Functional and Morphological Differentiation Induction of a Human Megakaryoblastic Leukemia Cell Line (MEG-01s) by Phorbol Diesters

By Michinori Ogura, Yasuo Morishima, Masao Okumura, Tomomitsu Hotta, Shigeru Takamoto, Ryuzo Ohno, Norio Hirabayashi, Hiroshi Nagura, and Hidehiko Saito

The effect of differentiation induction by a tumor-promoting phorbol diester, 12-0-tetradecanoylphorbol-13-acetate (TPA) on a clonal human megakaryoblastic cell line, MEG-01s, was investigated, and a prominent response was demonstrated. The cells became weakly adherent, developed conspicuous cytoplasmic blebs, and displayed mature megakaryocytic characteristics by light microscopy such as the development of azurophilic cytoplasmic granules and a mosaic pattern of oxyphilic patches. Multiplication of nuclei, and enhancement of the PAS reaction and alpha-naphthyl acetate esterase staining. Ultrastructural studies demonstrated the development of prominent cytoplasmic blebs, budding of blebs, and multiplication of nuclei. Numerous granules with central nucleoids that are similar to α granules had developed as well as granules with high electron density and clearly demarcated zones. Surface marker analysis revealed a moderate increase in IgG Fe receptor levels and a profound decrease in C3 receptor sites. By an immunofluorescent technique using monoclonal and polyclonal antibodies, a dramatic change in the expression of several megakaryocyte-platelet-specific proteins was demonstrated. All the proteins that had been expressed before induction such as platelet glycoprotein (GP) IIb/IIIa, fibrinogen, von Willebrand factor, factor XIIIa, β-thromboglobulin (β-TG), and HLA class 1 antigen were profoundly enhanced after induction by TPA. Induction by TPA led to the expression of fibronectin and factor V, which were not detected on nontreated cells. An ultrastructural immunoperoxidase study demonstrated platelet GP Ib and GP IIb/IIIa in both plasma membranes and protein synthesis areas such as perinuclear cisternae and endoplasmic reticulum after TPA induction. β-TG was also observed in some cytoplasmic granules of TPA-treated cells. TPA remarkably increased the secretion of β-TG into the culture medium of MEG-01s. Ploidy was also increased from 2C to 4C to 8C. Similar maturation of MEG-01s was induced by other phorbol diester analogues such as phorbol-12,13-dibutyrate, but not by phorbol itself. These results indicate that phorbol diester, TPA, can bring about differentiation and maturation of a human megakaryoblastic cell line (MEG-01s) and that MEG-01s cells will provide a useful model for studying megakaryocytic differentiation and numerous megakaryocyte-platelet-specific proteins.

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MATERIALS AND METHODS

Cells

The MEG-01 cells were established in our laboratory from a male patient in megakaryoblastic crisis of chronic myelogenous leukemia more than 3.5 years ago and have been maintained in continuous suspension culture for 340 passages. MEG-01 cells demonstrated many megakaryocytic characteristics and possessed no markers for B or T lymphocytes, myeloid cells, or erythroid cells. The cells were cultured in Falcon 3013 plastic flasks (Becton Dickenson, Oxnard, CA) in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS, 1988 by Grune & Stratton, Inc.)
Flow Laboratories, Stanmore, NSW, Australia) and aqueous penicillin G (100 U/mL) and streptomycin (50 μg/mL) at 37°C in 5% CO2 and air in a humidified incubator. The original MEG-01 cells had a tendency to adhere to the plastic culture flask under the usual culture conditions. After cloning, clonal cells, named MEG-01s, were obtained. MEG-01s cells had almost the same characteristics as MEG-01 cells except for two properties: (a) MEG-01s cells grow in single suspension without attachment to the culture dish, and (b) the expression of platelet glycoprotein (GP) IIb/IIIa in MEG-01s cells is weaker than that in MEG-01 cells. Platelet GP IIb/IIIa was demonstrated in 25% to 40% of MEG-01s cells, whereas it was expressed in over 90% of MEG-01 cells. This clonal MEG-01s line has been maintained over 1.5 years for over 140 passages, and these characteristics have been stable. MEG-01s cells were used for all the following experiments.

**Chemicals**

TPA, the phorbol ester, was purchased from Paesel GmbH & Co (Frankfurt, West Germany). Stock solutions (10⁻⁴ mol/L) of this compound were prepared in dimethyl sulphoxide (DMSO) (Dojin Chemical Institute, Kumamoto, Japan) and stored at −60°C. New stock solutions were prepared every 2 to 3 months. All stock solutions were diluted appropriately with RPMI 1640 medium just before use. The concentrations of DMSO present in the final solution of TPA stock solutions were prepared every 2 to 3 months. All stock solutions were prepared in dimethyl sulphoxide (DMSO) (Dojin Chemical Institute, Kumamoto, Japan) and stored at −60°C. New stock solutions were prepared every 2 to 3 months. All stock solutions were diluted appropriately with RPMI 1640 medium just before use.

**Cell Proliferation, Adhesion, and Morphological Studies**

Cell proliferation was studied by placing 3 x 10⁵ cells/mL in Falcon 3013 plastic flasks containing 10 mL of culture medium supplemented with 10% FCS containing TPA. Cultures were harvested on days 1, 3, 5, and 7 for viable cell counts. Viable cell counts were examined by using trypan blue dye exclusion. Adhesion was examined by placing 3 x 10⁵ cells/mL in 2 mL of culture medium in flat glass tissue culture chambers (Lab-Tek Products; Miles Laboratories, Inc, Naperville, IL). After incubation for one to seven days at 37°C, the slides were gently washed with warm phosphate-buffered saline (PBS), and the nonadherent cells were collected. The adherent cells were removed by exposure to 0.25% trypsin and collected separately. Viable cells were counted.

Morphological observations of live cultured cells were made with an Olympus inverted microscope. Cytospin slide preparations of 0.2-mL aliquots of cell suspensions were prepared by using a Shandon Cytospin 2 centrifuge (Shandon Southern Products, Ltd, Cheshire, England) and stained with May-Grünwald-Giemsa (MGG). For histochemical studies, the cells were also stained by published procedures as follows: naphthol ASD chloroacetate esterase, alphanaphthyl butyrate esterase, alphanaphthyl acetate esterase, myeloperoxidase, leukocyte alkaline phosphatase, acid phosphatase, and the PAS reaction.

**Ultrastructural Studies**

**Ultrastructural morphology.** Cell samples harvested on day 5 after the addition of TPA were centrifuged at 1,200 rpm for ten minutes and then washed three times with 0.01 mol/L PBS. Ultrastructural morphology was analyzed as described previously. Control MEG-01s cells cultured in the absence of TPA were processed similarly. Ultrastructural platelet peroxidase (PP0) was studied as described earlier.

**Immunoelectron microscopic observation.** The indirect peroxidase-labeled antibody technique, similar to that described before, was used. MEG-01s cells (control cells and TPA-treated day 3 cells) were harvested and washed in 0.01 mol/L PBS. The cells were pelleted by centrifugation, fixed in chilled periodate-lysine-paraformaldehyde, and washed in increasing concentrations of sucrose in PBS. The fixed cells were embedded in tissue Tek OCT compounds (Miles), frozen in a dry ice-ethanol slurry, and sectioned (6 to 8 μm) in a cryostat. The sections, air-dried on glass slides, were reacted with an antiplatelet GPIb/IIIa monoclonal antibody (MoAb) (HPL-3), an antiplatelet GPIb MoAb (HPL-9), or rabbit anti-human β-thromboglobulin (β-TG) polyclonal antibody (PoAb) (Amersham Corp, Buckinghamshire, England) for 12 hours at 4°C. HPL-3 and HPL-9 were kindly provided by Dr K. Furukawa at the Department of Medicine, Nagoya University Branch Hospital. After washing, the sections were reacted with horseradish peroxidase (HRP)-labeled antimouse IgG rabbit antibody (Fab') or HRP- labeled antirabbit antibody (Fab') for six hours. Then the sections were postfixed in 0.5% glutaraldehyde in PBS, washed in PBS, and incubated sequentially in incomplete Kavnovskys's solution. They were fixed in 2% osmium tetroxide in PBS, dehydrated in graded ethanol, and embedded in Epon. Ultrathin sections with or without lead stain were viewed under a Hitachi H-300 electron microscope.

**Cell Surface Markers**

Rosette markers. Sheep erythrocytes (SRBC), mouse erythrocytes (MRBC), SRBC sensitized with anti-SRBC rabbit IgG and IgM, and SRBC sensitized with IgM antibody complement were used for the study of cytoplasmic antigens of TPA-treated cells (day 3) using three kinds of MoAbs: (a) antiplatelet GPIb/IIIa MoAb (HPL-3), (b) antiplatelet GPIb MoAb (HPL-9), and (c) anti-HLA class I antigen MoAb (4E). Anti-vWF MoAb is the supernatant harvested from hybridoma cells, and other MoAbs are all ascites fluid.

Next, six rabbit PoAbs were also used: (a) anti-factor V (FY) PoAb (Dakopatts); (b) anti-vWF PoAb (Dakopatts); (c) anti-FXIIIa PoAb; (d) FXIIIa PoAb (Behringwerke AG, Marburg, West Germany); (e) antihuman fibrinogen PoAb (Dakopatts); and (f) antihuman fibrinectin PoAb (Dakopatts).

Binding of these antibodies was tested by an indirect immunofluorescence (IF) technique as described previously. Cell samples were analyzed by flow cytometry (FACS IV, Becton Dickinson). Normal mouse serum and normal rabbit serum were used as negative controls of primary antibody for MoAb and PoAb, respectively. Some samples were resuspended in a minimal amount of medium, which was deposited with one or two drops of 50% buffered glycerol on a coverslip. They were examined and photographed under an Olympus fluorescence microscope.

**Cytoplasmic Antigens**

The six PoAbs described earlier and anti-β-TG PoAb were used for the study of cytoplasmic antigens of TPA-treated cells (day 3) and control MEG-01s cells. After washing three times in cold PBS, the cells were centrifuged and resuspended in a small amount of PBS, and cytospin glass slide preparations were prepared. The cells were fixed with 4% paraformaldehyde at 4°C for 30 minutes, washed three times in PBS, and incubated in 0.1% Triton X-100 in PBS at room temperature for 15 minutes. After being washed three times in PBS, the specimens were incubated with 5% normal goat serum in PBS for 15 minutes. After being gently rinsed, the specimens were
incubated with each primary antibody at 4°C overnight. A negative control for the primary antibody was performed as described earlier. After being washed three times in cold PBS, a fluorescein isothiocyanate-labeled second antibody, as described earlier, was reacted at 4°C for one hour. The samples were then washed three times in cold PBS and deposited in one drop of 50% buffered glycerol on a coverslip. They were examined, and positive cells per 200 cells were counted.

Flow Cytometric DNA Analysis

The cell suspensions (5 x 10^5) of control and TPA-treated MEG-01s cells (days 1, 3, and 5) were mixed with normal human peripheral mononuclear cells (5 x 10^5), and the mixtures were stained with 50 μg/mL propidium iodide (Sigma) in 0.1% sodium citrate containing 0.1% Nonidet P40 (Sigma) and treated with ribonuclease (1 mg/mL) (Sigma) simultaneously. There was no cell clumping in both control and TPA-treated cells. The DNA content per cell of up to 2 x 10^5 cells were measured in a flow cytometer (Ortho Cytofluorograf 50H, Westwood, MA) after two to 24 hours of staining. Fluorescence of over 600 nm was measured at 200 mW and a wavelength of 488 nm. The internal standard was adjusted to 19 channels of 200 total channels to detect 16C, and the peak of the diploid cells was demonstrated in channel 25.

Analysis of β-TG Antigens

β-TG antigen was analyzed by a radioimmunoassay (RIA) using a monospecific rabbit antihuman β-TG antibody (β-TG RIA pack, Amersham). MEG-01s cells were seeded at 2 x 10^5/mL in 8 mL of RPMI 1640 medium containing 1% bovine serum albumin (BSA). Aliquots (2 mL) were removed every two to three days, and the same volume of fresh medium was replaced. Harvested medium was mixed with 2 μL of ethanol containing of 10 mmol/L phenylmethylsulfonilyfluoride (Sigma). The medium was then centrifuged, and the supernatant was frozen until use.

RESULTS

Effect of TPA on Cell Proliferation, Adhesion, and Morphology

Figure 1 shows the proliferation curve of MEG-01s cells in the presence of various concentrations of TPA. The cell numbers are the total of nonadherent and adherent cells. TPA at 10^{-5} to 10^{-9} mol/L inhibited the proliferation of the MEG-01s cell significantly. By the fifth day of TPA (10^{-7} to 10^{-9} mol/L) exposure, the net proliferation of cells decreased by approximately 28% (range, 24% to 39%). TPA at 10^{-10} mol/L, however, had almost no effect on cell proliferation.

A part of MEG-01s cell population began to adhere to the plastic surface 30 minutes after 10^{-5} to 10^{-7} mol/L TPA. When the concentration of TPA was reduced to 10^{-8} to 10^{-10} mol/L, however, almost all cells were nonadherent, even after 24 hours. At the concentration of 10^{-7} mol/L, a maximum adherence rate (29.1%) was obtained on the third day. The adhering cells, however, attached weakly to plastic or glass flasks; therefore, mechanical pipetting could release most of the adhering cells. Viability was approximately 75% to 83% of both suspension and adherent cells during the seven-day observation period. For further studies, 1 x 10^{-7} mol/L TPA was selected because this concentration induced considerable morphological changes in preliminary analysis.
Fig 2. (A) MGG staining of control MEG-01s cells with diameters of about 30 to 40 μm. The deeply basophilic cytoplasmas without any azurophilic granules and round to ovoid nuclei are demonstrated (B) MGG staining of day 5 cells treated with TPA (10^{-7} mol/L) with diameters of about 80 to 80 μm. The enlargement of cell size, the multiplication of nuclei and the increase in azurophilic granules are evident. (C) MGG staining of day 7 cells treated with TPA (10^{-7} mol/L). The diameter of the cells is more than three times that of the control cells. The azurophilic granules are diffusely distributed, and a mosaic pattern of the oxyphilic patches can be observed. Cytoplasmic basophilia is not observed (original magnification ×4,500).
whether these are typical demarcation membranes is unknown. PPO was expressed in the rough endoplasmic reticulum and perinuclear cisternae and not in the Golgi apparatus. Both control and TPA-treated MEG-01s cells showed a PPO reaction (data not shown).

Ultrastructural immunoperoxidase staining. Platelet GPIIb/IIIa was clearly demonstrated on the surface of cytoplasmic membranes, especially on the cytoplasmic blebs as HRP particles of both control cells and TPA-treated day 3 cells (data not shown). Endoplasmic reticulum, however, became labeled only in TPA-treated day 3 cells. Platelet GPIb was faintly demonstrated in the perinuclear space of control MEG-01s cells. In day 3 cells treated by TPA, however, this antigen was demonstrated not only in the perinuclear space, but also in the plasma membrane (data not shown). Negative control sections showed no specific staining. fl-TG was demonstrated in the rough endoplasmic reticulum of both control (Fig 4A) and TPA-treated cells (Fig 4B). However, positive staining of the perinuclear space and a few granules became evident only after TPA exposure.

Effect of TPA on Cell Surface Markers

Rosetting markers. E rosette, MRBC rosette, and EA tests for IgM Fc receptors were uniformly negative in both control cells and cells exposed to TPA (Table 1). The EAC test and EA tests for IgG Fc receptors were positive in 75% and 8% of control MEG-01s cells, respectively. After exposure to TPA, the positivity of the C3 receptor (EAC) rapidly decreased, whereas the positivity of the EA test for IgG Fc receptors increased.

Cell surface markers analyzed by antibodies. After exposure to TPA, the positivity of platelet GPIIb/IIIa and HLA class I antigen, vWF, and FXIIIa was dramatically increased (Tables 2 and 3). Although fibronectin and FX were not detected on the control MEG-01s cells, these antigens were clearly demonstrated after treatment with TPA. Fibrinogen levels were mildly increased following the exposure to TPA. These positivity rates were maintained during the seven-day observation period. Platelet GPIb, HLA-DR (human Ia-like) antigen, and FXIIIa were uniformly negative before and after TPA exposure. The increase in the expression of these proteins on TPA-treated cell surfaces is not due to the increased binding of these proteins to cell surfaces from the medium because similar changes were induced by TPA in MEG-01s cells cultured in serum-free medium (data not shown). Nonspecific binding of antibodies to the increased numbers of Fc receptors on TPA-treated MEG-01s cells was also unlikely since control experiments were negative that used, as primary antiserum, normal mouse or rabbit serum that contained the same amounts of IgG as did the specific antibodies. The positivity rate of cell surface antigens, as described earlier in control MEG-01s cells, was constant during the seven-day observation period without medium change.

Effect of TPA on Cytoplasmic Antigens

The positivity of vWF FXIIIa, β-TG, and fibrinogen was remarkably increased following exposure to TPA (Table 4). Although FV and fibronectin were not detected in control cells during the seven days of culture, these antigens were clearly demonstrated in TPA-treated cells. FXIIIa was not detected in control or TPA-treated cells.

Effect of TPA on Polyploidization

In control day 1 and day 3 cells, the pattern of DNA content was almost constant, and the peak of ploidy was in 2C (43%) and 4C (15.1%) (Fig 5A and B). After exposure to TPA, the peak of 4C increased to 29.3%, and small numbers of cells with 8C (8.4%) and 16C (7.4%) appeared.
Effect of TPA on the Secretion of $\beta$-TG

The concentration of $\beta$-TG in the medium increased slightly with time from $<1$ ng/mL on day 0 to 11 ng/mL on day 7 in the control culture without TPA (Fig 6). TPA remarkably enhanced the accumulation of secreted $\beta$-TG in the culture medium, and the quantity of secreted $\beta$-TG was dependent on the concentration of added TPA. When TPA was added at the concentrations of $10^{-10}$, $10^{-9}$, $10^{-8}$, and $10^{-7}$ mol/L, the quantities of $\beta$-TG on day 7 were 12, 37, 140, and 149 ng/mL, respectively.

DISCUSSION

The present paper demonstrates that TPA ($10^{-7}$ mol/L) can stimulate a human megakaryoblastic cell line (MEG-01s) to differentiate into more mature megakaryocytes as judged by several morphological and biochemical criteria. Similar maturation of MEG-01s was induced by other phorbol diester analogs such as PDBu, but not by phorbol itself. Since our study was completed, a similar study on the effect of phorbol esters on another human megakaryocytic cell line (EST-IU) has appeared in abstract form.24 These results are consistent with recent observations of Long et al in in vitro marine megakaryocyte colony formation.25 They showed that phorbol diesters can bring about murine megakaryocyte differentiation by substituting for megakaryocyte potentiator activity.

The inhibition of growth of MEG-01s cells by TPA was similar to that of other human leukemic cell lines in which
the differentiation was induced by TPA; the concentrations of TPA used in our experiments are similar to those reported in other cell lines. The sequence of maturation of the bone marrow megakaryocytes has been divided into three stages: promegakaryoblast or megakaryoblast (stage I), promegakaryocyte or basophilic megakaryocyte (stage II), and granular megakaryocyte or mature megakaryocyte (stage III). The morphological process of cytoplasmic maturation of MEG-01s after TPA stimulation, as examined by MGG staining, resembles the process of in vivo human megakaryocyte maturation as follows: (a) control MEG-01s cells, megakaryoblast; (b) day 1 to day 3 cells treated with TPA, promegakaryocyte; (c) day 5 to day 7 cells, granular megakaryocyte. The PAS reaction, which is positive in
Fig 4. (A) Electron micrograph of day 3 control MEG-01s cells reacted with anti-β-TG PoAb. Rough endoplasmic reticulum (rER) is positive for β-TG. Filament-like cytoplasmic protrusions are observed. (B) Electron micrograph of day 3 MEG-01s cells treated with TPA (10^{-7} mol/L) reacted with anti-β-TG PoAb. In addition to more developed rough endoplasmic reticulum (rER) and perinuclear space, a few granules are positive for β-TG (open arrowheads). Cytoplasmic blebs are evident (closed arrowheads). Original magnification x11,000.

Table 1. Effect of TPA on Rosetting Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Control Cells*</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>MRBC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EAC (C3 receptor)</td>
<td>75 ± 0.57</td>
<td>3 ± 1</td>
<td>2 ± 0</td>
<td>3 ± 0.57</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>EA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>IgM Fc</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>IgG Fc</td>
<td>8 ± 1</td>
<td>15 ± 2</td>
<td>30 ± 0.57</td>
<td>30 ± 1</td>
<td>32 ± 1.15</td>
</tr>
</tbody>
</table>

Values (percentage of positive cells) are the means ± SD of three separate experiments.
*Values for control cells are the mean positivity rates of the control cells on each harvesting day.
normal megakaryocytes and platelets, becomes more intense after the addition of TPA.

Ultrastructural changes in MEG-01s cells following TPA stimulation such as the formation of α granules and demarcation membranes are similar to those observed in the human megakaryocyte maturation in an in vitro colony. Ultrastructural immunoperoxidase studies showed that the synthesis of GPIIb/IIIa and GPIb was enhanced by TPA. The discrepancy in the detection of GPIb by different techniques may be due to the weak concentration of GPIb antigen in contrast with that of GPIIb/IIIa. β-TG, one of the platelet-specific proteins, is well known to be localized in the α granules of megakaryocytes and platelets and reported to be synthesized and secreted from MEG-01 cells. TPA remarkably enhanced the positivity of cytoplasmic β-TG and the secretion of β-TG in culture medium. Immunoelectron microscopic analysis revealed that after the addition of TPA β-TG appeared in a few granules in addition to the more developed rough endoplasmic reticulum and perinuclear space. This appears to be unequivocal evidence for cytoplasmic

Table 2. Effect of TPA on Cell Surface Antigens. I. Analyzed by MoAbs

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Control Cells</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPL-3</td>
<td>Platelet GPIIb/IIIa</td>
<td>25 ± 1.14</td>
<td>76 ± 2</td>
<td>97 ± 0.57</td>
<td>96 ± 1.5</td>
<td>40 ± 2.6</td>
</tr>
<tr>
<td>HPL-9</td>
<td>Platelet GPIb</td>
<td>Neg†</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Anti-vWF (Dakopatts)</td>
<td>vWF</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Antifibronectin (BRL)</td>
<td>Human fibronectin</td>
<td>Neg</td>
<td>16 ± 1.15</td>
<td>12 ± 0.57</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
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<tr>
<td>4D1</td>
<td>Human HLA antigen</td>
<td>Neg</td>
<td>Neg</td>
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<td>Neg</td>
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<td>4E</td>
<td>HLA class I antigen</td>
<td>60 ± 2</td>
<td>93 ± 1.52</td>
<td>97 ± 2</td>
<td>94 ± 1</td>
<td>94 ± 1.52</td>
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</table>

Values (percentage of positive cells) are the means ± SD of three separate experiments.
*Values for control cells are the mean positivity rates of the control cells on each harvesting day.
†Negative, <8% of the cells were positive under conditions in which the negative control showed a 5% positive background.

Table 3. Effect of TPA on Cell Surface Antigens. II. Analyzed by PoAbs

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Control Cells</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-FV (Behring)</td>
<td>FV</td>
<td>Neg</td>
<td>26 ± 2</td>
<td>23 ± 1</td>
<td>34 ± 1</td>
<td>21 ± 1.52</td>
</tr>
<tr>
<td>Anti-vWF (Dakopatts)</td>
<td>vWF</td>
<td>55 ± 1</td>
<td>91 ± 2.08</td>
<td>81 ± 1</td>
<td>78 ± 1.52</td>
<td>64 ± 3.05</td>
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<tr>
<td>Anti-FXIIIa (Behring)</td>
<td>FXIIIa</td>
<td>34 ± 3.6</td>
<td>68 ± 3.05</td>
<td>65 ± 2.08</td>
<td>67 ± 2</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>Anti-FXIIIb (Behring)</td>
<td>FXIIIb</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Antifibrinogen (Dakopatts)</td>
<td>Human fibrinogen</td>
<td>29 ± 1.52</td>
<td>32 ± 2.52</td>
<td>30 ± 1</td>
<td>40 ± 1.73</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Antifibronectin (Dakopatts)</td>
<td>Human fibronectin</td>
<td>Neg</td>
<td>27 ± 1</td>
<td>25 ± 1.52</td>
<td>31 ± 2</td>
<td>21 ± 1</td>
</tr>
</tbody>
</table>

Values (percentage of positive cells) are the means ± SD of three separate experiments.
*Values for control cells are the mean positivity rates of the control cells on each harvesting day.

Fig 5. (A) The pattern of the ploidy of control MEG-01s cells. The high peak of 2C and moderate peak of 4C are evident. (B) The pattern of the ploidy of day 5 MEG-01s cells treated with TPA (10^{-7} mol/L). The increase in the peak of 4C and the new small peak of 8C are demonstrated.
mic α-granule formation in MEG-01s cells following TPA exposure.

Megakaryocytic maturation of the nucleus involves nuclear endoreduplication, a process in which nuclear material reduplicates itself without nuclear division. In humans, 65% of the recognizable megakaryocytes were reported to be of the 8N type as judged by microscopic nuclear counts. However, cytoplasmic maturation and development of polyploidy are neither totally concurrent nor consecutive processes; the appearance of cytoplasmic organelles begins during ploidization and continues after DNA synthesis has stopped. In our study, the cells of 4C to 8C moderately increased in number after TPA stimulation, which suggests that TPA induced MEG-01s cells to differentiate along the normal megakaryocytic pathway. It is noteworthy that the expression of FXIIIa in MEG-01s cells may be used as one of the early markers of megakaryocytic lineage. In TPA-treated day 7 cells, the positivity of platelet GPIIb/IIIa was decreased to 40% ± 2.6%. This may be due to the more fragile cytoplasmic membranes with numerous buddings. However, the cause of this decrease remains to be investigated since HLA antigen levels were not decreased in the same cells. MEG-01s cells had HLA class 1 antigen on their cell’s surfaces, and TPA enhanced the expression of this antigen. Although the origin of HLA antigen on platelets has been a subject of debate, our observation may suggest the possibility that the HLA class 1 antigen of platelets may not be acquired from plasma but may be synthesized by megakaryocytes.

Our investigation will be useful for studying the biosynthesis of proteins unique to human megakaryocyte-platelets and the process of controlling megakaryopoiesis. It should be emphasized, however, that TPA induction of MEG-01s may be of limited use as a model for normal maturation mechanisms since MEG-01s cells are of leukemic origin and in vitro differentiation under these conditions does not appear to be complete in that shedding of platelets was not confirmed. Although the mechanisms by which TPA induced the differentiation of MEG-01s is not known, recent studies suggest that protein kinase C may play a role in mediating cellular membrane signals and may serve as a TPA receptor. If the effect of TPA is related to protein kinase C activation, the induction of MEG-01s differentiation by TPA will be also a useful model to elucidate general mechanisms of cellular differentiation.

ACKNOWLEDGMENT

We wish to thank Sayoko Sugiura and Makoto Kondo for their skillful technical assistance.

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