Megakaryoblastic Leukemia: The Characterization and Identification of Megakaryoblasts

By Tadashi Koike

This article describes three patients with megakaryoblastic leukemia, in whom the blast cells were identified as megakaryoblasts by the platelet peroxidase (PPO) reaction. More than 70% of the blasts in these patients were positive for the PPO reaction. Ultrastructurally, acid phosphatase activity in the megakaryoblasts was detected in the nuclear envelope, the endoplasmic reticulum, and in a few granules, but not in the Golgi cisternae. Some blast cells were identified by immunofluorescence or immunohistochemical stains, using monoclonal antiplatelet glycoprotein Ib/IIa antibody. In one patient, most of the blasts were positive for anti-HLA-DR monoclonal antibody. The possible order of the appearance of markers in the maturation of the megakaryocytic cell lineage is postulated, based on the data from the present cases and those previously reported. PPO activity appears in very immature cells, which retain Ia-like antigens. Platelet-specific glycoprotein Ib/IIa is seen in immature cells that are only recognized by PPO activity.

Although it is almost impossible to identify blast cells (< 25 μ in size) as precursors of the megakaryocytic cell line using conventional light microscopic stains, the more mature cells, such as micromegakaryocytes, can be identified by such techniques. Recent advances in ultrastructural cytochemistry and monoclonal antibodies have made it possible to identify megakaryoblasts. Breton-Gorius and her colleagues reported that platelet peroxidase (PPO), which has some characteristics distinct from myeloperoxidase, is synthesized early during megakaryocytic maturation and appears to be a specific enzymatic marker of this cell line.1-3

Subsequently, there have been several reports of acute megakaryoblastic leukemia in which blast cells were recognized by the PPO reaction.4-11 However, in all these reports, except one,4 circulating blasts comprised less than 30% of the peripheral blood, making it impossible to characterize megakaryoblasts by approaches such as surface markers. This article describes three patients in whom most of the circulating blasts were recognized as megakaryoblasts by their PPO activity; in one patient, the blasts comprised more than 70% of the peripheral blood and the majority of them had PPO activity. In this investigation, ultrastructural cytochemistry and a monoclonal antiplatelet antibody were employed in the characterization of megakaryoblasts, or promegakaryoblasts, as described by Breton-Gorius.12 In addition, the order of the appearance of markers in the maturation of the megakaryocytic cell lineage is postulated from our studies and previous reports.

CASE REPORTS

Patient 1

A 55-year-old housewife was referred to our hospital in September 1982 because of suspected acute leukemia. She had suffered from easy fatiguability for one month before admission. She had undergone hysterectomy for cervical cancer 22 years ago. On physical examination, she appeared anemic and had petechiae on her extremities. Slight hepatosplenomegaly was noted. The blood profile was as follows: RBC 2.51 x 10¹²/L, hemoglobin 7.9 g/dL, hematocrit 21.8%, reticulocytes 2.8%, platelets 38 x 10⁹/L, WBC count 5.0 x 10⁹/L, with 3% bands, 8% segmented neutrophils, 4% monocytes, 11% lymphocytes, and 74% blasts. Erythroblast were occasionally seen. Red cell morphology showed mild anisopoikilocytosis with a few teardrop cells. Most of the blasts had a round or oval nucleus with a high nucleocytoplasmic ratio, fine chromatin, and a single prominent nucleolus. The cytoplasm rarely contained granules or blebs. Bone marrow specimens could not be obtained by aspiration. A trephine biopsy of hemopoietic tissue revealed infiltration by a large number of fibroblasts and a small number of undifferentiated mononuclear cells. Megakaryocytes were reduced in number, and the reticulin fibers were moderately increased.

The patient was treated with vincristine and prednisolone, which were not effective. Her condition was complicated with iheus and infection. She is currently being maintained on supportive therapy with blood and is receiving platelet transfusions as required.

Patient 2

A 14-year-old boy was admitted to our hospital in December 1981 for further examination of pancytopenia. He had suffered from general malaise for two months. On physical examination, he appeared anemic and had petechiae on the body. There was no lymphadenopathy or hepatosplenomegaly. The blood picture was as follows: RBC count 3.45 x 10¹²/L, Hb 10.1 g/dL, Hct 31.6%, reticulocytes 0.1%, platelet count 30 x 10⁹/L, WBC count 2.4 x 10⁹/L, with 1% metamyelocytes, 2% bands, 2% segmented neutrophils, and 95% lymphocytes. Occasional circulating blasts were seen. The blood film showed atypical erythrocyte morphology, with no teardrop cells. Blasts had a round or oval nucleus with fine chromatin and a prominent nucleolus, and some had cytoplasmic protrusions and blebs. The cytoplasm was scanty, basophilic, and agranular. Micromegakaryocytes were occasionally seen in the peripheral blood. The leukocyte alkaline phosphatase (LAP) score was within normal limits. Attempts at marrow aspiration were unsuccessful. A bone marrow needle biopsy disclosed that hematopoietic tissue was largely replaced by fibrotic tissue, which contained...
atypical megakaryocytes as well as a small number of immature mononuclear cells. Coarse reticulin fibers were increased. A diagnosis of malignant myelosclerosis (acute myelofibrosis) was made. The patient is being followed on an outpatient basis without antileukemic chemotherapy. Recently, the proportions of his circulating blast cells has been gradually increasing. In November 1983, the blood picture was as follows: RBC count 3.22 x 10^12/L, platelet count 53 x 10^9/L, WBC count 1.6 x 10^9/L, with 1% myelocytes, 1% metamyelocytes, 8% bands, 9% segmented neutrophils, 5% monocytes, 62% lymphocytes, and 14% blasts.

**Patient 3**

A 79-year-old man was admitted in May 1981 with a complaint of vertigo. On physical examination, there was slight splenomegaly (2 cm below the costal margin). The blood profile was as follows: RBC count 3.22 x 10^12/L, Hb 9.9 g/dL, Hct 30.5%, reticulocytes 4.8%.

At the time of his second admission in May 1983, his spleen was enlarged 5 cm below the costal margin. The blood profile was as follows: RBC count 2.36 x 10^12/L, Hb 7.2 g/dL, Hct 21.4%, reticulocytes 2.2%. The LAP score was within normal limits. Bone marrow could not be obtained by aspiration. A trephine biopsy showed both hypercellular and hypocellular areas, with infiltration of fibroblasts and an increase in reticulin fibers. Atypical megakaryocytes were increased focally. In view of the patient's age, he did not receive antileukemic agents. Anemia and thrombocytopenia progressed very slowly.

On the second admission in May 1983, his spleen was enlarged 5 cm below the costal margin. The blood profile was as follows: RBC count 3.22 x 10^12/L, Hb 9.9 g/dL, Hct 30.5%, reticulocytes 4.8%, platelet count 140 x 10^9/L, WBC count 4.4 x 10^9/L, with 1% myelocytes, 0.5% metamyelocytes, 17% bands, 47% segmented neutrophils, 5.5% basophils, 19% lymphocytes, and 4% blasts. The ratio of erythroblasts to WBC was 2:100. Red cell morphology showed moderate anisopoikilocytosis. The LAP score was within normal limits. Bone marrow could not be obtained by aspiration. A trephine biopsy showed both hypercellular and hypocellular areas, with infiltration of fibroblasts and an increase in reticulin fibers. Atypical megakaryocytes were increased focally. In view of the patient's age, he did not receive antileukemic agents. Anemia and thrombocytopenia progressed very slowly.

The patient has remained reasonably well on supportive treatment in the 30 months since presentation. The data presented below were obtained during his second admission.

**MATERIALS AND METHODS**

**Blood Smears and Cytochemical Studies**

Blood smears were treated with May-Grünwald-Giemsa stain. Cytochemical reactions for myeloperoxidase, Sudan black B reaction, naphthol-ASD-chloroacetate esterase, alpha-naphthyl acetate esterase, alpha-naphthyl butyrate esterase, acid phosphatase, and periodic acid-Schiff reaction were performed.

**Electron Microscopic Studies**

Conventional thin electron microscopy. Heparinized venous blood was centrifuged, and the plasma was then removed and replaced with glutaraldehyde fixative. Theuffy coat was removed as a solid disc, sliced, fixed with 1.25% glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.2, for two hours, and postfixed in 1% osmium tetroxide. The specimens were then dehydrated in alcohol and embedded in Araldite (Nisshin Co, Tokyo). Ultrathin sections were stained with uranyl acetate, followed by lead citrate, and examined in a Hitachi (Tokyo) H-300 electron microscope.

Myeloperoxidase (MPO). Buffy coat cells were fixed for 30 minutes at 4°C with 1.25% glutaraldehyde in 0.1 mol/L phosphate buffer, then washed three times in 0.1 mol/L phosphate buffer. They were incubated for 30 minutes in the dark, at room temperature in DAB medium prepared as follows: 5 mg 3,3-diaminobenzidine tetrahydrochloride (Wako Fine Chemicals, Japan) in 10 mL Tris-HCl buffer containing 0.01% hydrogen peroxide, pH 7.6. The samples were washed twice in 0.1 mol/L phosphate buffer, postfixed for 30 minutes in 1% osmium tetroxide solution, and then processed as above. In order to evaluate the dense reaction product of oxidized diaminobenzidine (DAB), the sections were examined unstained.

Platelet peroxidase (PPO). PPO was detected by a modification of Anderson's method. After centrifugation of heparinized blood, plasma was removed and replaced with 0.5% glutaraldehyde in 0.1 mol/L phosphate buffer. After ten minutes, the glutaraldehyde was removed and replaced with a fixative consisting of 1% tannic acid, 2% paraformaldehyde, and 0.5% glutaraldehyde in 0.1 mol/L phosphate buffer at pH 7.2. Cells were fixed for 50 minutes at 4°C and washed in phosphate buffer. The DAB medium for PPO was as follows: 20 mg DAB in 10 mL 0.05 mol/L Tris-HCl buffer containing 0.1 mL hydrogen peroxide. Incubation was carried out in the dark, at room temperature for one hour. The samples were then processed in the same manner as the MPO samples. For evaluation of PPO reaction, sections were examined first without staining. For testing the inhibition by 3-amino-1,2,4-triazole (AMT, Nakarai Chemicals, Japan), the specimens were kept in buffer containing 0.02 mol/L AMT for 30 minutes at 4°C prior to incubation and were subsequently incubated in the same DAB medium supplemented with 0.02 mol/L AMT.

Acid phosphatase. The ultrastructural cytochemical analysis for acid phosphatase was performed according to the method of Barka and Anderson. The cells were fixed in 1.5% glutaraldehyde in 1/15 mol/L cacodylate buffer pH 7.4 at 4°C for 30 minutes and then incubated for one hour at 37°C in the modified Gomori medium of Barka and Anderson. The samples were washed in 0.1 mol/L phosphate buffer and postfixed for 30 minutes in 1% osmium tetroxide. Sections were examined unstained.

**Surface Membrane Markers**

Cell separation. Mononuclear cells were separated from heparinized venous blood by density centrifugation in a Ficoll-metrizoate gradient (Lymphoprep, Nyegaard, Oslo; density = 1.077).

**Fc receptors.** Ox red blood cells coated with rabbit IgG (Japan Immunoresearch Lab Co, Ltd, Japan) were added to the test cell suspension (10:1), which was then centrifuged at 200 g at room temperature, followed by incubation for 30 minutes at 4°C. Next, the cells were gently resuspended, centrifuged, and placed on slides using an Autosmear (Tomy Co, Ltd, Japan). After staining with May-Grünwald-Giemsa solution, they were observed. The criterion for positivity was more than three red cells per test sample.

Monoclonal antibodies. The cells from the three patients were examined for the expression of platelet-specific antigens using a monoclonal antiplatelet glycoprotein (GP) IB/IIa complex (Cappel Laboratories, West Chester, Pa). Bothuffy coat cells and mononuclear cells were used for the antiplatelet antibody examination.
Mononuclear cells (more than 85% blastic cells) from patient 1 were also studied for J5 (Coulter Electronics, Hialeah, Fla), OKT3, OKM1, and OKIa1 (Ortho Pharmaceuticals, Raritan, NJ). Binding of monoclonal antibodies was studied by indirect immunofluorescence. The test cells were incubated with the monoclonal antibody at 4 °C for 30 minutes. They were then washed twice and incubated with FITC-labeled IgG F(ab')2, fragments of goat anti-mouse globulin (Cappel Laboratories) for another 30 minutes at 4 °C. The cells were washed again, centrifuged, and resuspended in a minimal amount of medium, which was deposited into a small drop of 50% buffered glycerol on a coverslip. The objective slide was applied and the coverslip was sealed with nail polish. The slides were studied under an Olympus fluorescence microscope equipped with an epifluorescence condenser. The fluorescence examination was combined with a careful analysis of the cells under phase-contrast microscopy. At least 200 cells per slide were examined.

The positivity for the monoclonal anti-GP IIb/IIIa antibody was also examined by an indirect immunocytochemical technique using alkaline phosphatase as the indicator, according to the method of Li,18 with slight modifications. Smears or cytosin preparations from peripheral blood were fixed for 30 seconds with a buffered formalin-acetone mixture and rinsed with phosphate-buffered saline. The fixed, air-dried specimens were first incubated with the monoclonal antibody (1/20 dilution) for 30 minutes at room temperature and then rinsed with phosphate-buffered saline. They were further incubated with alkaline phosphatase-conjugated goat anti-mouse F(ab')2 (Tago, Burlingame, Calif) (1/30 dilution) for 20 minutes and rinsed with phosphate-buffered saline. The specimens were incubated for 30 minutes at 37 °C in the following medium: 50 mL barbital buffer, pH 7.6, 15 mg naphthol-AS-TR phosphate, sodium salt (Sigma, St Louis), 20 mg Fast red TR salt (Sigma), and 12 mg Levamisole (Sigma). They were then counterstained with Carrazi's hematoxylin and mounted in glycerol gelatin.

**Determination of Terminal Deoxynucleotidyl Transferase (TdT)**

Peripheral blood smears were stained for the presence of TdT using an indirect immunofluorescence method (Bethesda Research Lab, Rockville, Md).

**Detection of Cytoplasmic Factor VIII Antigens**

Mononuclear cells from three patients were fixed with ethanol or methanol and stained for the presence of factor VIII (FVIII) using indirect immunofluorescence. Both a conventional rabbit antihuman factor VIII antibody (DAKO, Copenhagen) and a monoclonal anti-FVIII-related antigen antibody (Cappel Laboratories) were used for the primary antibody.

**RESULTS**

**Optical Cytochemistry**

The reactions of the blast cells from the three patients are shown in Table 1. Only those from patient 1 exhibited the “megakaryocytic” pattern of nonspecific esterase, that is, the combination of a positive reaction to alpha-naphthyl acetate esterase and a negative alpha-naphthyl butyrate esterase reaction. Most of the cells showed strong positivity with a diffuse pattern for acid phosphatase. The activity was tar-

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
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<tbody>
<tr>
<td><strong>Age/sex</strong></td>
<td>55/F</td>
<td>14/M</td>
</tr>
<tr>
<td><strong>Splenomegaly</strong></td>
<td>Slight</td>
<td>—</td>
</tr>
<tr>
<td><strong>WBC (10⁹/L)</strong></td>
<td>5.0</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Blast (%)</strong></td>
<td>75</td>
<td>14</td>
</tr>
<tr>
<td><strong>Pro/myel (%)</strong></td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td><strong>Platelets (10⁹/L)</strong></td>
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<td>53</td>
</tr>
<tr>
<td><strong>Teardrop</strong></td>
<td>Slight</td>
<td>Nil</td>
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<tr>
<td><strong>Myelofibrosis</strong></td>
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<td>++</td>
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<tr>
<td><strong>Optical cytochemistry</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>MPO</strong></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>NAE</strong></td>
<td>+</td>
<td>+/—</td>
</tr>
<tr>
<td><strong>NBE</strong></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>ACID</strong></td>
<td>++</td>
<td>+/+</td>
</tr>
<tr>
<td><strong>PAS</strong></td>
<td>—</td>
<td>+/—</td>
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<td><strong>MPO (%)</strong></td>
<td>—</td>
<td>—</td>
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<tr>
<td><strong>Surface markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GP IIb/IIIa (%)</strong></td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>HLA-DR (%)</strong></td>
<td>88</td>
<td>NT</td>
</tr>
<tr>
<td><strong>Fc receptors (IgG)</strong></td>
<td>—</td>
<td>NT</td>
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</table>

NT, not tested.

NAE, alpha-naphthyl acetate esterase; NBE, alpha-naphthyl butyrate esterase.

*Percent in all blastoid cells.
†Percent in mononuclear cells.

Most of the blast cells from the three patients showed identical features. They had a round or oval nucleus with a single large nucleolus, a smooth to slightly irregular outline, and moderate chromatin condensation. There were various numbers of mitochondria, moderately long profiles of rough endoplasmic reticulum, a small Golgi apparatus, and a few granules. These granules differed in morphology from alpha granules; they were smaller than alpha granules and had homogeneous density. The plasma membrane was rather smooth. These findings were consistent with those of “undifferentiated” or “lymphoid” blasts. The presence of a few micromegakaryocytes, which had alpha granules and/or demarcation membranes, was confirmed in patients 2 and 3.

**Conventional Ultrastructure**

Most of the blasts from the three patients showed identical features. They had a round or oval nucleus with a single large nucleolus, a smooth to slightly irregular outline, and moderate chromatin condensation. There were various numbers of mitochondria, moderately long profiles of rough endoplasmic reticulum, a small Golgi apparatus, and a few granules. These granules differed in morphology from alpha granules; they were smaller than alpha granules and had homogeneous density. The plasma membrane was rather smooth. These findings were consistent with those of “undifferentiated” or “lymphoid” blasts. The presence of a few micromegakaryocytes, which had alpha granules and/or demarcation membranes, was confirmed in patients 2 and 3.

**Ultrastructural Cytochemistry**

All three patients demonstrated PPO positivity in the nuclear envelope and endoplasmic reticulum for more than 70% of blast cells (Table 1, Figs 1 through 3). None of the blasts from patients 1 and 2 showed peroxidase activity by the method of Graham-
Table 2. Previous Reports of Acute Megakaryoblastic Leukemia in Which Blast Cells Were Recognized as Megakaryoblasts by PPO Activity

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>Breton-Gorius</th>
<th>Bain</th>
<th>Bain</th>
<th>Bain</th>
<th>Bevan</th>
<th>Breton-Gorius</th>
<th>Mirchandani</th>
<th>Chan</th>
<th>Chan</th>
<th>Sasaki</th>
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<tbody>
<tr>
<td>Age/sex</td>
<td>80/M</td>
<td>60/M</td>
<td>74/M</td>
<td>45/M</td>
<td>14/F</td>
<td>2/M</td>
<td>4/F</td>
<td>66/M</td>
<td>15 mo/F</td>
<td>18 mo/F</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Slight</td>
<td>Slight</td>
<td>ND</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>WBC count (10³/L)</td>
<td>2.2</td>
<td>1.9</td>
<td>3.5</td>
<td>5.0</td>
<td>4.1</td>
<td>6.3</td>
<td>7.2</td>
<td>2.8</td>
<td>13.0</td>
<td>8.0</td>
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<tr>
<td>Blast (%)</td>
<td>80</td>
<td>10</td>
<td>20</td>
<td>24</td>
<td>30</td>
<td>10</td>
<td>13</td>
<td>19</td>
<td>19</td>
<td>Occasional</td>
</tr>
<tr>
<td>Pro/myel (%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Platelet (10⁹/L)</td>
<td>12</td>
<td>16</td>
<td>40</td>
<td>131</td>
<td>1,200</td>
<td>12</td>
<td>15</td>
<td>85</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Teardrop poikilocytes</td>
<td>ND</td>
<td>Slight</td>
<td>Nil</td>
<td>Slight</td>
<td>Marked</td>
<td>Nil</td>
<td>ND</td>
<td>ND</td>
<td>Slight</td>
<td>ND</td>
</tr>
</tbody>
</table>

Cytochemistry

| NAE       | ND | ND | + | + | + | ++ | ND | + | + | + | +^ |
| NBE       | ND | ND | ND | ND | – | +^ | ND | – | – | – | –^ |
| ACID      | ND | ND | +/+/+ | + | + | ND | ND | + | + | + | + |
| PAS       | –/+ | –/+ | –/+ | –/+ | – | + | ND | – | /+ | ND | –/+ |

Detection of PPO

<table>
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<tr>
<th>Method</th>
<th>Anderson</th>
<th>Roels</th>
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<th>Roels</th>
<th>Roels</th>
<th>Anderson</th>
<th>Anderson</th>
<th>Roels</th>
<th>Roels</th>
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<tr>
<td>% Positivity in blasts</td>
<td>80</td>
<td>all</td>
<td>all</td>
<td>all</td>
<td>all</td>
<td>all</td>
<td>Majority</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Demonstration in Golgi area</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Surface markers

| GP IIb | + %? | + ^ 75% |
| HLA-DR | —    | —     |
| Fc receptors | + ? | + ? |

ND, not described.
+ ^, weakly positive.

Fig 1. (Left) Blasts in the peripheral blood from patient 1 tested for PPO reaction. The section was not stained with lead citrate or uranyl acetate. The dense reaction of oxidized diaminobenzidine is located in the nuclear envelope and endoplasmic reticulum of the majority of blasts. Mitochondria also exhibit a reaction due to endogenous cytochrome oxidase. Gc indicates a Golgi cisternae negative for PPO (4,700x). (Inset) Enlargement of the Golgi zone (Gc) (9,600x). (Right) Peroxidase activity is not seen in blasts (patient 1), using the method of Graham-Karnovsky. PMN indicates polymorphonuclear leukocytes (4,700x).
MEGAKARYOBLASTIC LEUKEMIA

Karnovsky, and the PPO activity was inhibited by the addition of 0.02 mol/L AMT. In patient 3, about 20% of the blasts had peroxidase activity in a few granules, in addition to perinuclear cisternae and endoplasmic reticulum, when either the method of Anderson or Graham-Karnovsky was used. In patient 1, acid phosphatase activity was detected in the perinuclear space, rough endoplasmic reticulum, and a very few granules, but not in the Golgi cisternae (Fig 4).

Surface Membrane Markers

The monoclonal antiplatelet glycoprotein IIb/IIa complex antibody was present on a few of the cells from all three patients (Table 1). The results of both immunofluorescence and immunoalkaline phosphatase were identical. Some positive cells appeared to be micromegakaryocytes and matured megakaryocytes; others were clearly mononuclear round blastic cells (Figs 5 and 6). The platelets from a patient with thrombathenia type II were only partially positive for this antibody by the immunoalkaline phosphatase method (Fig 5). This antibody stained only mature and immature megakaryocytes in bone marrow and did not stain the blaslic cells from 12 patients with other types of leukemia, including three common acute lymphoblastic leukemias (ALL), four acute myeloblastic leukemias (AML), two acute prolymphoblastic leukemias (APL), two chronic myeloblastic leukemias (CML) in lymphoid blast crisis, and one myeloid blast crisis.

In patient 1, the blast cells were unreactive with J5, OKT3, and OKM1, but the majority reacted with OK1a (Fig 7). Fc receptors for IgG were not detected in the blasts from patients 1 and 3. No TdT-positive cells were detected in the peripheral blood of the three patients, and no positive cells for factor VIII were detected in the mononuclear cells. Normal mature megakaryocytes stained strongly for the conventional antibody and weakly, but clearly, for the monoclonal antibody.

DISCUSSION

Acute leukemia is usually classified on the basis of light microscopic morphology, with the occasional aid of cytochemical techniques. According to the uniform system of the French-American-British (FAB) Cooperative group,19 two groups of acute leukemias, "lymphoblastic" and "myeloid," are further subdivided into three (L1–L3) and six groups (M1–M6), respectively.
M1, M2, and M3 undergo predominantly granulocytic differentiation; M4 shows both granulocytic and monocytic differentiation; M5, predominantly monocytic differentiation; and M6, predominantly erythroid differentiation. There is no category of acute megakaryoblastic leukemia in this classification, which is probably due to the fact that recognition of megakaryoblasts has so far been impossible by routine light microscopic techniques.

Using conventional electron microscopy, it is also difficult to recognize megakaryoblasts unless characteristic structures, such as alpha granules and demarcation membranes, are observed; but these structures are rarely found. On the other hand, micromegakaryocytes, which are much smaller than normal megakaryocytes, have characteristic cytoplasmic features of mature megakaryocytes on both conventional electron microscopy and cytochemistry. The ploidy of micromegakaryocytes is known to be reduced to 8N or 4N, in contrast with that of mature normal megakaryocytes, which have 16N, 32N, or 64N. Several studies have described micromegakaryocytes in both the chronic stage and the transformed stage of chronic granulocytic leukemia, preleukemia, refractory anemia with an excess of myeloblasts, and acute leukemia.

Although there are no specific markers for identifying megakaryoblasts, Breton-Gorius and colleagues reported that platelet peroxidase (PPO), which has some characteristics distinct from myeloperoxidase (MPO), is synthesized early during megakaryocyte maturation and can be a specific marker for identifying megakaryoblasts. They later reported a case of chronic granulocytic leukemia in blast crisis, which they designated "megakaryoblastic crisis," and a case classified as "pure megakaryoblastic leukemia"; the majority of the blasts from these two patients exhibited peroxidase activity that was similar to PPO in all respects.

The PPO reaction is readily inhibited by aldehyde fixatives under conditions in which MPO activity is unaffected. Therefore, PPO activity is rarely demon-
MEGAKARYOBLASTIC LEUKEMIA

Fig 7. Mononuclear cells from patient 1 tested for monoclonal OKla antibody and observed by immunofluorescence. Majority of blasts are positive.

Fig 6. A mature megakaryocyte (top) and a megakaryoblast (bottom) in the peripheral blood from patient 3 positive for anti-GP Ila/IIla monoclonal antibody. P indicates platelets. Immunofluorescence (left) and phase-contrast (right).

reported the importance of nonspecific esterase reactions in recognizing the megakaryocytic precursors. They showed that the combination of a positive reaction to alpha-naphthyl acetate esterase and a negative alpha-naphthyl butyrate esterase reaction strongly suggests the megakaryocytic origin of the cell. This difference in nonspecific esterase reactions was confirmed by Castoldi, den Ottolander, and others. However, Hayhoe reported that this discrimination pattern was not always unequivocal. In this study, some blasts from patient 1 were clearly reactive for alpha-naphthyl acetate esterase, although not as strongly as that seen in monocytes or mature megakaryocytes. The blasts of patients 2 and 3 were too weakly positive to be recognized after nuclear stains. Alpha-naphthyl butyrate esterase was negative for all the blasts from these cases. Although the combination of positive acetate esterase and negative butyrate esterase, when found, may be almost pathognomonic, the presence of megakaryoblasts cannot be ruled out, even if alpha-naphthyl acetate esterase is not clearly positive.

In our patient 1, almost all of the blasts showed very strong activity, and exhibited the diffuse pattern described by others. Acid phosphatase is detected in blasts from various types of acute leukemia, and the pattern and intensity are sometimes useful for classifying acute leukemia. It is well known that acute lymphocytic leukemia of T cell origin is characterized by focal paranuclear acid phosphatase activity. Intensive acid phosphatase reaction is known to appear in diffuse patterns in acute monocytic leukemia and rarely in acute myelogenous leukemia. A distinctly positive acid phosphatase reaction in a diffuse pattern,
The presence of HLA-DR or Ia-like antigens in blast cells has been examined in only a few cases. In case 2 (P.P.) of Bevan et al., the HLA-DR antigen was not detected on the blasts that had PPO activity. However, in that patient, the majority of blasts also had Fc receptors and were stained with monoclonal antiplatelet glycoprotein I (AN 51). Therefore, those cells may have been more mature compared to those in patient 1. HLA-DR or Ia-like antigens are found not only on B cells, but also on the cells in the very early stages of erythropoiesis and granulopoiesis. Megakaryocytes and platelets are devoid of this antigen. However, it is reasonable to assume that cells at the early stage of megakaryocytic development also possess this antigen, as in the erythrocytic or granulocytic series.

Figure 8 illustrates the possible order of appearance of markers in the maturation of megakaryocytic cell lineage based on the present data and those previously reported. PPO activity appears in very immature cells that retain Ia-like (HLA-DR) antigens. Platelet-specific glycoprotein IIb/IIIa is seen at the level of round cells, which are only recognized by PPO activity. Glycoprotein Ib and Fc receptors appear in the later stage of maturation. It is reasonable to consider that FVIII-related antigens do not appear in blastic cells where alpha granules are not detected, although Innes et al., utilizing the anti-FVIII immunoperoxidase technique, described FVIII in small megakaryocyte precursors. As to the cytochemical reactions, acid phosphatase appears to be the earliest, with PAS activity occurring at a later stage.

Although the proliferating blasts were recognized as megakaryoblasts, the clinical and pathophysiologic...
MEGAKARYOBLASTIC LEUKEMIA

features were different among these three patients. The various clinical diagnoses in these patients run a broad spectrum from agnogenic myeloid metaplasia to acute myelofibrosis (malignant myelosclerosis) and acute leukemia; but, from our results, it is clear that selected cases in this spectrum are, in fact, diseases of platelet precursors.

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Megakaryoblastic leukemia: the characterization and identification of megakaryoblasts

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