Macrophage Differentiation of Human Precursor B-Cell Line by Phorbol Ester and Colony-Stimulating Factor

By Atsunobu Hiraoka, Kazuo Kubota, Harvey D. Preisler, and Jun Minowada

A human round cell line, KLM-2, is considered to be precursor B-cell by immunologic surface marker analysis and histochemical studies. The effect of 12-O-tetradecanoyl phorbol-13-acetate (TPA) and/or colony-stimulating factor (CSF) on KLM-2 cells was investigated. KLM-2 cells became adherent to the bottom of the flask on day 2 after liquid culture with TPA, and the number of macrophage-like adherent cells (mAC) with pseudopodia showed a TPA dose-dependent increase to the peak level on day 3 or 4. The mAC had a phagocytic activity. The suspending cells

were positive for α-naphthyl acetate esterase (NAE) up to about 50% in culture with TPA. A TPA concentration of 100 ng/ml was optimal to provide a maximum number of mAC and NAE-positive suspending cells. Particularly, such effects of TPA on KLM-2 cells were markedly accelerated in the presence of CSF. These findings indicate macrophage differentiation of KLM-2 cells in the presence of TPA and CSF. KLM-2 cells may be a bipotential stem cell line differentiating into B-cell and monocytic-macrophage lineages.

RESULTS AND DISCUSSION

KLM-2 cells became firmly adherent to the bottom of the flask on day 2 when cultured in the presence of TPA. Those mAC had long pseudopodia (Fig. 1A) and were not detached by shaking vigorously. The mAC were able to phagocytize latex beads. Number of mAC increased to the peak level on day 3 or 4 of culture in a TPA dose-dependent manner in the presence of 10% CSF (Fig. 2) and gradually declined by day 7. This decrease after day 3 or 4 reflected detachment of mAC into the medium, because absolute number of NAE-positive suspending cells, calculated from multiplication of the total suspending cell counts by percentage of NAE-positive cells, progressively increased after day 3 or 4. TPA of 100 ng/ml was optimal in providing

hiostiocytoma, was also added at a concentration of 10%. Macrophage-like adherent cells (mAC) with pseudopodia firmly adherent to the bottom of the flask were serially counted at random under an inverted microscope (x60 magnification). Total adherent cells were counted using a hemocytometer after treatment with 0.25% trypsin in magnesium (Mg) and calcium (Ca) free Earle’s balanced salt solution at 37°C for 10 min. Phagocytic activity of mAC was tested by uptake of 1.091-μm white latex beads (Sigma Chemicals). α-Naphthyl acetate esterase (NAE) and naphthol AS-D chloroacetate esterase (CAE) of the slide preparations of the suspending cells prepared using Shandon Elliot Cytospin centrifuge were stained using a double NAE and CAE staining kit (Sigma Chemicals).

MATERIALS AND METHODS

KLM-2 cells (10⁶) were cultured in Falcon plastic tissue culture flasks, containing 10 ml of RPMI 1640 medium and 20% FCS (Microbiological Associates, Walkersville, Md., lot no. 96944) with or without varying concentrations of TPA (Chemicals for Cancer Research, Eden Prairie, Minn.) at 37°C in a fully humidified atmosphere of 5% CO₂ for 7 days. TPA was dissolved in dimethylsulfoxide, and the final concentration of this solvent in culture medium was 0.1%. This concentration of the solvent had no effect on cell growth or development of adherent cells. Colony-stimulating factor (CSF; GCT-C-CM), partially purified conditioned medium derived from a cell line established from a lung metastasis of fibrous
Fig. 1. Adherent cells (x 150) (A) and suspending cells positive for NAE (x 400) (B) developed on day 3 of culture of KLM-2 cells with TPA (100 ng/ml) and 10% CSF. Adherent cells with long pseudopodia should be noted.

Fig. 2. A change in number of adherent cells with pseudopodia per one inverted microscopic field (x 60). Each plot indicates a mean ± SD of four separate experiments. TPA 200 ng/ml + CSF (○), TPA 100 ng/ml + CSF (□), TPA 50 ng/ml + CSF (△), TPA 25 ng/ml + CSF (Δ), TPA 5 ng/ml + CSF (■), TPA 100 ng/ml in the absence of CSF (□), no TPA with or without CSF (x).
mAC over 300 per an inverted microscopic field (×60) in the presence of CSF. However, fewer mAC were developed at the same dose of TPA in the absence of CSF on day 3, which corresponded to the level observed at TPA of 25 ng/ml with CSF. In the TPA (100 ng/ml) treated group without CSF, mAC increased in number later up to day 7. A high concentration of TPA (200 ng/ml) was toxic for the growth of mAC. In contrast to the TPA-treated groups, culture of KLM-2 cells in the absence of TPA was not affected in the low TPA concentration groups (5–25 ng/ml), and culture without TPA showed an exponential cell growth. Original KLM-2 cells negative for NAE progressively grew after day 3 of culture. Addition of only CSF had no significant influence on cell proliferation.

About half of the suspending cells in the culture with TPA (100 ng/ml) and 10% CSF were positive for NAE on day 3 (Table 1 and Fig. 1B). Suspending cells had a varying ratio of positive NAE activity, depending on concentration of TPA and culture periods, and mAC were also positive for NAE. The NAE activity of suspending cells appeared first not at 12 hr, but on day 1, suggesting that acquisition of NAE activity by KLM-2 cells required at least 24 hr of contact with TPA. However, there were no NAE-positive cells in the CSF control culture without TPA. Any suspending cells in the presence of TPA and CSF on day 3 or 4. These percentages of adherent cells were lower than that observed in a TPA-treated leukemic myeloid cell line.8,10,12

Figure 3 shows a change in suspending cellularity in each group. A high concentration of TPA (200 ng/ml) was toxic, and suspending cellularity fell to about 40% of initial level throughout. In contrast, cell counts in the TPA (50–100 ng/ml) treated groups rapidly decreased to the lowest level on day 2, which reflected a growth of adherent cells, and recovered thereafter until day 7. This 2-day dip in cellularity was less affected in the low TPA concentration groups (5–25 ng/ml), and culture without TPA showed an exponential cell growth. Original KLM-2 cells negative for NAE progressively grew after day 3 of culture. Addition of only CSF had no significant influence on cell proliferation.

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Table 1. Percentage of Suspending Cells Positive for NAE and Number of Total Adherent Cells After Trypsinization (Mean ± SD of Four Separate Experiments)

<table>
<thead>
<tr>
<th>Culture Condition</th>
<th>Culture Period</th>
<th>12 hr (%)</th>
<th>1 Day (%)</th>
<th>3 Days (%)</th>
<th>5 Days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPA (ng/ml)</td>
<td>CSF(10%)</td>
<td>12 hr (%)</td>
<td>1 Day (%)</td>
<td>3 Days (%)</td>
<td>5 Days (%)</td>
</tr>
<tr>
<td>(1) 200 (+)</td>
<td>ND</td>
<td>0±0</td>
<td>1.6±0.6</td>
<td>16.4±2.2</td>
<td>12.3±0.7</td>
</tr>
<tr>
<td>(2) 100 (+)</td>
<td>ND</td>
<td>0.1±0.1</td>
<td>12.0±2.7</td>
<td>46.9±3.3</td>
<td>18.8±3.1</td>
</tr>
<tr>
<td>(3) 50 (+)</td>
<td>ND</td>
<td>0±0</td>
<td>6.6±1.9</td>
<td>38.7±5.3</td>
<td>21.1±3.5</td>
</tr>
<tr>
<td>(4) 25 (+)</td>
<td>ND</td>
<td>0±0</td>
<td>6.6±2.0</td>
<td>30.0±7.3</td>
<td>16.4±2.1</td>
</tr>
<tr>
<td>(5) 5 (+)</td>
<td>ND</td>
<td>0.7±0.8</td>
<td>15.4±2.0</td>
<td>9.4±1.2</td>
<td></td>
</tr>
<tr>
<td>(6) 100 (-)</td>
<td>ND</td>
<td>0±0</td>
<td>6.3±2.1</td>
<td>23.9±1.9</td>
<td>20.2±4.7</td>
</tr>
<tr>
<td>(7) (-) (-)</td>
<td>ND</td>
<td>0±0</td>
<td>5.6±1.7</td>
<td>2.0±1.1</td>
<td>0.6±0.2</td>
</tr>
</tbody>
</table>

Figures in the parenthesis show the percentage of total number of adherent cells.

CSF (+): addition of 10% CSF; ND, not done.

Fig. 3. A change in suspending cellularity per flask at varying culture periods. Viable suspending cells were counted using a hemocytometer. Each plot indicates a mean of four separate experiments. TPA 200 ng/ml + CSF (○), TPA 100 ng/ml + CSF (●), TPA 50 ng/ml + CSF (△), TPA 25 ng/ml + CSF (□), TPA 5 ng/ml + CSF (■). TPA 100 ng/ml in the absence of CSF (□), no TPA with CSF (x-x), no TPA without CSF (x-x-x).
10% CSF showed a phagocytic index of 35.5% ± 6.0% on day 3.

The appearance of phagocytic NAE-positive mAC with long pseudopodia by treatment with TPA apparently indicated macrophage differentiation of KLM-2 cells. Furthermore, such a differentiation was synergistically accelerated in response to both TPA and CSF. Differentiation of the precursor B-cell line cells into macrophages suggested that KLM-2 cells can be considered to be a more immature bipotential stem cell line, maturing along two cell lineages, i.e., lymphocyte (B-cell) and monocyte-macrophage, although such differentiation might be a nonspecific effect of TPA.

ACKNOWLEDGMENT

Various monoclonal antibodies were provided by Dr. Gideon Goldstein, Ortho Pharmaceutical Corporation.

REFERENCES

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Macrophage differentiation of human precursor B-cell line by phorbol ester and colony-stimulating factor

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