Establishment and Characterization of Four Human Monocytoid Leukemia Cell Lines (JOSK-I, -S, -M and -K) with Capabilities of Monocyte-Macrophage Lineage Differentiation and Constitutive Production of Interleukin 1

Masatsugu Ohta, Yusuke Furukawa, Chizuka Ide, Nobuaki Akiyama, Tadashi Utakoji, Yasusada Miura, and Masaki Saito

Division of Hemopoeisis, Institute of Hematology [M. O., M. S.], and Department of Hematology [Y. F., Y. M.], Jichi Medical School, Minamikawachi-machi, Kawachi-gun, Tochigi, Japan 329-04; The 2nd Department of Anatomy, Faculty of Medicine, Iwate Medical School, Uchimaru, Morioka-shi, Iwate, Japan 010 [C. I.]; Institute of Medical Science, University of Tokyo, Shiroganedai, Minato-ku [N. A.]; and Department of Cell Biology, Cancer Institute, Kamikirihikari, Toshima-ku [T. U.], Tokyo, Japan 170

ABSTRACT

Four monocytoid cell lines, JOSK-I, -S, -M, and -K, were newly established successfully from peripheral blood of two cases of acute monocytic leukemia and one case each of acute myelomonocytic leukemia and chronic myelogenous leukemia in myelomonocytic blast crisis. In order to establish permanent cell lines, cultures of leukemic blasts were initiated in 96-well microtiter plates. Each cell line grew in a suspension culture with a doubling time of 24-32 h and has been serially maintained for over 20 mo. Each line had immature monocytic properties as judged from the results of cytological, immunochemical, and functional analyses. The cells showed a positive reaction for α-naphthyl butyrate esterase which was completely inhibited by sodium fluoride and exhibited immature monocytic features on electron microscopic observation. They also had surface markers specific for the monocyte-macrophage lineage. Chromosome analyses showed that each line had a variety of marker chromosomes; furthermore, these established lines exhibited high potentials involving morphological and functional differentiation into more mature monocytic cells when induced by several chemical inducers. We also found that two of the established cell lines produced interleukin 1 activity without any stimuli. These new lines might be valuable for studying the regulation of monocyte-macrophage differentiation and host defense mechanisms.

INTRODUCTION

Human monocyte-macrophage lineage has unique properties as to phagocytic functions and immune regulatory systems (1), but large amounts of homogeneous monocyte or macrophage populations are usually difficult to obtain for in vitro studies. In view of this, in addition to clarification of the mechanism of monocyte-macrophage differentiation, human monocytic leukemia lines might be important and attractive model systems, but the establishment of human monocytic leukemia lines has been in general difficult.

We have recently established four human monoblastic leukemia lines successfully after many attempts to culture human nonlymphoid leukemia cells obtained from peripheral blood or bone marrow of more than 40 patients with untreated leukemias. The present report deals with the establishment and characterization of four mononoblastoid cell lines designated as JOSK-I, from a case of acute myelomonocytic leukemia, JOSK-S, from one of acute monocytic leukemia, JOSK-M, from one of chronic myelogenous leukemia in myelomonocytic blast crisis, and JOSK-K, from another one of acute monocytic leukemia. These lines exhibited typical immature monocytic properties morphologically and functionally; furthermore, we studied the effects of several chemical inducers on differentiation induction and the productivity of biologically active substances of these cell lines.

MATERIALS AND METHODS

Clinical Data. Leukemias were diagnosed morphologically and histochromically on the basis of the French-American-British classification scheme (2).

JOSK-I was derived from a 72-year-old woman with acute myelomonocytic leukemia. On admission Sept. 14, 1983, her peripheral WBC was 9100/mm³ and more than 65% of the cells were blast cells which were positive for peroxidase and Sudan black B stainings; in addition, some of the blast cells were positive for naphthol AS-D chloroacetate esterase staining, and the other cells were positive for α-naphthyl butyrate esterase activity which was completely inhibited by sodium fluoride.

JOSK-S was derived from a 66-year-old woman and JOSK-K from a 54-year-old man, both with acute monocytic leukemia. On her admission Nov. 11, 1983, the peripheral blood WBC was 29,000/mm³ with 70% blast cells, and on his admission Jan. 5, 1984, the peripheral blood WBC was 75,400/mm³ with 58% blast cells. These blast cells were positive for peroxidase and Sudan black B and also positive for α-naphthyl butyrate esterase activity which was completely inhibited by sodium fluoride.

JOSK-M was derived from a 37-year-old man with chronic myelogenous leukemia in blast crisis. On admission Jan. 18, 1984, his peripheral WBC was 137,000/mm³ with 40% blast cells. Blast cells were positive for peroxidase, weakly positive for acid phosphatase stainings, and also positive for specific and nonspecific esterase double stainings; about 24% of the blast cells were positive for naphthol AS-D chloroacetate esterase and about 70% of them were positive for α-naphthyl butyrate esterase which was inhibited by sodium fluoride. Chromosome analysis showed that the Ph¹ chromosome was present in the blast cells. We diagnosed this case as chronic myelogenous leukemia in myelomonocytic blast crisis.

Culture Methods. Heparinized peripheral blood was obtained from each patient at the time of admission. Mononuclear cells were isolated from the blood specimens by the Ficoll-Hypaque method (3).

Mononuclear cells were diluted with α medium (α-MEM; Fl) and incubated for 1—2 h at 37°C in a humidified atmosphere of 5% carbon dioxide. Then the nonadherent cells were collected, washed twice with FCS-containing α-MEM, and resuspended at the density of 1 x 10⁶ cells/ml. Of these nonadherent cells 90% or more were leukemic as judged by morphological assessment on staining with Wright-Giemsa solution and the viability of the cells determined by the trypan blue dye exclusion test was 98% or more. A cell suspension was distributed...
in a 96-well microplate (No. 25860; Corning, NY) at 0.2 ml/well followed by culturing at 37°C in 5% CO₂ in humid air. The medium was changed 1–3 times/wk. After cell growth had been confirmed in the microwells, the cells were transferred to 24-well microplates (No. 25820; Corning) and finally, growing cells were maintained in 5-ml cultures in flasks (No. 3013; Falcon). After establishment had been confirmed, a subculture of each line was performed at 5- to 6-day intervals with seeding at an initial density of 2 × 10⁵ cells/ml.

Morphological and Cytochemical Studies. The morphology of the cells in culture was constantly examined under an inverted microscope. Cytospin slide preparations of the cell suspensions were prepared with a Shandon cytopsin centrifuge (Shandon Southern Products Ltd., Cheshire, United Kingdom) and subjected to the following regular cytological and cytochemical tests in addition to the Wright-Giemsa staining method: Sudan black B; alkaline phosphatase; acid phosphatase; and periodic acid-Schiff; in addition, cells were examined by specific and nonspecific esterase double stainings (4) including that for sodium fluoride-inhibitable α-naphthyl butyrate esterase.

Sheep erythrocytes were used for the erythrocyte rosette-preservation assay and the direct cell membrane immunofluorescence assay and the direct cell membrane immunofluorescence was investigated using fluorescein isothiocyanate-labeled rabbit anti-human immunoglobulin. The presence of surface antigens reactive with monoclonal antibodies such as OKIα, OKM1, OKM5 (Ortho Pharmaceutical Corp., Raritan, NJ) and Mo2 and J5 (CALLA) (Coulter Immunology, Hialeah, FL) was investigated by the indirect immunofluorescence method using Ortho Spectrum III.

Terminal deoxynucleotidyl transferase activity was assayed by the indirect immunofluorescence technique (5), and Epstein-Barr virus-associated nuclear antigen was assayed by the method of Reedman and Klein (6).

Ultrastructural Examination. For observation by transmission electron microscopy, cell pellets were fixed with 2.0% paraformaldehyde-2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 37°C for 3–5 h, postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) at 4°C for 2 h, dehydrated through a graded series of ethanol and propylene oxide, and finally embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a Hitachi 700-H electron microscope. For scanning electron microscopy, cells were fixed and postfixed as described above. They were then treated with isoamyl acetate and liquid carbon dioxide and dried by the critical point method. They were coated with platinum with an ion coater and examined under a Hitachi 430 scanning electron microscope.

Lysozyme and Phagocytic Activity and NBT Reduction Assay. Lysozyme activity in the culture media and the cell lysate of each line was assayed according to the lysoplate technique (7) by measuring the appearance of a lytic area of Micrococcus lysodeikticus (Sigma Chemicals, St. Louis, MO). Egg lysozyme (Sigma) was used as a standard. Phagocytic activity was determined by counting the number of cells that phagocytosed more than one yeast particle (8). The ability of cells to reduce NBT dye (Wako Pure Chemicals Co., Tokyo, Japan) was also added to the cell suspensions at 2 × 10⁵ cells/mL to a final concentration of 1 or 4 mM. After incubation for appropriate times, the morphology of the cells was observed under an inverted microscope.

Establishment of Four Monoblastoid Cell Lines. About 6–8 wk after the culture had been started, active proliferation was observable in each case. Each cell line was considered to be established by wk 8–11 when continuous growth in plastic culture flasks was maintained throughout all the subculture manipulations.

Each cell line grew in suspension and did not attach to the bottom of plastic culture flasks, and no aggregates were found. The cells of each cell line reached a saturation density of 1–2 × 10⁶ cells/ml when seeded at 2 × 10⁵ cells/ml with a doubling time of 24–32 h (Table 1). The established lines have been maintained in α-MEM supplemented with 10% FCS for more than 20 mo without any significant changes in the growth rate or in the morphology as well as some biological characteristics.

Morphological and Cytological Characteristics. In Wright-Giemsa-stained preparations, the established cells were round and polygonal in shape, and some cells showed small bleb-like formations. The cytoplasm of the cells was basophilic with a few granules and small vacuoles. The nuclei were round or indented with fine chromatin structures and with one to three large prominent nucleoli (Fig. 1A). Under an inverted microscope, the cells of each line in culture were round and somewhat polygonal in shape (Fig. 2A). On ultrastructural examination, scanning electron microscopy showed that each line had ruffled surface membranes with several small blebs and thin microvilli. No smooth-surfaced cells were found (Fig. 1D). Transmission

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electron microscopy showed that each line had some properties specific to immature monoblastoid cells. Each line had clear nuclei with well-defined nucleoli, and some cells had highly lobulated nuclei. There were a few heterochromatin structures in the nuclei and cytoplasmic microorganelles were sparse, but a few mitochondria, small azurophilic granules, rough endoplasmic reticulum, or lipid droplets were found; also ribosomes, especially polyribosomes, were frequently seen (Fig. 1E).

The cytochemical staining results were as follows: negative for peroxidase, Sudan black B, alkaline phosphatase, and naphthol AS-D chloroacetate esterase; faintly positive for acid phosphatase; and significantly positive for a-naphthyl butyrate esterase with ++++ sodium fluoride (Table 1; Fig. 1 B and C). Each line was negative for erythrocyte rosette formation and lacked surface immunoglobulins. The reactions to several monoclonal antibodies showed that the cytoplasmic ratio and in cytoplasmic basophilia. Cytoplasmic vacuoles increased and nuclei became more indented or reniform in shape. Especially in RA-treated cells, the nuclei had a stab- or segmented form-like shape (Fig. 2 D—F). The induced cells showed a-naphthyl butyrate esterase activity when stained by the esterase double-staining method.

Surface marker analyses showed that some surface antigens specific for monocyte-macrophage lineage were induced markedly with chemical inducers. The plating efficiencies of 10-day cultures, at which time the number of colonies was maximum, were in the range of 8.1—11.3% in each cell line. Each colony consisted of 100—500 cells. The cells were morphologically the same as the originally established cells.

Lysozyme activity in the range of 1.1—1.9 µg/ml was detected in the supernatants obtained from 5-day cultures when the cells were seeded at 2 × 10^4 cells/ml. The cell lysates showed lysozyme activity in the range of 290—350 ng/10^6 cells. A small population of each cell line in the logarithmic growth phase phagocytosed yeast particles without any stimulation, 10.5% of JOSK-I, 5.5% of JOSK-S, 4.0% of JOSK-M, and 3.0% of JOSK-K cells. The phagocytosing cells usually contained 1—4 yeast particles/cell.

Chromosome analysis was performed on a total of about 100 metaphases for each cell line. The modal chromosome number was around 55. Most of the cells had hyperdiploid chromosome number, and each line had several marker chromosomes. For each cell line, there were no cells showing normal diploid karyotypes. Prior to establishment of the cell lines, chromosomal analysis of each patient could not be fully examined but revealed that patient N. I. (JOSK-I) had a female karyotype with a 46 modal chromosome number, and patient M. S. (JOSK-S) also had a female karyotype and a 47 modal chromosome number with an excess of chromosome groups A and C. Representative Giemsa-banded karyotypes and the distribution of the number of chromosomes were shown in JOSK-I and -S cells. JOSK-S cells had tetrasomy of chromosome 7 (Fig. 3). Both cell lines showed HLA-A3, -B51, and -CW1.

Each of the established cell lines formed colonies in semisolid cultures containing 1.0% methylcellulose without any stimuli such as that of colony-stimulating factor. The plating efficiencies of 10-day cultures, at which time the number of colonies was maximum, were in the range of 8.1—11.3% in each cell line. Each colony consisted of 100—500 cells. The cells were morphologically the same as the originally established cells.

**Table 1 Characteristics of the established leukemia lines**

<table>
<thead>
<tr>
<th>Donor</th>
<th>JOSK-I</th>
<th>JOSK-S</th>
<th>JOSK-M</th>
<th>JOSK-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)/sex</td>
<td>N. I.</td>
<td>M. S.</td>
<td>K. M.</td>
<td>M. K.</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>AMoL*</td>
<td>AMoL</td>
<td>CML-BC</td>
<td>AMoL</td>
</tr>
<tr>
<td>Material</td>
<td>PB</td>
<td>PB</td>
<td>PB</td>
<td>PB</td>
</tr>
<tr>
<td>Growth pattern</td>
<td>Suspension</td>
<td>Suspension</td>
<td>Suspension</td>
<td>Suspension</td>
</tr>
<tr>
<td>Doubling time (h)</td>
<td>24–28</td>
<td>24–27</td>
<td>24–26</td>
<td>28–32</td>
</tr>
<tr>
<td>Morphology</td>
<td>Monoblastoid</td>
<td>Monoblastoid</td>
<td>Monoblastoid</td>
<td>Monoblastoid</td>
</tr>
<tr>
<td>Cytochemical staining</td>
<td>Peroxidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>–</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>Naphthol AS-D chloroacetate esterase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Naphthyl butyrate esterase with sodium fluoride</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Acid phosphatase</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Periodic acid-Schiff</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Nuclear antigen</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Epstein-Barr virus-associated nuclear antigen</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Terminal deoxynucleotidyl transferase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Surface marker</td>
<td>–</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>Surface immunoglobulin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sheep erythrocyte rosette</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>J5</td>
<td>0.9</td>
<td>1.8</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>OKIa1</td>
<td>95.7</td>
<td>97.5</td>
<td>98.5</td>
<td>83.9</td>
</tr>
<tr>
<td>OKM1</td>
<td>62.9</td>
<td>27.6</td>
<td>69.1</td>
<td>30.5</td>
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<tr>
<td>OKM5</td>
<td>90.9</td>
<td>86.8</td>
<td>97.7</td>
<td>28.3</td>
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<tr>
<td>Mo2</td>
<td>8.5</td>
<td>2.7</td>
<td>3.4</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*AMoL, acute myelomonocytic leukemia; AMoL, acute monocytic leukemia; CML-BC, chronic myelogenous leukemia in blast crisis; PB, peripheral blood.

- , negative reaction; ±, faintly positive reaction; +, positive reaction; ++, strongly positive reaction.

Percentage of positive cells determined by indirect immunofluorescence using Ortho Spectrum III.
reactive with the monoclonal antibodies OKM1 and Mo2 increased remarkably after treatment with the inducers examined. The OKM5-reactive cells showed little or no increase in percentage compared with untreated cells. There was a high percentage of OKIa1-reactive cells whether or not the cells were treated with chemical inducers (Table 3).

Cells cultured with VD3 became adherent to plastic culture dishes with over 90% of them being adherent by culture day 4. RA- or DMSO-induced cells did not show remarkable cell adherency. When treated with TPA, the cells became adherent to plastic culture dishes more rapidly, starting within 3 h of incubation, with over 90% of the cells being adherent within 24 h and with a marked enhancement of phagocytic activity (Figs. 2B and 4, B and C). The TPA-induced cells exhibited more mature monocytic morphology (Fig. 2C). On incubation with VD3 or RA, the cells also functionally differentiated, showing a marked increase in phagocytic activity and a parallel increase in the number of cells positive for NBT reduction. The DMSO-induced cells also showed marked enhancement of phagocytic activity but did not show a parallel increase in the number of NBT reduction-positive cells. Of these inducers, VD3 was the most active as to differentiation of JOSK-I cells into more mature monocytic cells (Table 2).

The other three cell lines also showed marked abilities to differentiate into more mature monocytic cells morphologically, immunochemically, and functionally.

Constitutive Production of Interleukin 1. The capacity of JOSK-I cells to produce IL-1 was assayed under several culture

Fig. 1. Morphological changes of JOSK-I cell line. A, control culture stained with Wright-Giemsa. × 250. B, esterase double stainings of control culture of JOSK-I cells. Cells are positive for α-naphthyl butyrate esterase activity. × 250. C, esterase double stainings with sodium fluoride. a-Naphthyl butyrate activity was completely inhibited. × 250. D, scanning electron microscopy. × 6200. E, transmission electron microscopy. × 5200.
conditions with regard to various cell growth phases. When the cells reached the maximal growth phase ($2 \times 10^6$ cells/ml) in the regular culture medium, the conditioned medium obtained from the cultivation of these cells in 1% FCS-RPMI 1640 medium for 48 h had high IL-1 activity; on the other hand, when the cells were at logarithmic growth phase ($3 \times 10^5$ cells/ml), conditioned medium gave low IL-1 activity (Fig. 5). JOSK-K cells also gave high IL-1 activity almost corresponding to that of JOSK-I, but neither JOSK-S nor JOSK-M did.

DISCUSSION

We have established four new monoblastoid leukemia lines, JOSK-I, -S, -M, and -K from peripheral blood mononuclear cells of patients with monocytoid or myelomonocytoid leukemias. These cell lines grew in suspension cultures and have been serially maintained for over 20 mo successfully. Morphological, cytochemical, and immunochemical studies revealed that each cell line exhibited immature monoblastoid characteristics. For the establishment of leukemic lines, many attempts at culturing human leukemic cells in culture flasks or dishes were made in vain in our laboratory, so we devised a culture system method using 96-well microplates. This method was found to be suitable for selecting clones having proliferation potential. With this method, the number of wells in which active proliferation was detectable was 1 or 2/microplate (96 wells).

Attempts have been made to establish permanent cultures of human nonlymphoid leukemia cells, and the established cell lines such as K562 (13), ML-1 (14), HL-60 (15), KG-1 (16), HL-92 (17), and PL-21 (18) have been useful for investigating the regulation of myelogenous leukemic cell growth and differentiation; but even so, successful establishment of human nonlymphoid monocytic leukemia lines has been rare. The established monocytoid lines, U-937 (19), SU-DHL-1, and SU-DHL-2 (20) were all derived from histiocytic lymphomas, whereas THP-1 (21) was reported as the cell line derived from an acute monocytic leukemia case and RC-2A (22) from an acute myelomonocytic leukemia case. All these cell lines showed some properties specific for the early stage of monocyte lineage. In our present study, both JOSK-S and -K cells originated from leukemic monoblasts, and the JOSK-I monoblastoid cell line was exclusively established from the mixture of myeloblasts and monoblasts which coexisted in the original leukemic state.

In the chromosome analysis, each line had a more hyperdiploid karyotype with marker chromosomes, compared with that of leukemic cells prior to establishment. It seemed probable that these chromosomal changes might occur during the establishment processes of each cell line. In the JOSK-M line, there was found no distinct Ph¹ chromosome. This condition might
be due to a preferential selection of the Ph' negative blast cells in blast crisis of chronic myelogenous leukemia during establishment of this line; however, a possibility of the presence of a masked Ph' chromosome would not be completely excluded for the present time in the new monocytoid cell line JOSK-M. More precise cytological and cytogenetic studies on this cell line are now in progress. HLA typing studies showed that the JOSK-1 line expressed HLA-A3, -B51, and -CW1 antigens, which were the common appearances in cultures in vitro of the established leukemia cells that were actively proliferating. This phenomenon remains to be proved.

Various chemical agents have been reported to be highly potent for inducing cell differentiation in some leukemic lines.

It is well known that either DMSO or RA stimulates granulocyte lineage differentiation of the HL-60, HL-92, and ML-1 lines (17, 23–25), and TPA induces macrophage-monoocyte lineage differentiation in a variety of leukemia lines such as KG-1, HL-60, and U937 (26–28), and also in fresh leukemia cells from myeloid leukemia patients (29–32). Recently, vitamin D3 has been shown to be an active inducer of differentiation of HL-60 or U937 cells into more mature monocyte lineage (33, 34). In the previously established monoblastic leukemia lines,

Table 2 Differentiation of JOSK-1 cells induced by VD3, RA, or DMSO

<table>
<thead>
<tr>
<th>Inducers, culture period (days)</th>
<th>Cell density (10^6) cells/ml</th>
<th>Adherent cells (%)</th>
<th>Phagocytosing cells (%)</th>
<th>NBT reduction-positive cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.5</td>
<td>0.0</td>
<td>10.0</td>
<td>4.2</td>
</tr>
<tr>
<td>VD3 (10 nm)</td>
<td>1.2</td>
<td>8.4</td>
<td>42.0</td>
<td>34.5</td>
</tr>
<tr>
<td>RA (1 μM)</td>
<td>0.8</td>
<td>5.2</td>
<td>39.0</td>
<td>17.2</td>
</tr>
<tr>
<td>DMSO (1.5%)</td>
<td>1.0</td>
<td>0.0</td>
<td>33.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Table 3 Changes in surface markers during differentiation induction of JOSK-1 cells

<table>
<thead>
<tr>
<th>Inducers, final concentration</th>
<th>Monoclonal antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>J5</td>
<td>OKL1</td>
</tr>
<tr>
<td>None</td>
<td>0.9*</td>
</tr>
<tr>
<td>VD3 (10 nm)</td>
<td>1.8</td>
</tr>
<tr>
<td>RA (1 μM)</td>
<td>0.4</td>
</tr>
<tr>
<td>DMSO (1.3%)</td>
<td>1.1</td>
</tr>
<tr>
<td>TPA (4 nm)</td>
<td>7.7</td>
</tr>
</tbody>
</table>

* Percentage of positive cells determined by indirect immunofluorescence using an Ortho Spectrum III.
conditioned medium from the culture of JOSK-I cells at the maximal growth was obtained as described in "Materials and Methods." •, control (conditioned medium was determined by the thymocyte proliferation assay method. Results are expressed as mean counts per well of triplicate cultures. Each conditioned medium at logarithmic growth phase (3 x 10^9 cells/ml); □, conditioned medium from the culture of human adherent peripheral blood leukocytes); ○, conditioned medium from the culture of JOSK-I cells at maximal growth phase (2 x 10^9 cells/ml); △, conditioned medium from the culture of JOSK-I cells at logarithmic growth phase (3 x 10^9 cells/ml). Bars, SE.

except for U-937 and THP-1 cells, the detailed evaluation of inducer-oriented differentiation inductions was not fully performed. Our newly established lines differentiated morphologically, phenotypically, and functionally into more mature mononuclear cells when induced by DMSO, RA, VD3, and TPA; however, the induced properties did not always parallel each other.

It is of much interest that these new cell lines differentiated into the monocyte-macrophage lineage when treated with DMSO or RA, both of which have been reported to be potent inducers of the granulocytic differentiation. U-937 cells were demonstrated to differentiate into macrophage-like cells on the addition of RA (35), and P388D1, a murine macrophage-like cell line, was induced functionally by DMSO to differentiate into a more mature form of macrophage-like cell (36). The detailed mechanism of action of each chemical inducer is now being examined in our laboratory with reference to potentialities to induce different cell lineages and to constitutional changes of membrane components, especially some molecular species of glycosphingolipids, which were recently reported to change dramatically depending on differentiation directions as well as differentiation stages (37, 38) and to play a primary role as a trigger for differentiation induction (39).

Monocyte-macrophage lineage has been considered to be a valuable source of biological response modifiers such as colony-stimulating factor (40), interleukin 1, (41) or other biologically active substances (1). We preliminarily investigated such active substances in the conditioned media of the established lines and found that the JOSK-I cell line showed high IL-1 activity production; this IL-1 production was constitutive because no stimuli were required. Details will be published elsewhere.

These established cell lines may offer an attractive model system for clarifying the processes of monocyte-macrophage differentiation and furthermore for investigating the roles of the cytokine-producing monocyte-macrophage lineage.

ACKNOWLEDGMENTS

We thank Drs. H. Nojiri, T. Suda, M. Akashi, and K. Motoyoshi, Division of Hemopoiesis, Institute of Hematology, Jichi Medical School, for their valuable advice during this work. We are also grateful to Y. Kobayashi for excellent technical assistance, N. Tokuhashi for skillful assistance in chromosome analysis, and N. Kuroo for secretarial assistance in preparing this manuscript.

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