Establishment of a New Peroxidase-Positive Human Myeloid Cell Line, PL-21

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A myeloid cell line, designated PL-21, was established from the peripheral blood of a patient with acute promyelocytic leukemia. The PL-21 cell line grew in single-cell suspension, with a doubling time of 48–64 hr, and consisted of promyelocytes with fine immature nuclei and prominent azurophilic granules in the cytoplasm. PL-21 cells were positive for peroxidase, naphthol AS-D chloroacetate esterase, and Sudan Black B staining. Under the usual culture conditions, a small proportion of these cells differentiated into mature granulocytes, and this differentiation was enhanced by the addition of dimethyl sulfoxide in the culture medium. PL-21 cells had receptors for the Fc portion of IgG and complement, intracytoplasmic lysozyme and phagocytic activity, but lacked Epstein-Barr virus-associated nuclear antigen. Chromosome analysis of this cell line revealed a human male polyploid karyotype with 1q+ and double minute chromosomes. This new myeloid cell line may provide useful material for the study of proliferation and differentiation of human leukemia cells.

Establishment of human myeloid cell lines has been difficult. Many attempts to grow leukemia cells from patients with myeloid leukemia resulted in the growth of lymphoblastoid cells that carried Epstein-Barr (EB) virus.1-2 Collins et al.3 first reported the establishment of acute promyelocytic leukemia (APL) cell line HL-60 in 1977, and in the following year, Koefler and Golde4 succeeded in establishing the acute myelocytic leukemia (AML) cell line KG-1. These myeloid cell lines have contributed to the understanding of the proliferation and differentiation of human myeloid leukemia cells.5 Recently, we have established a continuous culture line of peroxidase-positive myeloid cells from an APL patient. This article describes the establishment and characterization of this new cell line, PL-21. Some properties of PL-21 have been briefly reported previously.6

CASE REPORT

First Admission

A 23-yr-old man was referred to Kochi Municipal Central Hospital with a diagnosis of mediastinal tumor on February 14, 1980. On admission, physical examination revealed neither lymphadenopathy nor hepatosplenomegaly. His peripheral blood showed hemoglobin (Hb) 15 g/dl, hematocrit (Ht) 45%, RBC 564 × 10^6/cu mm, platelets 18 × 10^6/cu mm, and WBC 7,800/cu mm with 3% bands, 62% segmented neutrophils, 1% eosinophils, 1% monocytes, and 33% lymphocytes. After mediastinal irradiation of 2,000 rads, which induced a marked decrease in tumor size on chest x-ray films, resection of the tumor was performed. The tumor was located anteriorly to the upper part of the heart between both lungs, with slight adhesion to the contiguous tissues. The histologic examination of the tumor revealed only dense fibrous tissue with little cellular component. Postoperatively, the patient was given an additional irradiation of 3,000 rads and was discharged on May 19, 1980.

Second Admission

The patient had been well until January 1981, when he developed chest pain. Chest x-ray films revealed pleural effusion on the right. Cytologic examination showed that more than 90% of pleural effusion cells were immature myeloid cells, with many azurophilic granules in the basophilic cytoplasm. However, the peripheral blood picture at that time showed no abnormality. Chemotherapeutic agents were injected into the thoracic cavity, and the pleural effusion diminished. On March 31, 1981, his WBC was 5,200/cu mm with 1% myeloblasts, 33% promyelocytes, 8% myelocytes, 4% metamyelocytes, 1% bands, 4% segmented neutrophils, 1% eosinophils, 1% basophils, 9% monocytes, and 38% lymphocytes. Combination chemotherapy with daunorubicin, cytosine arabinoside, 6-mercaptopurine, and prednisolone was ineffective, and his WBC rose to 43,800/cu mm; the patient died of massive pericardial effusion on April 24, 1981. No evidence of disseminated intravascular coagulation (DIC) was observed throughout the course.

MATERIALS AND METHODS

Cell Culture

On April 18, 1981, the WBC rose to 35,200/cu mm with 17% myeloblasts and 50% promyelocytes. At that time, 5 ml of peripheral blood was obtained, and leukocytes were separated by Ficoll-Conray gradient centrifugation. The cells were cultured at 5 × 10^6/ml in 35-mm Petri dishes using RPMI 1640 supplemented with 15% fetal calf serum (FCS) plus 15% human cord blood serum (HCS). The cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 in air and fed once or twice a week.

Cytochemical Staining

The cells were stained with May-Grünwald-Giemsa (MGG) and evaluated for peroxidase, naphthol AS-D chloroacetate esterase, α-naphthyl butyrate esterase, Sudan black B, acid phosphatase, neutrophil alkaline phosphatase, and periodic acid-Schiff (PAS).

Cell Surface Markers

Sheep erythrocytes, bovine erythrocytes sensitized with IgG antibody, and bovine erythrocytes sensitized with IgM antibody–complement complex were used for E, EA, and EAC rosetting, respectively. Direct cell membrane immunofluorescence was per-
formed using FITC-labeled rabbit anti-human immunoglobulin, \(\kappa, \lambda, \alpha, \beta, \gamma, \mu\). The cells were also tested by indirect immunofluorescence for the presence of \(\lambda\) and common ALL (CALLA) antigens.

**Lysozyme Activity and Phagocytosis**

Intracytoplasmic lysozyme activity was examined by the immunoperoxidase method, as reported previously. Phagocytosis was evaluated by light microscopy on MGG-stained smears of PL-21 cells after incubation with *Candida albicans* and *Staphylococcus aureus* at 37\(^\circ\)C for 60 min.

**EB Virus Nuclear Antigen (EBNA) and Terminal Deoxynucleotidyl Transferase (TdT)**

EBNA was tested by a slight modification of the method of Reedman and Klein. TdT was assayed by indirect immunofluorescence using reagents available from Bethesda Research Laboratories, Bethesda, MD.

**Chromosome Analysis**

Metaphase preparations were prepared by hypotonic treatment with 0.075\( M\) KCl for 20 min and fixed with methanol-acetic acid. The cells were then spread on slides and dried and stained with Giemsa. Giemsa banding analysis was carried out according to the method of Seabright.

**RESULTS**

**Cell Culture**

A gradual proliferation of cells was noted within a few days of culture, and the first subculture was made after 3 wk. The cells were then maintained in continuous culture by serial cell transfers and were designated PL-21. The PL-21 cell line was initially grown in RPMI 1640 medium supplemented with 15\% FCS and 15\% HCS without any conditioned media or feeder layers. This cell line is presently adapted to grow in RPMI 1640 medium supplemented with 15\% FCS alone, with a saturation density of 1.6 \( \times 10^6/\)ml and a doubling time of 48–64 hr.

**Cytologic Characteristics of Fresh Leukemia Cells and Cell Line**

*Original leukemia cells.* The patient’s leukemia cells were relatively large and had fine chromatin and lobulated nuclei with prominent nucleoli (Fig. 1). The cytoplasm was basophilic and contained azurophilic granules. Almost all of these cells were positive for peroxidase, naphthol AS-D chloroacetate esterase and acid phosphatase, and partially positive for PAS and \(\alpha\)-naphthyl butyrate esterase staining. Neutrophil alkaline phosphatase staining was negative. Lysozyme activity was demonstrated in 100\% of PL-21 cells by the immunoperoxidase method (Fig. 2E), and 5\%–10\% of PL-21 cells were shown to have phagocytic activity for *Candida albicans* (Fig. 2F) and *Staphylococcus aureus*.

Under the usual culture conditions, PL-21 cells differentiated along the myeloid cell series; a small number of myelocytes, metamyelocytes, and segmented neutrophils were observed (Fig. 2B and C). To examine the effect on cell differentiation, various concentrations of dimethyl sulfoxide (DMSO), which is known to stimulate the differentiation of mouse Friend erythroleukemia\(^{10}\) and human leukemia cell line HL-60,\(^{11}\) were added in the culture medium of PL-21. Growth of PL-21 cells began to plateau at day 5, with DMSO concentrations of 1.5\% and below. A concentration of 1.7\% of DMSO inhibited cell growth (Fig. 3). On day 5, with 1.5\% DMSO concentration, the most remarkable cell differentiation was observed, as shown in Table I. Half of the cells showed more mature myeloid cell morphology (Fig. 4) and 50\%–60\% of the induced cells phagocytized *Candida albicans*, whereas only 5\%–10\% of uninduced cells had phagocytic activity (Table 1).
Fig. 2. (A) Inverted microscopic observation of P1-21 cells growing in single-cell suspension culture (x90). (B) Smear of P1-21 cells stained with MGG. A few mature neutrophils are presented among the predominantly promyelocyte population (x720). (C) Peroxidase staining of P1-21 cells (x720). (D) Sudan Black B staining of P1-21 cells (x900). (E) Lysozyme staining of P1-21 cells (x180). (F) Phagocytosis of Candida albicans by P1-21 cells. Of P1-21 cells, 5%-10% showed phagocytic activity (MGG staining, x900).

Table 1. Differentiation of PL-21 Cells in Culture With or Without 1.5% DMSO

<table>
<thead>
<tr>
<th>Duration of Culture</th>
<th>Concentration of DMSO (%)</th>
<th>Differentiation (%)</th>
<th>Phagocytosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mybl</td>
<td>Pro</td>
</tr>
<tr>
<td>Day 0</td>
<td>0</td>
<td>2</td>
<td>96</td>
</tr>
<tr>
<td>Day 5</td>
<td>0</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0</td>
<td>54</td>
</tr>
</tbody>
</table>

Mybl, myeloblasts; Pro, promyelocytes; My, myelocytes; Met, metamyelocytes; Bnd, bands; Seg, segmented neutrophils.
**Cell Surface Markers**

The results of surface marker analysis are summarized in Table 2. The original leukemia cells were E rosette-negative and lacked surface immunoglobulin. The PL-21 line was E rosette-negative, 50% EA-positive, 6% EAC-positive, surface immunoglobulin-negative, 10% Ia-positive, and CALLA negative.

**EBNA and TdT**

Neither EBNA nor TdT was detected in the fresh leukemia cells and PL-21 line by indirect immunofluorescence.

**Chromosome Analysis**

Chromosome analysis of a total of 34 PL-21 cells showed that all the cells had a polyploid male karyotype with 13q + chromosomes (Fig. 5). Seven of the 34 cells also had double minute chromosomes (Fig. 5). The number of chromosomes ranged from 59 to 120, and 2 modal numbers were observed, as shown in Table 3. A translocation between chromosomes 15 and 17 was not identified in the PL-21 cell line.

**DISCUSSION**

We have reported the establishment of a myeloid leukemia cell line, PL-21, from the peripheral blood of a 24-yr-old man with APL. Cytochemical staining clearly demonstrated that PL-21 originated from the patient’s myeloid leukemia cells. Moreover, surface and antigen marker studies showed this cell line to lack lymphoid characteristics and to be EBNA-negative. The majority of PL-21 cells were promyelocytes with azurophilic granules. A few myeloblasts and more mature myeloid cells, such as myelocytes, metamyelocytes, bands, and segmented neutrophils, were also observed.

In 1977, Collins et al. reported for the first time the establishment of human myeloid cell line HL-60 from a female with APL. HL-60 proliferated continuously and differentiated along myeloid cell lineage under the usual culture conditions. HL-60 cells were induced to mature to granulocytes or monocytes in the presence of DMSO, retinoic acid, and other pharmacologic substances. In 1978, Koeffler and Golde reported the establishment of AML cell line KG-1, which responded to colony-stimulating factor (CSF). Compared to these previously established cell lines, the PL-21 cell line reported here has many characteristics similar to those of HL-60, rather than to KG-1.

As shown in Table 2, PL-21 has receptors for the Fc portion of IgG. Fc receptors were reported to appear at the promyelocyte stage of myeloid cell differentiation, and leukemic promyelocytes were also reported to have these receptors. PL-21 also contains azurophilic granules that are formed at the promyelocytic stage of maturation. These granules are reported to possess myeloperoxidase, lysozyme, and many acid hyd-
lases\textsuperscript{19} and play important roles in the killing and digesting of the ingested microorganism. PL-21 was shown to have phagocytic activity, peroxidase, intracytoplasmic lysozyme, and acid phosphatase. These characteristics of PL-21 were considered to be consistent with properties of myeloid cells.

The patient from whom PL-21 was established presented with a mediastinal mass, and 1 yr later, pleural effusion developed. Pleural effusion cells had lobulated nuclei with fine chromatin and many azurophilic granules in the cytoplasm, which were strongly positive for peroxidase staining. Two months after the appearance of pleural effusion, morphologically similar peroxidase-positive cells also appeared in the peripheral blood. Our diagnosis on this case, therefore, was mediastinal granulocytic sarcoma, terminating in APL with pleural involvement, although the surgically removed mediastinal tumor was shown to be fibrous tissue apparently due to radiation therapy.

On occasion, myeloid tumors preceded by months or

Table 3. Distribution of Chromosome Number of the PL-21 Cell Line

<table>
<thead>
<tr>
<th>Chromosome Numbers</th>
<th>59-89</th>
<th>90</th>
<th>91</th>
<th>92</th>
<th>93</th>
<th>94</th>
<th>95-111</th>
<th>115</th>
<th>116</th>
<th>117</th>
<th>118</th>
<th>120</th>
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<tbody>
<tr>
<td>Cell number</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

A total of 34 PL-21 cells was analyzed.
years the onset of myeloid leukemia; however, the incidence of such cases is considered to be low. Muss and Moloney reported that 3.1% of AML and chronic myelocytic leukemias (CML) showed myeloblastic or granulocytic tumors, the majority of which were discovered at autopsy. In our hospital, this is the only case that presented as tumorous disease among 60 cases of acute nonlymphocytic leukemia and CML for the last 10 yr. DIC is a common complication of APL, but our patient had no hemorrhagic tendency and died of heart failure with pericardial and myocardial involvement.

APL was reported to have a characteristic chromosomal abnormality, a translocation involving chromosomes 15 and 17. Chromosome analysis of this cell line failed to reveal such a translocation. The cell line showed a very complex karyotype, with chromosome numbers ranging from 59 to 120 and abnormal chromosomes identified as 13q+; 7 of 34 cells had double minute chromosomes, which were also found in HL-60.

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REFERENCES
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