Acute Leukemia With Megakaryocytic Differentiation: A Study of 12 Cases Identified Immunocytochemically

By Ming-jeer Huang, Chin-Yang Li, William L. Nichols, Ji-Hsiung Young, and Jerry A. Katzmann

Acute leukemia with megakaryocytic differentiation has been an uncommonly recognized disorder. We used specific monoclonal and polyclonal antibody reagents (HP1-1D antibody and anti-factor VIII antibody, respectively) and an immunocytochemical staining technique to identify the megakaryocytic nature of the leukemic cells of 12 patients who presented with acute leukemia. The leukemic cells of our patients demonstrated the presence of one or both of these platelet- and megakaryocyte-related antigens, but were negative for all of the commonly employed cytochemical and immunocytochemical staining reactions, except for diffuse acid phosphatase activity and granular PAS positivity. Morphologically, the leukemic cells varied in size from 10 to 40 μm in diameter, frequently had cytoplasmic budding, and contained occasional vacuoles and/or peroxidase-negative azurophilic granules. Five patients presented with syndromes of acute myelofibrosis, and seven patients had otherwise unclassifiable acute leukemias, including three patients who had secondary leukemias.

A CUTE MEGAKARYOCYTIC or megakaryoblastic leukemia is an uncommonly recognized disorder that is characterized by rapidly progressive proliferation of atypical megakaryocytes and their precursor cells. Although this entity was described more than 50 years ago, its clinicopathologic features have remained poorly defined. Accurate diagnosis of acute megakaryocytic leukemia has been hampered by the lack of well-defined morphological characteristics and of readily employed, specific cytochemical or immunocytochemical markers.

Following the development of murine monoclonal antibodies directed at human platelet surface antigens, we used one of these antibodies (designated HP1-1D), and a polyclonal anti-factor VIII (factor VIII-related antigen) antibody, in conjunction with an immunocytochemical staining technique to further investigate the leukemic cell lineage in patients whose leukemic cells were otherwise unclassifiable by currently accepted morphological, cytochemical, and immunocytochemical techniques. Among the cases subjected to additional study with these reagents, we identified 12 patients who had acute leukemia with megakaryocytic differentiation. This report evaluates the characteristics observed in these cases.

MATERIALS AND METHODS

Case Acquisition

Among all patients with acute leukemia who were examined in our institution during the 36-month interval extending from May 1980 through April 1983, patients who had acute leukemia with megakaryocytic differentiation were identified and classified by application of the cytochemical and immunocytochemical techniques outlined below. The first patient (case 1 in Table 1) presented in May 1980 and was studied prior to the availability of monoclonal antibody HP1-1D. The second patient (case 2) presented and was studied during the process of development of this monoclonal antibody by observing the reactivity of the antibody contained in hybridoma cell culture supernatant with the leukemic blasts in the patient's formalin/acetone-fixed peripheral blood smear. This reactivity was detected by immunofluorescence microscopy, following additional incubation of the blood smear with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG. The remaining ten patients were subsequently identified during the 12-month interval from May 1982 through April 1983, when ascites fluid containing HP1-1D antibody was available.

Each of these 12 patients manifested clinical and hematologic features commonly associated with acute leukemia: acute or subacute clinical course; progressive cytopenia of one or more peripheral blood cell lines (erythrocytes, leukocytes, and/or platelets); progressive leukostasis in the peripheral blood. These features of our patients are outlined in Table 1.

Detailed retrospective review of each case was undertaken with regard to the clinical presentation, physical findings at the time of diagnosis, hematologic and coagulation and cytogenetic data, mor-
Table 1. Presenting Clinical and Hematologic Features of 12 Patients With Acute Megakaryocytic Leukemia

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/Sex</th>
<th>Preceding Disease and Duration</th>
<th>Presenting Symptoms and Duration</th>
<th>Physical Examination†</th>
<th>Peripheral Blood</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hepato-megaly</td>
<td>Spleno-megaly</td>
</tr>
<tr>
<td>1</td>
<td>18/F</td>
<td>None</td>
<td>Fever, weakness, gastrointestinal bleeding—4 months</td>
<td>+ + + +</td>
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<tr>
<td>2</td>
<td>59/M</td>
<td>None</td>
<td>Fever, night sweats, pleurisy, weight loss, epistaxis, bruising—6 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>39/M</td>
<td>None</td>
<td>Fever, weakness, bruising, epistaxis, gastrointestinal bleeding—3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>22/M</td>
<td>None</td>
<td>Left abdominal pain and fullness, weakness, dyspnea, and pleural effusion—4 months</td>
<td></td>
<td>+ + +</td>
</tr>
<tr>
<td>5</td>
<td>77/M</td>
<td>None</td>
<td>Fatigue, lightheadedness, hematuria—2 weeks</td>
<td></td>
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<tr>
<td>6</td>
<td>58/F</td>
<td>None</td>
<td>Skin infections, bruising—3 weeks</td>
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</tr>
<tr>
<td>7</td>
<td>64/M</td>
<td>None</td>
<td>Fatigue, dyspnea, lymphedema, bruising, hematomas—6 months</td>
<td>+ + + +</td>
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<tr>
<td>8</td>
<td>29/F</td>
<td>None</td>
<td>Fever, sweating, fatigue, gingival and vaginal bleeding—5 months</td>
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<td>9</td>
<td>61/F</td>
<td>None</td>
<td>Fever, night sweats, fatigue, dyspnea, bruising, vaginal bleeding—3 months</td>
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<tr>
<td>10</td>
<td>73/F</td>
<td>Hodgkin’s disease, remission after chemotherapy—5 years</td>
<td>Fever, skin infiltrates, gingival bleeding after tooth extraction—6 weeks</td>
<td>+ + + +</td>
<td></td>
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<tr>
<td>11</td>
<td>40/M</td>
<td>Polycythemia vera, remission after chlorambucil and 32P therapy—7 years</td>
<td>Fatigue, bruising, epistaxis, melena—6 weeks</td>
<td></td>
<td></td>
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<tr>
<td>12</td>
<td>40/M</td>
<td>Multiple myeloma, melphalan therapy—6 years</td>
<td>Fatigue, transfusion requirement, fever—3 months</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

*Cases 1, 3, 7, 8, and 9 had primary acute myelofibrosis; cases 2, 4, 5, and 6 had primary acute megakaryocytic leukemia; cases 10, 11, and 12 had secondary (therapy-related) acute megakaryocytic leukemia.

†Symbols: -, no enlargement detected; +, palpable up to 3 cm below costal margin; + +, palpable more than 3 cm below costal margin; + + +, palpable at or below the level of the umbilicus.
phological and cytochemical and immunocytochemical characteristics, clinical course (including results of treatment), and survival.

To estimate the incidence of acute leukemia with megakaryocytic differentiation, we reviewed the bone marrow diagnosis of all new acute leukemia cases studied morphologically and cytochemically in our laboratory during the 36 months from May 1980 through April 1983.

**Cytochemical and Immunocytochemical Stains for Acute Leukemia in General**

Wright staining of bone marrow aspirates and peripheral blood smears was performed for morphological analysis of all cases of acute leukemia. In 1980 and 1981, light microscopic cytochemical stains, including those for peroxidase, Sudan black B, nonspecific esterase, and acid phosphatase, were used to further study each case of acute leukemia and to differentiate acute lymphocytic from acute nonlymphocytic leukemia.1

During 1982 and 1983, cytochemical studies, including peroxidase, chloroacacetate esterase, nonspecific esterase, toluidine blue, and acid phosphatase, were applied in our laboratory to all the cases of acute leukemia for identification or confirmation of cell type. Immunocytochemical stains, including immunoperoxidase stain for nuclear terminal deoxynucleotidyl transferase (TdT) and indirect immunoalkaline phosphatase stain for myeloid antigen (OKM1), common ALL antigen (J5), T cell antigen (Leu-1), and B cell antigen (B1), were applied to the cases in which the leukemic cell type could not be identified by cytochemical stains. Those cases of acute leukemia in which the cell type could not be established by the above cytochemical and immunocytochemical methods were further studied by indirect immunoalkaline phosphatase staining for factor VIII antigen in bone marrow biopsy sections and/or by indirect immunoalkaline phosphatase staining of marrow aspirate and peripheral blood smears for platelet- and megakaryocyte-specific antigen, as described below.

**Antibody Reagents**

Rabbit anti-human factor VIII antiserum (Dako) was purchased from Accurate Chemical and Scientific Co (Westbury, NY). Alkaline phosphatase conjugates of goat anti-rabbit IgG and goat anti-mouse IgG were obtained from Tago, Inc (Burlingame, Calif). These reagents were stored at 4 °C. Normal rabbit, goat, and mouse sera were prepared from the blood of nonimmunized animals or purchased from commercial sources, and stored at −20 °C. Normal mouse ascites was obtained from mice inoculated intraperitoneally with NS-1 murine myeloma cells and was stored at −20 °C. Murine monoclonal antibody HP1-ID, of IgG2B isotype, was derived by hybridization of NS-1 mouse myeloma cells with splenocytes obtained from a BALB/c mouse three days following the second monthly intraperitoneal immunization with washed normal human platelets. Cell fusion, cloning, and subcloning at limiting dilution were performed as described by Foster et al.10 To detect the clones secreting platelet-specific antibody, diluted hybridoma cell culture supernatants were incubated with human peripheral blood smears fixed with formalin-acetone. The smears were examined by fluorescence microscopy, following additional incubation with FITC-conjugated goat anti-mouse IgG. Secondary screening of subcloned hybridoma supernatants was accomplished by using a modification of the double antibody radioimmunoassay technique described by McEver et al.11 Selected supernatants, reactive with platelets in these assays, were further studied for their ability to inhibit or enhance the aggregation or procoagulant functions of platelets in citrate-anticoagulated plasma. Negative controls used in each of these assays included fresh hybridoma culture medium with and without the addition of normal mouse serum. Based on results obtained from these assays, 16 hybrid cell lines, subcloned at limiting dilution, were selected for expansion, passage as mouse peritoneal tumors, and cryopreservation.

The hybridoma cell line secreting antibody HP1-ID has thus far been passed four times in mice and appears to be a stable cell line. Ascites fluids containing this antibody had IgG concentrations ranging from 2.8 to 5.4 mg/mL, as determined by radial immunodiffusion, and were stored in small aliquots at −70 °C for the studies reported here. HP1-ID antibody has been found, by double antibody immunofluorescence and by enzyme-linked immunocytochemical analysis, to react only with human platelets and megakaryocytes and not with other peripheral blood or bone marrow cells. The antibody immunoprecipitates the radioliodinated plasma membrane glycoprotein Ib/IIa complex from Triton X-100-solubilized human platelets, as determined by sodium dodecyl sulfate (SDS) gel autoradiography of the material bound to HP1-ID IgG immobilized either on plastic tubes coated with rabbit anti-mouse IgG or on protein A-Sepharose beads. When incubated at saturating concentrations with intact normal human platelets, HP1-ID antibody (ascites fluid or purified IgG) completely inhibits platelet aggregation in response to stimulation with adenosine diphosphate, epinephrine, sodium arachidonate, or collagen, producing a thrombosthenic platelet functional defect. The antibody displays markedly reduced binding to platelets obtained from three subjects with Glanzmann’s thrombasthenia, as determined quantitatively by double-antibody radioimmunoassay or fluorescence-activated cell sorting, but reacts extensively with normal platelets or with platelets and megakaryocytes obtained from subjects with other types of hematologic disorders. An account of the preliminary characterization of HP1-ID antibody has been presented in abstract form.12

**Immunohistochemical Staining Technique**

The immunohistochemical stain for factor VIII antigen was performed as follows:

1. Bone marrow biopsy sections were deparaffinized with xylene, alcohol, and distilled water (xylol three minutes twice; absolute alcohol ten dips, 95% alcohol ten dips, 80% alcohol ten dips, distilled water ten dips).
2. Sections were treated with pepsin solution (Sigma Chemical Co, St Louis; P-7000: 4 mg/mL in 0.01 N HCl) for 20 minutes, then rinsed with phosphate-buffered saline (PBS).
3. Sections were flooded with normal goat serum (1/20 diluted in PBS) and incubated for ten minutes, then the excess serum was drained.
4. Sections were overlaid with rabbit anti-human factor VIII (1/80 in PBS) or with nonimmune rabbit serum (1/80 in PBS; negative control) and incubated for 60 minutes, then rinsed in PBS.
5. Sections were incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG (1/30 in PBS) for 30 minutes, then rinsed in PBS.
6. Alkaline phosphatase activity was detected by use of Sigma reagents in a staining procedure employing naphthol AS phosphate as substrate, fast red violet LB salt as coupler, and Levamisole as inhibitor in a 20-minute reaction.
7. Following counterstaining with Mayer’s hematoxylin (Sigma), the sections were coverslipped with glycerol gelatin (Sigma).

**Immunocytochemical Staining Technique**

The immunocytochemical stain with platelet- and megakaryocyte-specific monoclonal antibody (HP1-ID) was performed as follows:

1. Air-dried marrow and blood smears were fixed with cold buffered formalin-acetone for 30 seconds, then rinsed in PBS.
2. The fixed smears were overlaid with specific monoclonal
antibody (HP1-1D ascites fluid 1/100 in PBS) and incubated for 30 minutes, then rinsed in PBS. Normal blood or marrow smears treated in the same fashion served as positive controls in every case studied, and morphologically recognizable cells of other lineage (nonplatelet, nonmegakaryocyte) served as negatives.

3. Smears were incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (1/30 in PBS) for 20 minutes, then rinsed in PBS.

4. Staining for alkaline phosphatase activity and counterstaining with Mayer’s hematoxylin was performed in the same fashion as for the immunohistochemical staining procedure outlined above (steps 6 and 7), following which the slides were coverslipped.

**Marrow Chromosome Analysis**

Bone marrow aspirates were processed by direct and 24-hour culture techniques using methods reported by Dewald et al. Peripheral blood specimens were processed without phytohemagglutinin stimulation. Metaphases were examined by GTG- and QFQ-banding.

**Coagulation Studies**

In the clinical hematology laboratory, semiautomated determinations of prothrombin and activated partial thromboplastin times were performed on citrated plasma specimens using reagents obtained from General Diagnostics (Morris Plains, NJ). In the special coagulation laboratory, blood samples were processed and assayed for coagulant activities using methods described by Bowie et al.

**RESULTS**

**Clinical and Hematologic Features at Presentation**

The clinical features and hematologic data of our patients at the time of diagnosis are outlined in Table 1. Seven patients were males and five were females. Their mean age was 48 years, and their range in age was from 18 to 77 years. Of these 12 patients, nine represented de novo leukemia (cases 1 through 9) and three had secondary leukemia (cases 10 through 12) associated with previous histories of chemotherapy and/or radiotherapy for Hodgkin’s disease, polycythemia vera, and multiple myeloma. Clinical presentations were similar in several respects to those observed in patients with other types of acute leukemia and included acute or subacute onset of fatigue, fever, and/or bleeding. Fever, not attributable to infection, was a prominent symptom in seven patients. Abnormal bleeding (easy bruising, hematomas, epistaxis, hematuria, menorrhagia and/or gastrointestinal bleeding) had occurred in ten of our patients during the generally short interval between the onset of symptoms attributable to leukemia and the confirmation of this diagnosis. Mild splenomegaly was detected at the time of diagnosis in two cases, substantial splenomegaly in three cases, and hepatomegaly in four cases. One patient had generalized palpable lymphadenopathy and another had localized inguinal lymphadenopathy. Diffuse osteosclerosis was noted on radiographic examination of one patient (case 7) but was not present in the remaining patients. Hypothyroidism was discovered at the time of diagnosis in two patients (cases 5 and 7).

Anemia was present in every case (hemoglobin ranged from 6 to 11.7 g/100 mL). Peripheral blood leukocyte counts ranged from 1,400 to 64,000/μL, and morphologically recognizable circulating blasts ranged from 3.5% to 78.5% of total leukocytes at the time of diagnosis. Significant thrombocytopenia was seen in six cases, and platelet counts ranging from nearly normal to markedly increased were observed in six cases. Abnormal giant platelets were observed in each case. A few teardrop poikilocytes (dacrocytes) were noted in the peripheral blood smears of five patients (cases 1, 6, 8, 9, 10), but were not a prominent finding in any case.

Although each of our patients was found to have acute leukemia with megakaryocytic differentiation, at the time of first evaluation, our patients presented with differing distinct patterns of clinical and hematologic features. Following initial examinations, and prior to study with HP1-1D and factor VIII antibodies, seven patients had features of undifferentiated (unclassifiable) acute leukemia and five had features of acute myelofibrosis.

Among patients with the former presentation, four (cases 2, 4, 5, and 6) had de novo acute leukemia and three (cases 10 through 12) had secondary or therapy-related leukemia. Organomegaly, including splenomegaly, was prominent in cases 4 and 10, and became prominent in case 5 during observation for three months, but was not a major clinical feature of the remaining patients, who initially presented with unclassifiable acute leukemia. Patients with de novo acute megakaryocytic leukemia had a relatively high percentage of morphologically recognizable leukemic cells in their blood and frequently presented with normal or increased numbers of platelets in the peripheral blood. With the exception of case 10, who presented in preterminal condition, patients with secondary acute megakaryocytic leukemia presented with pancytopenia accompanied by relatively few peripheral blood leukoblasts and had minimal or moderate myelofibrosis.

Five of our patients were recognized initially as having acute myelofibrosis. Among these, three patients (cases 3, 8, and 9) had no organomegaly, and each of these presented with pancytopenia and relatively few morphologically recognizable leukemic blast cells in their peripheral blood. In contrast, two patients (cases 1 and 7) had substantial organomegaly, accompanied by a large percentage of morphologically recognizable leukemic cells in the blood. Although these
latter two patients presented some features suggestive of chronic myelofibrosis (agnogenic myeloid metaplasia), their acute course and the results of hematologic studies allowed their classification within the syndrome of acute megakaryocytic leukemia accompanied by myelofibrosis. Fever or sweats were prominent symptoms in four of the five patients with this syndrome.

**Morphological Features of Leukemic Cells**

The leukemic cells in each case were angular in contour and generally had high nuclear-to-cytoplasmic ratios. The degree of differentiation of the leukemic cells varied from case to case and also frequently varied between the peripheral blood and bone marrow of the same patient. Whereas two cases showed a high percentage of primitive blast cells in the peripheral blood (Fig 1A), the blasts in other cases revealed some degree of differentiation, including cytoplasmic budding or cytoplasmic granules (Fig 1B and C). The size of the blast cells was quite variable, ranging from 10 to 40 μm in diameter (Fig 1C). Occasional giant blasts with multiple nuclei were found in six cases. The cytoplasm of the blasts was basophilic, with a few blasts containing cytoplasmic vacuoles (Fig 1B), while the nuclei were rounded or lobulated, and the nuclear chromatin was acidophilic with finely dispersed strands. Nucleoli ranged in number from none to as many as six in the larger blasts, but were not prominent in Wright-stained preparations. In none of the cases were Auer rods noted in the blasts.

Marrow aspiration revealed variable cellularity. Nine cases manifested hypocellularity, one case normal cellularity (case 5), and two cases hypercellularity (cases 6 and 12). Bone marrow biopsies were hypercellular in all cases, and scattered atypical megakaryo-
cytes, including mononuclear micromegakaryocytes, were recognizable between the sheets of blasts or fibrosis in all cases (Fig 2). Diffuse reticulin fibrosis (Fig 3) was present in 11 cases in which it was sought, although fibrosis was sometimes not conspicuous in marrow biopsy sections stained with hematoxylin and eosin. Morphological changes in other cell lines, including the pseudo–Pelger-Huet anomaly of neutrophils and dyserythropoietic morphological abnormalities of the erythroid lineage, were seen in two cases of secondary acute megakaryocytic leukemia (cases 11 and 12).

Cytochemical Features of Leukemic Cells
Among ten cases with peripheral blood and/or bone marrow smears available for cytochemical studies, the leukemic cells revealed uniform negativity with myeloperoxidase, chloroacetate esterase, alpha-naphthyl butyrate esterase, and toluidine blue stains (Table 2). The leukemic cells demonstrated various degrees of diffuse acid phosphatase activity. Coarse cytoplasmic granules were stained by the periodic acid-Schiff reaction, predominantly in the cells displaying cytoplasmic budding. Ringed sideroblasts were seen in the marrow of one patient (case 9) who had been treated with azathioprine for two weeks before the diagnosis was made.

Immunocytochemical Characteristics of Leukemic Cells
The leukemic cells of acute megakaryocytic leukemia were negative for nuclear TdT, myeloid (OKM1), T cell (Leu-1), and B cell (B1) antigens as determined in ten cases by immunocytochemical stains. Indirect immunoalkaline phosphatase staining of factor VIII antigen performed on marrow biopsy sections revealed positive red, granular cytoplasmic staining in blast cells, micromegakaryocytes, large atypical megakaryocytes, and platelets. The intensity of staining paralleled the degree of differentiation, with weaker staining in blasts and more intense staining in mature megakaryocytes (Fig 1, D and E). There was no staining of morphologically recognizable erythroid, myeloid, or lymphoid cells.

Indirect immunoalkaline phosphatase staining of platelet-specific surface antigen, using monoclonal antibody HP1-1D, revealed strong reddish granular staining around and overlying the platelets and leukemic cells, including the morphologically undifferentiated blasts and the atypical megakaryocytes that displayed various degrees of differentiation (Fig 1F). Among the nine cases of megakaryocytic leukemia in which HP1-1D antibody staining was employed, significant reactivity with the majority of the leukemic blast cells was observed in each case. HP1-1D monoclonal antibody has also been tested in normal conditions and in a variety of hematologic disorders. Positive staining of platelets and megakaryocytes occurs, but no staining of other hematopoietic cells has been observed. Thirty-seven cases of various types of acute leukemias and other hematologic malignancies, confirmed by cytochemical and immunocytochemical stains, were also included for additional study with HP1-1D antibody. Included were 14 cases of acute lymphoblastic leukemia, seven cases of acute granulocytic leukemia, three cases of acute monocytic leukemia, two cases of acute myelomonocytic leukemia, one case of erythroleukemia, four cases of acute undifferentiated leukemia, three cases of chronic granulocytic leukemia with lymphoid blast transformation, one case of myeloma, one case of malignant histiocytosis, and one case of hairy cell leukemia. These neoplastic cells did not show reaction with the platelet- and megakaryocyte-specific antibody, HP1-1D.

Chromosome Analysis
Cytogenetic studies of aspirated marrow or peripheral blood cells were performed in ten cases (all except cases 7 and 10). No suitable metaphases were obtained in cases 1 and 6. No apparent chromosome abnormalities were identified in cases 2, 4, and 5 (20, 20, and 11...
ACUTE MEGAKARYOCYTIC LEUKEMIA

Table 2. Histochcmical, Cytological, and Immunocytochemical Features of Leukemic Cells

<table>
<thead>
<tr>
<th>Case No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>9</th>
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<td>Factor VIII antigen*</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>HP1-1D antigen</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloroacetate esterase</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>-</td>
<td>-</td>
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<td>Butyrate esterase</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>ND</td>
<td>-</td>
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<td>Acid phosphatase</td>
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<td>Reticulin fibrosis*</td>
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<td>+</td>
<td>ND</td>
<td>+</td>
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</tbody>
</table>

ND, not done.
+ , Positive; - , negative.
*Performed on biopsy section.

Two cases with secondary leukemia (cases 11 and 12) had hypodiploidy with abnormal markers in 20 or more marrow metaphases examined. In case 11, the most frequent karyotype had absence of chromosomes 17 and Y, monosomy of chromosomes 2, 5, 7, 16, and 21, and the presence of six marker chromosomes, including 3p− and 14p+. In case 12, the most consistently identified chromosomal abnormalities included 5q−, −7 or 7p+, 14q+, −18, and 19q+. Chromosome abnormalities common to both cases included 5q− or −5, −7, and 14p+ or 14q+.

Among the cases of primary acute leukemia with megakaryocytic differentiation, multiple chromosomal abnormalities were found in three patients, each of whom presented with an acute myelofibrosis syndrome. Four analyzable peripheral blood metaphases were obtained from case 3, and all manifested hypodiploidy and contained multiple marker chromosomes. Case 9 had three normal marrow metaphases and ten abnormal metaphases that were hyperdiploid (47 or 49 chromosomes) and contained four or more marker chromosomes, two of which were identified as 2q− and 5q−. All 20 marrow metaphases obtained from case 8 were hypodiploid and frequently had monosomy of chromosomes 2, 3, 5, 7, 8, and 12, trisomy of chromosome 18, and three marker chromosomes, including 14q+. This latter patient received marrow transplanted from a brother. Four weeks following bone marrow transplantation, although her marrow aspirate contained no metaphases suitable for karyotyping, Y chromatin was demonstrable in 43% of 436 interphase marrow cell nuclei examined. Her donor’s marrow cells contained Y chromatin in 56% of the interphase nuclei examined by the same technique.

Coagulation Studies

Two of our patients (cases 5 and 7) were found to have deficiency of plasma coagulation factor V activity (Table 3). In each case, the prothrombin and partial thromboplastin times were moderately prolonged. These patients did not have liver dysfunction, as they did not have abnormalities of the serum alkaline phosphatase, bilirubin, or albumin and did not have major deficiencies of the other coagulation factors that were assayed. Neither did they appear to have increased intravascular coagulation and fibrinolysis (disseminated intravascular coagulation). Both patients had experienced significant clinical bleeding problems, but we could not conclude that their bleeding problems were more frequent or more severe than those that occurred in the remaining ten patients, many of whom had thrombocytopenia.

In one patient (case 5), coagulation factor V activity failed to improve following blood product transfusion, but increased into the normal range following reduction of his leukemic cell mass with chemotherapy. His plasma factor V activity was normal when he was in remission following chemotherapy. A third patient (case 10) had moderately prolonged prothrombin and activated partial thromboplastin times, but coagulation factors were not assayed. A fourth patient (case 2) was found to have normal plasma coagulation factor V, even though his coagulation times were mildly prolonged. Six additional patients had normal prothrombin times. The incidence of coagulation factor V deficiency was 17% among all of our patients with acute megakaryocytic leukemia, whereas its incidence was 20% among those patients who had prothrombin time determinations.

Tests reflecting platelet function were performed in one patient who was not thrombocytopenic (case 7). Ivy bleeding times were prolonged (mean of three determinations was ten minutes; normal range one to six minutes), and platelet aggregation was totally absent in response to stimulation with sodium arachidonate, epinephrine, or collagen suspension, and was grossly impaired in response to stimulation with adenosine diphosphate. Platelet procoagulant activity did not appear to be impaired, as the recalcification time of the
Table 3. Coagulation and Hematologic Studies of Two Patients With Coagulation Factor V Deficiency

<table>
<thead>
<tr>
<th>Determination</th>
<th>Case 7 At Diagnosis</th>
<th>Case 5 After Transfusion and Chemotherapy†</th>
<th>Case 5 In Remission§</th>
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<tbody>
<tr>
<td>Prothrombin time</td>
<td>17-20 s</td>
<td>22 s</td>
<td>24 s</td>
</tr>
<tr>
<td>Partial thromboplastin time</td>
<td>45-60 s</td>
<td>23 s</td>
<td>24 s</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>20-22 s</td>
<td>23 s</td>
<td>24 s</td>
</tr>
<tr>
<td>Factor V</td>
<td>50%-150%</td>
<td>26%</td>
<td>22%</td>
</tr>
<tr>
<td>Factor VII</td>
<td>65%-135%</td>
<td>58%</td>
<td>ND</td>
</tr>
<tr>
<td>Factor X</td>
<td>45%-155%</td>
<td>58%</td>
<td>ND</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>55%-145%</td>
<td>110%</td>
<td>ND</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>190-365 mg/dL</td>
<td>235 mg/dL</td>
<td>325 mg/dL</td>
</tr>
<tr>
<td>Fibrin degradation products</td>
<td>0-3 μg/mL</td>
<td>0 μg/mL</td>
<td>0 μg/mL</td>
</tr>
<tr>
<td>Blood leukocytes</td>
<td>4,100-10,900/μL</td>
<td>18,400/μL</td>
<td>6,200/μL</td>
</tr>
<tr>
<td>Percent leukoblasts</td>
<td>0%</td>
<td>52%</td>
<td>0%</td>
</tr>
</tbody>
</table>

* Determined ten hours following initial transfusion with 5 U platelet concentrate. Patient's weight: 75 kg.
† Determined following daily transfusion for two days with fresh frozen plasma (2 U daily), platelet concentrate (5 U daily), and packed erythrocytes (2 U daily).
§ Determined when in complete remission for six months.
ND, not done.

The patient’s platelet-rich plasma was nearly normal (94 seconds; normal range 70 to 90 seconds), despite plasma coagulation factor V deficiency, which was reflected by a more remarkable prolongation of the prothrombin and partial thromboplastin times.

Frequency of Acute Megakaryocytic Leukemia

During the 36-month interval from May 1980 through April 1983, these 12 cases of acute megakaryocytic leukemia represented 3.6% of all the new cases of acute leukemia (330 cases) whose diagnosis was confirmed by bone marrow examination in our institution. During the 12-month interval from May 1982 through April 1983, when HP1-1D and factor VIII antibody studies were prospectively employed for the classification of acute leukemia cases, the ten patients with acute megakaryocytic leukemia represented 8.3% of the total cases of acute leukemia studied concomitantly (121 cases). We did not routinely perform immunocytochemical studies with HP1-1D and factor VIII antibodies in those cases that had acute blastic transformation of chronic granulocytic leukemia. If these 13 cases are excluded from our 12-month series, acute leukemia with megakaryocytic differentiation represented 9.3% of our acute leukemia cases. Among the latter group, 85 cases represented acute nonlymphocytic leukemia, and of these, 11.8% were found by additional study to have acute leukemia with megakaryocytic differentiation.

Clinical Course, Response to Treatment, and Survival

Table 4 outlines our patients’ clinical course, treatment, response, and survival subsequent to diagnosis of acute leukemia with megakaryocytic differentiation. The mean survival of these 12 patients was 25.4 weeks from the onset of symptoms attributable to leukemia, and 14½ weeks from the date of diagnosis in our institution. In the absence of cytoxic therapy, a rapidly lethal course was observed in four patients (cases 1, 7, 9, and 10).

When remission induction was attempted, in most cases, the response was poor and survival was not improved. Among three patients with primary acute megakaryocytic leukemia (cases 2, 3, and 6), we observed no significant reduction of marrow hypercellularity following the administration of chemotherapy which frequently is effective in inducing remission of acute nonlymphocytic leukemia.

Patient 5, who initially was thought to have smoldering acute undifferentiated leukemia, was observed without specific therapy (except for treatment with thyroid hormone) for three months following diagnosis. During this interval, there was progression of his leukemia, manifested by increasing thrombocytopenia and leukoblastosis in the peripheral blood, increased marrow hypercellularity and fibrosis, increasing splenomegaly, deficiency of plasma coagulation factor V, and recurring hematuria and gastrointestinal bleeding. He consented to participate in the investigational use of etoposide (VP-16) treatment for acute nonlymphocytic leukemia in patients 65 years of age or older. Bone marrow remission, including reversal of myelofibrosis, was documented following one course of treatment with etoposide (160 mg/m² body surface area, infused intravenously daily for five days). The patient has received periodic retreatment with etoposide and has remained in complete hematologic and bone mar-
row remission from acute megakaryocytic leukemia for more than 12 months.

Patient 8, who was initially recognized as having acute myelofibrosis, received corticosteroid therapy for three months following diagnosis. During this interval, there was progression of her disorder, manifested by increasing thrombocytopenia and leukoblastosis in the peripheral blood, recurrent vaginal bleeding, and increased awareness of fever and night sweats. She was prepared for bone marrow transplantation with intravenous cyclophosphamide (60 mg/kg daily on the fifth and fourth day preceding transplantation) and total body irradiation (225 rad per fraction twice daily during the three days immediately preceding transplantation). One month following transplantation of marrow from her histocompatibility-matched brother, her peripheral blood granulocytes were quantitatively normal, her platelets had substantially improved quantitatively, and bone marrow examination confirmed the complete reversal of severe myelofibrosis and the engraftment of donor cells, as revealed by chromosome analysis. Unfortunately, this patient suffered from moderately severe (grade 3) graft-versus-host disease, and she developed therapy-resistant disseminated aspergillosis, dying three months following marrow transplantation despite a continuing marrow remission from acute megakaryocytic leukemia.

DISCUSSION

Acute megakaryocytic leukemia has been reported under several names, including acute megakaryoblastic leukemia, acute megakaryocytic leukemia with megakaryocytic predominance, and malignant megakaryocytosis. It was first reported by Von Boros and Korenyi in 1931, and subsequently, in the American scientific literature by McDonald and Hamrick in 1948. Most of the earlier reports described cases that had morphologically recognizable megakaryocytic differentiation. It is reasonable to expect that less differentiated forms of acute megakaryocytic leukemia may exist and can be difficult to recognize by morphology alone, as in other types of acute leukemias.

In recent years, various cytochemical stains, including myeloperoxidase, chloroacetate esterase, nonspecific esterase, and toluidine blue, have been used successfully for identification and confirmation of acute leukemias of granulocytic, monocytic, or basophilic cell type. Although megakaryocytes are known to stain positively by the PAS reaction and to have alpha-naphthyl acetate esterase and acid phosphatase activity, these
features are not unique to the megakaryocytic cell type. More specific markers for megakaryocytes have been recognized recently, including (1) platelet peroxidase demonstrable by ultrastructural cytochemistry;23,24 (2) factor VIII antigen demonstrable by immunohistochemical techniques;25 and (3) platelet membrane glycoproteins that can be identified by immunofluorescent or immunoultrastructural assay with monoclonal antibodies (eg, AN51 or J15).26-28 Each of these markers has been used for the study of acute megakaryocytic leukemia.16,23,28

In this study, we selected two simple and practical methods for identification and confirmation of acute leukemia with megakaryocytic differentiation. These two methods can be applied to the most readily available specimens, including blood and marrow smears, and paraffin-embedded sections of marrow biopsy. Both methods provided a permanent preparation with excellent preservation of cellular morphology, allowing morphological correlation of the immunologic reaction by ordinary light microscopy. When air-dried smears are available, the immunocytochemical stain for platelet-specific antigen using monoclonal antibody HP1-1D appears to be a sensitive and simple technique for identification of megakaryocytic precursors, including morphologically undifferentiated blasts. HP1-1D antibody recognizes a surface antigen that seems to be expressed relatively early during the differentiation of megakaryocytes from precursor cells.27 We found that adequate antigenic reactivity with HP1-1D antibody persisted for at least several days in air-dried nonfixed blood or marrow smears stored at room temperature. This antigenic reactivity was not preserved in decalcified paraffin-embedded marrow biopsies. Therefore, the immunohistochemical stain for factor VIII antigen was of particular value for analyzing marrow biopsy sections, especially in those cases that had very few circulating leukemic cells, or in which the cellular content of the marrow aspirate was inadequate due to diffuse marrow fibrosis. Factor VIII (von Willebrand’s factor) antigen has been demonstrated immunocytochemically in human endothelial cells, platelets, and megakaryocytes, including early marrow megakaryocytes,29 and appears to be a sensitive marker serving to distinguish these cells from other types of marrow cells.25

Using these specific immunologic reagents to define the megakaryocytic nature of our patients’ leukemic cells, we observed that the degree of differentiation of the leukemic cells varied from case to case and also between different sites of specimens obtained from the same patient. In some cases, although most of the leukemic cells in the peripheral blood appeared as morphologically undifferentiated blasts, aggregates of recognizable dysmegakaryocytes could be easily identified morphologically in the bone marrow, suggesting acute leukemia with megakaryocytic differentiation. Thus, proper examination of all the specimens available is particularly important in correctly diagnosing this disease.

Eleven of the cases included in our study had a diffuse increase of reticulin fibers in the marrow. Five had clinical, hematologic, and bone marrow features of acute myelofibrosis. Among these, three had the principal features of malignant myelosclerosis, as described in 1963 by Lewis and Szur40 and later reviewed by Bearman, Pangalis, and Rappaport.31 In contrast to the pancytopenia and the absence of organomegaly often observed in malignant myelosclerosis, two of our patients had substantial leukoblastosis and organomegaly at the time they were found to have acute myelofibrosis associated with megakaryocytic acute leukemia. During the period of this study, all of our patients recognized as having acute myelofibrosis (five cases) were found to have acute megakaryocytic proliferations, as determined immunocytochemically.

In 1973, Breton-Gorius and colleagues, using ultrastructural cytochemical study of the platelet peroxidase reaction, recognized that the blast cells in the peripheral blood of a patient with acute myelofibrosis were megakaryoblasts.21 Den Ottolander and associates later reported three cases of acute megakaryoblastic leukemia that presented as acute myelofibrosis.32 By employing the platelet peroxidase reaction to study four cases of acute leukemia presenting as acute myelofibrosis, Bain et al concluded in 1981 that megakaryoblastic leukemia is probably the most frequent underlying cause of the clinical syndrome of acute myelofibrosis,28 which, therefore, should be classified as a form of acute myeloid or nonlymphocytic leukemia. Rather than an intrinsic part of the disease, the frequent association of neoplastic megakaryocytic proliferation with marrow fibrosis is thought to be mediated by platelet- or megakaryocyte-derived substances that may stimulate the proliferation of fibroblasts33,34 or other marrow matrix cells, or which may inhibit the resorption or remodeling of bone marrow connective tissue. Candidate substances include a platelet-derived mitogenic factor35 and platelet factor 4.36 The resolution of marrow fibrosis in some cases following chemotherapy29,37 or bone marrow transplantation38,40 the demonstration of a normal karyotype in marrow fibroblasts,41 and the morphological association of marrow fibroblastic proliferation with clusters of megakaryocytes lacking α-granules42 are in support of this hypothesis. Thus, the intense myelofibrosis...
sometimes accompanying acute megakaryocytic leukemia may dominate the clinical presentation of patients with this disorder.

Acute myelofibrosis or acute megakaryocytic leukemia may also represent manifestations of secondary or therapy-related acute leukemia. Following chemotherapy and/or radiotherapy, this entity characteristically occurs after a long interval, often displays a brief preleukemic or dysmyelopoietic period, and frequently evolves into a panmyelosis that is marked by increasing blasts, progressive morphological abnormalities, major chromosomal aberrations, poor response to antileukemic therapy, and a short survival.\(^43\)\(^-\)\(^45\) Classification of individual cases of therapy-related acute nonlymphocytic leukemia according to FAB criteria\(^46\) is often difficult. Morphological findings in cases of therapy-related acute leukemia are strikingly similar, frequently suggesting involvement of all myeloid cell lines.

In two of the 15 cases of therapy-related acute leukemia reported by Foucar and colleagues,\(^44\) the megakaryocytic abnormality was the predominant finding. Ali and Janes\(^47\) observed two cases of acute myelofibrosis following cytotoxic chemotherapy. Sultan and coworkers\(^48\) reported four cases of therapy-related leukemia that had acute myelofibrosis and significant numbers of marrow megakaryoblasts. Three of our cases presented with therapy-related acute leukemia with megakaryocytic differentiation. These patients' bone marrows revealed a preponderance of megakaryoblasts and megakaryocytes, as well as variable myelofibrosis that was not conspicuous except in case 10. Morphological abnormalities in a small population of granulocytes and erythroblasts were observed in two patients from whom adequate marrow aspirates were obtained, suggesting abnormality affecting all myeloid cell lines.

In addition to its presentations as de novo or therapy-related acute leukemia or acute myelofibrosis, acute leukemia with megakaryocytic differentiation can evolve from preceding chronic myeloproliferative disorders, including chronic granulocytic leukemia,\(^49\)\(^-\)\(^51\) agnogenic myelofibrosis with myeloid metaplasia,\(^51\)\(^,\)\(^52\) and idiopathic preleukemic or myelodysplastic syndromes.\(^54\)\(^,\)\(^55\) Acute megakaryocytic proliferations can occur in children and in adolescents as well as in adults, manifest as acute myelofibrosis and/or acute megakaryocytic leukemia.\(^26\)\(^,\)\(^28\)\(^,\)\(^37\)\(^,\)\(^42\)\(^,\)\(^54\)\(^,\)\(^55\) Of the 11 cases of acute megakaryoblastic leukemia reported by Flandrin and colleagues,\(^54\) seven were children or adolescents. Acute myelofibrosis or acute megakaryocytic leukemia has occurred in association with constitutional anomalies of chromosome 21, including Down's syndrome\(^26\)\(^,\)\(^56\) with congenital megakaryocytic leukemia\(^53\)\(^,\)\(^,\)\(^57\)\(^,\)\(^58\) and constitutional ring chromosome 21.\(^38\) It therefore appears that neoplastic megakaryocytic proliferations may: (1) occur in any age group; (2) evolve from a preceding myeloproliferation, arise de novo, or occur as complications of constitutional chromosome abnormalities or of drug or radiation exposure; and (3) present clinically in the marrow as a cellular fibrotic reaction or in the blood as abnormal numbers of megakaryocytes, precursors, or progeny, or as varying combinations of these two principal manifestations.

With the use of specific immunologic reagents, acute leukemia with megakaryocytic differentiation was found to comprise as much as 11.8% of our cases of acute nonlymphocytic leukemia studied during a 12-month period. While this incidence estimate may in part result from fortuitous circumstances and/or the pattern of case referral to our institution, based on our observations and those of other investigators, it would appear that acute leukemia with megakaryocytic differentiation does constitute a significant proportion of the acute leukemias. As a result of its frequent presentation with the cytologic appearance of undifferentiated or poorly differentiated acute leukemia, acute megakaryocytic leukemia may potentially mimic the less well-differentiated acute myeloid or lymphoid leukemias, such as the M1 or L2 FAB subtypes, and may therefore have been previously unrecognized. Because of its frequently poor response to treatment, it seems important to correctly identify acute leukemia with megakaryocytic differentiation so that more effective treatment programs may be developed and evaluated.

The results of conventional chemotherapy of acute megakaryocytic leukemia have generally been disappointing. Although complete remissions have been reported in occasional patients treated with chemotherapy appropriate for acute lymphocytic or nonlymphocytic leukemia, our patients who received cytoreductive chemotherapy usually responded poorly. However, one of our patients achieved and has remained in complete remission for more than 12 months following treatment with etoposide (VP-16). Another patient (case 4), treated elsewhere with 62.5% of the etoposide dose that induced remission in the aforementioned patient, did not appear to respond. Further study of this treatment modality seems indicated, particularly as an alternative to bone marrow transplantation, which presently may offer the best opportunity for remission from acute leukemia with megakaryocytic differentiation.\(^36\)\(^-\)\(^40\)

Our identification and study of 12 cases of acute leukemia with megakaryocytic differentiation in-
cluded five cases with acute myelofibrosis and seven cases with otherwise unclassifiable acute leukemia, three of which represented secondary acute leukemia. Although the clinical presentation of these patients was similar in several respects to that of patients with other types of acute leukemia, we noted certain characteristics that may be helpful in suspecting acute leukemia with megakaryocytic differentiation. These characteristics include frequent presentation with hemorrhagic manifestations and/or fever not attributable to infection; prolongation of coagulation times due to factor V deficiency; near-normal or even increased platelet counts and the presence of dysplastic giant platelets in the blood at the time of diagnosis; wide variation in the size of leukemic cells that frequently display cytoplasmic vacuoles or budding and peroxidase-negative azurophilic granules; and the association with diffuse reticulin myelofibrosis and aggregates of dysplastic megakaryocytes among sheets of blasts or fibrotic reaction in the marrow. Acute megakaryocytic proliferations may occur more frequently than has been appreciated and may masquerade as relatively undifferentiated leukemias or as acute myelofibrosis.

Simple indirect immunoalkaline phosphatase stains for platelet-specific surface antigen and factor VIII antigen have proved most practical in confirming the diagnosis of acute leukemia with megakaryocytic differentiation.

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NOTE ADDED IN PROOF

Since conclusion of the study reported here, during the subsequent 12 months (May 1983 through April 1984), we have immunocytochemically identified nine additional cases of acute megakaryocytic leukemia/acute myelofibrosis, including four cases of secondary acute leukemia (previous histories of Down’s syndrome, essential thrombocytopenia, myelodysplastic syndrome, and melphalan-treated multiple myeloma, respectively).

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Acute leukemia with megakaryocytic differentiation: a study of 12 cases identified immunocytochemically

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