Evidence for Early Hematopoietic Progenitor Cell Involvement in Acute Promyelocytic Leukemia

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Key Words: Acute promyelocytic leukemia; Immunophenotype; t(15;17) Translocation; Fluorescence-activated cell sorting; FACS; Fluorescence in situ hybridization; FISH

Abstract

Acute promyelocytic leukemia (APL) represents a subtype of acute myeloid leukemia with characteristic morphologic, molecular, and immunophenotypic features. Previous immunophenotypic analyses have shown that leukemic cells in APL typically express the myeloid markers CD33 and CD13 but lack expression of the early hematopoietic progenitor cell antigens CD34 and HLA-DR. We analyzed selected immunophenotypic features of APL by flow cytometry and showed that 7 (41%) of 17 cases contained significant subsets of CD34+ leukemic cells; CD34+ myeloid cells predominated in 2 APL cases. By using a fluorescence-activated cell sorter-fluorescence in situ hybridization approach, we confirmed that the CD34+ cells harbored the t(15;17) translocation characteristic of APL. By using the same experimental approach, CD34+ populations were stratified into primitive CD34+ CD38- and committed CD34+ CD38+ progenitor cell subpopulations; cells in both subsets contained the t(15;17) translocation. The knowledge that APL may be partly or largely CD34+ is important for proper diagnosis. Furthermore, identification of the t(15;17) translocation in CD34+ CD38- blasts indicates that, in at least some cases, the leukemogenic mutation in APL occurs within primitive hematopoietic progenitor cells.

Acute promyelocytic leukemia (APL) is perhaps the foremost example of a leukemia in which the correct diagnosis is necessary for appropriate treatment. The highly specific t(15;17)(q22;q11-21) translocation, seen in the majority of cases, fuses the 5' portion of the PML (promyelocytic leukemia) gene on chromosome 15 with the 3' portion of the retinoic acid receptor-α (RARA) gene on chromosome 17 and generates a PML-RARA fusion protein.1-3 This fusion confers the exquisite sensitivity of this tumor to differentiation by all-trans-retinoic acid (ATRA).4 Treatment of APL with ATRA has short- and long-term benefits5-6 and, as a result, has become a mainstay of therapy for APL. More recently, arsenic trioxide has been identified as a potential alternative therapeutic agent in relapsed APL.7 For these reasons, accurate diagnosis of APL is critical.

The diagnosis of APL relies on the synthesis of specific morphologic, molecular, and immunophenotypic features. The French-American-British (FAB) criteria categorize APL into 2 subtypes: M3 (hypergranular) and M3v (microgranular variant).8-9 While M3 morphologic features are highly characteristic, the morphologic features of M3v are less specific, and this subtype may be difficult to diagnose.10 In most cases, the diagnosis ultimately is confirmed by identification of the t(15;17) translocation using standard cytogenetics or fluorescence in situ hybridization (FISH),11,12 by identification of the PML-RARA fusion using reverse transcriptase-polymerase chain reaction (RT-PCR), or both.13-15 The typical turnaround times for these methods and their lack of availability at some institutions may preclude rapid confirmation of the diagnosis, resulting in a greater initial dependence on morphologic and immunophenotypic features.

The immunophenotype of APL has been studied previously.16-22 Leukemic cells have been found to express high levels of the myeloid marker CD33 and to largely lack...
expression of myeloid progenitor cell antigens CD34 and HLA-DR. This classic immunophenotype has been considered helpful in the diagnosis of APL, especially in cases of M3v. We recently noted several cases of CD34+ APL, prompting us to analyze CD34 expression in APL immunophenotyped at our institution. In contrast with previous studies, we found that a substantial percentage of cases contain a sizable subset of CD34+ progenitor cells, and, occasionally, APL may be composed largely of CD34+ blasts. By using a fluorescence-activated cell sorter (FACS)-FISH approach, we detected the t(15;17) translocation in the CD34+ cells, including the very primitive CD34+ CD38- subset, indicating that, in at least some cases, the transforming event in APL occurs at the early progenitor cell level.

Materials and Methods

Patients

Seventeen patients diagnosed with APL at the Hospital of the University of Pennsylvania, Philadelphia, between 1985 and 1995 form the basis of this study. All cases met FAB criteria for APL or showed evidence of the t(15;17) by cytogenetic analysis or by molecular methods (FISH or RT-PCR). FAB subclassifications were confirmed by review of Wright-stained peripheral blood or bone marrow aspirate smears, bone marrow core biopsy specimens, and cytochemistry stains of blood or bone marrow (Sudan black B, myeloperoxidase, nonspecific [a-naphthyl butyrate] esterase, and periodic acid–Schiff). All cases were classified as acute myeloid leukemia (AML)–M3 (10) or AML-M3v (6) by FAB criteria, except for 1 leukemia originally classified as AML-M2 based on morphologic features and cytochemical stains. In the light of finding a t(15;17) translocation, this case was reclassified as AML-M3v.

Flow Cytometry (Immunophenotyping)

The leukemic cells were analyzed with antibodies directed against CD34 (HPCA-2), HLA-DR, CD33, CD13, CD2, CD4, CD7, and, in select cases, CD38 (Leu-17). Cells from peripheral blood or bone marrow were purified by Ficoll-Hypaque gradient and stained according to the manufacturer’s protocol. Antibodies were obtained from Immunotech, Westbrook, ME (CD34) and Becton Dickinson, San Jose, CA (all others), and were fluorescently labeled with phycoerythrin or fluorescein isothiocyanate. Gated populations of leukemic cells were selected based on forward and side scatter properties, which varied depending on the degree of granularity of a particular tumor. Samples were analyzed on a FACSsort flow cytometer (Becton Dickinson) using CellQuest software (Becton Dickinson). Ten thousand events were analyzed for each surface marker.

Cell Sorting

Tumor samples from peripheral blood of patients 1 and 16 were stained with CD34 alone or with CD34 and CD38. Cells were incubated with murine anti-human CD34 conjugated with phycoerythrin (Becton Dickinson) and murine anti-human CD38 conjugated with fluorescein isothiocyanate (Becton Dickinson) or Cy-chrome (Pharmingen, San Diego, CA) in staining buffer (phosphate-buffered saline + 10% fetal calf serum) on ice for 30 minutes and washed in staining buffer 3 times. Samples were sorted on a FACStar Plus flow cytometer (Becton Dickinson) initially into CD34+ and CD34- populations. Subsequent samples from the same patients were sorted into CD34+ CD38- and CD34+ CD38+ populations. For the CD34/CD38 sorts, sort purities were confirmed by reanalyzing the sorted populations and ranged from 90% to 98%.

Cytogenetic Analysis and Fluorescence In Situ Hybridization

Standard metaphase cytogenetic analysis was performed by trypsin G banding. Slides for FISH were prepared by various methods. For the CD34 sort, cells were collected at concentrations of 0.2 to 4 x 10^5 cells per milliliter. Cells from patient 1 and from the negative control were then cytocentrifuged onto slides at a concentration of 10^4 cells per slide, air dried, fixed in Carnoy for 20 minutes, and air dried overnight. Cells from patient 16 were spun, fixed twice in glass tubes, deposited onto slides, air dried, refixed, and air dried again. All specimens were analyzed with the ONCOR t(15;17) probe according to the manufacturer’s FISH protocol (ONCOR, Gaithersburg, MD).

For the CD34/CD38 sort, 2 x 10^3 cells from each population were sorted directly onto Cell-TAK-coated glass slides (Collaborative Biomedical Products, Becton Dickinson Labware, Bedford, MA). For unsorted populations, 3 x 10^6 cells were cytocentrifuged onto Cell-TAK slides. Cells were promptly hypotonized by addition of 100 μL of a 0.075-mol/L concentration of potassium chloride onto the slides, followed by a 20-minute incubation at 37°C. Then, 150 μL of Carnoy fixative (25°C) was slowly added to each slide. Slides were air dried. FISH on specimens from patient 16 was performed with the ONCOR t(15;17) probe according to the manufacturer’s protocol with the exception that the slide pretreatment (37°C, 2 x SSC [sodium chloride sodium citrate]) was deleted. FISH was performed on specimens from patient 1 and on the negative control with the t(15;17) probe obtained from VYSIS (Downer’s Grove, IL) according to the manufacturer's protocol.
turer’s protocol. Slides were counterstained with 4′-6-
diamidino-2-phenylindole dichloride for visualization with
a triple bandpass filter on a Nikon (Rockville, MD)
Optiphot II fluorescence microscope, and selected images
were digitized using Cytovision (Applied Imaging, Santa
Clar, CA).

Sorting directly onto Cell-TAK–coated slides proved to
be the most successful method of collecting and retaining
very small numbers of sorted cells for FISH. For all FISH
experiments, a nucleus was scored as positive if the red and
green signals were fused, were touching, or were separated
by less than 1 signal width. Scoring was performed in a
blinded fashion to eliminate bias.

RNA Extraction and RT-PCR

Mononuclear cells were separated from bone marrow
and peripheral blood by using Lymphocyte Separation
Media (Organon Teknika, Durham, NC), and RNA was
extracted using RNAzol Reagent (TEL-Test, Friendswood,
TX) according to the manufacturer’s protocol. Complimentary
DNA (cDNA) was synthesized from 1 μg of total RNA using 200 ng of a reverse transcription–specific primer (NRTR) for PML-RARA, 5′-TCACCTTGTGTGAT-
GATGCA-3′; or for β2-microglobulin, 5′-GCAACCT-
GCTCAGATACATCAA-3′, 40 units of RNase
inhibitor (RNasin, Promega, Madison, WI), 200 units of
Maloney murine leukemia virus reverse transcriptase (M-
MLV-RT, GibcoBRL, Grand Island, NY), and lx RT
buffer with a 1.5-mmol/L concentration of magnesium chlo-
ride (Perkin-Elmer), a 200-μmol/L concentration of
deoxyribonucleotide triphosphate (Pharmacia, Piscataway, NJ),
5% dimethyl sulfoxide, and 1.5 U of Taq polymerase (Perkin-Elmer) in a final volume of 50 μL. PCR was
performed with initial denaturation at 95°C for 5 minutes
followed by 30 cycles of 95°C for 30 seconds, 60°C for 30
seconds, and 72°C for 30 seconds, with a final 72°C 5-
minute extension. A 1-μL aliquot of the primary product was
used as the template for the second round of PCR, which
proceeded for 35 cycles using the same amplification condi-
tions, with primers as described. PCR products were visual-
ized by UV light following electrophoresis through an
ethidium bromide–containing agarose gel.

Results

APL May Be a CD34+ Leukemia

A summary of the morphologic, immunophenotypic,
karyotypic, and molecular data and FAB classifications for
the 17 patients is shown in Table 1 and Table 2. An
immunophenotyping result was scored as positive if more
than 20% of the gated cell population stained positively with
the antibody being studied. As shown, 7 (41%) of 17 cases
demonstrated CD34+ populations. Of note, in 2 cases (1 and
16), the majority of the leukemic cells were CD34+ (88% and
66%, respectively). Immunophenotyping data from one
such case (patient 16) is compared with that from a représen-
tative CD34- case in Figure 1. HLA-DR was coexpressed
in 3 of the 7 CD34+ APLs but by none of the CD34- APLs.
HLA-DR expression further supports a progenitor cell
derivation of the leukemic cells in these cases.

To confirm that the CD34+ cells were part of the
leukemic clone, cells from 2 CD34+ cases (1 and 16) were
sorted into CD34+ and CD34- populations by FACS, and
FISH analysis for the t(15;17) was performed. Tumor cells
from a patient with AML-M0 with a complex karyotype but
no evidence of a t(15;17) served as the negative control.
Results of these analyses are shown in Table 3. For both
patients with APL, the majority of cells in the CD34+ popula-
tions (78% and 92.2%) showed evidence of the t(15;17) by
FISH, confirming that the CD34+ cells are part of the
leukemic clone. Levels of positivity in the negative control
(AML-M0) ranged from 4% to 8% in the sorted and
unsorted populations, respectively, consistent with back-
ground levels of positivity.

CD34+ APL Shows Variable Degrees of Myeloid
Progenitor Cell Differentiation

Analysis of coexpression of CD34 and CD38 was used to
determine the degree of myeloid progenitor cell
Table II
Summary of Immunophenotyping Data*

<table>
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<th>CD33</th>
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<th>CD2</th>
<th>CD4</th>
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</tr>
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<td>+ (26)</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
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<td>16</td>
<td>M3v</td>
<td>+ (66)</td>
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<td>+</td>
<td>+</td>
<td>+ (65)</td>
<td>+ (26)</td>
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<tr>
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<td>M3v</td>
<td>-</td>
<td></td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>NA</td>
</tr>
</tbody>
</table>

FAB = French-American-British classification; TdT = terminal deoxynucleotidyl transferase; NA = not available.
* Percentage of positivity for CD34, HLA-DR, CD2, CD4, and TdT is given in parentheses. Positive results (+) without associated percentile values indicate >90% positivity for the relevant marker. A negative result indicates <20% positivity for the relevant marker.
† Originally classified as M2 by FAB criteria, but reclassified as M3v after documentation of t(15;17) by cytogenticss.

Figure 11 Immunophenotyping data comparing CD34+ case (A) with CD34+ case (B) (patient 16). The position of the gates in each case is determined by the level of nonspecific staining present in the auto control (tumor cells stained with irrelevant antibody).

differentiation within individual tumors. Flow cytometry data from the ungated cell populations of 4 tumors are shown in Figure 2. Figures 2A through 2C are CD34+ tumors encompassing a range of CD34 positivity (46%–88%); Figure 2D is an example of a CD34- case, shown for comparison. As demonstrated, the level of differentiation of the leukemic cells varies considerably; case 1 (Figure 2A) shows a predominant, less mature CD34+CD38- population with a smaller, more differentiated CD34+CD38+ population, whereas case 2 (Figure 2C) exemplifies a more differentiated leukemia with a predominant CD34+CD38+ population. Case 8 (Figure 2D) is the most differentiated tumor, as shown by the lack of CD34 expression and the predominant CD38 positivity. Thus, similar to other subtypes of AML, APL may show variable levels of myeloid differentiation.

Primitive Progenitor Populations Are Part of the Leukemic Clone in APL

Two CD34+ APL cases (1 and 16) were sorted into CD34+CD38+ and CD34+CD38- populations by FACS. FISH analysis was performed on each population to identify the t(15;17) translocation. Tumor cells from a patient with acute biphenotypic leukemia with a t(9;22) translocation served as the negative control. Results are presented in Table IV and Image II. As indicated, the large majority (94.0%–97.6%) of
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**Table 2**
Summary of Karyotypic and Molecular Data*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>FAB</th>
<th>Karyotype</th>
<th>FISH</th>
<th>RT-PCR</th>
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<tr>
<td>1</td>
<td>M3v</td>
<td>t(15;17)</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>M3v</td>
<td>Normal</td>
<td>+ (46)</td>
<td>+</td>
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<tr>
<td>3</td>
<td>M3v</td>
<td>add(7)(q36)t(15;17)</td>
<td>+ (58)</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>M3v</td>
<td>t(15;17),+M1,+M2</td>
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<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>M3</td>
<td>t(15;17)</td>
<td>NA</td>
<td>NA</td>
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<td>6</td>
<td>M3</td>
<td>Failed</td>
<td>+ (53)</td>
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</tr>
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<td>t(15;17)</td>
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<td>+</td>
</tr>
<tr>
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<td>t(15;17)</td>
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<td>t(15;17)</td>
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<td>M3</td>
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<tr>
<td>17</td>
<td>M3v</td>
<td>t(15;17)</td>
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FAB = French-American-British classification; FISH = fluorescence in situ hybridization; RT-PCR = reverse transcriptase polymerase chain reaction; NA = not available.

* For positive FISH results, the percentage of nuclei showing fusion signal is given in parentheses. A positive RT-PCR result indicates a promyelocytic leukemia breakpoint in intron 3, intron 6, or exon 6.

**Table 3**
Fluorescence In Situ Hybridization Analysis for t(15;17) in CD34 Sorted Cells*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Unsorted</th>
<th>CD34*</th>
<th>CD34-</th>
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<tbody>
<tr>
<td>1</td>
<td>NA</td>
<td>78 (18/23)</td>
<td>NA</td>
</tr>
<tr>
<td>16</td>
<td>94.0 (282/300)</td>
<td>92.2 (461/500)</td>
<td>92.2 (369/400)</td>
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<tr>
<td>Negative control (AML-M0)</td>
<td>9 (4/46)</td>
<td>4 (3/69)</td>
<td>NA</td>
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</table>

AML = acute myeloid leukemia; NA = not available.

* Data are given as the percentage of nuclei showing fusion signal (number of positive nuclei/total number analyzed).

**Table 4**
Fluorescence In Situ Hybridization Analysis for t(15;17) in CD34/CD38 Sorted Cells*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Unsorted</th>
<th>CD34+/CD38+</th>
<th>CD34-/CD38-</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NA</td>
<td>11.0 (33/300)</td>
<td>11.0 (33/300)</td>
</tr>
<tr>
<td>16</td>
<td>9.3 (28/300)</td>
<td>14.0 (125/128)</td>
<td>14.0 (125/128)</td>
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<tr>
<td>Negative control (acute biphenotypic leukemia)</td>
<td>97.6 (488/500)</td>
<td>97.0 (194/200)</td>
<td>97.0 (194/200)</td>
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</table>

NA = not available.

* Data are given as the percentage of nuclei showing fusion signal (number of positive nuclei/total number analyzed).

nuclei examined in both populations from the patients with APL showed the t(15;17) translocation, confirming that both the primitive CD34+ CD38- and the more committed CD34+ CD38+ progenitor populations are part of the leukemic clone. The background level of positivity in the negative control (acute biphenotypic leukemia) ranged from 9.2% to 11.0%.

**Immunophenotypic Correlations**

CD34 status was correlated with FAB classifications of M3 or M3v. Of 7 M3v cases, 5 (71%) were CD34+, whereas only 2 (20%) of 10 cases of classic M3 were CD34+, suggesting an association between expression of CD34 and M3v morphologic features. We also correlated CD2 expression status with FAB subtype and CD34 expression. Aberrant expression of CD2 on leukemic promyelocytes previously has been associated with M3v morphologic features. We found CD2 expression in 8 of 16 APLs, but only 4 of these were M3v. On the other hand, our data suggest an association between expression of CD2 and CD34 in that CD2 was coexpressed on 6 (86%) of 7 CD34+ APLs but on only 2 (20%) of 10 CD34- APLs. The relationship among CD34 expression, CD2 expression, and M3v morphologic features needs to be confirmed in a larger series.

**CD34 Status and Response to Therapy**

Clinical data were available for 11 of the 17 patients.
Patients were divided into 4 groups based on the CD34 status of their leukemias and whether they received ATRA therapy during induction with conventional chemotherapy. Time to response was measured by number of days to achieve a normal bone marrow or days to a CBC count meeting specific criteria (platelet count, > 100 × 10^3/μL [> 100 × 10^9/L]; WBC count > 4,000/μL [> 4 × 10^9/L]; hemoglobin, > 10 g/dL [> 100 g/L]). The period of follow-up ranged from 119 to 2,844 days. The individual data points are listed in **Table 5** (along with status at follow-up), and are shown graphically in **Figure 3**. While larger scale studies are needed to address the effect of CD34 status on response to therapy and survival, these preliminary data show no significant differences between CD34+ and CD34− APL in their response to ATRA.

**Discussion**

In the present study, we demonstrated the presence of a significant subset of CD34+ leukemic cells in 7 (41%) of 17 cases of APL; the majority of leukemic cells were CD34+ in 2 cases (12%). FISH analysis documented the t(15;17) translocation in sorted CD34+ cells, confirming that they are part of the leukemic clone. These findings contrast with those of previous studies that have shown APL to be...
Interphase fluorescence in situ hybridization analysis of CD34/CD38 sorted populations from CD34+ acute promyelocytic leukemia. Red signal indicates promyelocytic leukemia (PML) sequence (chromosome 15). Green signal indicates retinoic acid receptor-α (RARA) sequence (chromosome 17). Yellow signal bordered by green and red indicates the derivative 15 fusion (PML-RARA). The fusion signal is present in CD34+ CD38- (A) and CD34+ CD38+ (B) populations, indicating the presence of the fusion gene in primitive and more differentiated progenitor populations (4′,6-diamidino-2-phenylindole dichloride counterstain, x1000).

CD34-18-20 although some series have cited occasional CD34+ cases,16-17,21-22 Piedras et al26 report CD34 positivity in 5 of 16 APL cases, when the criteria for a positive result are less stringent (10% positive cells in gated population), and a single case report describes an APL case in which the majority of the cells were CD34+.27 Interestingly, our data suggest an association between CD34 expression and M3v. The fact, then, that a significant proportion of our cases have CD34 positivity may reflect in part a higher percentage of M3v cases (7/17) in our cohort than in those described in previous studies.

The immunologic and morphologic variability in phenotype between tumors demonstrated in the present series, in conjunction with the determination of very early progenitor cell involvement in 2 cases, raises questions about the biology of APL development and progression. One model for APL development is that the cell of origin is variable and that features specific to the cell of origin dictate the phenotype of the tumor. Turhan et al28 found that in 3 cases of CD34- APL, the primitive CD34+ CD38- progenitor population isolated by FACS did not show evidence of the PML-RARA fusion at the level of transcription, suggesting that this primitive progenitor population may not be part of the leukemic clone. By using a FACS-FISH approach, we demonstrated in the present study the t(15;17) in CD34+ CD38- progenitor cells in CD34+ APL, indicating that the leukemogenic mutation in APL occurs in very primitive myeloid progenitor cells in at least some cases.

Figure 3I CD34 status vs time to response. Time to response was measured by number of days to achieve a normal bone marrow or days to a CBC count meeting specific criteria (see text). Results are presented as individual data points according to CD34 status and the use of all-trans-retinoic acid (ATRA) in treatment.

An alternative model for APL development is that the cell of origin is the same for all tumors and that the phenotypic and morphologic variability derives from genetic or epigenetic factors that determine the level of differentiation of individual tumors. The recent observation that the type of PML-RARA fusion (dictated by the PML breakpoint) correlates with the presence or absence of myeloblasts29 supports this model. Factors that control the particular myeloid pro-
Table 51  
Response of Acute Promyelocytic Leukemia to Therapy According to CD34 Status*  

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>CD34 Status</th>
<th>All-trans-Retinoic Acid</th>
<th>Time to Response (d)</th>
<th>Status at Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>28</td>
<td>Remission</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>41</td>
<td>Remission</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>–</td>
<td>102</td>
<td>Remission</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>–</td>
<td>44</td>
<td>Second remission</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>+</td>
<td>25</td>
<td>Remission</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>+</td>
<td>43</td>
<td>Second remission</td>
</tr>
<tr>
<td>9</td>
<td>–</td>
<td>+</td>
<td>21</td>
<td>Remission</td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>–</td>
<td>38</td>
<td>Remission</td>
</tr>
<tr>
<td>11</td>
<td>–</td>
<td>–</td>
<td>53</td>
<td>Remission</td>
</tr>
<tr>
<td>13</td>
<td>–</td>
<td>–</td>
<td>62</td>
<td>Remission</td>
</tr>
<tr>
<td>14</td>
<td>–</td>
<td>–</td>
<td>26</td>
<td>Remission</td>
</tr>
</tbody>
</table>

* Time to response defined by days to normal bone marrow or CBC count meeting specific criteria (see text). Follow-up ranged from 119 to 2,844 days.

genitor in which transcription of PML-RARA is permitted also may have a role in this determination. For example, it previously has been shown in chronic myelogenous leukemia that not all progenitors that show evidence of the BCR-ABL fusion by cytogenetic analysis are transcribing the gene.30 A similar situation may exist in APL, where in the more differentiated (CD34+) cases, the t(15;17) is present in primitive progenitors but is not transcriptionally active. This then could explain why the CD34+ cases studied by Turhan et al28 using RT-PCR, which detects RNA but not DNA, did not show evidence of the PML-RARA fusion in CD34+ CD38- progenitors, while the CD34+ cases examined by FACS and FISH in the present series showed evidence of the t(15;17) at the genomic DNA level in almost all CD34+ CD38- progenitors examined.

Tumor evolution also may have a role in phenotypic variability. In transgenic mouse models, the PML-RARA fusion protein has been shown to recapitulate the phenotype of APL by generating, first, preleukemic and, later, overtly leukemic states,31 supporting the hypothesis that tumor progression has a role in the development of overt leukemia and that the PML-RARA fusion product may be necessary but not sufficient for development of the full leukemic phenotype. Analogous to the situation in other forms of acute leukemia,32 tumor progression also may have a role in generating a more genetically evolved lesion. The fact that the CD34+ cases are less differentiated immunophenotypically and morphologically (M3v morphologic features) raises the possibility that these cases represent more genetically evolved forms of APL. Correlation among morphologic features, immunophenotype, and cytogenetic findings in a larger series might be helpful for addressing this issue.

The recognition that a subset of APLs express CD34 in CD34+ APL, as shown in the present study, is similar to findings of previous studies in other forms of AML, which have shown cytogenetic abnormalities within early progenitors33,34 and to studies in APL; Haferlach et al35 used the so-called FICTION method to demonstrate the t(15;17) in CD34+ cells in cases of CD34+ APL, and Takatsuki et al36 demonstrated by RT-PCR for PML-RARA that this fusion was detectable in colony-forming units–granulocyte macrophage and burst-forming units–erythroid, suggesting that the origin of the translocation was a pluripotent stem cell. By using a FACS-FISH approach, we now provide direct evidence that CD34+ CD38- progenitors can harbor the t(15;17), indicating that in at least some cases, APL is a disease of very primitive hematopoietic progenitor cells.

References


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This work was supported in part by a McCabe Fund Award (Dr Salhany) and by grant CA 42232 from the National Institutes of Health, Bethesda, MD.

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Acknowledgments: We thank Rich Schretzenmair for contributions to the immunophenotyping work; and Roberta J. Lamb and Charles H. Fletcher, Jr, for contributions to the cell-sorting studies.

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† Deceased.


