Prognostic Value of Lymphocyte Surface Markers in Acute Myeloid Leukemia


We studied the expression of cell surface antigens associated with myeloid and lymphoid leukemias on bone marrow-derived blast cells from 339 patients with newly diagnosed de novo acute myeloid leukemia (AML) enrolled on Cancer and Leukemia Group B (CALGB) chemotherapy protocols. Surprisingly, of 211 cases studied for the expression of CD2 (T-cell marker, sheep erythrocyte binding receptor for T lymphocytes) 45 were positive (21%). In addition, of 298 patients studied for CD19 (B-lymphocyte marker), 41 were positive (14%). Overall, of 170 patients studied for both CD2 and CD19, 56 (33%) were positive. Interestingly, central review of the French-American-British (FAB) morphology of the CD2- and CD19-positive cases showed that FAB M3 was twice as frequent, and M4 eight times as frequent compared with the CD2- and CD19-negative cases. Of 22 lymphocyte antigen-positive cases in which cells were available for studies of Ig or T-cell antigen receptor (TCR) gene rearrangement, 20 were germline, one had a rearranged Ig heavy chain gene, and one had rearranged TRββ and Ig heavy chain genes. The presence of messenger RNA for CD2 was demonstrated in four CD2 surface antigen-positive cases, thus validating the cell surface data. Lymphocyte-antigen-positive cases had karyotypes commonly seen in AML; 71% of cases with an abnormal clone had t(8;21)(q22;q22), inversion 16(p13q22), t(15;17)(q22;q12), or t(9;11)(p22;q23). The patients with lymphocyte markers had a significantly higher incidence of these karyotypic abnormalities compared with patients with lymphocyte antigen-negative AML (34% vs 15%, P < .02). When the outcome to therapy of the lymphocyte antigen-positive cases was compared with that for the CD2, CD19-negative cases, we found that the CD2, CD19-positive cases actually had higher complete remission rates (75% vs 59%, P = .04), and significantly longer time to failure (P = .02; 32.4% ± 6.0% vs 18.0% ± 4.1% at 2 years) and overall survival (P = .02; 43.5% ± 6.3% vs 28.0% ± 4.5% at 2 years). CD2 antigen-positive cases also had a significantly superior survival (P = .02; 43.8% ± 7.9% vs 29.8% ± 3.8% at 2 years). There were no significant differences (P < .05) between the two groups in age, leukocyte count at diagnosis, incidence of extramedullary disease, or FAB classification. Taken together, these findings indicate that a significant proportion of adults with AML have blasts that express lymphoid cell-associated antigens (CD2 and CD19) in the absence of other features of lymphocytic leukemia and that patients whose cells manifest this phenotype have a more favorable prognosis.

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THE TREATMENT OF acute myeloid leukemia (AML) has improved steadily over the past 10 years. Sixty to seventy percent of patients achieve a complete remission (CR) after induction therapy, which usually includes cytosine arabinoside (ARA-C) and daunorubicin. Unfortunately, up to 75% of these patients eventually relapse and ultimately die of their disease. However, approximately 15% to 20% of patients enjoy prolonged disease-free survival and it is assumed that many of these patients are cured of their disease.

Considerable effort has been spent in identifying prognostic variables that might predict how patients will fare with therapy. Groups of patients stratified by examination of morphology, cytogenetics, and surface antigens have been identified and associated with response to therapy and survival. Numerous independent prognostic variables have thus been identified for patients with AML. However, no single variable can accurately predict prognosis.

The Cancer and Leukemia Group B (CALGB) began a prospective study of the expression of surface antigens defined by monoclonal antibodies (MoAbs) on AML cells in 1982. An interim analysis of 196 patients showed that there were certain immunologically defined subgroups that had a better prognosis, or alternatively, worse prognosis, based on the expression of several myeloid antigens. This study continued with the addition of several additional MoAbs. During a recent updated analysis of 339 evaluable patients, we noticed that a high proportion of patients expressed lymphocyte-associated surface antigens on cells classified as AML. This report describes the morphologic, immunologic, cytogenetic, molecular genetic, and clinical features of these lymphocyte marker-positive AML patients. Surprisingly, we have found that this subgroup of patients survived longer than patients whose blast cells lacked lymphocyte antigens on their cell surfaces.

MATERIALS AND METHODS

Patients. All patients in this study were newly diagnosed adults (age ≥ 16 years) with de novo AML (except for 14 patients with an antecedent hematologic disorder) who were treated on CALGB protocols from 1982 until June 1, 1988. After informed consent had been obtained, a sample of heparinized bone marrow and peripheral blood (if the percentage of blasts in the peripheral blood was greater than 50%) was sent by overnight express mail to the CALGB reference immunophenotyping laboratory. From 1982 until September 1986, the laboratory was that of James D. Griffin at the Dana Farber Cancer Institute, Boston, MA. After September 1986, the reference laboratory was that of Edward D. Ball at the Dartmouth Medical School, Hanover, NH. Samples of bone marrow from 339 patients were evaluated for surface markers in this study.

Morphology and cytochemistry. Unstained smears of bone marrow were sent to the central CALGB morphology laboratory at the
was based on the criteria established by the French-American-British (FAB) Cooperative Study Group. At least a 500-cell differential count of the bone marrow slides were performed in all cases. Cytochemical stains included myeloperoxidase, Sudan black B, ASD-chloroacetate esterase combined with α-naphthyl butyrate esterase, and α-naphthyl acetate esterase with sodium fluoride inhibition according to standard methods. Any case meeting the FAB criteria for lymphocytic leukemia was excluded from this analysis.

**Treatment.** All patients were treated according to CALGB protocols for AML, which usually included a standard remission induction regimen of ARA-C and daunorubicin (DNR). Two hundred and forty-eight patients were treated on the CALGB 8221 and 8525 protocols (DNR 45 mg/m²/d × 3d [30 mg/m² for patients > 60 years of age], ARA-C 200 mg/m²/d × 7d). After attaining CR, patients were then randomized to four courses of low, mid, or high-dose ARA-C as postremission intensification therapy, followed by four courses of DNR and subcutaneous vindesine. Sixty-five patients were treated on protocol 8321, which used ARA-C/DNR induction and a five-drug postinduction program for 8 months. Twenty-one patients were induced into CR with either ARA-C/asparaginase (n = 14) or ARA-C/mitoxantrone (n = 7) followed by postinduction therapy with ARA-C, with or without DNR.

**Cells.** Leukemia cells were isolated by Ficoll-Hypaque gradient centrifugation as previously described. The cells were then frozen at 1°C per minute in medium RPMI 1640 (GIBCO, Grand Island, NY) containing 50% fetal bovine serum (HyClone, Logan, UT) and 10% dimethyl sulfoxide (DMSO; Sigma Chemical Co, St Louis, MO) using a controlled rate freezer, and then stored in liquid nitrogen. For complete description of the most recent leukocyte antigen cluster designations, see reference 12.

**MoAbs.** A panel of MoAbs was designed to include those reactive with commonly expressed myeloid cell-associated antigens (CD11b, CD13, CD14, CD15, CD16, CD32, CD33, and CD64), lymphoid cell-associated antigens (CD2, CD5, CD10, and CD19), megakaryocyte-associated antigen (CD41), glycoporphin A-associated antigen (no CD yet assigned), and antigens associated with both myeloid and lymphoid cells (CD34 and HLA-DR) (Table 1). Some MoAbs were used as dilutions of ascites, some as purified antibody, and some as hybridoma supernatants. Fluorescein isothiocyanate (FITC)-labeled My9 and phycoerythrin-conjugated T11 and B4 were purchased from Coulter (Hialeah, FL). Appropriate isotype-matched negative controls labeled with the same fluorochrome were also used. Although not used routinely in the prospective study, MoAb OKT3 (Ortho Pharmaceuticals, Raritan, NJ) was used to phenotype cells from selected patients. All MoAbs were titrated on appropriate target cells to determine optimal dilutions to use for immunophenotyping.

**Immunophenotyping.** For indirect immunofluorescence analysis, cells were thawed in phosphate-buffered saline (PBS) (pH 7.4) containing 0.1% bovine serum albumin (BSA) and DNAase (Sigma) and washed from the freezing medium. Samples containing less than 50% viable cells at this stage were not analyzed. Viability was generally in the range of 90%. Aliquots of 10⁶ cells were incubated with each of 18 different MoAbs, as shown in Table 1. After 1 hour of incubation, cells were washed in PBS/BSA and 0.05% sodium azide (AZ). FITC-labeled goat antimouse Ig F(ab')₂, (Boehringer-Mannheim, Indianapolis, IN) was added and further incubated for 1 hour. Following this step, the cells were washed again in PBS/BSA/AZ and fixed with 1% para-formaldehyde. The cells were then analyzed on an Ortho Cytofluorograph (Westwood, MA) or a Coulter Epics C (Coulter) within 2 days of staining. Analysis was performed using a log amplification scale. Staining of nonviable cells was minimized by gating on viable cells as estimated by forward- and right-angle light scatter. Controls for these experiments included irrelevant isotype-matched MoAbs. Positive was defined as cells fluorescing greater than control MoAb-stained cells on > 20% of cells.

Simultaneous analysis of two cell surface antigens (CD33 and CD19) by two color immunofluorescence was performed with an Epics C flow cytometer (Coulter) using a dual fluorescence protocol.

Cells from one patient whose cells expressed CD2 were examined for their ability to rosette with sheep erythrocytes. Sheep erythrocytes (Kroy Wilfer Labs, Stillwater, MN) were treated with

### Table 1. Panel of MoAbs Used in This Study

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Lineage</th>
<th>Antigen</th>
<th>Cluster*</th>
<th>Cellular Reactivity</th>
<th>Reference</th>
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<tr>
<td>Mo1</td>
<td>Myeloid</td>
<td>CR3</td>
<td>CD11b</td>
<td>Monos, PMNS</td>
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<td>gp150</td>
<td>CD13</td>
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<td>gp55</td>
<td>CD14</td>
<td>Monos, PMNS</td>
<td>14, 15</td>
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<td>Monos, B</td>
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<td>Monos</td>
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<td>LNF-III</td>
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<td>Illa/llb</td>
<td>CD41</td>
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<td>CD34</td>
<td>Progenitor cells</td>
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<td>gp50</td>
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<td>gp45</td>
<td>CD19</td>
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<td>28</td>
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</table>

**Abbreviations:** Monos, monocytes; PMNS, polymorphonuclear leukocytes; B, B lymphocytes; T, T lymphocytes; LNF-III, lactofucopentaose III; CALLA, common acute lymphoblastic leukemia antigen.

*For complete description of the most recent leukocyte antigen cluster designations see reference 12.
2-amino-ethylisothiouronium bromide (AET; 140 mmol/L, pH 9) for 15 minutes at 37°C. After washing, the erythrocytes were added to a suspension of AML cells at a ratio of 1 AML cell to 50 erythrocytes, incubated for 15 minutes, and centrifuged for 10 minutes at 300g. Cells in the pellet were gently transferred into 6-well plates, incubated overnight at 4°C, and then transferred to slides for viewing or staining.

Cytogenetic analysis. All cytogenetic studies were performed as part of a CALGB prospective study of chromosome analysis in acute leukemia (CALGB 8461). Banded chromosome studies of bone marrow obtained at diagnosis were performed in CALGB institutional cytogenetic laboratories and the karyotypes were centrally reviewed. Specimens were processed using primarily short-term (24–72-hour) unstimulated cultures. G-banding was most commonly used. A minimum of 20 metaphase cells were analyzed in each case. Karyotypes were designated according to the ISCN (1985): An International System for Human Cytogenetic Nomenclature.29

Ig and T-cell antigen receptor (TCR) gene rearrangement studies. High molecular weight DNA was prepared30 from frozen samples of 22 lymphocyte antigen-positive AML cases and digested with restriction enzymes (BamHI, EcoRI, HindIII, Xba I) from New England Biolabs, Beverly, MA, according to their instructions. Aliquots containing approximately 5 μg digested DNA were loaded on to 20-cm, 0.8% agarose gels and electrophoresed for approximately 18 hours. After denaturation and neutralization, the DNA was transferred to nylon membranes (Zetabind; CUNO, Inc, Meridian, CT) by the method of Southern.31 Following washing at 65°C for 30 minutes in 0.1X SSC, 0.5% sodium dodecyl sulfate (SDS), 0.2 mol/L Tris, pH 8.0, the membranes were prehybridized at 42°C for 2 hours in mixtures containing 50% formamide, 10% dextran sulfate, 1 mol/L sodium chloride, 0.1 mol/L Tris, pH 7.5, 5 mmol/L EDTA, 0.5 mg/mL heparin, 0.1% sodium pyrophosphate, 0.5% sarcosyl, and 0.1 mg/mL sonicated salmon sperm DNA. DNA probes labeled with 32P to a specific activity of 2 to 3 × 106 cpm/μg by random primer extension were added to the mixtures (1 × 106 cpms/mL) and hybridized for 16 to 18 hours at 42°C. Following a wash in 2X SSC (25°C) for 15 minutes and two washes in 0.1X SSC, 0.2% SDS (65°C) for 30 minutes, the membranes were blotted dry and exposed to x-ray film with an intensifying screen for 3 to 7 days.

The following probes were used: JH, germline μ gene, 2.5-kb Sau3AI/Sau3AI fragment; Cκ, germline κ gene, 2.5-kb EcoRI/EcoRI fragment; and Cγ germline λ gene, 3.8-kb EcoRI/HindIII fragment32 were obtained from Dr Philip Leder (Harvard University, Boston, MA). Tα Jurkat cDNA, pγ1.43; Tβ Jurkat cDNA, 770-bp Pvu I/Pvu I fragment34; and Tγ γ1, 800-bp HindIII/EcoRI fragment35 were obtained from Dr Tak Mak (University of Toronto, Canada). Tγ Jγ1, 1.6-kb Xba I/Xba I fragment36 was obtained from Dr Stanley Korsmeyer (Washington University, St Louis, MO). These fragments were cut from their vectors and gel-purified before labeling.

Northern analysis of CD2 mRNA expression. Glyoxylated-total RNA from cryopreserved AML cells (20 μg) along with RNA with molecular weight markers were electrophoresed in 1% agarose.37 The RNA was transferred to nylon filters (Schleicher and Schuell, Kennes, NH) and hybridized to 32P-labeled cDNA probes for 18 hours. The blots were then washed twice with 2X SSC for 5 minutes each at room temperature, once with 0.1X SSC containing 1% SDS at 42°C for 30 minutes, once with 0.1X SSC at room temperature for 5 minutes and then applied to Kodak XAR x-ray film for 48 to 60 hours. Each Northern blot was hybridized with a 32P-labeled CD2 cDNA probe (kindly provided by Brian Seed, Boston, MA).46 subsequently stripped, and rehybridized with the actin probe. The extent of hybridization was quantified by densitometry of autoradiographs using an E-C Apparatus Corp (St Petersburg, FL) instrument interfaced to a Hewlett-Packard (Palo Alto, CA) integrator. The data were expressed as a ratio of the signal obtained with the CD2 probe divided by the signal obtained with the actin probe to standardize for lane loading.

Statistical analysis. The primary goal of the statistical analysis performed was to determine whether there are any differences in patient characteristics or outcomes between patients whose marrow blasts express lymphocyte antigens and those whose marrow blasts do not. Survival curves were estimated using the product-limit method.38 Survival curves were estimated from entry onto the treatment protocol, which was also the time the marrow samples were drawn and the patient characteristics recorded. Standard CALGB criteria were used to assess response to therapy. Time to treatment failure was defined for all patients from the date of entry onto treatment protocol until the first of the following events: removal from therapy or failure to respond; relapse for patients who achieved a CR; or death from any cause. Disease-free survival was defined only for patients who achieved a CR and was measured from the date of CR to the date of relapse or death, whichever occurred first. P values are based on the logrank test for survival,39 Fisher's exact test for sex, FAB, extramedullary involvement, and response,40 and the Wilcoxon test for age, leukocyte count, platelets, and hemoglobin.41

RESULTS

Patients. Three hundred and thirty-nine consecutive patients with evaluable bone marrow specimens were included in this study. The clinical/pathologic features of these patients are shown in Table 2. Of a subset of 170 cases in which CD2 and CD19 were both evaluable, cells from 56 patients (33%) were found to have the T-lymphocyte marker, CD2, or the B-lymphocyte marker, CD19. An additional 12 cases were studied for only one or the other of CD2 or CD19 and were positive for the marker studied. The 56 patients studied for both antigens and positive for CD2 or CD19 and the 12 patients positive for CD2 or CD19 (when only one marker was studied) were defined as the lymphocyte antigen-positive population. To be considered lymphocyte antigen-negative, patients had to have both CD2 and CD19 evaluated. One hundred and fourteen patients were in this category. Review of the morphology in the CD2- or CD19-positive cases showed the following distribution: 27 cases were FAB M1 or M2, eight cases were M3, 26 cases were M4 or M5, three were M6, two were acute undifferentiated leukemias, and two were acute unclassifiable leukemia. Interestingly, of 12 patients with M4Eos who could be classified for CD2 and CD19 status in this study, 10 were positive. Table 2 displays these data in contrast to the findings with lymphocyte antigen-negative patients. Although FAB M4Eos was eight times and M3 twice as common among the CD2- or CD19-positive cases compared with CD2- and CD19-negative cases, there was no significant difference overall (P = .08) between the FAB subtypes in the two groups. Central review of the morphology of the bone marrow slides submitted from each patient showed that the median percentage of leukemia cells, myeloblasts, promyelocytes, and/or monoblasts was 81% for both lymphocyte antigen-positive and -negative cases. The median percentage of lymphocytes was 3% in both the lymphocyte antigen-positive and -negative cases.
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were also expressed on these cases. CD19 MoAb can also be found on lymphocytic leukemia cells. The FAB subclasses of these four cases are M4 (two cases), M2, and M6.

Other myeloid-associated antigens, such as CD11b, CD15, and CD64, were also expressed on these cases.

If restricted to only cases in which both markers were studied, the frequency of CD2 or CD19 positivity was 56 cases of 170 (33%). All cases were not studied for each marker because the CD2 MoAb was added to the panel at a later time than CD19 MoAb.

CD2, defined by OKT11, was found on 45 of 211 cases (21%), and CD19, defined by MoAb B4 was found on 41 of 298 cases (14%). The median percentages of positive cells for CD2 and CD19, respectively, were 38% and 38% and the ranges were 20% to 85% and 20% to 92%. Of interest, two other lymphoid markers (CD5 and CD10) included in the MoAb panel were infrequently positive. CD10 was only found on one case (of 141 studied) and CD5, defined by T101, was found on seven of 238 cases (3%).

Cells from five of the 68 patients (FAB subclasses M1 [n = 2], M2 [n = 1], and M6 [n = 2]) that expressed CD2 or CD19 did not express any of several highly myeloid-specific markers including CD13, CD14, CD33, or CD64 (Table 3). However, four of these cases did express CD11b or CD15, which are both myeloid-cell associated markers. Sixty-three cases expressed both myeloid and lymphoid markers. Seventeen of these cases expressed both CD19 and CD2, while 20 cases were CD2 positive, CD19 negative and 14 cases were CD19 positive, CD2 negative. The remaining 12 lymphocyte antigen-positive cases were classified on the basis of having only one of these markers positive; the other marker was not studied. Studies on stored cells from five cases were found to be negative for cell surface CD3, despite being positive for CD2.

To consider whether CD2, CD19 positive cases were those with either an “early” hematopoietic cell (CD34 positive) or larger granular lymphocyte (LGL)/natural killer (NK) phenotype (CD16 positive), the expression of these antigens on CD2- and CD19-positive cases was analyzed. There were significant correlations between expression of CD34 and both CD2 (P = .04) and CD19 (P = .01) and between CD16 and both CD2 (P = .04) and CD19 (P = .01). From 122 cases in which all of these markers

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Table 2. Clinical/Pathologic Characteristics of Patients

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>N</th>
<th>Percent of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid only</td>
<td>5</td>
<td>7%</td>
</tr>
<tr>
<td>Myeloid and T</td>
<td>23</td>
<td>34%</td>
</tr>
<tr>
<td>Myeloid and B</td>
<td>23</td>
<td>34%</td>
</tr>
<tr>
<td>Myeloid, T and B</td>
<td>17</td>
<td>25%</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>100%</td>
</tr>
</tbody>
</table>

Lymphoid refers to expression of either CD2 or CD19, but not CD13, CD14, CD33, or CD64. Four of the five cases in this category did express CD11b, and CD16, both of which are myeloid cell-associated but which can also be found on lymphocytic leukemia cells. The FAB subcategories of these four cases are M1 (two cases), M2, and M6.

Myeloid refers to expression of either CD13, CD14, CD33, or CD64. Other myeloid-associated antigens, such as CD11b, CD15, and CD64, were also expressed on these cases.

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Table 3. Phenotypes of Lymphoid Antigen- (CD2 or CD19) Positive AML Cases

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>N</th>
<th>Percent of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid only</td>
<td>5</td>
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<td>25%</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>100%</td>
</tr>
</tbody>
</table>

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*Number of patients in category and the percentage of the total in either the lymphocyte antigen-positive or -negative groups.

†Of the 12 patients classified as M4E whose lymphoid antigen status was evaluable, 10 were lymphoid antigen-negative.
were measured, 10 cases expressed CD34, CD2, and CD19 while seven cases expressed CD34, CD16, and either CD2 or CD19.

Two-color flow cytometry was performed on 10 cases that manifested expression of CD33 and CD2 or CD19. In five cases, this analysis was unrevealing because the phycoerythrin-conjugated anti-CD2 MoAb detected CD2 less well than did the original indirect immunofluorescence method used to identify the case. In the other five cases, the two-color analysis showed a subpopulation of cells co-expressing both CD33 and CD2 (three cases) or CD33 and CD19 (two cases). Two representative cases are shown in Fig 1. In Fig 1A, a distinct population of cells is seen that co-expressed both CD33 and CD19, while another population of cells expressed only CD33. Displayed in Fig 1B is a case in which essentially all of the cells expressed both CD33 and CD2.

Cells from one patient that expressed high levels of surface CD2 demonstrated an ability to rosette with sheep erythrocytes (data not shown).

Cytogenetics. Adequate cytogenetic analysis was performed on 178 patients. Of these, CD2 and CD19 status was determined for 129 patients, 44 of whom were CD2- or CD19-positive and 85 of whom were CD2 and CD19-negative (Table 4). Only normal karyotypes were seen in about 50% of patients in both groups. One-fourth (23%) of the lymphocyte antigen-positive patients had either t(8;21)(q22;q22) (n = 6) or inversion 16(p13q22) (n = 5). The remaining cases also had clonal chromosome abnormalities associated with AML including t(15;17)(q22;q12) in three cases, and t(9;11)(p22;q23) in one case. The patients with lymphocyte markers had a significantly higher incidence of these markers compared with patients with lymphocyte antigen-negative AML (34% v 15%, P = .02).

Ig and TCR gene rearrangement studies. DNA was extracted from cryopreserved cells from 22 patients in the lymphocyte antigen-positive group. Bone marrow samples were available from 10 patients while only peripheral blood-derived cells were available for study in 12 patients. Gene rearrangements were demonstrated in cells from two of the 22 patients with lymphocyte antigen expression. One patient's cells (CD2 = 38%; CD19 = 37%) had a rearrangement of one allele of the Ig heavy chain gene, while another patient's cells (CD2 = 0; CD19 = 92%) had rearrangements of both alleles of the Ig heavy chain as well as rearrangement of the TCR beta chain gene. The k, lambda, T- alpha, T- beta, and T-gamma genes were in germline configuration. No evidence of gene rearrangement was present in the other 20 cases. Of the 22 cases studied, 15 were positive for CD2 surface expression and 15 were positive for CD19. Thus, none of the 15 CD2-positive patients studied and one of the 15 CD19-positive patients studied showed rearrangements of the T- or B-cell-associated genome, respectively.

In both cases in which gene rearrangements were identified, the density of the rearranged band(s) compared with the germline band(s) was less than that expected for a leukocyte preparation containing a homogeneous population of myeloblasts representing 73% and 92% (percentages of blasts in the two samples with rearrangements) of the total cells in the sample. This finding suggests that the myeloblast population were not homogeneous and that the gene rearrangements were present in sub-populations of these cells.

Northern analysis. Cells from eight patients with AML (four CD2-positive and four CD2-negative) were examined for expression of CD2 mRNA by Northern blotting. The sensitivity of this assay was determined by mixing various percentages of normal lymphocytes with a nonlymphoid cell line, HL-60 (which is CD2-negative) and performing the same experiments. Cells from three of four CD2-positive patients were found to have detectable levels of CD2 mRNA transcripts, levels of which correlated with the degree of cell surface antigen expression as determined by flow cytometry (see Table 5). Both 1.65- and 1.3-kb species of mRNA were found in AML blasts, as has been found in normal T lymphocytes. The lymphocyte mixing experiments indicated that 5% of cells could be normal lymphocytes without being detected by Northern blotting. Thus, it is unlikely that the hybridization observed in the three CD2-positive patients could be attributed to normal lymphocyte contamination in the AML samples because all of the samples that had detectable CD2 mRNA contained less than 5% lymphocytes (Fig 2).

Response and survival analysis. Correlations between expression of individual surface markers and clinical parameters were made. Expression of at least one of the lymphocyte antigens, CD2 or CD19, was associated with a higher probability of induction of CR. Lymphocyte antigen-positive and -negative cases had CR rates of 75% and 59%,
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Patients 1 through 8 correspond to lanes F, H, G, B, E, D, C, and A, respectively, in the Northern blot shown in Fig 2.

†Numbers shown are the mean fluorescence intensity values from flow cytometric analysis. Patients 5 through 8 had greater than 20% of cells positive for CD2 and were therefore considered CD2-positive in all of the analyses in this study.

‡Values shown are semiquantitative levels of mRNA for CD2 derived from densitometric scanning of a Northern blot.

respectively ($P = .04$). When examined by individual markers, expression of CD2 alone tended to correlate with achievement of CR; 76% for CD2-positive cases versus 60% for CD2-negative cases ($P = .08$). Expression of CD19 alone did not correlate with achievement of CR (66% vs 63% CR rate for CD19-positive vs -negative cases; $P = .73$).

Survival of the entire group of lymphocyte antigen-positive patients was superior to the group of patients whose cells did not express these antigens ($P = .02$) (Fig 3A). One- and 2-year survival for the lymphocyte antigen-positive cases was 61.8% ± 5.9% (standard error) and 43.5% ± 6.3%. The corresponding figures for lymphoid antigen-negative patients were 47.2% ± 4.7% and 26.0% ± 4.5%, respectively. Moreover, time to failure ($P = .02$) and disease-free survival ($P = .08$) (patients with CR only) were superior for the lymphocyte antigen-positive cases (Fig 3B and C). Although all survival curves were plotted to 3 years, there were several surviving patients in each group beyond 3 years (see legend for Fig 3 for complete details).

Patients whose cells were CD2-positive had a significantly better survival ($P = .02$), with 68.9% ± 6.9% and 43.8% ± 7.9% of patients surviving at 1 and 2 years (Fig 3D). Time to failure and disease-free survival also tended to be superior for CD2-positive patients ($P = .06$ and $P = .07$, respectively) (Fig 3E and F). One- and 2-year disease-free survival for CD2-positive patients was 64.7% ± 8.2% and 48.3% ± 8.9%, while these figures for CD2-negative patients were 54.2% ± 5.0% and 31.8% ± 5.0%.

There was no statistically significant difference ($P < .05$) between the lymphocyte-positive and -negative patients according to age, sex, FAB type, white blood cell count (WBC), platelet count, extramedullary involvement, or other clinical parameters (Table 2). There was a statistically significant difference ($P = .03$) between the hemoglobin values (at diagnosis) in the two groups, the importance of which is unknown. The impact on response of the differences in cytogenetic findings between lymphocyte antigen-positive and -negative patients could not be assessed because adequate cytogenetic analyses were available on only 70% of cases.

DISCUSSION

The purpose of this study was to determine if the pattern of expression of surface antigens on AML cells had prognostic significance. The panel of MoAbs chosen was designed to encompass the known myeloid cell-associated antigens and selected pan-lymphocyte antigens associated with B cells and T cells, the latter for the purpose of excluding cases of acute lymphocytic leukemia (ALL). Over a 6-year time period, bone marrow-derived blast cells from 339 patients were examined by immunofluorescence with the panel of MoAbs. The expression of cell surface antigens was then correlated with response to therapy, time to failure, length of remission, and survival. Unexpectedly, we found that a large number of cases that met the FAB criteria for AML expressed T- (CD2) or B- (CD19) cell-associated antigens. Moreover, as a group, the patients with CD2- or CD19-positive leukemia cells had a higher frequency of remission induction, longer remission duration, and survival than the patients without lymphocyte antigens. When analyzed separately for CD2 or CD19 expression, only CD2 expression correlated with outcome.

The most common lymphocyte-associated antigen found on AML cells in this study was the CD2 antigen, defined by MoAb OKT11. This antigen, known previously as the sheep erythrocyte receptor, is found on all mature T cells and the majority of T-cell malignancies. It has been previously reported that cells from some children with AML also
Better outcome to standard AML therapy, both in terms of remission induction and survival. The possible reasons for the apparent differences in these studies are numerous and include (1) different patient populations (adult versus children), (2) different treatment regimens, (3) greater statistical power resulting from the larger number of patients in our series, and (4) identification of different patient subsets in the two studies.

Mixed phenotypic leukemia can manifest in at least two different ways. There can be two or more distinct cellular subsets with different antigenic phenotypes or there can be one cell population that co-expresses the antigens of both myeloid and lymphocyte cell lineages. Most of the cases identified in this study are probably in the former category based on the following reasoning. Many of our cases (all with very high blast counts) were found to be greater than 90% positive for a myeloid antigen, and simultaneously between 20% and 50% positive for a lymphocyte antigen. This finding suggests that there were blasts that were both myeloid and lymphocyte antigen-positive and, in addition, blast cells that were only myeloid antigen-positive. However, one of the cases studied (Fig 1B) by two-color flow cytometry showed a dominant population that co-expressed both a myeloid and a lymphocyte antigen, thus falling into the latter category.

There was an association between lymphocyte antigen-positivity and expression of the hematopoietic progenitor cell antigen, CD34, and the NK cell-associated antigen, CD16. This association implies that many of the cases identified in this study arose from relatively undifferentiated cells (hence, CD34 positivity) or, alternatively, from cells destined to express an NK cell phenotype. Several cases (n = 10) expressed CD34, CD2, and CD19; five of these cases were M1 or undifferentiated (AUL) by FAB classification, implying that the leukemias with this phenotype tend to be morphologically undifferentiated.

Interestingly, five cases that were CD2-positive were found to be CD3-negative. Although expression of both CD2 and CD3 are features of normal T lymphocytes, the...
lack of detectable CD3, a protein associated with the TCR, suggests that the cells bearing CD2, but not CD3, are not T cells. Thus, our inability to detect TCR rearrangements in 15 of 15 CD2-positive cases studied is not surprising. Therefore, it is not clear that concomitant CD2 and myeloid antigen positivity within a leukemia cell population is an example of a mixed-lineage phenotype. It is possible that CD2-positive AML may reflect an expansion of a normal bone marrow precursor cell that is capable of expressing myeloid antigens and the CD2 antigen. CD2 functions as an adhesion molecule and is believed to be a ligand for the lymphocyte function-associated molecule (LFA-3).\(^4\) LFA-3 is expressed on all cells, including cytotoxic effector cells.\(^5\) It is interesting to speculate that one of the reasons that patients whose cells expressed CD2 had a better outcome might have a biologic basis relating to antileukemia cellular-mediated cytotoxicity stabilized by the expression of CD2.

Relating to the Ig and TCR gene rearrangement studies, there is the possibility that we have underestimated the frequency of rearrangement in lymphocyte antigen-positive AML cells because we were only able to study a small number of the positive cases. Moreover, many cases with high percentages of lymphocyte antigen-positive cells were not studied because cells were not available. However, one case that expressed CD2 on greater than 90% of cells did not have a detectable rearrangement of TCR \(\beta\), \(\gamma\), or \(\delta\)-chain genes and was negative for surface expression of CD3, thus suggesting that these cells were not truly T cells by phenotype.

This study demonstrates that cells from patients with AML have yet another level of phenotypic heterogeneity, namely, differential expression of unusual antigens for myeloid cells. That this finding had better, rather than worse, implications for the patients’ outcomes to therapy was surprising. Further study is necessary to understand the mechanism by which myeloid leukemia cells express lymphoid cell-associated antigens and why this may be beneficial to the patient.

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