Establishment of a Novel Human Megakaryoblastic Leukemia Cell Line, MEG-01, With Positive Philadelphia Chromosome

By Michinori Ogura, Yasuo Morishima, Ryuzo Ohno, Yukio Kato, Norio Hirabayashi, Hiroshi Nagura, and Hidehiko Saito

A megakaryoblastic cell line, designated MEG-01, was established from the bone marrow of a patient with blast crisis of Philadelphia (Ph\(^1\)) chromosome-positive chronic myelogenous leukemia. MEG-01 cells grew in single-cell suspension with a doubling time of 36 to 48 hours. Under the usual culture conditions, approximately half of the cells adhered to the culture flask with extension of pseudopods. MEG-01 cells were positive for the periodic acid-Schiff reaction, \(\alpha\)-naphthyl acetate esterase, and acid phosphatase, and negative for myeloperoxidase, \(\alpha\)-naphthyl butyrate esterase, naphthol AS-D chloroacetate esterase, and alkaline phosphatase. Ultrastructural platelet peroxidase was positive in MEG-01 cells. Cytoplasmic factor VIII (FVIII)-related antigen was weakly positive in larger MEG-01 cells by both an indirect immunofluorescent technique and a direct immunoperoxidase technique using monoclonal antibodies and a direct immunoperoxidase technique using horseradish peroxidase-conjugated conventional rabbit anti-human FVIII antibody. Platelet glycoprotein (GP) Ib/IIa antigen was uniformly demonstrated on the surface of MEG-01 cells by both indirect immunofluorescent and immunoperoxidase techniques using antiplatelet GP Ib/IIa monoclonal antibodies; platelet GP Ib antigen was demonstrated only in the cytoplasm of larger MEG-01 cells. MEG-01 cells possessed no markers for B or T lymphocytes or for myeloid cells. Chromosome analysis of this cell line revealed a human male hyperdiploid karyotype with a modal chromosome number of 56 to 58. The Ph\(^1\) chromosome was observed in all karyotypes analyzed. This novel human megakaryoblastic cell line may provide a useful model for the study of human megakaryopoiesis and of the biosynthetic mechanisms of proteins unique to megakaryocytic lineage.

THE PHYSIOLOGIC mechanisms governing the differentiation of human megakaryocyte and platelet release remain largely unknown. In order to study these mechanisms, a permanent cell line of human megakaryocytic lineage has been long awaited, since the collection of a sufficient number of megakaryocytes from bone marrow as well as their long-term cultures is difficult. Recently, an occurrence of megakaryoblastic crisis of chronic myelogenous leukemia (CML) has been reported that was recognized by the presence of characteristic ultrastructural platelet peroxidase (PPO) or by the presence of platelet glycoprotein (GP) or factor VIII (FVIII) related antigen detected by immunofluorescent or immunoperoxidase techniques using monoclonal or conventional antibodies.\(^1\)\(^4\) We report here the establishment of a novel human megakaryoblastic leukemia cell line (MEG-01) from a patient with Philadelphia chromosome (Ph\(^1\))-positive CML in blast crisis. To the best of our knowledge, this is the first human cell line of megakaryocytic lineage ever reported in a full paper, and we believe that MEG-01 will provide a useful model for the study not only of human megakaryocytic differentiation and maturation, but of production and release of platelets or proteins such as FVIII-related antigen, platelet glycoproteins, or platelet-derived growth factor (PDGF).

MATERIALS AND METHODS

Case History

The cell line described in this report was derived from a bone marrow sample from a 55-year-old male with blast crisis of Ph\(^1\).

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buthylate esterase, α-naphthyl acetate esterase, myeloperoxidase (MPO), leukocyte alkaline phosphatase, acid phosphatase, and the periodic acid-Schiff (PAS) reaction.

Ultrastructural Studies

Ultrastructural morphology (conventional thin electron microscopy). Cultured cells were centrifuged at 1,200 rpm for ten minutes, then washed three times in 0.01 mol/L phosphate buffer, fixed in 2.5% glutaraldehyde for one hour, and post-fixed in 1% osmium tetroxide for one hour. The samples were dehydrated in a graded series of ethanol and embedded in Araldite (Nissin Co, Tokyo). Ultrathin sections were then stained with 1% uranyl acetate and Reynolds's lead citrate, and examined in a Hitachi (Tokyo) H-300 electron microscope.

Ultrastructural MPO. Cells obtained after centrifugation were washed three times in 0.01 mol/L phosphate buffer, fixed for 30 minutes at 4°C with 1.25% glutaraldehyde in 0.1 mol/L phosphate buffer, and then washed three times in 0.01 mol/L PBS. They were incubated for 30 minutes in a dark room at room temperature in diaminobenzidine tetrahydrochloride (DAB) medium prepared as follows: 5 mg of 3.3-DAB (Sigma Chemical Co, St Louis) in 10 mL of Tris-HCl buffer containing 0.01% hydrogen peroxide, pH 7.6. All preparations were washed twice in 0.1 mol/L phosphate buffer, post-fixed in 1% osmium tetroxide solution, and then processed as indicated. No post-staining was performed to evaluate the dense reaction product of oxidized DAB.

Ultrastructural PPO. After being washed in Hanks's balanced salt solution, cells were fixed in a tannic acid aldehyde mixture by a modification of the method of Anderson et al for one hour at 4°C. The fixative consisted of 1% tannic acid, 2% paraformaldehyde, and 0.5% glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.2. Cells were then washed in 0.01 mol/L phosphate buffer, and incubated in DAB medium as described by Breton-Gorius and Guichard, in the dark at room temperature for one hour. DAB medium for PPO was as follows: 20 mg of DAB in 10 mL of 0.05 mol/L Tris-HCl buffer containing 0.1 mL of hydrogen peroxide. All preparations were then processed in the same manner as the MPO samples. No post-staining was done to evaluate PPO reaction.

Cell Surface Markers

Rosette markers. Sheep erythrocytes (SRBCs), mouse erythrocytes (MRBCs), SRBCs sensitized with anti-SRBCs rabbit IgG and IgM, and SRBCs sensitized with IgM antibody–complement complex were used for E, MRBC, EA, and EAC rosetting, respectively.

Surface immunoglobulins. Cell surface immunoglobulins on living cells were analyzed by incubating cells with fluorescein isothiocyanate (FITC)-labeled polyvalent goat anti-human immunoglobulin or monospecific goat anti-human IgG, IgA, IgM, IgD, anti-k, or anti-λ (Cappel Laboratories, West Chester, Pa) for one hour at 0°C after the cells had been treated with acetous buffer.

Monoclonal antibodies. The expression of platelet-specific antigens of cells was analyzed by using monoclonal antibodies against the platelet GP Ib/IIa complex (one purchased from Cappel Laboratories and the other, HPL-3, prepared by Dr K. Furukawa at the Department of Medicine, Nagoya University Branch Hospital), platelet GP Ib (HPL-9, prepared by Dr Furukawa), and FVIII-related antigen (Cappel Laboratories). The cells were also analyzed for 13 other kinds of monoclonal antibodies directed to differentiation antigens of hematopoietic cells. The specificity of each antibody is described in Table 2.

Binding of monoclonal antibodies was tested by indirect immunofluorescence technique. Cells were incubated with each monoclonal antibody at 4°C for 30 minutes, then washed twice by cold PBS with 1% bovine serum albumin and 0.4% sodium azide, and then incubated with FITC-labeled IgGF(ab')2, fragments of goat anti-mouse IgG or IgM (Cappel Laboratories) for 30 minutes at 4°C. The cells were again washed and analyzed by flow cytometry ( Spectrum III, Ortho Diagnostic Systems, Raritan, NJ). Some samples were resuspended in a minimal amount of medium, which was deposited into one or two drops of 50% buffered glycerol on a coverslip. The samples were examined and photographed under an Olympus fluorescence microscope.

The reactivity of MEG-01 cells to the monoclonal anti-GP Ib/IIa or GP Ib antibodies was also examined by an indirect peroxidase-labeled antibody technique as previously described. Cells were centrifuged and washed, then the cell pellet was fixed for four hours at 4°C with periodate-lysine–parafomaldehyde (PLP) solution containing 2% parafomaldehyde. The cell pellet was washed in increasing concentrations of sucrose in PBS, frozen in OCT compound (Lab Tek Products, Naperville, Ill), and sectioned (8 μm thick) on a cryostat microscope. The sections were placed on albumin-coated slides, then air-dried for 30 minutes and rinsed with cold PBS. They were reacted with 10% non-immune goat serum and then with each monoclonal antibody for 12 hours at 4°C. After rinsing with cold PBS, they were incubated with horseradish peroxidase (HRP)-conjugated anti-mouse F(ab')2 (Cappel Laboratories) for six hours at 4°C. After excess antibody reagents were washed off with cold PBS, the sections were reacted with 0.25% DAB solution in 50 mmol/L Tris-HCl buffer, pH 7.6, containing 10 mmol/L hydrogen peroxide. They were washed with distilled water and counterstained with veronal-acetate–buffered 1% methyl green solution, pH 4.0. Fresh leukemic blast cells (mononuclear cells) from the bone marrow which was separated by Ficoll-Conray gradient centrifugation on May 9, 1983, were also analyzed for the expression of platelet GP Ib/IIa antigen by an indirect IF technique using the same two kinds of monoclonal antibodies. Normal mouse serum was used as a negative control of the first antibody in all experiments.

Cytoplasmic FVIII Antigens

Two kinds of monoclonal antibodies (one purchased from Cappel Laboratories and the other from Dako, Glostrup, Denmark) and HRP-conjugated conventional rabbit anti-human FVIII antibody (Dako) were used for the study of cytoplasmic FVIII-related antigens. Cells were centrifuged, washed, then fixed with either ethanol, acetone, or methanol, and analyzed by an indirect IF technique. A direct peroxidase-labeled antibody technique was also performed as described previously. For the former experiment, normal mouse serum was used as a negative control of the first antibody and for the latter experiment, thin sections of small intestine were used as control tissues. Cytoplasmic FVIII-related antigen of fresh leukemic blast cells from the patient's bone marrow was analyzed by an indirect immunofluorescence technique using two kinds of monoclonal antibodies as described above.

Epstein-Barr Virus Nuclear Antigen (EBNA) and Terminal Deoxynucleotidyl Transferase (TdT)

EBNA was tested by the method of Reedman and Klein with a slight modification. TdT was assayed by an indirect immunofluorescence technique using a reagent kit (Bethesda Research Lab, Rockville, Md).

Lysozyme Activity and Phagocytosis

Test for intracytoplasmic lysozyme activity was performed by the immunoperoxidase method (Bethesda Research Lab). Phagocytosis was tested by immunophagocytosis and determined by light microscopy on MGG-stained slides after incubation of MEG-01 cells with opsonized zymosan (Sigma Chemical Co) at 37°C for 30 minutes.
Chromosome Analysis

The cultured cells were incubated in the presence of Colcemid (Gibco Laboratories, Grand Island, NY) at 0.05μg/mL, for one hour at 37 °C, then treated with 75 mmol/L KCl hypotonic solution for 30 minutes at 37 °C, and fixed by methanol-acetic acid (3:1). Chromosomes were banded by the trypsin–Giemsa methods. Karyotypes were constructed from photographic enlargements according to the Paris Conference classification scheme.

RESULTS

Cell Culture

Approximately four weeks after the beginning of the culture, cells consistently proliferated as single suspension with a doubling time of 36 to 48 hours. At present, the cells have been in culture for more than 18 months and were designated as MEG-01. Cultures are free from Epstein-Barr virus and mycoplasma contamination.

Morphology and Cytochemistry of Fresh Leukemia Cells

Figure 1A illustrates the leukemic blasts observed in MGG-stained preparation from the patient’s fresh bone marrow specimen. Blasts were mostly round or ovoid with a modal diameter of about 30 to 40 μm, and had large round to ovoid nuclei with fine chromatin and few prominent nucleoli. Cytoplasms were basophilic and contained several vacuoles. Several cytoplasmic protrusions were frequently observed.

Fig 1. (A) MGG staining of fresh leukemic blasts, showing round to ovoid nuclei and peripheral cytoplasmic protrusions (original magnification ×100; current magnification ×132). (B) MGG staining of the patient’s bone marrow, showing numerous micromegakaryocytes (original magnification ×100; current magnification ×132).

Fig 2. (A) Inverted photomicrograph of MEG-01 cells cultured for two days. MEG-01 cells were primarily grown in single-suspension cultures, but a few large cells attached to the plastic culture dish with elongated cytoplasmic extension are seen. Budding is evident at the tips of the cytoplasmic extension (original magnification ×100; current magnification ×132). (B) Smear of MEG-01 cells stained with MGG staining. Abundant cytoplasmic vacuoles and cytoplasmic protrusions are demonstrated. Almost no granules are observed (original magnification ×100; current magnification ×132). (C) The PAS reaction of smeared MEG-01 cells. In a positive cell, cytoplasmic protrusions are granularly positive (original magnification ×100; current magnification ×132). (D) Alpha-naphthyl acetate esterase staining of MEG-01 cells. Almost all cells are granularly positive (original magnification ×100; current magnification ×132). (E) Acid phosphatase stainings of MEG-01 cells. All cells are strongly positive (original magnification ×100; current magnification ×132). (F) MPO staining of MEG-01 cells. All cells are uniformly negative (original magnification ×100; current magnification ×132). (G) Dual esterase staining for naphthol ASD chloroacetate and α-naphthyl butyrate esterases of MEG-01 cells. No positivity is observed for dual esterases (original magnification ×100; current magnification ×132). (H) Alkaline phosphatase staining of MEG-01 cells. All cells are negative (original magnification ×100; current magnification ×132).
No Auer rods were observed. Positivity was noted with \(\alpha\)-naphthyl acetate esterase and acid phosphatase; weak positivity was noted with the PAS reaction. However, MPO, naphthol ASD chloroacetate and \(\alpha\)-naphthyl butyrate esterases, and neutrophil alkaline phosphatase were uniformly negative. Figure 1B shows abundant micromegakaryocytes in the patient's bone marrow.

Morphology and Cytochemistry of MEG-01 Line Cells

MEG-01 line proliferated in single suspension without forming cell clumps. Approximately one half of the cells adhered to plastics or glass, extending pseudopods. Among these adherent cells, multinucleated cells whose diameters were approximately two to three times larger than single suspended cells were observed (Fig 2A). Like the original blasts, MEG-01 cells were mostly round to ovoid with almost the same modal diameter, and had large round to ovoid nuclei with fine chromatin and one or more prominent nucleoli. Cytoplasms were relatively basophilic and contained a few vacuoles. Cytoplasmic protrusions were also observed (Fig 2B). The PAS reaction was granularly positive in about 20% to 40% of MEG-01 cells in their peripheral cytoplasms and cytoplasmic protrusions (Fig 2C). Fifty to sixty percent of the cells were positive for \(\alpha\)-naphthyl acetate esterase (Fig 2D). These cytochemical reactions were stronger in the adherent cells. All cells were strongly positive for acid phosphatase in a diffuse and finely granular fashion (Fig 2E). No positivity was noted for MPO, naphthol AS-D

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**Fig 3.** (A) Electron microscopic photograph of MEG-01 cells. A peripherally located nucleus and several mature cytoplasmic structures, including mitochondria, rough endoplasmic reticulum, and Golgi apparatus, are noted. No demarcation membranes and \(\alpha\)-granules are evident (original magnification \(\times 7,020\); current magnification \(\times 50,544\)). (B) Ultrastructural demonstration of PPO of the MEG-01 cell. The nuclear envelope and short segments of endoplasmic reticulum contain the dense reaction of oxidized DAB. The Golgi apparatus appears unreactive (original magnification \(\times 6,500\); current magnification \(\times 46,800\)).
chloroacetate and α-naphthyl butyrate esterases, or alkaline phosphatase (Fig 2F through H).

**Ultrastructural Morphology (Fig 3A)**

MEG-01 cells had one round to ovoid indolob nucleus with a smooth to slightly irregular outline, moderately condensed chromatin, and one or more large nucleoli. The cytoplasm contained numerous mitochondria, moderately long segments of rough endoplasmic reticulum, a small Golgi apparatus, and a few granules. Neither demarcation membranes nor α-granules were observed.

**Ultrastructural Cytochemistry**

PPO positivity was noted in the nuclear envelope and in the segments of endoplasmic reticulum in MEG-01 cells (Fig 3B). The Golgi apparatus appeared unreactive for PPO. MPO was uniformly negative by the method of Graham-Karnovsky.

**Surface and Cytoplasmic Markers**

The results of cell surface and cytoplasmic marker tests are summarized in Tables 1 through 3. E rosette, MRBC rosette, and surface immunoglobulins were uniformly negative in both original blasts and MEG-01 cells. EA tests for IgG-Fc receptor and IgM-Fc receptor were positive in 12% and 0% of MEG-01, respectively. The EAC test for the C3 receptor was positive in 53% of MEG-01.

By an indirect immunofluorescence technique using the two kinds of monoclonal antibodies, platelet GP IIb/IIIa antigen was demonstrated strongly on the cell membrane of more than 92% of MEG-01 cells (Fig 4). By an indirect immunoperoxidase technique of fixed cryostat sections of cells, platelet GP IIb/IIIa was strongly positive both on cell membranes and in the cytoplasm of >94% of MEG-01 cells (Fig 5A). Platelet GP IIb/IIIa antigen was also expressed on the cell surface of >90% of fresh leukemic blasts. Platelet GP Ib antigen was not detected on the surface by an indirect immunofluorescence technique, but was demonstrated in the cytoplasm of approximately 20% of MEG-01 cells by an indirect immunoperoxidase technique. The positive cells were mainly larger cells (Fig 5B). BA-I antigen was expressed on almost all MEG-01 cells. CDw14 (20.3 antigen) was weakly expressed on the surface of 25% of MEG-01 cells, especially large cells. Although FVIII-related antigen was negative on the surface of MEG-01 cells by an indirect immunofluorescence assay with the monoclonal antibody, this antigen was positive in the cytoplasm of MEG-01 cells, especially large cells, by both indirect immunofluorescence

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**Table 1. Rosetting Markers of MEG-01 Cells**

<table>
<thead>
<tr>
<th>Rosetting</th>
<th>Positive Cells (%)</th>
</tr>
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<tbody>
<tr>
<td>E</td>
<td>0</td>
</tr>
<tr>
<td>MRBC</td>
<td>0</td>
</tr>
<tr>
<td>EA</td>
<td>12</td>
</tr>
<tr>
<td>IgG-Fc</td>
<td>0</td>
</tr>
<tr>
<td>IgM-Fc</td>
<td>53</td>
</tr>
</tbody>
</table>

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**Table 2. Cell Surface Antigens on MEG-01 Cells**

<table>
<thead>
<tr>
<th>Monoclonal Antibody (from Cappel Laboratories)</th>
<th>Specificity</th>
<th>Positive Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiplatelet GP IIb/IIIa</td>
<td>Platelet GP IIb/IIIa</td>
<td>92</td>
</tr>
<tr>
<td>HPL-3</td>
<td>Platelet GP IIb/IIIa</td>
<td>93</td>
</tr>
<tr>
<td>HPL-9</td>
<td>Platelet GP Ib</td>
<td>Negative</td>
</tr>
<tr>
<td>anti-FVIII</td>
<td>FVIII-related antigen</td>
<td>Negative</td>
</tr>
<tr>
<td>4D-1</td>
<td>Human la antigen</td>
<td>Negative</td>
</tr>
<tr>
<td>NL-1</td>
<td>Common acute lymphocytic leukemia antigen (CD10)</td>
<td>Negative</td>
</tr>
<tr>
<td>BA-1</td>
<td>B cell, granulocyte</td>
<td>100</td>
</tr>
<tr>
<td>BA-2</td>
<td>Monocyte (CD9)</td>
<td>Negative</td>
</tr>
<tr>
<td>B1</td>
<td>Pan-B cell (CD19)</td>
<td>Negative</td>
</tr>
<tr>
<td>35.1</td>
<td>SRBC receptor (CD2)</td>
<td>Negative</td>
</tr>
<tr>
<td>10.2</td>
<td>Pan-T cell (CD5)</td>
<td>Negative</td>
</tr>
<tr>
<td>12.1</td>
<td>Mature T cell (CD6)</td>
<td>Negative</td>
</tr>
<tr>
<td>4A</td>
<td>Pan-T cell (CD7)</td>
<td>Negative</td>
</tr>
<tr>
<td>Leu 7</td>
<td>Natural killer cell</td>
<td>Negative</td>
</tr>
<tr>
<td>20.2</td>
<td>Monocyte, granulocyte (CD12)</td>
<td>Negative</td>
</tr>
<tr>
<td>20.3</td>
<td>Monocyte, platelet (CD14)</td>
<td>25</td>
</tr>
<tr>
<td>1G10</td>
<td>Granulocyte (CD16)</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Negative, <8% of the cells was positive under the condition in which the negative control showed 5% of positive background; CD, the cluster designation by First and Second International Workshops on Human Leucocyte Differentiation Antigens.15

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**Table 3. Cytoplasmic Antigens in MEG-01 Cells**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Positive Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiplatelet GP IIb/IIIa*</td>
<td>94</td>
</tr>
<tr>
<td>Cappel Laboratories</td>
<td></td>
</tr>
<tr>
<td>HPL-3</td>
<td>95</td>
</tr>
<tr>
<td>Antiplatelet GP Ib*</td>
<td>20</td>
</tr>
<tr>
<td>HPL-9</td>
<td></td>
</tr>
<tr>
<td>Anti-FVIII-related antigen</td>
<td>25</td>
</tr>
<tr>
<td>Cappel: monoclonal†</td>
<td></td>
</tr>
<tr>
<td>Dako: monoclonal†</td>
<td>24</td>
</tr>
<tr>
<td>Dako: HRP-conjugated conventional‡</td>
<td>26</td>
</tr>
</tbody>
</table>

*Analyzed by an indirect immunoperoxidase technique.†Analyzed by an indirect immunofluorescent technique.‡Analyzed by a direct immunoperoxidase technique.
with two kinds of monoclonal antibodies and a direct immunoperoxidase technique with HRP-conjugated conventional rabbit anti-human FVIII antibody (Fig 5C). Cytoplasmic FVIII-related antigen was expressed in approximately 40% of fresh leukemic blasts. Ia antigens, T cell differentiation antigens such as CD2, CD5, CD6, and CD7, and B cell-related antigens such as CD9, CD10, CD19, and granulocyte/monocyte differentiation antigens such as CD12 and CDw15 were not demonstrated on the cell surface of MEG-01.

**Other Activity**

Neither EBNA nor TdT was detected in the fresh leukemia and MEG-O1 cells. MEG-O1 cells had no lysozyme and phagocytic activities.

**Chromosome Studies**

Chromosomal analysis of a total of 44 MEG-O1 cells revealed that the karyotype was hyperdiploid, with a modal chromosome number of 56 to 58 (range, 50 to 113). The karyotype of the modal number was 57XY, +2, +3?, +9q+, +11, +19, +19, +21, t(9;22), +4mar (Fig 6). The Ph1 chromosome [(t(9;22))] was positive in all karyotypes analyzed. A few double minute chromosomes were also observed.

**DISCUSSION**

Attempts to develop permanent suspension cultures of human leukemia cells have been made by many investigators and a considerable number of permanent lymphoid, myeloid, or erythroid cell lines have been established. Nevertheless, there have been no full reports of the successful establishment of a human cell line with characteristics of megakaryocytic lineage, in spite of recent identification of megakaryoblastic crisis of CML and acute megakaryoblastic leukemia. Morgan et al, however, reported the establishment of human megakaryocyte cell lines in abstract forms. We report here the establishment of a novel human megakaryoblastic leukemia cell line (MEG-O1) from the bone marrow of a 55-year-old man with Ph1-positive CML in megakaryoblastic crisis.

By routine light microscopic examinations, it is quite difficult to identify megakaryoblasts, even though megakaryocytes are known to stain positively by the PAS reaction and to have α-naphthyl acetate esterase and acid phosphatase activity. By conventional electron microscopy, it is also very difficult to recognize megakaryoblasts unless characteristic structures such as α-granules and demarcation membranes are identified, and these structures are seldom found in early megakaryoblasts. However, more specific markers for megakaryocytic lineage have been recognized recently, including (1) PPO demonstrable by ultrastructural cytochemistry, (2) platelet membrane GP identified by immunofluorescent or immunoutrastructural assay with monoclonal antibodies (eg, AN51 or J15), and (3) FVIII-related antigens identified by immunofluorescent or...
immunoperoxidase assays with monoclonal or conventional polyclonal antibodies.

In MEG-01 cells, a combination of a strongly positive reaction to α-naphthyl acetate esterase and a negative reaction to α-naphthyl butyrate esterase was demonstrated, and a strongly positive reaction to acid phosphatase was also observed with a diffuse staining pattern. The PAS reaction was positive in 40% to 50% of the cells in suspension culture and in most of the adherent cells, and the staining pattern was coarsely granular in the peripheral cytoplasm. The reaction was very strong in the cytoplasmic protrusions of the adherent cells. MPO was uniformly negative. These cytochemical reactions and staining patterns of MEG-01 cells, especially of the adherent cells, are compatible with those of normal megakaryocytes. Some of the adherent cells are multinucleated and two to five times larger than single suspension cells, and morphologically resemble megakaryocytes. The findings strongly suggest a possibility that MEG-01 is of megakaryocytic origin.

Breton-Gorius and colleagues reported that PPO, which has distinct characteristics and localization pattern from MPO, was synthesized during early megakaryocytic differentiation and could be a specific enzymatic marker for the recognition of megakaryoblasts. MEG-01 cells exhibited the ultrastructural peroxidase activity characteristic of PPO and did not show that of MPO. Rabellino et al reported that both human megakaryocytes and platelets had IgG-Fc receptors at 95% ± 3% and 85% ± 3%, respectively, and Koike reported that IgG-Fc receptors appeared in the later stage of megakaryocytic maturation. This receptor was demonstrated in 12% of the MEG-01 cells. Vainchenker et al reported the valuable utilization of monoclonal antiplatelet antibodies for the detection of antigenic markers of human megakaryocytic maturation and differentiation. According to their reports, GP Ib recognized by AN51 is progressively expressed during megakaryocytic maturation and serves as a marker of the later stage of megakaryopoiesis. On the other hand, the GP IIb/IIIa complex recognized by J15 is reported to be an early antigenic marker of megakaryocytic maturation. We used two kinds of antiplatelet GP IIb/IIIa monoclonal antibodies; one was purchased from Cappel Laboratories and the other (HPL-3) was provided by Dr Furukawa. Both antibodies react with normal platelets but not with platelets from patients with Glanzmann's thrombasthenia. The antibody from Cappel Laboratories inhibits fibrinogen binding and aggregation induced by collagen and epinephrine in citrated platelet-rich plasma, and HPL-3 also inhibits fibrinogen binding and aggregation induced by collagen and adenosine diphosphate (ADP). We also used an antiplatelet GP Ib monoclonal antibody (HPL-9), which reacts with normal platelets but not with platelets from patients with Bernard-Soulier syndrome and which blocks platelet aggregation induced by ristocetin. More than 92% of the MEG-01 cells showed strongly positive reactions to both antibodies on their cell surfaces. On the other hand, platelet GP Ib, defined by HPL-9, was demonstrated in the cytoplasm mainly of the large adherent MEG-01 cells but not on the cell surfaces.
These results indicate that MEG-01 are cells at the early stage of megakaryopoiesis and that the large adherent MEG-01 are cells that have differentiated into a slightly later stage.

Vinci et al reported that FVIII-related antigen was detected as a diffuse and often weak labeling pattern in promegakaryoblasts. Innes et al also reported a case of megakaryocytic leukemia in which FVIII was detected in the megakaryoblasts, micromegakaryocytes, and mature megakaryocytes by an immunoperoxidase technique. However, Koike reported three cases of megakaryoblastic leukemia in which no positive blasts for FVIII were demonstrated. In MEG-01 cells, we detected positive reaction to FVIII antigen in the cytoplasm, especially of large adherent cells by both an indirect immunofluorescence technique and a direct immunoperoxidase technique. Although Vinci et al reported that HLA-DR or Ia-like antigens were absent in mature megakaryocytes or platelets but present in most of promegakaryoblasts, MEG-01 cells demonstrated no HLA-DR or Ia-like antigens.

BA-1 antigen, reportedly positive on B lymphocytes and granulocytes, was granulocytic on almost all MEG-01 cells. Abramson et al reported positive BA-1 antigen on an erythroleukemia cell line, K-562. We also found BA-1 antigen on a MPO-positive cell line from CML in blast crisis which was established in our laboratory (unpublished data). Thus, BA-1 antigen seems to be present on a rather wide variety of hematopoietic cells, including some of megakaryocytic lineage.

CDw14 (20.3 antigen), reportedly expressed on platelets and monocytes, was demonstrated on approximately 25% of MEG-01 cells, most of which were the large adherent cells. Thus, CDw14 antigen seemed to be expressed on the relatively mature MEG-01 cells.

The presence of platelet GP IIb/IIIa antigen and PPO and the absence of α-granules and demarcation membranes suggest that MEG-01 cells are megakaryoblasts in a relatively early stage. However, strong reactivity to the PAS reaction and the presence of cytoplasmic FVIII-related antigen and platelet GP Ib mainly in the large adherent cells, all of which become positive in the cells at the later stage of megakaryopoiesis, may indicate a heterogeneity of MEG-01 cells and/or the differentiating or maturing possibility, with a morphological change in vitro. More precise cloning of this cell line may clarify this point.

Chromosomal analysis of MEG-01 showed a complicated karyotype, with chromosome numbers ranging from 50 to 113. The Ph' chromosome and XY chromosomes were observed in all karyotypes analyzed. This supports the idea that MEG-01 originates from a male patient with Ph' positive CML in blast crisis.

The K562 cell line, which originated from CML in myeloid blast crisis, is known as a human proerythroblastic cell line. Although Gewirtz et al reported the spontaneous expression of platelet GP antigen on the surface of undifferentiated K562 cells, using a rabbit antiserum directed against purified platelet GP, Bai et al reported that platelet GP IIb/IIIa was not detected on K562 cells. In contrast to the K562 line, almost all MEG-01 cells constantly demonstrated platelet GP IIb/IIIa and had no markers of myeloid lineage. These findings support the idea that the MEG-01 line is a human megakaryoblastic leukemia cell line.

Weinstein et al reported a line of rat promegakaryoblasts which contained FVIII-related antigen and fibrinogen in their cytoplasm and was capable of maturing in vitro to the megakaryocyte stage. Like the rat promegakaryoblast line, MEG-01 cells gradually attached to plastic culture dishes, exhibiting elongated cytoplasmic extensions spontaneously in the RPMI 1640 medium containing 10% FCS. We are now studying whether these adherent and elongated MEG-01 cells with cytoplasmic extensions are really more mature megakaryoblasts or megakaryocytes, and whether these cells possess coagulation factors such as FVIII, fibrinogen, or platelet factor 4.

We believe that the MEG-01 line will provide a useful model for the study of human megakaryocytic maturation and differentiation. It may also contribute to the clarification of the biosynthetic mechanisms of proteins unique to the megakaryocytic lineage such as PDGF, and may provide a useful tool for the genetic biosynthesis of platelet GP and FVIII. MEG-01 will also contribute to the pathophysiologic analysis of megakaryoblastic leukemia and other diseases involving megakaryopoiesis.

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Establishment of a novel human megakaryoblastic leukemia cell line, MEG-01, with positive Philadelphia chromosome

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