The Immunophenotype of Adult Acute Myeloid Leukemia

High Frequency of Lymphoid Antigen Expression and Comparison of Immunophenotype, French-American-British Classification, and Karyotypic Abnormalities

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Immunophenotyping has become common in the diagnosis and classification of acute leukemias and is particularly important in the proper identification of cases of minimally differentiated acute myeloid leukemia (AML-M0). To evaluate the immunophenotype of adult AML, 106 cases were studied by cytochemical analysis and by flow cytometry with a panel of 22 antibodies. The results were compared with the French-American-British (FAB) Cooperative Group classification, as well as with available cytogenetic data on each case. CD45, CD33, and CD13 were the most commonly expressed antigens (97.2%, 95.3%, and 94.3%, respectively). Lymphoid-associated antigens were expressed in 48.1% of cases. CD20 was the most commonly expressed lymphoid antigen (17%), although often expressed in only a subpopulation of leukemic cells, followed by CD7 (16%), CD19 (9.8%), CD2 (7.5%), CD3 (6.7%), CD5 (4.8%), and CD10 (2.9%). Some immunophenotypes correlated with FAB type, including increased frequency of CD2 expression in AML-M3; lack of CD4, CD11c, CD36, CD117, and HLA-DR expression in AML-M3; increased frequency of CD20 and CD36 expression and lack of CD34 expression in AML-M5; increased frequency of CD5 expression in AML-M5a; and increased frequency of CD14 expression in AML-M5b, when compared with all other AMLs ($P < .05$). When compared with AML-M5b, AML-M5a demonstrated a lack of CD4 expression and a high frequency of CD117 expression. Complete morphologic and cytogenetic agreement between AML-M3 and t(15;17) was present, and four of five cases of AML-M4Eo demonstrated inv(16). The remaining case of M4Eo was characterized by a 6;9 translocation, and two other inv(16) cases were not classified as M4Eo. Expression of CD2 was present in two t(15;17) cases and in one inv(16) case, but expression of this antigen was not restricted to AML cases with these karyotypic abnormalities. Similarly, expression of CD19 was not specific for t(8;21) AML. All t(8;21) leukemias demonstrated M2 morphology. With the exception of M3, M4Eo, and a subgroup of M2 leukemias, the FAB classification does not appear to define cytogenetically distinct disease groups in adult AML. Immunophenotypically distinct profiles were identified in the M3 and M5 morphologic groups of the FAB classification. Immunophenotyping studies are helpful in the determination of myeloid lineage. In general, however, they are not sufficiently specific alone to be useful in precisely identifying either FAB or cytogenetically defined disease subtypes. (Key words: Acute myeloid leukemia; Immunophenotyping; Flow cytometry, Cytogenetics; French-American-British Cooperative Group classification) Am J Clin Pathol 1998;109:211–220.

Acute leukemias are traditionally classified by a combination of morphologic and cytochemical features on the basis of criteria of the French-American-British (FAB) Cooperative Group.[1,2] The addition of immunophenotypic studies is valuable in both the confirmation and precise lineage classification of acute lymphoblastic leukemia, as well as in the detection of minimally differentiated acute myeloid leukemia (AML-M0). The value of these studies is reflected by the addition of myeloperoxidase cytochemistry–negative AML to the FAB classification as AML-M0.[3] Other leukemia classification systems also have attempted to integrate immunologic studies,[4–6] and, recently, an immunologic classification of acute leukemias was proposed.[7] The increased use of immunophenotypic studies in acute leukemias, however, has led to the identification of leukemias with aberrant immunophenotypes.[8–12] Although some of these cases are considered to be biphenotypic or mixed-lineage leukemias, most leukemias with aberrant immunophenotypes do not fulfill proposed criteria for mixed-lineage leukemia.[7] Furthermore, the clinical significance of aberrant lineage expression in adult leukemias is controversial, and the clinical significance of a diagnosis of acute

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TABLE 1. FREQUENCY OF NONLYMPHOID ANTIGEN

AML (no.)[*]	*CD45 (%)*[†]	*CD4 (%)*	*CD11c (%)*	*CD13 (%)*	*CD14 (%)*	*CD15 (%)*	*CD33 (%)*
M0 (7)	7/7 (100)	4/7 (57)	6/6 (100)	6/7 (86)	0/7	4/7 (57)	6/7 (86)
M1 (15)	13/15 (87)	8/15 (53)	14/15 (93)	14/15 (93)	0/15	10/15 (67)	13/15 (87)
M2 (21)	21/21 (100)	10/21 (48)	20/21 (95)	19/20 (95)	3/21 (14)	17/20 (85)	20/21 (95)
M3 (7)	7/7 (100)	0/7	1/7 (14)	7/7 (100)	0/7	5/7 (71)	7/7 (100)
M4 (11)	11/11 (100)	7/11 (64)	11/11 (100)	11/11 (100)	2/11 (18)	7/11 (64)	11/11 (100)
M4Eo (5)	5/5 (100)	1/5 (20)	3/5 (60)	5/5 (100)	1/5 (20)	5/5 (100)	5/5 (100)
M5a (3)	2/3 (67)	0/3	3/3 (100)	2/3 (67)	0/3	3/3 (100)	3/3 (100)
M5b (6)	6/6 (100)	5/6 (83)	6/6 (100)	6/6 (100)	4/6 (67)	6/6 (100)	6/6 (100)
M6 (2)	2/2 (100)	0/2	2/2 (100)	2/2 (100)	0/2	2/2 (100)	2/2 (100)
M7 (1)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	0/1	0/1	1/1 (100)
MDS/AML (17)	17/17 (100)	9/17 (53)	16/17 (94)	16/17 (94)	5/17 (29)	12/17 (71)	17/17 (100)
AML, NOS (11)	11/11 (100)	6/11 (55)	9/11 (82)	10/11 (91)	2/11 (18)	7/11 (64)	10/11 (91)
Total (106)	103/106 (97.2)	51/106 (48.1)	92/105 (87.6)	99/105 (94.3)	17/106 (16.0)	78/105 (74.3)	101/106 (95.3)

AML, NOS = not otherwise specified AML French-American-British subtype; Glyco A = glycophorin A; MDS/ AML = AML associated with myelodysplastic syndrome; MPO = myeloperoxidase.
[*]Number of cases tested.
[†]Percent of cases expressing the antigen.

mixed-lineage leukemia, using proposed criteria, is still unproved.

Although widely accepted, the FAB classification of AML has been the subject of recent criticism.[13] The FAB criteria identify one clinically significant type of AML, acute promyelocytic leukemia; however, the remaining AML subtypes appear to be a cytogenetically and immunologically heterogeneous group of diseases. Cytogenetic evaluation of AML, however, identifies numerous prognostically significant types of AML. Previous reports suggest that patterns of immunophenotypic antigen expression are useful in predicting some of these clinically significant cytogenetic abnormalities.[11,14–19]

In the current study, we used a large panel of immunophenotypic markers to evaluate antigen expression in 106 cases of adult AML. The primary goals of the study were to (1) determine which myeloid antigens were most often expressed in adult AML, (2) determine the frequency of lymphoid antigen expression in adult AML, (3) evaluate immunophenotypic correlations with FAB subtypes of AML, and (4) evaluate the ability to predict specific cytogenetic abnormalities reliably on the basis of morphologic and immunophenotypic results.

MATERIALS AND METHODS

We reviewed consecutive cases of acute leukemia seen in the Division of Pathology at the City of Hope National Medical Center between July 1995 and November 1996. Of 176 patients with acute leukemia who underwent sufficient immunophenotyping studies, 106 adults and 4 children were classified as having AML using morphologic, cytochemical (myeloperoxidase and/or Sudan black B, and α-naphthyl butyrate esterase), and immunophenotypic studies. Pediatric (< 18 years of age) cases of AML were excluded from the study, as were cases of chronic myelogenous leukemia in blast crisis. Cases of AML included those that were newly diagnosed and those that were relapses. The FAB criteria were used for diagnosis and subclassification of all cases,[1–3,20] and the recently proposed immunologic classification of leukemias was used as a guide for identifying and excluding mixed-lineage leukemias.[7]

Immunophenotyping was primarily performed by three-color flow cytometric analysis of bone marrow aspirate or peripheral blood specimens with a Becton Dickinson (Mountain View, Calif) FACScan instrument. After mononuclear cell enrichment by centrifugation over Histopaque-1077 (Sigma, St Louis, Mo), the peripheral blood or bone marrow samples were studied for surface antigen expression using a panel of 22 monoclonal antibodies. Blasts were gated for analysis with the use of CD45 antigen expression and right-angle light scatter, as described by Borowitz et al.[21] The antigens detected and antibodies used were CD45/HLE-1, CD2/Leu-5b, CD3/Leu-4, CD4/Leu-3a and Leu-3b, CD5/Leu-1, CD7/Leu-9, CD8/Leu-2a, CD10/CALLA, CD19/Leu-12, CD20/Leu-16, CD11c/Leu-M5, CD13/Leu-M7, CD14/Leu-M3, CD15/Leu-M1, CD33/Leu-M9, CD34/HPCA-1, CD56/Leu-19, CD61, HLA-DR (Becton Dickinson, San Jose, Calif), CD36, CD117/c-kit, and glycophorin A (Immunotech, Marseille, France). For all the above markers, blasts were considered positive if 20% or more expressed an antigen.

EXPRESSION IN ACUTE MYELOID LEUKEMIA (AML)

CD36 (%)	*CD117 (%)*	*Glyco A (%)*	*CD56 (%)*	*CD61 (%)*	*HLA-DR (%)*	*CD34 (%)*	*MPO (%)*
3/7 (43)	4/7 (57)	0/7	4/7 (57)	2/7 (29)	4/7 (57)	7/7 (100)	0/7
7/15 (47)	10/15 (67)	2/15 (13)	2/15 (13)	3/15 (20)	12/15 (80)	9/15 (60)	15/15 (100)
11/21 (52)	15/21 (71)	1/21 (5)	9/21 (43)	4/20 (20)	18/21 (86)	11/21 (52)	20/20 (100)
1/6 (17)	2/7 (29)	0/6	0/7	0/7	2/7 (29)	2/7 (29)	7/7 (100)
10/11 (91)	9/11 (82)	1/11 (9)	2/11 (18)	1/10 (10)	11/11 (100)	5/11 (45)	10/10 (100)
1/5 (20)	4/5 (80)	0/5	0/5	0/5	5/5 (100)	3/5 (60)	5/5 (100)
3/3 (100)	3/3 (100)	0/3	2/3 (67)	0/3	3/3 (100)	0/3	0/3
6/6 (100)	0/6	0/6	4/6 (67)	3/6 (50)	6/6 (100)	1/6 (17)	3/6 (50)
0/2	2/2 (100)	0/2	0/2	0/2	2/2 (100)	2/2 (100)	1/1 (100)
1/1 (100)	1/1 (100)	0/1	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
12/17 (71)	14/17 (82)	1/17 (6)	4/17 (24)	5/17 (29)	16/17 (94)	14/17 (82)	14/14 (100)
7/11 (64)	4/11 (36)	2/11 (18)	3/11 (27)	3/11 (27)	10/11 (91)	7/11 (64)	7/11 (64)
62/105 (59.0)	68/106 (64.2)	7/105 (6.7)	31/106 (29.2)	22/104 (21.2)	90/106 (84.9)	62/106 (58.5)	83/100 (83.0)

Cases of acute mixed-lineage leukemia were excluded using previously described immunologic criteria.[7]

Karyotypic data were available for 68 cases. Banded chromosomes were analyzed using standard methods and identified using GTG banding. At least 20 metaphases were examined from each case. Karyotyping was determined on the basis of the 1995 criteria of the International System for Human Cytogenetic Nomenclature.[22]

We used the χ^2 test to perform a statistical comparison of the association between expression of a specific antibody and a single FAB subtype. The comparison was done for a specific antibody in an FAB subgroup with expression of the antigen in all other AML cases or expression of the antigen in another specific FAB leukemia subtype.

RESULTS

One hundred-six specimens from 52 men and 54 women were studied (male-female ratio, 1:1.04). The patients were aged 19 to 85 years (mean, 56 years; median, 57 years). Seventy-eight cases (73.6%) were subclassified with the use of FAB criteria. Eleven cases were designated "AML, unclassified," and 17 were not subclassified because they evolved from a pre-existing myelodysplastic syndrome (MDS).

TABLE 2. FREQUENCY OF LYMPHOID ANTIGEN EXPRESSION IN ACUTE MYELOID LEUKEMIA (AML)*

AML (no.)†	*CD2(%)*‡	*CD3 (%)*	*CD5 (%)*	*CD7 (%)*	*CD10 (%)*	*CD19 (%)*	*CD20 (%)*
M0 (7)	0/7 (0)	0/7 (0)	0/6 (0)	2/7 (29)	0/7 (0)	0/7 (0)	0/7 (0)
M1 (15)	1/15 (7)	1/15 (7)	0/15 (0)	3/15 (20)	0/15 (0)	1/14 (7)	1/15 (7)
M2 (21)	1/21 (5)	0/21 (0)	1/21 (5)	3/21 (14)	1/20 (5)	4/19 (21)	2/21(10)
M3 (7)	2/7 (29)	0/7 (0)	0/7 (0)	0/7 (0)	0/7 (0)	1/7 (14)	0/7 (0)
M4 (11)	1/11 (9)	0/11 (0)	0/11 (0)	1/11 (9)	0/11 (0)	0/11 (0)	1/11 (9)
M4Eo (5)	0/5 (0)	1/4 (25)	1/5 (20)	0/5 (0)	0/5 (0)	0/4 (0)	1/5 (20)
M5a (3)	0/3 (0)	1/3 (33)	2/3 (67)	0/3 (0)	0/3 (0)	0/3 (0)	2/3 (67)
M5b (6)	1/6 (17)	1/6 (17)	1/6 (17)	1/6 (17)	0/6 (0)	0/6 (0)	3/6 (50)
M6 (2)	0/2 (0)	0/2 (0)	0/2 (0)	1/2 (50)	0/2 (0)	0/2 (0)	0/2 (0)
M7 (1)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
MDS/AML (17)	2/17 (12)	1/17 (6)	0/17 (0)	4/17 (24)	0/17 (0)	0/17 (0)	3/17 (17)
AML, NOS (11)	0/11 (0)	2/11 (18)	0/11 (0)	2/11 (18)	2/11 (18)	4/11 (36)	5/11 (45)
Total (106)	8/106 (7.5)	7/105 (6.7)	5/105 (4.8)	17/106 (16.0)	3/105 (2.9)	10/102 (9.8)	18/106 (17.0)

AML, NOS = not otherwise specified AML French-American-British subtype; MDS/ AML = AML associated with myelodysplastic syndrome.
*All 106 cases were CD8 negative.
†Number of cases tested.
‡Percent of cases expressing the antigen.

The results of immunophenotyping and myeloperoxidase cytochemical analysis are summarized in Tables 1 and 2. CD45, CD33, CD13, and CD11c were the most commonly expressed antigens (97.2%, 95.3%, 94.3%, and 87.6%, respectively). All cases were positive for either CD13 or CD33. Myeloperoxidase was detected cytochemically in 83% of all cases studied and in 89.3% of non–AML-M0 cases.

Lymphoid-Associated Antigen Expression

Fifty-one cases (48.1%) expressed lymphoid-associated antigens (CD2, CD3, CD5, CD7, CD8, CD10, CD19, or CD20). Thirty-eight cases (35.8%) expressed a single lymphoid antigen, 10 cases (9.4%) expressed 2 lymphoid antigens, and 3 cases (2.8%) expressed 3 lymphoid-associated antigens without fulfilling criteria for acute mixed-lineage leukemia. Of the cases with 2 or more lymphoid markers, 11 simultaneously expressed T- and B-cell–associated markers and 2 expressed two B-cell–associated markers. All AML subtypes demonstrated lymphoid-associated antigens, except for M7 (Table 2), and lymphoid antigen expression was most common in acute monocytic leukemias and in the unclassified group of AMLs. CD20 was the most frequently expressed lymphoid marker; it was present in 18 of 106 cases (17%). In 15 of the CD20-positive cases (83.3%), expression of CD20 was present in a subset of gated cells, ranging from 21% to 31%. The next most frequently expressed lymphoid-associated marker was CD7, present in 17 cases (16%), followed by CD19 (9.8%), CD2 (7.5%), CD3 (6.7%), CD5 (4.8%), and CD10 (2.9%). Expression of CD8 was not detected in any case. The helper T-cell–associated antigen CD4 was studied but was not considered as a lymphoid-specific antigen, as it has been frequently reported in normal and leukemic myeloid cells.[23]

Acute Myeloid Leukemia Subtypes

Table 3 lists the immunophenotypes that differed substantially for a particular FAB morphologic type of AML when compared with all other cases of AML or with all cases of another specific AML subtype. Although there were few cases per subgroup, trends for M3 and M5 could be established.

Acute myeloid leukemia, M0—Seven cases (6.6%) were classified as minimally differentiated AML. By

TABLE 3. STATISTICALLY SIGNIFICANT FRENCH-AMERICAN-BRITISH (FAB)-TYPE IMMUNOPHENOTYPIC CORRELATIONS

Antigen	*FAB Type*	*No. Positive*		*Type of AML Compared*	*No. Positive*	P *value*
CD34	M0	7/7	*vs*	All other AMLs	55/99	.0211
CD2	M3	2/7	*vs*	All other AMLs	6/99	.0293
CD4	M3	0/7	*vs*	All other AMLs	51/99	.0084
CD11c	M3	1/7	*vs*	All other AMLs	91/98	< .0001
CD36	M3	1/6	*vs*	All other AMLs	61/99	.0297
CD117	M3	2/7	*vs*	All other AMLs	66/99	.0422
HLA-DR	M3	2/7	*vs*	All other AMLs	88/99	< .0001
CD5	M5a	2/3	*vs*	All other AMLs	3/102	< .0001
CD20	M5a	2/3	*vs*	All other AMLs	16/103	.0201
CD34	M5a	0/3	*vs*	All other AMLs	62/103	.037
MPO	M5a	0/3	*vs*	All other AMLs	83/97	.0001
CD4	M5a	0/3	*vs*	M5b	5/6	.0177
CD117	M5a	3/3	*vs*	M5b	0/6	.0027
CD14	M5b	4/6	*vs*	All other AMLs	13/100	.0005
CD34	M5b	1/6	*vs*	All other AMLs	61/100	.032
MPO	M5b	3/6	*vs*	All other AMLs	80/94	.0265
CD20	M5b	3/6	*vs*	All other AMLs	15/100	.0266
CD20	All M5	5/9	*vs*	All other AMLs	13/97	.0013
CD34	All M5	1/9	*vs*	All other AMLs	61/97	.0026
CD36	All M5	9/9	*vs*	All other AMLs	53/96	.009
MPO	All M5	3/9	*vs*	All other AMLs	80/91	.0001
CD34	MDS/AML	14/17	*vs*	All other AMLs	48/89	.0293
CD19	AML,NOS	4/11	*vs*	All other AMLs	6/91	.0017
CD20	AML,NOS	5/11	*vs*	All other AMLs	13/95	.0079

AML = acute myeloid leukemia; AML, NOS = not otherwise specified AML FAB subtype; MDS/AML = AML associated with myelodysplastic syndrome; MPO = myeloperoxidase.

definition, all were cytochemically negative for myeloperoxidase and Sudan black B, and all six cases studied were negative for butyrate esterase. CD13 and/or CD33 were expressed in all cases, and all cases expressed CD45, CD34, and CD11c. According to FAB criteria for AML-M0, myeloperoxidase-negative AMLs that expressed lymphoid antigens were not diagnosed as AML-M0 and were placed in the unclassified category. Expression of CD7 alone, however, was not considered a sufficiently specific lymphoid marker to preclude a diagnosis of M0, and two CD7-positive cases were classified as AML-M0.

Acute myeloid leukemia, M1—Fifteen cases (14.2%) were classified as acute myeloblastic leukemia without maturation. All cases were positive for myeloperoxidase; CD11c (93.3%), CD13 (93.3%), CD45 (86.7%), and CD33 (86.7%) were the next most frequently expressed antigens. Almost half (46.7%) the M1 cases expressed one lymphoid-associated marker (CD7, CD3, CD2, CD19, or CD20).

Acute myeloid leukemia, M2—Twenty-one cases (19.8%) were classified as acute myeloblastic leukemia with maturation. Myeloperoxidase was detected in all cases. CD45 (100%), CD11c (95.2%), CD33 (95.2%), and CD13 (95.0%) were the most commonly expressed antigens. Seven cases (33.3%) expressed one or more lymphoid-associated marker (CD2, CD5, CD7, CD10, CD19, and CD20), with two cases demonstrating two markers (CD7/CD19 and CD5/CD10) and another demonstrating three markers (CD2/CD7/CD20) without fulfilling criteria for acute mixed-lineage leukemia. CD19 expression was present in four cases (21%).

Acute myeloid leukemia, M3—Seven cases (6.6%), including two microgranular variants, were classified as acute promyelocytic leukemia. All were positive for myeloperoxidase, CD45, CD13, and CD33. CD4 (0%), CD11c (14.3%), CD36 (16.7%), CD56 (0%), and HLA-DR (28.6%) were present less frequently in cases of M3 compared with cases of non-M3 AMLs that expressed CD4 (51.5%), CD11c (92.3%), CD36 (61.6%), CD56 (41.4%), and HLA-DR (88.9%) commonly. The lack of expression of CD4, CD11c, CD36, and HLA-DR was statistically significant (see Table 3). The cases of AML-M3 expressed the least number of lymphoid antigens, and only two of seven cases (28.6%) expressed lymphoid-associated antigens. One case expressed both CD2 and CD19, and another was CD2 positive. The frequency of CD2 expression in this type of AML was higher than in other AML types.

Acute myeloid leukemia, M4—Eleven cases (10.4%) were classified as the usual type of acute myelomonocytic leukemia. All cases studied were positive for myeloperoxidase, CD45, CD11c, CD13, CD33, and HLA-DR. CD36 (90.9%), CD117 (81.8%), CD4 (63.6%), and CD15 (63.6%) were also expressed frequently. Three cases (27.3%) expressed a single lymphoid-associated antigen (CD2, CD7, or CD20). No case of AML-M4 expressed more than one lymphoid antigen.

Acute myeloid leukemia, M4Eo—Five cases (4.7%) were classified as acute myelomonocytic leukemia with eosinophils. All were positive for myeloperoxidase, CD45, CD13, CD15, CD33, and HLA-DR. CD4 (20%) and CD36 (20%) were infrequently expressed when compared with all leukemias and especially when compared with the other cases of AML-M4, but this finding was not statistically significant. Three cases (60%) expressed one lymphoid-associated antigen (CD3, CD5, or CD20). No cases expressed more than one lymphoid antigen.

Acute myeloid leukemia, M5—Nine cases (8.5%) were classified as acute monocytic leukemia, including three as poorly differentiated/monoblastic (M5a) and six as differentiated (M5b). All cases were positive for CD11c, CD15, CD33, CD36, and HLA-DR, and 88.9% expressed CD45 and CD13. Two thirds of both subtypes expressed CD56. Cases of M5a were negative for myeloperoxidase, CD4, CD14, and CD34, whereas all three cases expressed CD117. In contrast, all cases of AML-M5b were CD117 negative, half were myeloperoxidase positive, 83.3% expressed CD4, and two thirds expressed CD14. The differences between CD4 and CD117 expression in the two types of monocytic leukemia were statistically significant. Only one case of M5b expressed CD34, and half the cases were CD61 positive. Lymphoid-associated antigen expression (CD2, CD3, CD5, CD7, or CD20) was relatively common in both types of M5 leukemia, with 5 cases expressing 1 lymphoid antigen, 2 cases expressing 2 lymphoid antigens (CD3/CD20 and CD5/CD20), and 1 case expressing 3 lymphoid antigens (CD3/CD5/CD20). CD20 was detected in five cases (55.5%), four of which showed expression on only a subpopulation of cells (21%–28%). Expression of CD5 was detected in two of the three cases of M5a. The increased frequency of expression of CD20 and CD36, as well as the decreased frequency of CD34 expression and cytochemical reactivity for myeloperoxidase, in both types of M5 was statistically significant when compared with non-M5 leukemias.

Acute myeloid leukemia, M6—Two cases (1.9%) were classified as erythroleukemia. Both expressed CD45, CD11c, CD13, CD15, CD33, CD34, CD117, and HLA-DR. One case expressed one lymphoid-associated antigen (CD7). The blasts did not express glycophorin A in either case.

Acute myeloid leukemia, M7—One case (0.9%) was classified as acute megakaryoblastic leukemia. The blasts were positive for CD45, CD4, CD11c, CD13, CD33, CD36, CD117, CD56, CD61, HLA-DR, CD34, and myeloperoxidase.

Acute myeloid leukemia associated with myelodysplastic syndrome (MDS/AML)—Seventeen cases (16%) were not classified according to FAB categories because they were associated with pre-existing myelodysplasia. All cases were positive for CD45, CD33, and myeloperoxidase. They also frequently expressed CD11c (94.1%), CD13 (94.1%), HLA-DR (94.1%), CD117 (82.4%), and CD34 (82.4%). Nine cases (47%) expressed lymphoid-associated antigens (CD2, CD3, CD7, or CD20), including eight with one lymphoid antigen and one with two (CD7/CD20). The immunophenotype of these cases was not substantially different from AMLs of non-M3 type not associated with MDS.

Acute myeloid leukemia, not otherwise specified (AML, NOS)—Eleven cases of AML (10.4%) were not further classified. This was a morphologically heterogeneous group, four of which had a myelomonocytic or monocytic morphologic appearance but lacked cytochemical evidence of monocytic differentiation. Four other cases had some features of M0, such as lack of cytochemical reactivity for myeloperoxidase with the immunophenotypic expression of several myelomonocytic markers, but were unclassified because of expression of lymphoid-associated markers. The cases of AML, NOS most frequently expressed CD45 (100%), CD13 (90.9%), CD33 (90.9%), HLA-DR (90.9%), and CD11c (81.8%). This group had the highest frequency of lymphoid-associated antigen expression (CD3, CD7, CD10, CD19, or CD20) compared with all other AML subtypes, with 4 cases expressing 1 lymphoid antigen, 4 expressing 2 lymphoid antigens (CD10/CD19, CD10/CD20 and 2 cases with CD3/CD20), and 1 case expressing 3 lymphoid antigens (CD7/CD19/CD20). Overall, the frequency of CD19 and CD20 expression in the AML, NOS group was substantially higher than in other types of AML.

Correlation Between Cytogenetic Findings and French-American-British Subtypes

Karyotypic analysis was performed on 68 cases (64.2%). Nineteen (27.9%) had a normal karyotype. Cytogenetic abnormalities appeared to correlate with only two FAB subtypes, M3 and M4Eo. All six cases of AML-M3 studied had t(15;17). The seventh case, which was studied only by the reverse transcriptase–polymerase chain reaction (RT-PCR), also demonstrated evidence of this translocation (data not shown). The t(15;17) was not found in any of the other AML groups. Four of the five cases of AML-M4Eo revealed inversion of chromosome 16. The fifth case was positive for t(6;9) and has been previously reported.[24] Two other cases, one classified as a usual type of AML-M4 and one as M5b, were also positive for inv(16). Three of 15 cases of AML-M2 (20%) demonstrated either karyotypic (two cases) or RT-PCR (one case) evidence of t(8;21). The RT-PCR–positive case (data not shown) was not studied by standard cytogenetics.

Correlations Between Cytogenetic and Immunophenotypic Findings

Table 4 lists the karyotypic abnormalities found in AML cases positive for CD2 and CD19. Expression of CD2 was present in eight cases (7.5%), including two AML-M3 cases and no M4Eo cases. Both CD2-positive M3 cases were characterized by t(15;17); one case also expressed CD19 aberrantly. No other CD2-positive cases demonstrated t(15;17), and five t(15;17)-positive leukemias were CD2 negative. One CD2-positive case demonstrated inv(16). This case was AML-M4 without eosinophilia.

In 16 cases (15.1%), HLA-DR was absent. Five of these were t(15;17) positive; two other t(15;17)-positive cases of AML were HLA-DR positive. CD19 was expressed in 10 cases (9.8%), including four M2 cases (21%). Three of the four CD19-positive cases of AML-M2 were also CD34 positive; one of these had t(8;21). The other two t(8;21)-positive cases were M2 leukemias without CD19 expression. No other immunophenotypic patterns were found to correlate with specific karyotypic abnormalities. Two cases with 11q23 abnormalities were identified. One case of AML-M1, which had no aberrant antigen expression, demonstrated t(4;11)(p16;q23) and 17p abnormalities. The second case was a myeloperoxidase-negative AML, NOS, with deletions of 6(q13q21), 7(q34q36), and 11(q14q23) and expression of CD7,

TABLE 4. KARYOTYPIC ABNORMALITIES IN CD2- AND CD19-POSITIVE CASES OF ACUTE MYELOID LEUKEMIA

Case No.	*Antigen*	*Other Lymphoid Antigens*	*AML Type*	*Karyotype*
1	CD2	CD19	M3	46,XX, t(15;17)(q24;q21)[21]
2	CD2	—	M3	Classic cytogenetics not performed*
3	CD2	—	M4	46,XX, inv(16)(p13q22)[20]
4	CD2	—	M5b	46,XX[20]
5	CD2	—	MDS/ AML	45,XX, –7[20]
6	CD2	—	MDS/ AML	44,XX, del(3)(q25q26.2), del(7)(q11.2q3?4), –16, dic(17;22)(p10;q10))[12]/ 44-45,XX, del(3)(q25q26.2), del(7)(q22q32), dic(17;22) (p10;q10)[4]/ 45,XX, del(1)(p22p36.1), del(3)(q25q26.2) del(8)(q22q24.1), der(10)t(1;10)(p13;q26), dic(17;22)(p10;q10)[3]
7	CD19	—	M1	46,XX[1]†
8	CD19	—	M1	46,XX,,t(3;14;21)(q26.2;q13;q22)[17]/ 46,idem, del(6)(q13q15)[3]
9	CD19	CD7	M2	46,XX[30]
10	CD19	—	M2	46,XX, der(8)t(8;21)(q22;q22), der(14)t(8;14;21)(8qter->8q22::14q11.2->cen>14q32::21q22->21qter), der(21)t(14;21)(q32;q22)[20]‡
11	CD19	—	M2	46,XX,inv(3)(q21q26.2)[20]
1	CD19	CD2	M3	46,XX,t(15,17)(q24;q21)[21]
12	CD19	CD7 and CD20	AML, NOS	46,XX[19]/46,XX,t(9;17)(q22;q25)[1]

AML = acute myeloid leukemia; AML, NOS = not otherwise specified AML French-American-British subtype; MDS/ AML = AML associated with myelodysplastic syndrome.
*PML/RARα fusion transcripts of t(15;17) detected by reverse transcriptase-polymerase chain reaction (RT-PCR).
†BCR/ABL fusion gene negative by fluorescence in situ hybridization analysis, incomplete classic cytogenetic study.
‡AML1/ETO fusion transcripts of t(8;21) detected by RT-PCR.

CD13, CD33, and CD56. No cases in this series showed the 9;22 translocation.

DISCUSSION

Immunophenotypic studies are now recognized to be essential for the accurate diagnosis of AML-M0, as well as for the confirmation of a diagnosis of acute lymphoblastic leukemia. Of the myeloid-associated antigens used in the current study, CD13 and CD33 were the most commonly expressed in adult cases of AML; one of these antigens was expressed in every case studied. The use of antibodies against these two antigens is probably sufficient for an initial acute leukemia screening panel. The addition of CD11c and CD15 may help confirm the myeloid lineage of cases that are CD13 or CD33 negative. We also continue to perform myeloperoxidase cytochemistry routinely in the evaluation of all acute leukemia specimens because of rare CD13- and CD33-negative AML cases that we have seen previously.[25]

Immunophenotyping of acute leukemias with a large panel of antibodies, however, identifies seemingly aberrant expression of lymphoid antigens in some cases of AML. The significance of this expression is controversial. Some studies have found no clinical significance to this finding in patients with AML.[26] Some have associated expression of CD7 with a poor prognosis.[10,27–30] Some have reported that expression of CD2 and CD19 in AML is a favorable prognostic indicator,[31] whereas others have found that expression of CD2 and CD19 in AML is associated with a poor prognosis.[32,33] In one large review, the frequency of lymphoid antigen expression in AML was estimated to be between 10% and 30%.[10] In addition, a previous study from our institution, performed on specimens received between 1987 and 1992, found lymphoid antigen expression to occur in approximately 20% of cases.[9] In most studies, CD7 has been the most commonly expressed lymphoid antigen; other T- or B-cell–associated antigens have been expressed in fewer than 10%, and usually in fewer than 5%, of cases of AML.[9,10,26,27,30] Our finding of lymphoid antigen expression in 48.1% of adult cases of AML, therefore, differs from some reports. Others, however, have found a high incidence of lymphoid antigen expression in AML.[30,34] Del Poeta et al[30] reported an overall frequency of lymphoid marker expression in AML identical to the current report but found a higher incidence of CD7 expression and included terminal deoxynucleotidyltransferase as a lymphoid marker. In the current study, we found CD20 to be the lymphoid marker most frequently expressed, although at fairly low levels of intensity. The presence of CD20 on only a subpopulation of blast cells suggests that this type of aberrant expression of an antigen may be easily missed, or

assumed to represent admixed B lymphocytes. The lack of CD19 expression on the same population, however, supports the interpretation that CD20 is really present on blast cells. We cannot exclude the possibility that differences in the frequency of detection of lymphoid antigens in AML are related to technical or patient population differences between the studies.

Some immunophenotypes correlated significantly with the FAB subtype of AML. In particular, CD2 expression, as well as lack of CD4, CD11c, CD36, CD117, and HLA-DR expression, correlated with acute promyelocytic leukemia. Also, the presence of CD20 and CD36 expression and the lack of CD34 and myeloperoxidase reactivity correlated with M5 leukemias. Furthermore, CD4 and CD117 expression differed substantially between M5a and M5b leukemia. Despite these differences, enough overlap in expression of antigens occurred in most types of AML to make purely immunologic classification of leukemia subtypes impractical. In addition, some antigens offered little or no diagnostic information. Although it might be expected that expression of glycophorin A and CD61 would correlate with FAB M6 and M7 subtypes, respectively, this was not the case. Both erythroleukemia specimens were negative for glycophorin A, whereas the blasts of seven other cases of AML expressed this antigen. The single case of acute megakaryocytic leukemia was CD61 positive, but more than 20% of other AML cases also expressed CD61. Platelet glycoprotein IIb/IIIa (CD41) expression in nonmegakaryocytic leukemias has been previously reported to be falsely positive because of platelet adherence to the blast cells,[35] and it would seem probable that a similar false-positive result could occur for CD61. Such adherence of platelets to blast cells, however, was not noted on smears or cytospin preparations of our cases. A combination of morphology, with the addition of other platelet-associated antigens (including CD41), and platelet peroxidase detection is helpful in properly identifying cases of acute megakaryocytic leukemia.

Detection of the CD56 antigen, a neural adhesion cell molecule, was not diagnostically useful in our cases. This antigen is reportedly expressed by a subtype of AML that may be confused with acute promyelocytic leukemia.[36] More than 29.2% of our cases, however, expressed this antigen, and none appeared to mimic M3 leukemia. Although expression of CD56 also has been reported to be associated with monocytic leukemias,[29] we found no such correlation. Expression of CD56 appears to occur in a variety of different FAB AML types[37] and offered no additional diagnostic information.

Although a correlation between FAB type and immunophenotype has been found by some,[6] Drexler[38] found a general lack of correlation. We found some correlation between immunophenotype and M3 and M5 leukemia; however, we do not believe that these immunophenotypes are sufficiently specific to make a diagnosis solely on the basis of these data in most cases of AML. Brandt et al[39] recently reported an association between CD19 expression in AML and monocytic lineage. We found no such association using the Leu-12 antibody directed against CD19, but the previous report found such an association only with the B4 antibody.

Because the detection of t(15;17) in AML dictates a particular line of therapy, and other specific karyotypic abnormalities identify prognostically significant groups of AML, it is essential to define the cytogenetic changes of a particular leukemia. Previous reports have found associations between the immunophenotype of some morphologic AML types and specific cytogenetic translocations, particularly t(15;17),[11,15,16] t(8;21)[11,17,18] and inv(16).[14] In the current study, expression of CD2 was present in some t(15;17) AML-M3 cases, and most t(15;17) leukemias lacked expression of HLA-DR; however, other cases had these immunphenotypes and did not demonstrate t(15;17). Similarly, expression of CD2, which has been associated with inv(16) as well as with t(15;17),[11,14,15] was not restricted to patients with inv(16). The association between CD19 and CD34 expression in AML-M2 and t(8;21) also has been described,[11,17,18,39] and we have previously reported a high frequency of this immunophenotype in leukemias with that translocation.[25] Despite this finding, most cases of CD19-positive AML in the current study did not have this karyotypic abnormality, and most cases of t(8;21) leukemia did not have this immunophenotype. The frequency of abnormalities of chromosome 11q23, as well as of t(9;22), was not increased in our patients, as has been previously reported in adult patients with AML who had lymphoid antigen expression.[10,40–42] This finding could have resulted from our elimination of cases that fulfilled immunologic criteria for mixed-lineage leukemia. In the past, many of those cases would have been classified as AML because of cytochemical reactivity for myeloperoxidase. Immunologic correlations, in our opinion, are not sufficiently sensitive or specific to substitute for standard karyotypic analysis.

When the cytogenetic findings were compared with FAB subtypes, only M3, M4Eo and a subset of M2 cases correlated. All M3 cases demonstrated evidence

of t(15;17) and four of five M4Eo cases had inv(16). The M4Eo case that did not correlate, however, demonstrated t(6;9), a cytogenetic translocation known to be associated with poor outcome.[24] Two other non-M4Eo cases also demonstrated inv(16). Although all cases of t(8;21) AML were M2, most cases of M2 leukemia did not have this translocation. A comparison of the morphologic, immunophenotypic, and cytogenetic findings of these patients appears to support the concept that, apart from M3 and M4Eo leukemias, the FAB classification does not define biologically distinct groups of AML.[13] Unless aberrant expression of lineage antigens in AML can be proved to be clinically significant, it would appear that elaborate immunologic classifications of AML are also of limited value. With the exception of identifying cases of mixed-lineage leukemia, therefore, it would appear that identification of aberrant lymphoid antigen expression in AML is not of diagnostic importance. Such identification, however, may be helpful in the follow-up examination of patients for minimal residual disease.[34] Future classification systems of AML should include both diagnostically and prognostically significant morphologic, cytochemical, immunophenotypic, and cytogenetic features.

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