous Evaluation of Terminal Deoxynucleotidyl Transferase and Myeloperoxidase in Acute Leukemias Using an Immunocytochemical Method

SANDRA S. KAPLAN, M.D., LILA PENCHANSKY, M.D., JOHN R. KRAUSE, M.D., R.E. BASFORD, PH.D., AND URSULA ZDZIARSKI, B.S.

Departments of Pathology and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

The classification of acute leukemia is important for the selection of optimal therapy. Classification often rests on morphologic, cytochemical, and immunologic criteria, and the marker enzyme terminal deoxynucleotidyl transferase (TdT) has been considered to be a reliable indicator of lymphoblastic leukemias. Because TdT-positive cells sometimes are seen in leukemias otherwise identified as myeloblastic, the authors evaluated blasts identified as myeloid by the presence of myeloperoxidase (MPO) for the simultaneous expression of TdT. The blasts in the bone marrow aspirate or peripheral blood of unselected patients with hematologic malignancies were evaluated and 60 cases are shown. The French–American–British system and, in some patients, cytochemical and immunologic studies were used to classify the leukemias. The authors demonstrated that blasts simultaneously contained MPO and TdT in 29% of patients with acute myeloblastic leukemia and 3% of patients with acute lymphocytic leukemia (ALL). This finding supports the hypothesis that TdT is an expression of cell primitivity rather than a marker for lymphoblastic cells. (Key words: Leukemia; Terminal transferase; Myeloperoxidase) Am J Clin Pathol 1987; 87: 732–738

THE CLASSIFICATION of acute leukemias is based largely upon the examination of Romanovsky-stained peripheral blood and bone marrow aspirate smears, standardized for the past ten years according to the French–American–British (FAB) system, which divides myeloblastic leukemias into six types and lymphoblastic leukemias into three.[2] This distinction of leukemias as myeloblastic or lymphoblastic has been of considerable importance clinically, since response to therapy and prognosis are significantly different for these two classes of leukemias.[14,39] Because classification based on morphologic characteristics alone sometimes is imprecise, cytochemical stains[12,14] and immunologic studies using a wide variety of monoclonal antibodies[8,24] have become widely used to aid in the identification of leukemia cells.

One of the most useful procedures for the classification of acute leukemia has been the determination of terminal deoxynucleotidyl transferase (TdT), a nuclear enzyme that had been considered to be specific for lymphoblastic cells.[5] This enzyme may be evaluated immunologically using heteroantisera linked with a fluorescence or immunoperoxidase visualization technique,[22] which makes it possible to identify this marker in individual cells. It soon became evident that many patients (5–44%) with morphologically and cytochemically defined acute myeloblastic leukemia (AML) also had cells positive for TdT.[6,7,12,18,22]

To resolve the question of whether patients with AML who showed TdT-positive cells had two cell types (one with myeloid and one with lymphoid characteristics) or a single cell expressing two markers, it was necessary that the myeloid lineage of the TdT-positive cells be established clearly. Several such studies have been reported where dual labeling was performed on selected cases. These studies initially indicated that cells labeled with more than one marker were quite unusual.[3,13,23,27,28,37] More recently, however, Mirro and associates used dual markers to evaluate 12 cases selected from a series of 123 patients with acute leukemia and found evidence for dual cell lineage in all 12. There was indirect evidence of dual lineage in another 13 cases.[26]

Although these studies provide strong evidence that, at least in some instances, a single cell may react with both myeloid and lymphoid markers, the question of how frequently this is found needed to be addressed.[25] To determine the frequency of dual markers in acute leukemias, patients newly diagnosed with a hematologic malignancy were routinely evaluated for the presence of myeloperoxidase (MPO) and TdT from November 1984 until November 1985. We determined that 29% of patients with AML had blasts containing both markers, while only one patient (an adult) with acute lymphocytic leukemia (ALL) expressed simultaneous markers.

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Address reprint requests to Dr. Kaplan: Department of Pathology, Magee Womens Hospital, Forbes Avenue at Halket Street, Pittsburgh, Pennsylvania 15213.

Methods

Patients

Eighty-six patients were evaluated. In 60 of the patients, a diagnosis of acute leukemia was established from bone marrow aspirate or buffy coat smears stained with the Romanovsky method. A minimum of 200 cells were counted, and nearly all cases also had a core biopsy evaluated (stained with hematoxylin and eosin and with Giemsa). Classification was determined using morphologic and, in many cases, cytochemical and immunologic characteristics. Cytochemical reactivity of blast cells was evaluated using periodic acid-Schiff (PAS), chloroacetate esterase (CAE), alpha-naphthyl acetate esterase (NSE) with and without fluoride inhibition, and acid phosphatase (AcP) reactions according to standard methods. Cytochemical MPO stains were done in some cases also. A case was designated positive if 3% of the blasts were positive.[2] The lymphoblastic leukemia cases were further subclassified using monoclonal antibody J5 (CALLA), E-rosettes, and surface and cytoplasmic immunoglobulins according to previously described technics.[15,19] Some of the cases were classified using some or all of a monoclonal antibody profile consisting of I2, My7, My9, B1, B4, T1, T11, T4, and T8 and evaluated using fluorescence activated cell sorting (FACS) technology or immunogold staining.[9,10] (Monoclonal antibodies were obtained from Coulter Immunology, Hialeah, FL.) A case was considered positive for the marker if 10% of the blasts displayed binding of the antibody.

MPO and MPO Antibody Preparation

MPO was purified from human polymorphonuclear (PMN) leukocytes (obtained from patients undergoing therapeutic phlebotomy for secondary polycythemia) according to Anderson and associates,[1] using gel filtration and column chromatography. Protein assays were done by the Schacterle-Pollack method,[33] using a bovine serum albumin (BSA) standard, and myeloperoxidase enzyme assays were done according to Himmelhoch and associates.[17] Purity of the preparations was established by gel electrophoresis, which demonstrated a single band. Rabbits were immunized by injecting 375 μg of MPO in complete Freund's adjuvant per rabbit in each thigh and subcutaneously in the back of the neck. Intravenous booster injections were given on days 10 and 17, using 75 μg MPO in saline. On day 27, the serum was tested for antibody and the gamma globulin fraction was purified by treatment with Cellex D® [20] (Bio-Rad, Rockville Centre, NY). The reactivity of the antibody was compared to cytochemical MPO methods and the optimal antibody concentration for immunocytochemical assays was determined by incubating dilutions of antiserum with methanol-fixed smears of normal peripheral blood. The primary antibody was visualized using rhodamine-conjugated goat antirabbit serum, and the smears were evaluated using a Nikon Labophat® equipped with fluorescein isothiocyanate (FITC) and rhodamine filters (excitation wave lengths 410–485 and a 50-W mercury lamp). The antibody to myeloperoxidase reacted strongly with neutrophils and precursors of neutrophils and weakly with monocytes. It did not react with eosinophils, platelets, or lymphocytes.

Dual Labeling

The double labeling was performed sequentially as described by Folds and associates.[13] Briefly, indirect immunofluorescence on methanol-fixed bone marrow aspirates or peripheral blood buffy coat smears for TdT was done according to Bollum and co-workers.[5] (The primary antibody to TdT, the secondary antibody, and reagents were obtained from Supertech Laboratories, Bethesda, MD). The TdT antibody does not react with cells in adult bone marrows but may react with up to 10% of cells in pediatric marrows (in patients younger than two years of age). The procedure then was repeated with the MPO antibody as described above. At least 200 cells per slide were evaluated to determine the percentage of the blasts that were positive for TdT or MPO. A cell reacting either with TdT or MPO antisera was evaluated immediately for the simultaneous presence of the other by switching the filter. At least 100 more of the TdT-positive cells were evaluated specifically to determine the percent TdT-positive cells that also were MPO positive. All of the cases were evaluated by one of the authors (S.S.K.), and nearly every case also was evaluated by L.P. or J.R.K.

Results

The reactivity of the antibody to MPO was compared with the standard cytochemical method in 21 cases of acute nonlymphocytic leukemia and 10 cases of ALL. Equivalent results were seen in the two methods, and the immunocytochemical method appeared to be more sensitive. No positive blasts were seen in the ALL cases, and three of the patients with acute nonlymphocytic leukemia were MPO negative by both methods.

There were 28 cases classified as AML. A brief clinical profile and their diagnostic laboratory studies are shown in Tables 1–3. There were 13 females and 15 males, and 6 were 18 years of age or younger. The mean age of the adults was 64 years. The mean age of the children was 12.8 years. Eleven of the patients with AML (39%), confirmed with cytochemical and/or immunologic data, showed no evidence of TdT activity (Table 1). Seventeen of the patients with AML (61%) had blast cells reacting with TdT. Nine of these cases, shown in Table 2, showed

Table 1. Laboratory Features of TdT-Negative Patients with Acute Myeloblastic Leukemia

Patient*	Age/Sex	WBC $\times 10^9$/L	Percentage Blasts		Immunofluorescence†			Cytochemistry‡	Surface Markers§	
			PB	ASP	MPO	TdT	Dual		Present	Absent
1. M2	62/M	48.8	76	ND	>50	Neg	No	Auer rods 10%	ND	ND
2. M2	78/F	98.0	97	90	>50	Neg	No	SBB, MPO	ND	ND
3. M2	12/M	6.4	36	35	>50	Neg	No	CAE, NSE, SBB	ND	ND
4. M1	78/F	1.3	0	50	30	Neg	No	MPO	ND	ND
5. M2	45/F	75.0	92	>90	>75	Neg	No	SBB, MPO	My9, My7	B1, J5, T11
6. M1	49/M	138.0	16	29	>75	Neg	No	CAE, Auer rods, MPO	ND	ND
7. M1	18/F	6.5	32	38	50W	Neg	No	SBB, MPO	My7, My4, Ia	B4, J5, T11
8. M2	8/M	23.9	4	42	35	Neg	No	CAE, SBB	My7, My9	B1, J5, T11
9. M5	65/M	2.9	9	90	Rare	Neg	No	NSE	ND	ND
10. M2	71/M	101.7	87	90	>75	Neg	No	NSE, Auer rods	ND	ND
11. M5	76/F	29.6	78	>90	<5	Neg	No	ND	My7, Ia	B1, B4, J5, T11, T1

* Patient number and FAB classification.
† Percent positive blasts.
‡ Cytochemistries supporting diagnosis of AML; MPO not done in cases 1, 3, 8–11.
§ Surface markers supporting diagnosis of AML by FACS unless otherwise indicated.

no evidence of simultaneous expression of MPO and TdT. Three of these patients had 13–20% TdT-positive cells. One of these, case 2, had no surface marker studies and conceiveably could be biphenotypic. The other two were not felt to be biphenotypic because of negative lymphoid surface markers. Two patients had numerous TdT-positive cells. Although neither of these patients showed dual labeling, they were believed to be myeloid leukemias because of the surface marker results. Eight of the 17 patients with TdT-positive results had cells that simultaneously exhibited MPO and TdT (Table 3), with a representative cell shown in Figures 1*A* and *B*. The percentage of doubly labeled cells was independent of the total number of TdT-positive cells. Only two of these patients (case 1 with 15% and case 6 with 14%) had what would be considered to be significant numbers (>10%) of TdT-positive cells. Case 1, however, had only a rare dual cell, while case 6 had 80% (of the TdT-positive cells) doubly marked. In addition, case 6 displayed especially prominent MPO activity in the TdT-positive blasts, such that the MPO fluorescence could be seen with the FITC filter (Fig. 2). Two patients, patients 3 and 8, who showed relatively few TdT-positive cells, exhibited double marking in most of these cells, strongly suggesting that the TdT-positive cells were not indicative of a second leukemic population but rather represented TdT-positive myeloid cells.

The diagnosis of ALL, based on morphologic and cytochemical and/or surface marker criteria, was made in 30 cases. Eighteen of these patients could be morphologically classified as L1 ALL, with 12 of 14 CALLA positive and 16 of 16 PAS positive. All had most of their blasts TdT positive (85 ± 4%) and showed only a rare MPO-positive cell that could easily be identified as a developing myeloid cell. None of these patients had cells simulta-

Table 2. Laboratory Features of TdT Positive Patients with Acute Myeloblastic Leukemia

Patient*	Age/Sex	WBC $\times 10^9$/L	Percentage Blasts		Immunofluorescence†			Cytochemistry‡	Surface Markers§	
			PB	ASP	MPO	TdT	Dual		Present	Absent
1. M1	66/F	4.6	65	>70	10	<1	No	ND	ND	ND
2. M2	75/F	2.5	20	69	>50	20	No	MPO	ND	ND
3. M4	65/M	5.1	83	>90	>80	<5	No	NSE	ND	ND
4. AUL	58/M	143.0	55	71	<5	<5	No	NSE	My7, My4, Ia	
5. M4	62/M	31.0	24	20	60	<5	No	ND	My9, My7, MO2, My4, Ia	B4, J5, T11
6. M1	17/F	48.4	11	ND	25	18	No	ND	My7, My9	B1, J5, T11
7. M1	72/M	126.4	77	64	25	13	No	ND	My7, My9, Ia	B1, B4, J5, T1
8. M5	50/F	1.5	64	81	<5	>75	No	NSE	My7, My9, Ia	J5
9. M4	41/M	7.8	12	>90	15	60	No	SBB	My9, My7, Ia, M4	T1, T11

* Patient number and FAB classification.
† Percent positive blasts.
‡ Cytochemistries supporting diagnosis of AML; MPO was negative in case 4; not done in cases 1, 3, 5–11.
§ Surface markers supporting diagnosis of AML by FACS unless otherwise indicated.

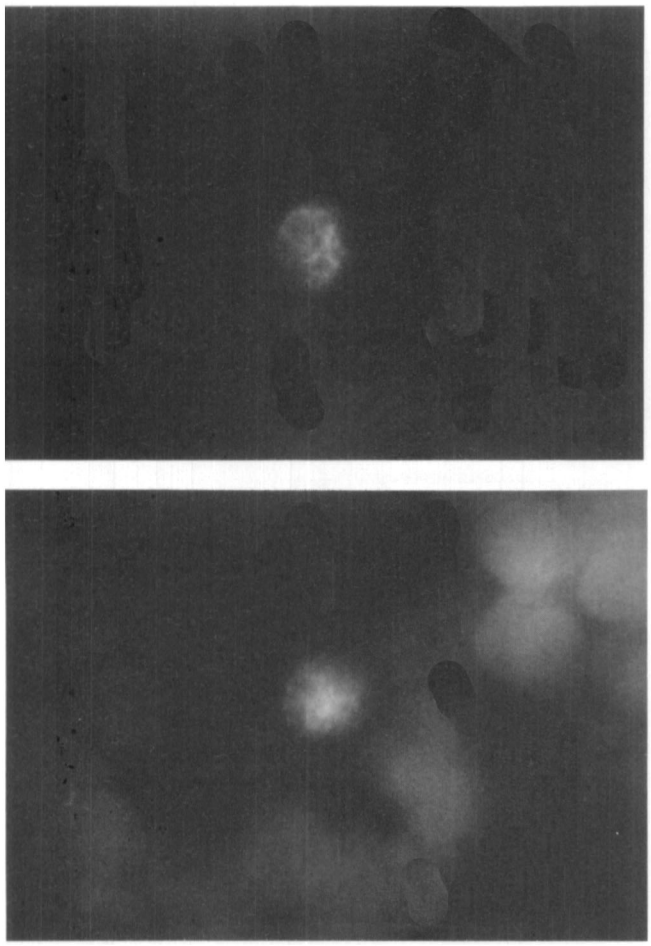
Table 3. Laboratory Features of Patients with Simultaneous MPO and TdT in Acute Myeloblastic Leukemia

Patient*	Age/Sex	WBC $\times 10^9$/L	Percentage Blasts		Immunofluorescence†			Cytochemistry§	Surface Markers¶	
			PB	ASP	MPO	TdT	Dual‡		Present	Absent
1. M2	71/M	20.8	54	50	>50	15	<2	NSE	ND	ND
2. M1	57/F	6.5	52	Dry	>50	5	50	CAE	ND	ND
3. M4	72/M	1.8	0	>90	>75	1	75	SBB, NSE, MPO	ND	ND
4. M2	63/M	29.7	90	>90	80	9	3	SBB, MPO	My9, My7, Ia	B4, J5, T11
5. M2	8/F	8.9	83	88	>75	10	100	CAE, MPO, NSE, Auer rods	ND	ND
6. M2	14/F	2.9	0	38	80	14	80	CAE	My7, My9	J5, T11
7. M1	84/F	76.0	93	ND	13	9	<2	ND	My7, Ia	B1, B4, J5, T11
8. M1 or AUL	54/F	1.4	Rare	67	16	>5	60	Neg	ND	ND

* Patient number and FAB classification.
† Percentage positive blasts.
‡ Percentage of TdT-positve cells that are MPO positive.
§ Cytochemistries supporting diagnosis of AML; MPO not done in cases 1, 2, 6, and 7.
¶ Surface markers supporting diagnosis of AML by FACS unless otherwise indicated.

neously positive for TdT and MPO. The clinical and laboratory data on the remaining 12 patients with ALL are shown in Table 4. One of these patients, patient 1, had blasts simultaneously positive for MPO and TdT. This patient's leukemia was classified as lymphoblastic because of the large number of TdT-positive cells. All cytochemistries were negative, and surface markers were not available. All of the patients achieved remission with administration of vincristine, L-asparaginase, and prednisone.

There were two patients with mixed acute leukemias. Both were children, and both were characterized by unusual case histories (reported separately).[30] One of the pa-



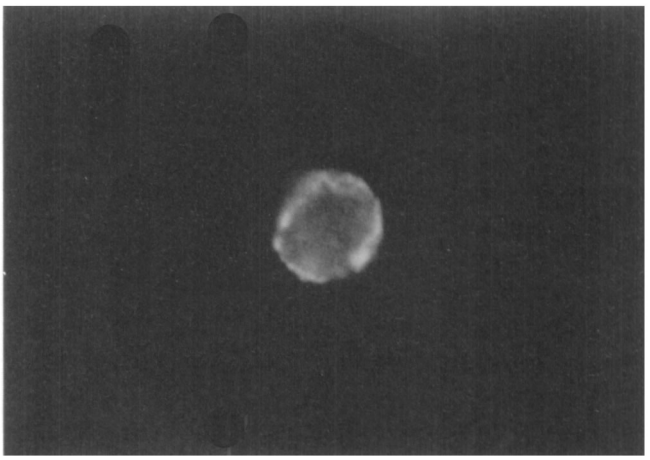


FIG. 1. A leukemic blast is shown with the fluorescein filter (*A, upper, left*) to exhibit TdT activity in the nucleus and with the rhodamine filter (*B, upper, right*) to exhibit myeloperoxidase positive granules in the cytoplasm (×1,000).

FIG. 2. A leukemic blast photographed with the fluorescein filter shows visible MPO granules in addition to the TdT-positive nucleus (×1,000).

Table 4. Laboratory Features of Patients with Acute Lymphoblastic Leukemia

Patient*	Age/Sex	WBC $\times 10^9$/L	Percentage Blasts		Immunofluorescence†			Cytochemistry‡	Surface Markers§
			PB	ASP	MPO	TdT	Dual		
1. L2/L1	19/M	6.7	60	90	Rare	>80	5–10	All Negative	ND
2. L1/L2	1/M	13.7	31	80	Neg	>10	No	PAS¶	ND
3. L1/L2	27/M	22.1	92	>90	Neg	>90	No	PAS	ND
4. L1/L2	10/F	9.2	1	76	Rare	60	No	PAS,	ND
5. L2/L1	17/M	10.2	95	>95	Rare	60	No	PAS, NSE**	T1, T11
6. L1/L2	16/M	43.6	48	74	25	61	No	PAS, NSE, ACP††	T11, T8
7. L2/L1	68/M	1.1	3	>90	10	90	No	ND	CALLA, Ia, B4, T1
8. L2/L1	75/M	5.8	34	>90	Neg	>75	No	PAS	CALLA, Ia, B4, B1, T1, T11
9. L1/L2	8/M	38.0	84	96	<2	>90	No	PAS	CALLA, Ia
10. L3	10/M	12.7	2	83	Many	0	No	ACP	CALLA, B1, Ia
11. L2	6/M	3.8	31	95	Neg	92	No	PAS	CALLA
12. L1/L2	7/M	8.8	78	90	Rare	90	No	PAS	CALLA

* Patient number and FAB classification.
† Percentage positive blasts.
‡ Cytochemistries supporting diagnosis of ALL.
§ Surface markers supporting diagnosis of ALL using immunogold method.
¶ Coarse block-like granules.
** NSE perinuclear dot.
†† Golgi zone granule.

tients had equal numbers of MPO-positive and TdT-positive blasts and had CALLA-positive as well as CALLA-negative blasts. Twenty-five percent of the TdT-positive blasts showed MPO-positive granules. The other child's leukemia was predominently lymphoblastic, B1 positive, with 80% TdT-positive blasts. Twenty-five percent of these blasts were MPO positive. None of the 13 cases with chronic myeloblastic leukemia (CML), including a juvenile CML, exhibited dual markers. Two of these patients were in myeloid blast crisis.

Discussion

Our study demonstrated, in an unselected series, that 8 of 28 (29%) patients with AML, 1 of 30 (3%) patients classified as having ALL, and 2 of 2 patients with mixed leukemias had cells with dual markers. The degree of dual positivity was variable and was not proportional to the number of TdT-positive cells. This series clearly establishes that TdT-positive nuclei occur in cells that possess MPO-positive granules, including cases where the total number of cells with any marker is small, and further shows that this occurrence is not uncommon in AML. None of our patients with CML, including two in blast crisis, exhibited dual markers. Bettelheim and associates reported two cases with mixed blast crises, but none had dual markers.[4] Although other reports have shown that lymphoid markers, including TdT, occurred in cells that are MPO-positive or exhibit myeloid-specific surface markers,[3,13,23,27,28,37,38] these studies evaluated only selected patients.

Although the explanation for the simultaneous presence of TdT and MPO or of other mixed lineage markers is not entirely clear, there is considerable evidence for the hypothesis that, at least in some cases, a common stem cell precursor undergoes leukemic transformation, with resulting biphenotypic expression. Support for this idea comes from Perentesis and associates,[31] who reported a case in which AML occurred in a patient who presented with a TdT+ CALLA+ BA-1+ BA-2+ Peanut agglutinin (PNA) and TA-1 neg ALL. Although dual labeling was not done in this study, this patient's cells continued to exhibit the BA-1 and BA-2 markers on cells that now reacted with TA-1, PNA, and NSE, while lysozyme levels became markedly elevated and TdT and CALLA reactivity no longer were present. The hypothesis is further supported by Tindle and associates,[38] who developed the monoclonal antibody, BI-3C5, from an AML cell line and found that it reacted both with 14 of 17 early myeloid (M1 and 2) leukemias and 12 of 16 acute lymphoblastic (TdT+ CALLA+) leukemias but failed to react with more differentiated acute leukemias. Kita and associates examined 15 non-T, non-B ALL patients and found that 5 of them expressed both TdT and myeloid surface markers simultaneously. This was interpreted as representing an immature stage of hematopoiesis corresponding to the bifurcation of lymphoid and myeloid pathways.[21] Other support comes from Fialkow and associates,[11] who evaluated G6PD in malignant blasts and determined that in some patients with AML a pluripotent stem cell was involved. Ha and colleagues[16] studied 60 cases of acute leukemia using the presence of immunoglobulin gene rearrangement to identify cells of B-lymphocyte lineage and found that, while CALLA+ leukemias all showed rearrangements, other acute leukemias did also. They found 2 of 9 T-cell ALLs, 2 of 3 acute undifferentiated leukemias (AULs), and 1 of 13 AMLs that exhibited gene rearrangement. Palumbo and colleagues[29] and Rovigatti and as-

sociates[32] also evaluated this parameter and found evidence for rearrangement of immunoglobulin heavy chain genes in some patients with myeloblastic leukemia and in some myeloblastic cell lines. Smith and co-workers,[34] however, interpreted the occurrence of bilineage marking to indicate abnormal gene expression in leukemia.

Since the first report of dual marking by Folds and associates in 1982, the sophisticated methods for the determination of identifying markers has come into wide use. Reports of doubly or multiply marked cells have rapidly increased, shedding light on the phenotypic characteristics of leukemic blasts and suggesting that mixed lineage cells may be a product of the differential evolution of the pleuripotent stem cell toward one cell line or another with respect to nuclear, cytoplasmic, and surface membrane characteristics. The therapeutic implications of this phenotypic identification will only become clearer when clinicopathologic correlations can be established. Preliminary evidence, however, suggests that phenotypic characteristics may be of relevance to response to treatment.[35] It is likely to be of some importance therefore to identify leukemic blasts using these immunocytochemical "fingerprints," not only at the time of diagnosis, but also at relapse, because it has been shown that phenotypic switching occurs[26,36] and the degree of heterogeneity recently described suggests that switching may not be uncommon.

Because MPO is specific for myeloid cells, while TdT positivity may occur both in lymphoid and in myeloid blasts, the presence of both of these markers in a single cell suggests a need for caution in a patient who otherwise appears to have lymphoblastic leukemia. Thus, patient 1 in Table 4, who was felt to have ALL clinically and morphologically, might more appropriately be re-classified as having AML or being biphenotypic; and two patients shown in Table 2 illustrate that large numbers of TdT-positive cells may be seen in leukemias felt to be nonlymphocytic on the basis of surface membrane studies.

Observations of the frequent expression of dual lineage markers in AML present a persuasive body of evidence for the presence of a common myeloid–lymphoid progenitor. The frequency of MPO-positive TdT reacting cells in AML and its rarity or absence in ALL suggests that in ALL the cells are biologically primitive but committed to lymphoid maturation. Thus, these cells could share a myeloid membrane epitope, as shown by Kita and associates,[21] but would not show a cytoplasmic enzyme. The presence of TdT positivity in AML then should not be taken as showing dual lineage but rather as an indicator of cell primitivity or as an indicator of asynchrony of nuclear and cytoplasmic development. These cells are committed to myeloid differentiation but retain TdT activity. The data further suggest that the presence of TdT antigen is not sufficient for a diagnosis of ALL, and the rarity of MPO positivity in well-documented ALL suggests that caution be exercised when classifying a TdT-positive leukemia when MPO-positive granules are simultaneously present.

The use of multilabel immunofluorescence for microscopy and flow cytometry, together with immunogold and cytochemistry, provide a rich resource for the characterization of leukemic cells. Their use to date has greatly expanded our understanding of the nature of acute leukemia and indicates the direction that must be taken for future study.

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References

1. Anderson MR, Atkin CL, Eyre HJ: Intact form of myeloperoxidase from normal human neutrophils. Arch Biochem Biophys 1982; 214:273–283
2. Bennett JM, Catovsky D, Daniel MT, et al: Proposals for the classification of the acute leukemias. Br J Haematol 1976; 33:451–458
3. Bettelheim P, Paietta E, Majec O, Godner H, Schwarzmeier J, Knapp W: Expression of a myeloid marker on TdT positive acute leukemic cells: Evidence by double fluorescence staining. Blood 1983; 60:1392–1396
4. Bettelheim P, Lutz D, Majic O, et al: Cell lineage heterogeneity in blast crisis of chronic myeloid leukemia. Br J Haematol 1985; 59:395–409.
5. Bollum FJ: Terminal deoxynucleotidyl transferase as a hematopoietic cell marker. Blood 1979; 54:1203–1215
6. Bradstock KF, Hoffbrand AV, Ganeshaguru K, et al: Terminal deoxynucleotidyl transferase expression in acute non-lymphoid leukemia: An analysis of immunofluorescence. Br J Haematol 1981; 47:133–143
7. Bradstock KF, Hewson J, Kerr A, Kabral A, Lee CH, Hughes WG: Expression of terminal deoxynucleotidyl transferase in malignant myeloblasts. Am J Clin Pathol 1983; 80:800–805
8. Chan LC, Pegram SM, Greaves MF: Contribution of immunophenotype to the classification and differential diagnosis of acute leukaemia. Lancet 1985; 1:475–479
9. Crockard A, Catovsky D: Cytochemistry of normal lymphocyte subsets defined by monoclonal antibodies and immunocolloidal gold. Scand J Haematol 1983; 30:433–443
10. DeWaelle M, DeMey J, Moeremans M, DeBrabander M, VanCamp B: Immunogold staining methods for the light microscopic detection of leukocyte cell surface antigen with monoclonal antibodies. J Histochem Cytochem 1983; 31:376–381
11. Fialkow PJ, Singer JW, Adamson JW, et al: Acute non-lymphocytic leukemia: Heterogeneity of stem cell origin. Blood 1981; 57:1068–1073
12. Flandrin G, Daniel MT: Cytochemistry in the classification of leukemias, The leukemic cell. Edited by D Catovsky. Edinburgh, Churchill Livingstone, 1981, pp 29–48
13. Folds JD, Bollum FJ, Dean L, Chan J, Stass SA: Simultaneous evaluation for terminal deoxynucleotidyl transferase and myeloperoxidase in leukemia. Am J Hematol 1982; 12:391–396
14. Galton DAG, Dacie JV: Classification of the acute leukemias. Blood Cells 1975; 1:17–24
15. Grey HM, Rabellino E, Pirofsky B: Immunoglobulins on the surface of lymphocytes. IV. Distribution in hypogammaglobulinemia, cellular immune deficiency and chronic lymphocytic leukemia. J Clin Invest 1971; 50:2368
16. Ha K, Hozumi N, Hrincu A, Gelfand EW: Lineage specific classi-

fication of leukemia: Results of the analysis of sixty cases of childhood leukemia. Br J Haematol 1985; 61:237–249
17. Himmelhoch RS, Evans WH, Mage MG, Peterson EA: Purification of myeloperoxidase from the bone marrow of the guinea pig. Biochemistry 1969; 8:914–921
18. Jani P, Verbi W, Greaves MF, Bevon D, Bollum F: Terminal deoxynucleotidyl transferase in acute myeloid leukemia. Leuk Res 1983; 7:17–29
19. Jondel M, Holm Z, Wigzel H: Surface markers on human T and B lymphocytes. J Exp Med 1972; 136:207
20. Kabat EA: Basic principles of antigen–antibody reactions, Methods in enzymology, vol 70, Immunochemical techniques, part A. VanVunakis H, Langor JJ. New York, Academic Press, 1980, pp 126–127
21. Kita K, Nasu K, Kamesaki H, et al: Phenotypic analysis of acute lymphoblastic leukemia (ALL) cells which are classified as non-T and non-B and negative for common ALL antigen. Blood 1985; 66:47–52
22. Krause JR, Brody JP, Kaplan SS, Penchansky L: Terminal deoxynucleotidyl transferase activity in acute leukemia. A study of 110 cases comparing an immunoperoxidase PAP versus immunofluorescence method. Am J Hematol 1986; 22:179–184
23. Lanham GR, Bollum FJ, Williams DL, Stass SA: Simultaneous occurrence of terminal deoxynucleotidyl transferase and myeloperoxidase in individual blasts. Blood 1984; 84:318–320
24. Mahen M, Baker MA, Falk JA, Taub RN: Immunologic diagnosis and monitoring of human acute leukemias: A review. Am J Pathol 1981; 103:137–158
25. McGraw TP, Folds JD, Bollum FJ, Stass SA: Terminal deoxynucleotidyl transferase positive acute myeloblastic leukemia. Am J Hematol 1981; 10:251–258
26. Mirro J, Zipf TF, Pui CH, et al: Acute mixed lineage leukemia: Clinicopathologic correlations and prognostic significance. Blood 1985; 66:1115–1123
27. Neame PB, Soamboonsrup P, Browman G, et al: Simultaneous or sequential expression of lymphoid and myeloid phenotypes in acute leukemia. Blood 1985; 65:142–148
28. Paietta E, Cutcher JP, Wiernik PH: Terminal transferase positive acute promyelocytic leukemia: In vitro differentiation of a T-lymphocyte/pro-myelocytic hybrid phenotype. Blood 1985; 65: 107–114
29. Palumbo A, Minowada J, Eriksen J, Croce CM, Rovera G: Lineage infidelity of a human myelogenous leukemic cell line. Blood 1984; 64:1059–1063
30. Penchansky L, Kaplan SS, Lochner J, Krause JR: Immunogold stain method for the study of surface markers in bone marrow aspirates of leukemic children. Scand J Haematol (in press)
31. Perentesis J, Ramsey NKC, Brunning R, Kersey JH, Filipovich AH: Biphenotypic leukemia: Immunologic and morphologic evidence for a common lymphoid–myeloid progenitor in humans. J Pediatr 1983; 102:63–67
32. Rovigatti U, Mirro J, Kitchingman G, et al: Heavy chain immunoglobulin gene rearrangement in acute non lymphocytic leukemia. Blood 1984; 63:1023–1027
33. Schacterle GR, Pollack RL: A simplified method for the quantitative assay of small amounts of protein in biologic material. Anal Biochem 1973; 51:654–655
34. Smith LJ, Curtis JE, Messner HA, Senn JS, Furthmoyr H, McCulloch EA: Lineage infidelity in acute leukemia. Blood 1983;1138–1145
35. Stass S, Mirro J: Unexpected heterogeneity in acute leukemia. Human Pathol 1985; 16:864–866
36. Stass SA, Mirro J, Melvin S, Pui CH, Murphy SB, Williams D: Lineage switch in acute leukemia. Blood 1984; 64:701
37. Terrin BN, Studer EJ, Wood N, Mohanakumar T, Russel EC: Childhood leukemia with simultaneously expressed myeloid and lymphoid markers suggesting stem cell origin. Am J Hematol 1985; 20:175–181
38. Tindle RW, Nichols RAB, Chan L, Campana D, Catovsky D, Birnie GD: A novel monoclonal antibody BI-3C5 recognizes myeloblasts and non-B non-T lymphoblasts in acute leukemias and CGL blast crisis, and reacts with immature cells in normal bone marrow. Leuk Res 1985; 9:1–9
39. Whiteside MG: Influence of classification system in the treatment of leukemia. Pathology 1982; 14:295–297