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Immunophenotypic Analysis of Acute Leukemia

S. THOMAS TRAWEEK, MD

Since the introduction of monoclonal antibodies for the study of hematopoietic disease in the early 1980s, techniques to aid in the identification of cell lineage have proliferated. Immunofluorescent flow-cytometric studies are particularly helpful in the characterization of acute leukemia, and when used in conjunction with light-microscopic findings, can make a significant contribution to the final diagnosis in 20–30% of cases.¹ The introduction of immunophenotypic studies in the characterization of acute leukemia is not without cost, however, because the degree of heterogeneity in these diseases has dramatically increased. In particular, the existence of multilineage antigen expression in acute leukemia is now well-accepted, and when present, may considerably frustrate the classification process. Despite this marked heterogeneity, accurate diagnosis of acute leukemia is extremely important, because therapy and prognosis depend primarily on proper characterization. The most widely accepted and applied acute leukemia classification scheme, developed by the French-American-British (FAB) Cooperative Group in 1976, initially used only morphologic and cytochemical findings.² Since then, the diagnostic criteria has been revised and expanded to include immunophenotypic and ultrastructural studies for certain acute leukemias.^{3–5} The increased use of immunophenotypic analyses in acute leukemia also precipitated a need for classification of

mixed lineage leukemia, and several proposals have been made,⁶ but none have been universally accepted.

In an attempt to further define the range of hematopoietic cell antigen reactivity in acute leukemia, this study was undertaken to establish immunophenotypic profiles on samples referred to The City of Hope National Medical Center. These cases were specifically submitted for characterization and diagnosis of acute leukemia, and were all studied in a relatively uniform manner in one department. The findings on this referral-based population will be compared with a number of previous immunophenotypic studies of acute leukemia.

METHODS AND MATERIALS

Study group. The findings on peripheral blood or bone marrow samples submitted to the James Irvine Center for the Study of Leukemia and Lymphoma at The City of Hope National Medical Center between December 1, 1987 and August 1, 1992 were reviewed. The accrual start date was determined by the initiation of flow-cytometric studies in the analysis of acute leukemia at this institution. The patient population was primarily adult.

Inclusion criteria for the study were:

1. That an unequivocal diagnosis of acute leukemia was made, based on examination of a peripheral blood or bone marrow specimen. In most cases, the acute leukemia was assigned an FAB category, based on the Wright-Giemsa-stained morphologic appearance of the blasts and on the results of cytochemical stains for sudan black-B, myeloperoxidase, nonspecific esterase (either α -naphthyl acetate esterase performed with and without fluoride inhibition or butyrate esterase), acid phosphatase, and periodic acid-Schiff, which were performed according to standard methods. An accurate FAB designation could not be reliably deduced in a few cases, however, due to either poor technical quality of the submitted slides, lack of a sufficient sample for a full cytochemical panel, or avail-

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Immunophenotypic Analysis of Acute Leukemia

TABLE 1. MONOCLONAL ANTIBODIES

Cluster Designation	Lineage Association	Name/Source
CD2	T-lymphoid	Leu 5/BD
CD3	T-lymphoid	Leu 4/BD
CD4	T helper/inducer	Leu-3/BD
CD5	T-lymphoid	Leu-1/BD
CD8	T suppressor	Leu-2/BD
CD7	T-lymphoid	Leu-9/BD
CD10	B-lymphoid	J5/Coulter
CD13	Myeloid/monocytic	My7/Coulter
CD14	Monocytic	My4/Coulter
CD15	Myeloid/monocytic	Leu-M1/BD
CD19	B-lymphoid	B4/Coulter
CD20	B-lymphoid	B1/Coulter
CD33	Myeloid/monocytic	My9/Coulter
CD34	Stem cell	HPCA-1/BD
CD45	Leukocyte common antigen	Leukocyte/BD
—	HLA-DR	I2/Coulter

BD = Becton Dickinson, San Jose, CA; Coulter, Hialeah, FL

ability only of cytocentrifuge preparations for morphologic evaluation.

2. That immunophenotypic studies were performed using flow cytometry on either peripheral blood or bone marrow containing at least 30% blast cells. If the phenotypic results of bone marrow were not characteristic of the disease as classified by FAB, at least 30% blasts were also required in the peripheral blood to eliminate the possibility of spurious phenotype results secondary to hemodilution.
3. That enough viable blast cells were recovered to perform an immunophenotypic panel that included at least two lineage-associated antigens each from the B-lymphoid, T-lymphoid, and myeloid cell lines.

Cell preparation and immunophenotypic analysis. After mononuclear cell enrichment by centrifugation over Ficoll-Paque (Pharmacia, Piscataway, NJ), the peripheral blood and bone marrow samples were studied for surface antigen expression with direct and indirect immunofluorescent techniques using a variety of commercially available monoclonal antibodies. (Table 1) In most cases, greater than 50% blast cells were present after mononuclear-cell enrichment, as determined by a Wright-Giemsa-stained cytocentrifuge preparation. The antibody panel used to evaluate specimens submitted for acute leukemia characterization varied only slightly over the 4.5-year study period. Specifically, CD45 and CD34 antibodies were added, whereas CD3 and CD5 were dropped during this time. In addition, a limited number of cells in some cases resulted in incomplete antibody panels. Analysis of the stained cells was performed initially with an Epics 5 flow cytometer (Coulter, Hialeah, FL), and later with a FACScan (Becton Dickinson, Mountain View, CA). Only cells within a mononuclear

cell gate, individually created for each acute leukemia, were considered in the final tally. Matched concentrations of immunoglobulin isotype controls were run concurrently with all cases. Terminal deoxynucleotidyl transferase (Tdt) determinations were not consistently done, and are reported here only for selected cases.

Criteria for antigen expression. Positivity for an individual antigen was defined as more than 20% fluorescent cells above the isotype control for all antibodies except CD34. CD34 was considered to be positive if there were 10% of fluorescent cells above the isotype control. Multilineage antigen expression was considered to be present if: (1) the sum of the most reliable lineage marker for the type of acute leukemia and the inappropriately expressed antigen was greater than 120%, or (2) coexpression of multilineage antigens was demonstrated in more than 20% of the gated cell population by dual-color immunofluorescence.

Classification of acute leukemia. The final classification of each acute leukemia was based on an integrated assessment of the morphologic, cytochemical, and immunophenotypic data. The phenotypic findings were chiefly confirmatory in the classification of AML-M1 through AML-M6, as the diagnosis was based primarily on morphologic and cytochemical findings. Immunophenotyping was instrumental however, in the final diagnosis and classification of acute lymphoblastic leukemia, minimally differentiated acute myelogenous leukemia (AML-MO), acute undifferentiated leukemia, and mixed lineage leukemia, because morphologic and cytochemical studies provide little information about the lineage of these diseases. After classification, immunophenotypic profiles were generated for each subtype of acute leukemia.

RESULTS

Acute Myelogenous Leukemia

Two hundred seven cases of AML were studied; the results are given in Table 2. Almost all cases (99%) expressed CD45. The HLA-DR antigen was present in the majority of AML, excluding AML-M3. CD33 was the myeloid lineage antigen most commonly present, followed in frequency by CD13 and CD15. The monocyte-associated antigen CD14 was detected in about one half of the AMLs with a monocytic component (as detected by nonspecific esterase activity), but was also occasionally present in subgroups of AML with no monocytic features. CD7 expression was primarily seen in AML-MO; AML-M1; and AML-M5a, the least mature AML; but also was detected sporadically in other types of

TABLE 2. ACUTE MYELOGENOUS LEUKEMIA PHENOTYPES.

Leukemia Type (No. of Patients)†	Phenotype													
	CD45	CD2	CD3	CD5	CD7	CD19	CD20	CD13	CD14	CD15	CD33	CD10	HLA-Dr	CD34
AML-M1 (n = 40)	18/19 (95%)	0/40	0/18	0/15	9/37 (24%)	1/40 (3%)	0/39	29/38 (76%)	0/27	14/26 (54%)	33/40 (83%)	1/38 (3%)	23/27 (85%)	22/36 (61%)
AML-M2 (n = 69)	22/22 (100%)	0/68	0/49	0/43	7/62 (11%)	4/67 (6%)	1/68 (1%)	44/66 (67%)	4/67 (6%)	18/36 (50%)	54/69 (78%)	1/61 (2%)	51/67 (81%)	34/53 (64%)
AML-M3 (n = 19)	11/11 (100%)	6/19 (31%)	0/8	0/10	0/19	0/19	0/19	16/18 (89%)	1/19 (5%)	5/12 (42%)	16/19 (84%)	0/19	1/18 (6%)	10/16 (63%)
AML-M4 (n = 39)	14/14 (100%)	0/39	0/25	0/25	3/38 (8%)	0/39	0/38	16/35 (46%)	17/38 (45%)	15/20 (75%)	36/39 (92%)	2/39 (5%)	33/38 (87%)	19/26 (73%)
AML-M5 (n = 18)	5/5	0/18	0/13	0/13	8/18 (44%)	0/17	0/18	12/17 (71%)	8/18 (44%)	5/8 (63%)	15/18 (83%)	1/18 (6%)	15/18 (83%)	8/13 (62%)
AML-M6 (n = 3)	1/1	0/3	0/2	0/2	1/3	0/3	0/3	2/3	0/3	1/1	0/3	0/3	2/3	3/3
AML-M7 (n = 2)	1/1	0/2	0/1	0/1	1/2	0/2	0/2	1/2	0/2	0/1	1/2	0/2	0/1	1/2
AML-MO (n = 9)	3/3	0/8	0/7	0/5	3/7	0/9	0/9	7/9	1/9	2/6	5/9	0/9	9/9	9/9
AML-NOS (n = 8)	3/3	0/8	0/3	0/2	0/4	0/7	0/8	6/7	0/8	3/6	7/8	0/6	7/7	5/7
AML Total	78/79 (99%)	6/205 (3%)‡	0/126	0/116	32/190 (17%)	5/203 (2%)	1/204 (<1%)	133/195 (68%)	35/191 (18%)	63/116 (54%)	167/207 (80%)	5/198 (3%)	143/169 (85%)§	111/165 (67%)

NOS = not otherwise specified; AML: acute myelogenous leukemia.

* Results are expressed for each antigen as number of positive cases/number of cases studied.

† Total AML studied = 207. Three cases, all M2, failed to express myeloid lineage antigens.

this equals 3/69 (4%) of M2 or 3/207 (1.5%) of all AML studied

‡ CD2 present only in M3.

§ Excludes M3 from total.

AML. Other than CD7, single lymphoid-associated antigen positivity was present in 3% or less of the AMLs, and was restricted to CD2, CD10, CD19, and CD20. The proportion of CD34-positive cases was 67% for all AML, and there was no significant difference in expression of this antigen between the AML subtypes.

AML-M1. All 40 cases of AML-M1 had detectable myeloperoxidase activity (as required by FAB criteria), and all expressed at least one myeloid lineage antigen. This subtype comprised 19% of AMLs and 10% of all acute leukemias. The single AML in this study that was not positive for CD45 was from the AML-M1 subgroup. One case was positive for the B-cell antigen CD19, and also contained a t(8;21) chromosomal translocation. One case expressed CD10, with no other lymphoid antigens.

AML-M2. Sixty-nine cases, representing 33% of AMLs and 18% of acute leukemias were studied. This subgroup had the highest rate of aberrant B-cell antigen expression among the AMLs; either CD19 or CD20 was expressed in 5 cases (7% of AML-M2). In no case was there more than one lymphoid antigen expressed. Cytogenetic studies were performed on three of the four CD19-positive AML-M2; all contained a t(8;21) chromosomal translocation. Significantly, three cases of clear-cut AML-M2 by morphologic and cytochemical evaluation were devoid of the myeloid antigens CD13, CD14, or CD33. CD15, however, was not examined on these

cases. One of these phenotypically null AML-M2 even contained a t(8;21) translocation, as demonstrated by cytogenetic examination.

AML-M3. Nineteen cases of acute promyelocytic leukemia were studied, representing 9% of AMLs and about 5% of all acute leukemias. The T-cell antigen CD2 was expressed in nearly one third of the cases, and AML-M3 was the only subtype of AML to be positive for this antigen. The results of cytogenetic studies were available in nine cases; all displayed the pathognomonic t(15;17), including all six CD2-positive cases of AML-M3. No cases were CD7- or CD10-positive, but one sample expressed CD14 with no evidence of nonspecific esterase activity. In marked contrast to the other types of AML, only one case of AML-M3 was positive for HLA-DR. The degree of positivity for this antigen was not great, however, only 26%, and contributions from nonblasts cannot be entirely excluded. The stem cell antigen CD34 was detected in 10 of 16 cases (63%), a level of positivity similar to the other AML subtypes.

AML-M4 and AML-M5. The 39 cases of acute myelomonocytic leukemia (19% of AMLs and 10% of acute leukemias) and 18 cases of acute monocytic leukemia (9% of AMLs and 5% of acute leukemias) were similar immunophenotypically. Almost one half of the cases from each subtype expressed CD14, a much higher percentage than was detected in any other group of AMLs. The proportion of cases that were positive for CD7 was

Immunophenotypic Analysis of Acute Leukemia

TABLE 3. ACUTE LYMPHOBLASTIC LEUKEMIA PHENOTYPES

Pre-B cell	Phenotype													
	CD45	CD2	CD3	CD5	CD7	CD19	CD20	CD13	CD14	CD15	CD33	CD10	HLA-Dr	CD34
ALL-L1* (n = 27)	6/9	0/26	0/16	0/13	0/23	27/27 (100%)	5/27 (19%)	4/24 (17%)	0/23	3/13 (23%)	5/25 (20%)	27/27 (100%)	26/26 (100%)	21/24 (88%)
ALL-L2* (n = 102)	27/32 (84%)	1/101 (1%)	0/69	1/58 (2%)	0/92	102/102 (100%)	37/101 (37%)	11/86 (13%)	0/95	6/48 (13%)	11/99 (11%)	95/102 (93%)	101/101 (100%)	60/75 (80%)
ALL-NOS* (n = 11)	1/1	0/11	0/10	0/7	0/9	11/11	4/11	0/11	0/11	2/5	1/11	9/11	11/11	8/9
Total	34/42 (81%)	1/138 (<1%)	0/95	1/78 (1%)	0/124	140/140 (100%)	46/139 (33%)	15/121 (12%)	0/129	11/66 (17%)	17/135 (13%)	131/140 (94%)	138/138 (100%)	89/108 (82%)
ALL-L3† (n = 6)	1/1	0/6	0/6	1/6 (17%)	0/6	6/6 (100%)	6/6 (100%)	0/5	0/5	0/3	0/5	4/6 (67%)	6/6 (100%)	0/4
ALL-T cell‡ (n = 11)	3/3	7/11 (64%)	3/10 (30%)	8/10 (80%)	9/11 (82%)	0/11	0/11	0/9	0/9	1/4	1/10	4/11	0/9	2/7

ALL = acute lymphoblastic leukemia; NOS = not otherwise specified.

* Total precursor-B cell ALL studied = 140. Results are expressed for each antigen as number of positive cases/number of cases studied. One myeloid antigen was detected in 19 samples (14%), and two myeloid antigens were detected in 12 samples (9%).

† All six cases of ALL-L3 expressed monoclonal surface immunoglobulins and were Tdt-negative.

‡ A myeloid antigen was present in 2/11 T-cell ALL. CD4 and CD8 were tested in 6 cases; 5 were negative for both antigens and 1 case was positive for both. HLA-DR was not detected in any of the 9 T-cell ALL studied.

especially high in AML-M5, but positivity was also evident to a lesser degree in AML-M4. Both types of AML had the highest percentage of CD 10-positive cases, but no other B-cell markers were detected.

AML-M6 and AML-M7. Only a few cases of erythroleukemia and megakaryocytic leukemia were studied—too few for meaningful immunophenotypic analysis. The cases studied, however, showed no obvious deviation phenotypically from other types of AML.

AML-M0. Nine cases of minimally differentiated AML were studied, representing 4% of AMLs and 2% of all acute leukemias. By definition, these acute leukemias were myeloperoxidase- and sudan black-B-negative, but expressed at least one myeloid associated antigen. CD7 was positive in nearly one half of the cases examined for this antigen. CD 14 was detected in one case, but nonspecific esterase activity was lacking. All nine cases were positive for HLA-DR and CD34.

Acute lymphoblastic leukemia. One hundred fifty-seven cases of ALL were studied; the results are given in Table 3. Because immunophenotype defines important prognostic and therapeutic subgroups in this type of acute leukemia, the ALLs were divided into three groups on the basis of lineage, as determined by surface antigen expression.

Precursor-B-cell ALL. One hundred forty precursor-B-cell ALL were studied. Included were 27 ALL-L1 (17% of ALLs and 7% of all acute leukemias), 102 ALL-L2 (65% of ALLs and 27% of all acute leukemias), and 11 cases (7% of ALLs and 3% of all acute leukemias) in which a morphologic subtype could not be assigned. By definition, all 140 cases were positive for CD 19. In addition, all expressed HLA-DR. CD20-positivity was de-

tected in one third of the cases, and was more prevalent in the ALL-L2 than in the ALL-L1 subtype. Only 6% of the cases failed to express CD10, and the majority, 82%, were positive for CD34. Precursor-B-cell ALL was the acute leukemia type most prone to mixed-lineage antigen expression. A single myeloid antigen was present in 14% of these ALLs, whereas two myeloid antigens were evident in 9%. Myeloid antigen positivity was more frequent in ALL-L1 than in ALL-L2. Precursor-B-cell ALL was the most likely acute leukemia in this study to be CD45-negative, with 19% of cases failing to express the common leukocyte antigen. A single T-cell antigen was detected in two cases. One of the ALL-L2s had monoclonal surface immunoglobulin light and heavy chains, and was also Tdt- and CD34-positive.

B-cell ALL. All six cases of B-cell ALL expressed a mature B-cell phenotype (CD19- and CD20-positive, CD34- and Tdt-negative) with monoclonal surface immunoglobulin light and heavy chains. Four of the six cases were positive for CD10, and one case expressed the pan-T-cell antigen CD5. Four of the six cases displayed typical FAB-L3 morphology. Despite a mature B-cell phenotype, however, two displayed non-L3 blast morphology.

T-cell ALL. Eleven cases of ALL with a T-cell phenotype were studied (7% of ALLs). CD5 and CD7 were the T-cell antigens most commonly expressed. An atypical T-cell phenotype, with aberrant expression of at least one pan-T-cell antigen, was detected in all but one case. CD3 and CD8 expression were analyzed in six cases; five were negative for both antigens and one was positive for both. CD10 was detected in four cases, but none of the nine T-cell ALLs studied for HLA-DR expression were

positive. A single myeloid antigen was present in two cases.

Acute undifferentiated leukemia. Four cases (1%) of the 382 acute leukemias studied offered no evidence of lineage commitment, either cytochemically or immunophenotypically. No lymphoid or myeloid-associated antigens were detected. Myeloperoxidase was not detected in any of the cases. Terminal deoxynucleotidyl transferase was positive in two of the three cases tested. HLA-DR was expressed by three cases of the acute undifferentiated leukemia, but CD34 was present in only one case.

Unclassifiable acute leukemia. Fourteen cases (4%) of acute leukemia were difficult to classify, primarily due to the presence of a confusing array of multilineage-associated antigens and no distinguishing morphologic or cytochemical features. Included in these were 12 cases of myeloperoxidase-negative acute leukemia with myeloid antigens (AML-MO) and coexpression of one or more B- or T-lymphoid antigens.

DISCUSSION

Immunophenotypic studies have an established place in the diagnosis and classification of acute leukemia.¹ The availability of murine monoclonal antibodies that are reactive with lymphoid- and myeloid-associated surface epitopes, coupled with relatively user-friendly and inexpensive flow cytometers, has made the use of immunophenotypic analyses in acute leukemia easily accessible to many laboratories. Numerous reports on the phenotypic characteristics of acute leukemia have been generated. Most of the studies have expanded our understanding of these diseases, and have facilitated the recognition of certain types of acute leukemia, such as minimally differentiated AML and B- and T-cell ALL. Applying extensive panels of antibodies to large numbers of acute leukemias, however, also has dramatically increased the heterogeneity of acute leukemia. At times this has created confusion and uncertainty about the significance of the relationship between phenotype and biologic behavior, and has complicated classification in a small proportion of cases.⁸ This study generates immunophenotypic profiles that may help to better define the range of antibody reactivity for many types of acute leukemia. The data were collected from samples referred for characterization and diagnosis of acute leukemia. The relatively uniform diagnostic approach and consistent antibody panel applied to the acute leukemias created a unique opportunity for an analysis of this sort.

Acute Myelogenous Leukemia

In this study, as in previous studies,⁹⁻¹² AML was a moderately heterogeneous group of diseases. The immunophenotypic signature of these acute leukemias is expression of one or more myeloid antigens, which was

seen in the nearly all cases. A lack of myeloid antigen positivity does not absolutely exclude AML, however, because three cases failed to mark as myeloid despite the presence of myeloperoxidase activity and morphologic features that were consistent with AML-M2. Although unusual, myeloid antigen negativity in *bona fide* AML has been reported previously.^{9,12,13} In general, little mixed-lineage antigen expression by a single lymphoid-associated antigen was seen in this series of AML. When present, these small expressions of lineage promiscuity should not be construed as sufficient evidence to warrant a diagnosis of mixed-lineage leukemia.¹⁴

Initially thought to be a T-cell antigen, CD7 is now known to be present in AML.¹⁵ CD7 expression appears to be associated with blast cell immaturity,¹⁵ because the percentage of positive cases was greatest in AML-MO, AML-M1, and AML-M5a. CD2 was the only other T-cell antigen detected. B-cell antigen expression was almost exclusively CD19, and was predominantly seen in AML-M2. This association has recently been reported by Kita and colleagues, and also has been linked with a specific cytogenetic abnormality, the t(8;21) translocation.¹⁶ This translocation was demonstrated in all four of the CD19-positive cases of AML that we studied karyotypically. CD19 has been associated previously with AML-M5,¹⁷ but none were detected in this series.

Almost one half of the cases of AML-M4 and AML-M5 expressed CD14. This antigen is not restricted to acute leukemia with monocytic differentiation, however, but was also present in 6% of AML-M2 and one case of AML-M3. Up to 25% of M1 and M2 AMLs have been previously reported to express this antigen,^{18,19} but CD14 positivity in AML-M3 is apparently rare.

Acute promyelocytic leukemia (AML-M3) is an unusual and distinctive disease clinically, morphologically, immunophenotypically, and even genotypically.²⁰ As previously reported, the majority of cases of AML express HLA-DR.^{8,9,11} The clear exception to this rule is AML-M3, in which only rare cases are reported to be positive.^{9,19} In this study, all CD2-positive AML were AML-M3 types, which was confirmed by cytogenetic findings. Positivity for this T-cell marker has been well-documented in AML-M3, and the presence of this antigen has even been associated with specific breakpoints within the t(15;17) translocation.²⁰ Other studies clearly indicate that expression of this antigen may occur in other subtypes of AML.²¹ For this group of acute leukemia, however, a CD2-positive/HLA-DR-negative myeloid phenotype was specific for AML-M3.

Acute Lymphocytic Leukemia

The separation of ALL into B- and T-cell types is clinically important, with both therapeutic and prognostic implications.^{22,23} The cases of ALL in this study were

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therefore divided into three subtypes, which were based primarily on immunophenotypic findings. The Precursor-B-cell ALL all expressed CD19, by definition. In addition, all were HLA-DR-positive. Otherwise, these acute leukemias were the most heterogeneous leukemias in this study. The majority of cases expressed CD10, which is a marker of longstanding and well-known prognostic value.²⁴ On the other hand, CD10-negativity has been associated with a poor prognosis.²⁵ As has been previously reported, CD20 was more common on ALL-L2 than on ALL-L1.²⁶ In marked contrast to AML, a significant minority of precursor-B-cell ALLs failed to express the common leukocyte antigen CD45, a finding of possible prognostic significance.²⁷ Myeloid antigens were commonly present, however, and were seen either singly or in pairs in 23% of cases. As in AML, the presence of these aberrantly expressed antigens should not preclude an otherwise straightforward diagnosis of precursor-B-cell ALL. The expression of T-cell antigens is infrequently detected in this type of leukemia.^{23,26}

B-cell ALL is a rare disease, and is the least common subtype of ALL. Classic FAB-L3 morphologic features are associated only with a B-cell phenotype. Morphologic features that are characteristic of non-L3 blasts however, also can be seen in this subtype of acute leukemia.²⁸ Two cases in this study expressed typical B-cell ALL phenotypes, but did not display an ALL-L3 appearance. Cytogenetic study was performed on one of these cases, and revealed a t(8;22) chromosomal abnormality. Immunophenotypic studies are few,²⁸⁻³⁰ but most cases previously reported and all of the cases of B-cell ALL in this study expressed monoclonal surface immunoglobulins and were Tdt- and CD34-negative. One case expressed CD5, an unusual finding, but none were positive for other T-cell or myeloid markers. CD10 was detected in the majority of cases, which is typical.^{28,29}

The phenotypic heterogeneity of T-cell neoplasms is well-documented, and covers the range of phenotypes expressed during thymic differentiation.³¹⁻³³ Most cases will express more than one T-lineage marker. Aberrant deletion of one or more pan-T-cell antigens is common in this disease, however, and may be a helpful diagnostic finding.³⁴ All but one of the 11 cases of T-cell ALL showed deletion of one or more of the 4 pan-T-cell antigens used. CD7 was the pan-T-cell antigen most often expressed by the T-cell ALL in this series and in others.³⁵ CD7 is also the antigen that is most commonly deleted in these acute leukemias.³⁶ No CD7-positivity was detected in any pre-B- or B-cell ALL, which is typical.³⁵ The HLA-DR antigen often is not expressed in T-cell ALL,³² which is another phenotypic feature that distinguishes this acute leukemia from pre-B-cell ALL and B-cell ALL. None of the cases of T-cell ALL in this study were

HLA-DR-positive. CD10 has been reported in 20-30% of T-cell ALLs,³¹ a frequency approximately similar to that seen in this series. Myeloid antigen expression also has been reported,³⁷ as was seen in one case in this study. Expression of myeloid antigens may be a potential source of confusion considering the occasional presence of CD2 and CD7 in AML.

Undifferentiated and Mixed-Lineage Leukemias

A null phenotype or an indeterminate phenotype, as seen in acute undifferentiated leukemia and mixed-lineage leukemia, respectively, comprised approximately 5% of the cases studied. All four cases of acute undifferentiated leukemia expressed no myeloid or lymphoid surface antigens, and were characterized only by variable expression of HLA-DR and CD34. The clinical consequence of this null phenotype is unclear.^{38,39} The mixed-lineage leukemia comprised a diverse group with phenotypes specifically excluded from classification by FAB criteria, such as AML-MO with single B- or T-lymphoid antigen expression, or with confusing multilineage phenotypes and no convincing morphologic or cytochemical evidence of lineage preference. The clinical significance of multilineage phenotypes in acute leukemia is also unknown, and because the definition of this condition varies, direct comparison with existing studies is difficult.

CD34 is an antigen normally expressed by immature hematopoietic cells. Normal bone marrow contains less than 3% CD34-positive cells, making this an excellent marker for monitoring blast cell populations. However, not all acute leukemias express this antigen. Approximately 45-65% of AMLs⁴⁰⁻⁴² and about 75% of pre-B-cell ALLs^{40,43} have been reported to be CD34-positive. Using a level of positivity that is similar to previous reports,^{42,43} a slightly greater proportion of CD34-positive acute leukemias were detected in this study, both for AML and ALL. Previous studies suggest CD34-positive AMLs are more likely to be AML-M1 or AML-M2 types,^{41,42} but this was not the case in our study. The level of CD34 expression was approximately equal in all subtypes of AML except AML-MO, which was consistently positive. Even AML-M3, with relatively differentiated morphologic and immunophenotypic features, expressed CD34 at levels similar to other AMLs. This intuitively unusual finding is similar to that reported by Merle-Beral and associates,⁴⁴ and may be an atavistic feature of leukemic cells, because CD34 expression in normal promyelocytes has not been documented. Similar to a large group of ALLs studied by Borowitz and co-workers,⁴³ CD34 expression in ALL was associated with features of immature blasts, such as ALL-L1 mor-

phology, absence of CD20 positivity, and myeloid antigen coexpression. Only cases of ALL-L3, composed of nearly terminally differentiated B cells, consistently failed to mark for this antigen. Large studies are few,⁴¹⁻⁴³ but the clinical significance of CD33 expression in acute leukemia appears to be a function of cell lineage, with positivity portending a poor prognosis in adult AML, and a good prognosis in pediatric ALL.

The utility of immunophenotypic analysis in acute leukemia is not limited to the characterization of cell lineage, but also may yield important prognostic information. For example, CD10-positivity in pre-B-cell ALL is clearly associated with a favorable outcome,²⁴ whereas lack of this antigen portends a poor prognosis.²⁵ CD7 expression in AML has been linked with an immature phenotype in AML, and consequently, a worse outcome.¹⁵ In many instances, however, conflicting reports make evaluation of the significance of antigen expression difficult. For example, the significance of HLA-DR in AML is unclear, because discrepant results have been reported.^{45,46} The significance of mixed-lineage antigen expression is particularly tortuous. Myeloid antigen expression in cases of ALL has been reported in numerous previous studies to have an adverse clinical influence,^{47,48} but evidence also exists that intensive multi-agent chemotherapy negates this effect.⁴⁹ Lymphocyte surface markers in AML has been reported to infer a poor response to therapy,⁵⁰ have no effect,⁵¹ or have favorable prognostic significance.⁵² The reason for these conflicting findings is unclear, but may be related to differences in patient populations (adult *versus* pediatric, referral *versus* community-based), in therapy, in antibodies used, and in definitions of what constitutes atypical lineage antigen expression. In general, mixed-lineage antigen expression is often, but not always, associated with other immunophenotypic and morphologic features of immaturity in both AML and ALL. Like CD34, this appears to confer a worse prognosis in AML, and either have no effect or predict a better response to therapy in ALL, especially in pediatric patients. Further studies using readily available reagents and uniform patient inclusion/response criteria will be needed before valid conclusions can be drawn. In the meantime, caution is urged in the application of immunophenotypic findings with purported clinical significance.

A few studies on the phenotypic changes associated with recurrent acute leukemia have been published. For AML, relapse of disease is associated with a tendency toward a more immature phenotype manifest as increased expression of CD33 and CD34 with decreased expression of CD15, and is associated with poor outcome.⁵³ Only small changes in phenotype have been re-

ported in the few studies performed on relapsed ALL.^{26,54} In both AML and ALL, the conversion from a CD34-negative phenotype at initial presentation to CD34-positive at relapse has been reported,⁵³ but the converse change, from CD34-positivity to CD34-negativity, is apparently rare. In addition to single antigen changes, dramatic shifts in lineage also may occur with the recurrence of acute leukemia.^{55,56} Examples of a wholesale lineage switch are uncommon, and the clinical significance of this occurrence is unclear.

The motivation to institute immunophenotypic studies on acute leukemia was fueled initially by the desire to improve the characterization of this heterogeneous group of neoplasms. Clearly this objective has been met, as immunophenotypic analyses have been shown repeatedly to improve the recognition of lineage in acute leukemia. French-American-British criteria now require phenotypic studies for some subsets of AML.^{4,5} These studies are also an essential component in the diagnosis of ALL.¹ Furthermore, the existence of mixed-lineage leukemia is essentially dependent on phenotypic studies. In addition to identifying cell lineage in acute leukemia, certain antigenic markers have been associated with patient outcome, and will undoubtedly play a bigger role in prognostication as more cases are studied and clinically significant parameters become clearer. Finally, immunophenotyping can play a role in the evaluation of treatment efficacy, by monitoring blast-associated markers such as CD34, and may also prove to be an important tool in assessing minimal residual disease.⁵⁷

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