Characterization, Growth, and Differentiation of a Human Myeloid Leukemia Cell Line, TI-1 Cell

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A cell line (TI-1) has been established from the peripheral blood of a patient with acute myeloid leukemia (M2). A typical TI-1 cell displayed many abnormalities of its chromosomes, but not the Philadelphia (Ph1) chromosome. Light and electron microscopic examination and histochemical analysis indicated that the TI-1 cells were undifferentiated blast cells, but immunologic marker studies suggested that these cells had myeloid characteristics. The proliferation of TI-1 cells was dependent on the concentration of fetal bovine serum (FBS). Their doubling time was 13.8 hours when they were cultured in a medium containing 10% FBS. Phorbol-12-myristate 13-acetate (PMA) induced the TI-1 cells to differentiate into monocyte-like cells, as judged by their morphologic similarity to monocytes, their adhesion to the culture dish, and their increase of both nitroblue tetrazolium (NBT)-reducing ability and nonspecific esterase (NSE)-activity. PMA significantly inhibited the proliferation and DNA synthesis of TI-1 cells in a dose-dependent manner. The PMA-induced differentiation was significantly inhibited by the protein kinase C inhibitors (H-7, staurosporine). Hemin induced the TI-1 cells to differentiate into erythroid cells. The number of hemoglobin-producing cells and hemoglobin production was increased by hemin treatment. Hemin also inhibited the proliferation of the TI-1 cells. Thus, the TI-1 cell represents a bipotant, granulo-monocytoid, and erythroid cell line. The TI-1 cell line will be a useful model for monocytoid and erythroid differentiation.

Attempts to develop long-term suspension cultures of human myeloid leukemia cells have met with limited success. Some human myeloid cell lines have been found to be useful models for studying cell differentiation because they are induced to differentiate into more mature cells by various inducers: The well-known examples are the HL-60, U-937, KG-1, and K-562 cell lines.

The HL-60 cell, a cell line established from the peripheral blood of a patient with acute myeloid leukemia, displays distinct morphologic and histochemical myeloid characteristics. HL-60 cells can be induced to differentiate into either monocyte-macrophage or granulocyte lineage by various inducers such as phorbol 12-myristate 13-acetate (PMA), 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], retinoic acid (RA), and dimethylsulfoxide (DMSO). Other cell lines, such as the U-937 cell and KG-1 cell, which express either monocyte or granulocytic characteristics, respectively, are also differentiated to more mature cells by various inducers.

Another important myeloid cell line is the K-562, which was established from the pleural fluid of a patient with chronic myelocytic leukemia in blast crisis. It might represent a cell line that is more immature than HL-60 cells because of its lack of morphologic and histologic differentiation. K-562 cells have chromosomal abnormalities including the Philadelphia (Ph1) chromosome. Hemin, DMSO, and butyrate induce these cells to differentiate into an erythroid lineage.

Recently, we have developed a unique human myeloid cell line (designated TI-1) obtained from the peripheral blood leukocytes of a patient with acute myelocytic leukemia. In the present study, we report our characterization of these TI-1 cells and present our evidence that the cells can be induced to differentiate into either monocyte-like cells or erythroid cells by PMA and hemin, respectively.

Materials and Methods

Cell culture and evaluation of TI-1 cell differentiation. The TI-1 cells were grown in RPMI-1640 medium containing 5% fetal bovine serum (FBS) at 37°C in a 5% CO2 humidified atmosphere. The cells were plated at a concentration of 1 x 10^6 cells/mL and then treated either with hemin or PMA (Sigma Chemical Co, St Louis, MO). The PMA was dissolved in ethanol, so that the final ethanol concentration in use was less than 0.1% at which concentration the proliferation and differentiation of TI-1 cells were not affected. The viability of the TI-1 cell was assessed by the trypan blue exclusion test. The number of cells was determined using a hemocytometer. The proliferation of TI-1 cells was investigated at the various concentration of FBS (0% to 20%). The number of hemoglobin-producing cells in each culture was determined by benzidine staining according to the methods of Rutherford et al. The cell differentiation into granulocyte-monocyte lineage was evaluated by the assay of nitroblue tetrazolium (NBT)-reducing ability and nonspecific esterase (NSE)-activity. NSE-activity was determined cytochemically with a-naphthylbutyrate as a substrate. In each assay at least 200 cells were examined.

Light microscopy and ultrastructural studies. The morphology of the TI-1 cell was studied throughout on cytopsin slide preparations stained with May-Giemsa. For ultrastructural studies, the TI-1 cells were fixed with 1.25% glutaraldehyde in 0.2 mol/L cacodylate-buffer for 30 minutes at 0°C and postfixed in 2% osmium tetroxide for 60 minutes at 0°C. The samples were dehydrated in a graded series of ethanol and embedded in epon. Ultrathin sections were stained with uranylacetate and lead citrate and examined with a JEOL JEM-1200EX electron microscope (Japan Electric Optical Laboratory Ltd, Tokyo, Japan).

Immunologic marker analysis and karyotypic analysis. The TI-1 cells were suspended in phosphate-buffered saline (PBS) containing 1% bovine serum albumin at a concentration of 2 x 10^7 cells/mL. Aliquots of 1 x 10^7 cells were stained with an equal volume of commercially available monoclonal antibodies (MoAbs) at 4°C for 30 minutes. In brief, both direct and indirect immunofluorescent staining procedures were used. Goat antihorse IgG secondary antibodies were used for indirect immunofluorescent staining. At the completion of the staining procedure, the cells were washed twice, resuspended in 1 mL PBS, and analyzed with...
an FCM-1D flow cytometer (Japan Spectroscopic Co, Tokyo, Japan).

For karyotypic analysis, the TI-1 cells were examined by the trypsin-Giemsa G-banding method. \(^{19}\)

**\[^{19}\]H\] thymidine incorporation.** An equal number of cells (5 \(\times\) 10^4 cells) in the same volume (250 \(\mu\)L) of each sample in RPMI 1640 medium containing 10% FBS were cultured with 0.25 \(\mu\)Ci \[^{19}\]H] thymidine for 3 hours at 37°C in the 96-well culture plate and harvested onto glass fiber filters. The radioactivity content was determined using a liquid scintillation spectrometer.

**Hemoglobin determination.** The produced hemoglobin of hemin-induced TI-1 cells was determined by the method of Cioe et al. \(^{20}\)

Briefly, the cells were washed twice with PBS and then the cells (1 \(\times\) 10^6) were resuspended in an equal volume of distilled water. Cells were lysed by three cycles of freeze-thawing and centrifuged once at 2,000g for 30 minutes at 4°C. The reading of the absorbance of the supernatant was taken at the range of 403 to 576 nm and hemoglobin was then quantitated at 414 nm.

Significant differences were determined by the Student's \(t\)-test.

**RESULTS**

**Establishment of a human myeloid cell line and serum dependence of TI-1 cell.** A human myeloid cell line (the TI-1 cell) was established from the peripheral blood of a patient with acute myelocytic leukemia (French-American-British [FAB] classification: M2). The number of white blood cells in the peripheral blood of the patient was 12,900/mm^3 before treatment. The surface marker analysis of the leukemia cell showed the increase of CD10 and CD13. The karotype of leukemia cell was normal pattern, 46XY. The histochemistry showed positive peroxidase activity and negative nonspecific esterase one. The mononuclear cells were isolated from the peripheral blood cells. The percentage of the leukemia cells in the mononuclear cell was 80%. Freshly isolated leukemia cells were cultured in the medium of RPMI 1640 containing 10% FBS at 37°C in a humidified 5% CO\(_2\) atmosphere. One half of the medium was replaced every 3 or 4 days. Almost all of the cells died. However, after 3 months' culture, some homogenous cells that were round and larger than normal lymphocyte cells began to proliferate. We have maintained these cells for another 6 months and have begun to characterize those cells. The proliferation of the TI-1 cells was dependent on the concentration of FBS. When the TI-1 cells were cultured in RPMI 1640 medium containing 5% and 10% FBS, their doubling time was 15.6 hours and 13.8 hours, respectively (see Fig 4).

**Morphology.** The morphologic characteristics of the cells were those of very undifferentiated cells. The cells were round and of 15- to 20- \(\mu\)m diameter. Their chromatin was fine and marked with one to three nucleoli. The cytoplasm was basophilic and sometimes contained some small granules (Fig 1A). Some mitotic figure and binucleated cells were observed. The TI-1 cells had no detectable \(\alpha\)-naphthylbutyrate esterase and naphthyl ASD chloroacetate esterase activity. Peroxidase activity was also not detected.

Electron microscopic examination of the TI-1 cells showed a single lobed nucleus with moderately condensed chromatin and two or more nucleoli (Fig 2). The cytoplasm was rich and contained numerous mitochondria, segments of rough endoplasmic reticulum, Golgi apparatus, and some granules.

**Immunologic analysis of cell surface marker.** Figure 3 summarizes the results of the immunologic marker assays. As this figure shows, no markers of T- and B-lymphoid cells were detected. The cell line was positive for CD13, which is a typical granulo-monocytic marker, while CD14 was negative, which is mainly expressed on monocyte. The antigens of CD33 and CD34 were also expressed on the surface of the cells.

**Chromosome analysis.** All cells showed a complex male karyotype. The number of chromosome was varying between 65 and 71 (60% of the analyzed cells had 69 chromosomes). The cells had chromosomal abnormalities as follows: 2q+, 6p+, 9p+, 9p-, 13p+, trisomy of no. 1, 4, 7, 8, 11, 12, and 19 chromosome, XXY (Fig 4). However, no Ph1 chromosome was observed.

**Effects of the concentration of FBS on the proliferation of TI-1 cells.** The proliferation of the TI-1 cells was dependent on the concentration of FBS. Without FBS, TI-1 cells did not grow. When TI-1 cells were cultured in RPMI 1640 medium containing 5% and 10% FBS, their doubling time was 15.6 hours and 13.8 hours, respectively. The growth rate of the TI-1 cells culturing in the medium containing 10% FBS was almost the same as that of 20% FBS (Fig 5).
Effects of PMA on the proliferation and the DNA synthesis of TI-1 cells. PMA significantly inhibited the cell proliferation in a dose-dependent manner (Fig 6A). PMA (4 × 10^{-7} g/L, 4 × 10^{-10} mol/L) inhibited cell proliferation by about 70% of the control at day 3. At the concentration of 10 × 10^{-7} g/L (1.62 × 10^{-10} mol/L), cell proliferation was only 20% of the control. At the concentration of 100 × 10^{-7} g/L (1.62 × 10^{-8}), PMA had a cytotoxic effect on the TI-1 cells. At less than 10 × 10^{-7} g/L, cell viability was more than 85%. Figure 6B shows the effects of PMA on the DNA synthesis of TI-1 cells at day 1. The DNA synthesis was evaluated using [3H]-thymidine uptake. PMA also inhibited the DNA synthesis of TI-1 cells, dose dependently. At the concentration of 4 × 10^{-7} g/L and 10 × 10^{-7} g/L, PMA inhibited the DNA synthesis of TI-1 cells by about 70% and 30% of the control, respectively. On days 2 and 3, the same tendencies were noted (data not shown).

Phenotypic differentiation of TI-1 cells induced by PMA in the presence or absence of protein kinase C inhibitors. As shown in Fig 7, PMA (8 × 10^{-7} g/L)-induced TI-1 cells showed the increase of NBT reducing ability and NSE activity. The NBT reducing ability was used to observe all differentiation into both granulocyte and monocyte lineage. The measurement of NSE-activity was used to distinguish the differentiation into monocyte lineage. The intensity of reactivity to CD13 was also augmented up to 1.4-fold of the control by PMA treatment. This evidence suggests that PMA-induced TI-1 cells into monocyte lineage. Morphologically, the PMA-induced TI-1 cells were differentiated into monocyte lineage because of the increase of cell size, the decrease of nucleus/cytoplasm ratio, more aggregated chrom
GROWTH AND DIFFERENTIATION OF TI-1 CELLS

Fig 4. TI-1 cells were examined by the trypsin-Giemsa G-banding method.

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With the result that hemoglobin F was produced by hemin treatment.

DISCUSSION

The cell surface antigens of TI-1 cells strongly reacted with the MoAb to the CD13 antigen that is expressed in the granulocytic and monocytic lineage, while the monocytic antigen marker, CD14, was not expressed. These data suggest that the TI-1 cell is a myeloid cell line. However, the TI-1 cells are more immature and closer to a stem cell than HL-60 cells because the TI-1 cells display fewer characteristics representative of morphologic and histochemical differentiation, as compared with HL-60 cells. Most importantly, the TI-1 cells can be induced to differentiate into two different cell lineages, either the monocytic or erythrocytic lineage by PMA and hemin, respectively. Taken together, the TI-1 cells are at a closely similar differentiation stage as the K-562 cells, because the K-562 cells are lacking in morphologic and histochemical differentiation characteristics and are reported to be differentiated into an erythroid cell lineage by hemin and butyrate. The K-562 cells can also be differentiated into a megakaryocytic lineage by PMA. However, the TI-1 cells are clearly different from K-562 cells because karyotype analysis of the TI-1 cells showed many differences from that of the K-562 cells, including having a Y chromosome and no Philadelphia chromosome.

The inhibition of cell proliferation has been reported to be closely related to the induction of cell differentiation in other cell lines. The inhibition of the proliferation of the
TI-1 cell was also accompanied with the terminal differentiation by the treatment with PMA. PMA induced the TI-1 cells into a monocytic lineage rather than a granulocytic one because they were morphologically similar to monocytes and displayed both NBT-reducing ability and NSE-activity. PMA directly activates Ca$^{2+}$- phospholipid protein kinase (protein kinase C). PMA-induced differentiation was significantly inhibited by the inhibitors of protein kinase C (H-7, staurosporine). The reason why the inhibition by staurosporine was stronger than that by H-7 might be explained by the evidence that staurosporine is a more potent inhibitor of protein kinase C than H-7 at the concentrations used in these studies. These results suggest that the activation of protein kinase C is involved in the differentiation of TI-1 cells.

Hemin not only inhibited cell proliferation but also induced TI-1 cells to differentiate into erythroid cells. The number of differentiated cells assessed by benzidine staining increased in a dose-dependent manner. We believed that, in the case of TI-1 cells treated with hemin, the increased percentage of benzidine-positive cells reflects an increase in hemoglobin synthesis because: (1) there was a positive correlation between the increased percentage of benzidine-positive cells after hemin treatment (see Fig 9) and the amount of hemoglobin as quantitated by the visible absorbance spectrum; and (2) the absorption bands of the
Fig 7. Effects of protein kinase C inhibitors of the TI-1 cells induced by PMA. TI-1 cells (2 x 10⁵ cells/mL) were treated with PMA (8 x 10⁻⁷ mol/L) in the presence or absence of protein kinase C inhibitors (H-7, staurosporine) for 3 days, and cell differentiation was determined by NBT-reducing ability (□) and NSE-activity (■). Data represent the mean ± SD. An asterisk (*) indicates P < .01 for the difference between the percentage of either NBT- and NSE-positive cells in cultures treated with PMA in the presence and absence of protein kinase C inhibitors. Asterisks (**) indicate P < .01 for the difference between the percentage of either NBT- and NSE-positive cells in the presence and absence of PMA.

supernatant of hemin-induced TI-1 cells were observed mainly at 414 nm and other small peaks were observed at 540 nm and 576 nm, which are patterns consistent with that of hemoglobin.

Fig 8. Effects of hemin on the proliferation of TI-1 cells. TI-1 cells were inoculated at 1 x 10⁵ cells/mL on day 0 in the presence (○) or absence (□) of hemin (0.03 mmol/L). The viability was always more than 90%. Each point represents the mean of triplicate counts (±SD).

Fig 9. The percentage of benzidine positive cells (○, ●) and the amount of hemoglobin production (△, ▲) in human-induced TI-1 cells were investigated. TI-1 cells (1 x 10⁵ cells/mL) were cultured in the presence (△, ▲) or absence (○, ●) of hemin (0.03 mmol/L) for 4 days. Each point represents the mean of triplicate counts (±SD). This result is representative of three experiments.

In the case of the K-562 cells, trisomy of chromosome 11 and tetrasomy of chromosome 16 were present. It has been reported that chromosome 11 contains the β-like globin gene and chromosome 16 contains the α-like globin gene. The TI-1 cells also have trisomy of chromosomes 11 and 16. The TI-1 cells and K-562 cells are induced to differentiate into erythroid cells by hemin. Taken together,
the abnormalities of chromosome 11 and 16 might be closely related to the evidence that these cells were induced to differentiate into erythroid cells synthesizing hemoglobin by hemin.

As described above, the TI-1 cell is a unique human myeloid cell line because it can be differentiated into two types of cell lineage by inducers and has many abnormalities of its karyotype. Studies are currently under way to define the mechanisms that contribute to the TI-1 cell differentiation.

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