Establishment of a Novel Acute Monoblastic Leukemia Cell Line (YK-M2) Having a Near-Triploid Karyotype

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ABSTRACT

The YK-M2 cell line was established from the peripheral blood of a patient with acute monoblastic leukemia in whom an anterior mediastinal tumor preceded the peripheral blood manifestation. The established cells grew in a single cell suspension with a doubling time of 60 h and consisted of primitive monoblastic cells. The cells were 52% positive for peroxidase staining and manifested strongly positive activity of α-naphthyl acetate esterase, which was completely inhibited by sodium fluoride. The cells showed strong expression of Fcy receptors and phagocytosed sensitized ox erythrocytes. When the cells were incubated with 1α,25-dihydroxy-cholecalciferol, they were induced to differentiate into mature monocyte-macrophage-like cells, which reduced the nitroblue tetrazolium dye and released a small amount of the superoxide anion. Cytogenetic studies revealed that the cells had a near-triploid karyotype with a modal chromosome number of 68, and the short arm of chromosome 17 was deleted [del(17)p11]). The YK-M2 cell line is particularly unique in that the cells retained the polyplody karyotype that may be an initial cytogenetic change in the malignant transformation of the parent leukemia cells.

INTRODUCTION

A number of investigators have established a close association of specific structural chromosome rearrangements with particular subtypes of ANLL (1) and remarkable concordance of cellular oncogenes and the breakpoints involved in the rearrangements (2). However, little is known about the importance of numerical chromosome alteration, especially of polyploidy, in ANLL. Tetraploid metaphases have been obtained from a few cases of ANLL (3–8). Since these metaphases were characterized by the duplication of marker chromosomes or specific translocations, tetraploidy has been considered to have occurred secondarily, excluding one case reported by Testa et al. (7).

Here we report a case of AMoL in which the leukemia cells had a polyploid karyotype and were successfully established into a continually growing cell line having a near-triploid karyotype. We describe the characterization of the newly established acute monoblastic leukemia cell line, designated YK-M2, as well as chromosome analysis of the parent leukemia cells. The importance of the formation of a polyploid clone will be discussed.

MATERIALS AND METHODS

Case Report. A 31-year-old Japanese man was admitted to our hospital for a left pleural effusion in July 1984. On examination, dullness to percussion was noted over most of the left hemithorax, with diminished breath sounds. No lymphadenopathy was found. The routine laboratory data were normal except for a slight elevation of the serum lactic dehydrogenase. A computer-assisted tomographic scan of the chest demonstrated a huge anterior mediastinal mass and large left pleural effusion. The pleural fluid contained a number of monoblasts that showed a positive reaction for peroxidase and nonspecific esterase staining. The lactic dehydrogenase of the pleural fluid was 3433 units/liter. A diagnosis of extramedullary tumor of monoblastic cells was made, and the patient was treated by combination chemotherapy and irradiation to the mediastinum. In October 1984, the bone marrow, which was normal at diagnosis, was infiltrated by monoblasts comprising 72.4% of the nucleated cells. In February 1985, the monoblasts appeared in the peripheral blood and rapidly increased in spite of therapy. The patient died of respiratory failure due to massive pleural effusion in March 1985.

Cell Culture. On March 12, 1985, the peripheral WBC rose to 11,000/µl with 81.0% blast cells. Mononuclear cells were prepared from the peripheral blood by routine Ficol-Conray gradient centrifugation. Cells were cultured at a concentration of 1 x 10⁶ cells/ml in a 24-well culture plate (No. 25820; Corning) using RPMI 1640 (Nissui Seiyaku Co., Tokyo, Japan) supplemented with 20% FCS (M. A. Bioproducts, Walkersville, MD), 2 mM L-glutamine, penicillin G (100 IU/ml), and gentamicin (50 µg/ml). The cells were incubated at 37°C in 5% CO₂ in humidified air and were refed with a half-change of fresh medium every 3 days.

Cell Characterization. Morphological and cytochemical examination of the cells included routine May-Giemsa staining, myeloperoxidase, α-naphthyl acetate esterase, inhibition of sodium fluoride, and naphthol AS-D chloroacetate esterase. Cell marker analyses were performed as described elsewhere (9). The cells were examined by rosette assay for sheep erythrocyte receptors, C3b receptors and Fcy receptors. Phagocytosis of IgG-coated ox erythrocyte (immunophagocytosis) were assessed by Cytospin slide preparation stained with May-Giemsa. Surface immunoglobulins were detected by the direct immunofluorescence method using fluorescein isothiocyanate-labeled F(ab')2 anti-human immunoglobulin antibodies (Tago, Inc., Burlingame, CA). MoAb used in this study were OKIa-I for HLA-DR antigens; OKT-3, 4, 6, 8 (Ortho Pharmaceuticals, Raritan, NJ); Leu-1 (Becton Dickinson, Mountain View, CA); J5 for common acute lymphoblastic leukemia antigens (10); B1 (Coulter Immunology, Hialeah, FL); WT-1 (11); MCS-1; MCS-2 (12); My4; and My7 (13). Assessment of the cells reacting with these MoAb was performed by the indirect immunofluorescence method. To block nonspecific binding with Fcy receptors, cells were incubated with an optimal amount of MoAb in the presence of normal AB serum which was treated at 56°C for 30 min. TdT was also examined by the indirect immunofluorescence using rabbit anti-TdT serum (Becton Dickinson, Gaithersburg, MD). Epstein-Barr virus-determined nuclear antigen was assayed by the anticomplement immunofluorescence test according to the method of Reedman and Klein (14). Mycoplasma contamination was assayed by Dr. J. Minowada of the Fujisaki Cell Center (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan). The samples were cultured in agar plate and Mycoplasma colonies were examined under microscope.

Electron Microscopy. The cell pellet was fixed in 2.5% glutaraldehyde and 1% OsO₄, dehydrated with serial ethanol, and embedded in an epoxy resin. Selected blocks were cut into thin sections, stained with uranyl acetate and lead citrate, and examined by a Hitachi H-560 electron microscope.

Cytogenetic Studies. The pleural fluid cells and leukemia cells were...
Fig. 1. Morphology of YK-M2 cells in culture. A, YK-M2 cells stained by May-Giemsa. B, peroxidase-positive YK-M2 cell. About one-half of the cells were positive for the staining. C, nonspecific esterase activity of YK-M2 cells using a-naphthyl acetate as a substrate. The activity was completely inhibited by sodium fluoride. D, YK-M2 cells cultured in 100 nM 1α,25-(OH)2D3 for 6 days. Left, mature monocyte-macrophage-like cell having fine granules in the cytoplasm; upper right, cell reducing NBT dye. Bar, 10 μm.

processed as described previously (15), after short-term culture in RPMI 1640 supplemented with 20% FCS. The established cells in the log phase of growth were exposed to Colcemid (0.02 μg/ml) and ethidium bromide (10 μg/ml) (Aldrich Chemical Co., Milwaukee, WI) simultaneously for 2 h (16). These cells were harvested and suspended in 0.075 M potassium chloride for 20 min at 37°C and then fixed with acetic acid:methanol (1:3). They were spread on slides by air-drying and the chromosomes were banded by trypsin-Giemsa.

Differentiation Induction by 1α,25-(OH)2D3. 1α,25-(OH)2D3 was a generous gift of Chugai Pharmaceutical Co., Tokyo, Japan. The established cells were seeded at a concentration of 3.0 x 10^5 cells/ml in RPMI 1640 supplemented with 10% FCS, and they were grown in the absence or presence of various concentrations of 1α,25-(OH)2D3 for culture periods of up to 6 days. The 1α,25-(OH)2D3 was dissolved in 95% ethanol and was added to the medium to make the final ethanol concentration 0.1% or less (17). After the culture periods indicated, the cells were harvested, and changes in cell morphology, viable cell counts, NBT reduction, and O2− release were determined. Morphological assessment of the cells was made on Cytospin slide preparations stained with May-Giemsa. Cell viability was determined by trypan blue dye exclusion. NBT reduction test was done according to the method of Breitman et al. (18). Briefly, the cells (2.0 x 10^6 cells/ml) were incubated for 25 min at 37°C with an equal volume of 0.2% NBT dissolved in phosphate-buffered saline containing freshly diluted 12-O-tetradecanoyl-phorbol-13-acetate (200 ng/ml; Sigma Chemical Co., St. Louis, MO). The percentage of cells containing intracellular blue-black formazan deposits was determined on May-Giemsa-stained Cytospin slide preparations. O2− release was quantitated as the superoxide dismutase-
Fig. 2. G-banded karyotype of the established YK-M2 cell line. The short arm of the chromosome 17 on the right is deleted. [68,X,-Y,3n± [+3,+4,+5,+6,+7,+10,+11,+16],del(17)(p11),+4 markers]. *Inset*, partial karyotype of chromosome 17 obtained by short-term culture of the fresh leukemia cells. The del(17)(p11) is also demonstrated.

Table 1 Cytogenetic findings of pleural fluid cells, leukemia cells, and YK-M2 cells

<table>
<thead>
<tr>
<th>Distribution of chromosome numbers</th>
<th>Pleural fluid cells</th>
<th>Leukemia cells</th>
<th>YK-M2 cells</th>
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<tbody>
<tr>
<td>51–60</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>61–70</td>
<td>0</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>71–80</td>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>81–90</td>
<td>14</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>91–100</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>101–110</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(Total metaphases counted)</td>
<td>(20)</td>
<td>(11)</td>
<td>(26)</td>
</tr>
<tr>
<td>Structural changes</td>
<td>Inadequate</td>
<td>del(17)(p11)</td>
<td>del(17)(p11)</td>
</tr>
<tr>
<td></td>
<td>for karyotyping</td>
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* Karyotyping was unsuccessful because of the fuzzy appearance of the chromatids.

*12-O-Tetradecanoylphorbol-13-acetate was added to the indicated assay tubes at 100 ng/ml. For each assay, duplicate reaction mixtures, one of which contained superoxide dismutase (30 μg/ml; Diagnostic Data, Mountain View, CA) as a blank, were prepared. After 1 h of incubation at 37°C, the reaction mixtures were cooled and centrifuged. The absorbance of the supernatant was determined, and the concentration of reduced cytochrome c was calculated using the equation $E_{550}$,nm $= 2.1 \times 10^4$ M$^{-1}$ cm$^{-1}$ (20).

RESULTS

Establishment of Cell Line. From the beginning of the cell culture, the cultured cells showed slow but definite proliferation. After 2 weeks of initial culture, the first transfer was carried out and the established cell line was designated YK-M2. The YK-M2 cell line has been maintained in 75-cm² tissue culture flasks (No. 25110; Corning) using RPMI 1640 supplemented with 10% FCS. The cells grow in a single cell suspension with a doubling time of about 60 h.

Cell Characterization. The YK-M2 cells were medium sized

inhibitable reduction of cytochrome c (19). The assay contained cells (2.5 × 10⁶ cells/ml), cytochrome c (0.06 mM) (horse heart, type 3; Sigma) and Hanks' balanced salt solution in a total volume of 500 μl.
to large, having an appearance of primitive monoblastic cell (Fig. 1A), resembling the pleural fluid cells and the parent leukemia cells of the patient. The relatively abundant cytoplasm was gray-blue and some cells contained azurophilic granules. No Auer rods were observed. The nuclear shapes were reniform or folded. The nuclear chromatin pattern was spongy and the presence of irregular nucleoli was characteristic. Most of the cells had clear cytoplasmic vacuoles. In electron micrographs, the YK-M2 cells showed mildly folded nuclei with distinct nucleoli, well-developed rough endoplasmic reticulum, and abundant mitochondria. Granules were rarely seen in the cytoplasm (Fig. 3A).

Cytochemical and immunological characteristics of the YK-M2 cell line were almost the same as those of pleural fluid cells and parent leukemia cells. The YK-M2 cells were 52% positive for peroxidase (Fig. 1B) and manifested strongly positive activity of α-naphthyl acetate esterase (Fig. 1C) which was susceptible to sodium fluoride inhibition. Some of the cells also had specific esterase activity when naphthol AS-D chloroacetate was used as a substrate. Both of the YK-M2 cells and pleural fluid cells showed strong expression of Fcγ receptors and phagocytosed sensitized ox erythrocytes. The YK-M2 cells strongly expressed myeloid-specific antigens identified by MCS-2 and My7 MoAb. MCS-1, My4, and OKIa-1 were weakly positive. The T-cell lineage-associated antigen markers such as sheep erythrocyte receptors, Leu-1, OKT series, and WT-1 were undetectable. C3b receptors, surface immunoglobulins, J5, B1 antigens, and TdT were also negative. The cells were negative for Epstein-Barr virus–determined nuclear antigens and Mycoplasma colonies were undetectable.

Cytogenetic Studies. Chromosome analysis of the YK-M2 cell line revealed it to be a near-triploid karyotype with a modal number of 68 chromosomes. Cells having a near-tetraploid karyotype were also observed. In all metaphases analyzed, the short arm of one No. 17 chromosome was deleted [del(17)(p11)], and several undefined marker chromosomes were found. However, there were no duplicated rearranged chromosomes. The representative karyotype was: 68,X,-Y,3n±,+3,-4,-4,-5,+6,+7,-10,-11,-16,del(17)(p11),+4 markers (Fig. 2).

Although the pleural fluid cells and leukemia cells of the patient had polyploid karyotypes, the modal chromosome numbers were different from that of the YK-M2 cells. The del(17)(p11) was also observed in the parent leukemia cells (Table 1; Fig. 2) and their representative karyotype was: 78,XX,-Y,4n±,-2,-4,-4,-4,-5,+7,-8,-9,-10,-11,-12,-14,-14,-14,-17,-20,-21,del(17)(p11),+3 markers.

Differentiation Induction Studies by 1α,25-(OH)2D3. With the addition of 1α,25-(OH)2D3 to the culture, the YK-M2 cells were induced to differentiate into mature monocyte–macrophage-like cells (Fig. 1D). The induced cells remained to grow in a single cell suspension. The cells were morphologically characterized by a decreased nuclear/cytoplasmic ratio, less basophilic cytoplasm, condensed nuclear chromatin, and appearance of fine granules. In the electron microscopic examinations, the differentiated cells after 3 days of incubation in 10 nm 1α,25-(OH)2D3 showed a decreased nuclear/cytoplasmic ratio, kidney-shaped nuclei, development of smooth endoplasmic reticulum, and the appearance of specific granules (Fig. 3B), which indicated that the YK-M2 cells were induced to differentiate into more mature cells and acquired monocytic features.
The growth rate of the YK-M2 cells was apparently altered by the 4-day treatment with 50 nM 1α,25-(OH)2D3, and growth was almost stopped by that with 100 nM 1α,25-(OH)2D3 (Fig. 4A). The percentage of YK-M2 cells reducing NBT increased with the days of culture at the concentration of 50 and 100 nM 1α,25-(OH)2D3. The percentage reached about 60% after 6 days of incubation in 100 nM 1α,25-(OH)2D3 (Fig. 4B). As shown in Table 2, exposure of the cells to TPA significantly increased the O2·− release when the cells were incubated with 1α,25-(OH)2D3. The extent of O2·− release increased 6-fold with the treatment of 100 nM 1α,25-(OH)2D3 compared with untreated cells. Therefore, the data of NBT reduction and O2·− release were in agreement.

DISCUSSION

Normal hematopoietic differentiation along the myeloid (granulocyte/monocyte) pathway is characterized by changes in morphology and cytochemistry, modification of membrane antigens, and acquisition of functional capacities. Compared with the normal myeloid counterpart cells, leukemic cell phenotypes may result from a combination of the expression of normal genes, coding for differentiation markers, and abnormal features (21). The YK-M2 cells presented here appear to have properties of primitive monocytes as far as judging from morphological and cytochemical studies. In addition, the cells seem to lack bacteriocidal capacity, because they did not reduce NBT or produce O2·− unless they were induced to differentiate by some compound. However, the cells exhibited strong expression of Fcγ receptors on the surface membrane. Moreover, some of the cells phagocytosed sensitized ox erythrocytes. These mature properties correspond to those of monocytes of peripheral blood and tissue macrophages (22). The asynchronism of the cell differentiation markers of the YK-M2 cells may result from malignant processes which disconnect orderly differentiation programs controlled by separate genes (23).

The patient described here was an unusual case of AML, in which an anterior mediastinal tumor and a malignant pleural effusion preceded the full-blown acute leukemia by 8 months. Although the mediastinal tumor was not examined histologically, there was no doubt that the tumor was composed of monocytic precursor cells appearing in the pleural fluids. Several investigators have indicated that extramedullary tumors of myeloid blast cells, in rare cases, precede peripheral blood or bone marrow manifestation of ANLL (24), and the disorders are considered to be part of a spectrum of ANLL. However, whether the leukemia cells which show tumor formation before the development of leukemia have the same chromosome changes as those of usual ANLL cells has not yet been confirmed.

Although the modal chromosome numbers of the pleural fluid cells and leukemia cells in this patient and of the established YK-M2 cells were different, these cells were associated with polyploid karyotypes in common. This phenomenon may be a reflection of the growth advantage of tumor cells having a particular chromosome number under each circumstance, i.e.,
in pleural fluids, peripheral blood, and in vitro culture. In spite of careful banding analysis, there were no duplicated marker chromosomes in the analyzed metaphases. Therefore, the polyploid cells could not have originated in vitro by fusion of diploid cells or by duplication of the chromosome complement. Instead, the polyploid clone could be an initial cytogenetic aberration of various types of hematological diseases. A deletion of the short arm of chromosome 17, which is the clonal marker chromosome of the YK-M2 cells as well as of the parent leukemia cells, has been reported in two cases of ANLL (25). However, implications of the rearrangement remain to be determined.

Continually proliferating human myeloid cell lines have been used for the studies of differentiation induction of leukemia cells by various compounds (18, 26) including 1α,25-(OH)2D3 (17, 27). The newly established YK-M2 cells also showed terminal differentiation to mature monocyte-macrophage-like cells by incubation with 1α,25-(OH)2D3. The ultrastructural detection of specific granules was persuasive evidence for the monocytic nature of the induced cells. Judging from the three parameters, inhibition of cell growth, NBT reduction, and O2~ production, the YK-M2 cells seem to be less sensitive to differentiation induction by 1α,25-(OH)2D3 than the human acute promyelocytic leukemia cell line HL-60. The optimal concentration of 1α,25-(OH)2D3 inducing differentiation of the YK-M2 cells was 100 nM, whereas the HL-60 cells has been reported to be differentiated to mature cells at concentrations ranging from 0.1 to 10 nM (17, 27). Differentiation induction studies of the YK-M2 cells using other compounds are in progress.

REFERENCES

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