Human Myelogenous Leukemia: Enhanced Clonal Proliferation in the Presence of Phorbol Diesters

By H. Phillip Koeffler

Phorbol diesters, including 12-O-tetradecanoyl-phorbol-13-acetate (TPA) at low concentrations, enhanced the clonal proliferation of a human myelogenous leukemia cell line (KG-1). TPA (1.0 x 10^-7 to 5.0 x 10^-9 M) increased KG-1 clonal growth by four to tenfold in the presence of suboptimal concentrations of colony stimulating factor (CSF) and by two to fourfold in the presence of maximally stimulating concentrations of CSF. The ability of TPA analogs to enhance KG-1 clonal growth paralleled their reported ability to promote tumors in mice. Only slight CSF activity was detected in the conditioned medium from KG-1 cells cultured in liquid medium with TPA. TPA was unable to increase myeloid clonal growth of CSF-stimulated normal human marrow cells. The KG-1 cells offer a model to study TPA enhancement of CSF-stimulated growth.

MATERIALS AND METHODS

Cells

The KG-1 cell line was derived from a patient with acute myelogenous leukemia (AML). The KG-1 cells are at the myeloblast and promyelocyte stage of differentiation and the cells retain their morphological and cytochemical characteristics of AML cells. A unique feature of the KG-1 cells is their requirement of CSF for clonal proliferation in soft-gel culture.

The KG-1 cells were grown in tissue culture flasks with a modified minimum essential medium (Flow Laboratories, Inc., Rockville, Md.), 20% fetal calf serum, penicillin, and streptomycin. During their logarithmic growth phase (2-4 days after subculturing in fresh medium), the KG-1 cells were plated at 1.5 x 10^6 cells/dish in 0.3% agar as described previously. The culture dishes were incubated at 37°C in 7.5% CO₂ in air, and the number of colonies (>40 cells) were enumerated with a dissecting microscope on day 14. The KG-1 cells used for the experiments were at about passage 20-40 (which is approximately 20-40 wk after the line was established). Normal human bone marrow cells were obtained by l Barcode aspiration from normal volunteers, and the nucleated cells (1.5 x 10^6 cells/dish) were cultured in agar as described previously.

Reagents

The CSF was partially purified, conditioned medium from a continuous line of human T-lymphocytes. The specific activity of the CSF was about 1.6 x 10^6 U/mg protein. (One unit of CSF is defined as the amount of CSF required to stimulate the growth of one human myeloid colony when 1 x 10^5 cells/ml, 20% calf serum for an additional 3 days. The conditioned media were tested for colony and cluster formation by 8 and less than 40 cells) formation of both KG-1 (1.5 x 10^5 cells/plate) and normal human mononuclear, marrow cells (1.5 x 10^5 cells/plate). The conditioned media were tested in triplicate at 3 concentrations (50, 100, and 200 μl) and the clonal growth was recorded for that concentration of conditioned media that stimulated maximal clonal growth and colony formation.

A series of phorbol diesters were tested for their effect on KG-1 and normal human marrow myeloid colony formation. Stock solutions of TPA, phorbol-12, 13-didecanoate (PDD), 14-0-methylphorbol 12-myristate 13-acetate (4-O-Me-TPA), 4α-phorbol 12,
Fig. 1. Effect of TPA on clonal proliferation of KG-1 myelogenous leukemia cells. Values represent the mean of 7–10 experiments done with triplicate or quadruplicate plates. The KG-1 cells were plated at 1.5 x 10^5 cells/plate. The standard deviations are given for the mean values of plates containing CSF alone. The concentration of TPA added to the plates is shown at the end of each CSF-dose response curve. CSF is partially purified Mo. CSF. Sym- bols: ■, 2.5 x 10^-6 M TPA; △, 1.0 x 10^-5 M TPA; ◊, 7.5 x 10^-6 M TPA; □, 5.0 x 10^-6 M TPA; △, 2.5 x 10^-6 M TPA; ◊, 1.0 x 10^-6 M TPA; ●, 5.0 x 10^-7 M TPA; ◤, CSF alone.

didecanoate and phorbol (all purchased from Consolidated Midland Co., Brewster, N.Y.) were made at 10^-4 M in acetone and were diluted for each experiment with α medium to the appropriate concentration and added to the culture plates in a 100 μl volume. Acetone represented less than 0.01% of the volume of individual culture plates and when added alone, had no effect on colony formation. Epidermal and fibroblast growth factors were a gift from Dr. C.F. Fox, Department of Microbiology, UCLA; insulin was purchased from Sigma (St. Louis, Mo.) erythropoietin was provided by the National Heart, Lung and Blood Institute, and purified Pseudomonas endotoxin was purchased from Flint Laboratories.

RESULTS

As previously reported,26,27 the KG-1 cells were responsive to CSF in soft-agar culture (Fig. 1). In the absence of CSF, only rare KG-1 colonies developed, but colony-formation increased as increasing CSF concentrations were added to the plates. The KG-1 cells had a cloning efficiency of 3.3% in the presence of maximally stimulating concentrations of CSF. The addition of TPA (1 x 10^-9 – 5 x 10^-11 M) to the CSF-
stimulated culture plates markedly enhanced KG-1 clonal growth (Fig. 1). The TPA increased KG-1 colony formation four to tenfold in cultures containing suboptimal concentrations of CSF and two to fourfold in cultures containing maximally stimulating concentrations of CSF. Maximal stimulations of KG-1 proliferation were seen in the plates containing \(5.0 \times 10^{-10} M\) TPA. TPA had little effect in clonal growth of KG-1 cells in plates containing no CSF.

The interaction of other sources of CSF with TPA was examined. TPA (\(5 \times 10^{-10} M\)) increased KG-1 clonal growth 3.1-fold when added to the plates containing maximally stimulating concentrations of CSF derived from conditioned medium of PHA-stimulated human peripheral blood leukocytes (50 \(\mu l\)). Likewise, TPA (\(5 \times 10^{-9} M\)) increased KG-1 colony formation 2.4-fold when added to plates containing maximally stimulating concentrations of CSF derived from partially purified human placent al conditioned medium (25 \(\mu l\)). (Both results represent a mean of 3 experiments performed in triplicate.)

The author tested the effect of other phorbol diesters and phorbol on KG-1 clonal growth (Fig. 2). The ability of the agents to induce proliferation paralleled their reported ability to promote tumors in mice.\(^{35,36}\) TPA was the most potent stimulator of growth. PDD, an active tumor-promoter in vivo was nearly as active as TPA. 4-0-Me TPA, a poor murine tumor-promoter, was the most effective stimulator of growth. The ability of the agents to induce proliferation paralleled their reported ability to promote tumors in mice.\(^{37,38}\) Growth promoters for other cell types were also tested. Epidermal and fibroblast growth factors (0.01-1 \(\mu g/ml\)), insulin (1-100 ng/ml), and erythropoietin (1-5 U/ml), had no effect on TPA-stimulated clonal growth of KG-1 cells (data not shown).

The effect of TPA on normal human marrow myeloid colony formation was also tested (Table 1). Colony-formation occurred in the presence of CSF and the number of colonies increased as higher concentrations of CSF were added to the culture plates. No colonies developed in the absence of CSF. Addition of TPA (\(5 \times 10^{-12}-1.0 \times 10^{-10} M\)) to the cultures containing CSF produced no significant change in human myeloid colony formation as compared to culture plates containing CSF alone. A few colonies developed in plates that contained TPA but no CSF (Table 1). TPA at \(10^{-9} M\) was inhibitory to normal human myeloid colony formation.

Normal human monocytes and macrophages produce CSF. Conditioned medium from KG-1 cells cultured with \(10^{-7}-10^{-9} M\) TPA stimulated a low number of both KG-1 and normal human myeloid colonies in the absence of CSF (Fig. 3). Colony and cluster formation was less than 15% of culture plates that contained maximally stimulating concentrations of CSF. The conditioned media from KG-1 cells cultured with \(10^{-9} M\) TPA induced no KG-1 or normal human myeloid colonies. Endotoxin activates macrophages to produce or secrete CSF.\(^{39,40}\) The addition of endotoxin (0.1-10 \(\mu g/ml\)) to the TPA-exposed KG-1 cells did not increase the colony-stimulating activity of the KG-1 conditioned medium.

### Table 1. Effect of TPA on Normal Human Myeloid Colony Formation

<table>
<thead>
<tr>
<th>TPA Concentration (M)</th>
<th>Myeloid Colony Formation* (Units of Mo. CSF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>--</td>
<td>0</td>
</tr>
<tr>
<td>(1.0 \times 10^{-9})</td>
<td>0</td>
</tr>
<tr>
<td>(5.0 \times 10^{-10})</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>(2.5 \times 10^{-10})</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>(1.0 \times 10^{-10})</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>(5.0 \times 10^{-11})</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>(1.0 \times 10^{-11})</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>(1.0 \times 10^{-12})</td>
<td>0</td>
</tr>
</tbody>
</table>

*Plates contained 1.5 \(\times 10^6\) normal human nucleated bone marrow cells. Colony number is expressed as the mean ± SD of 7 experiments done in triplicate or quadruplicate.

**Fig. 3.** Effect of TPA on the production of CSF activity by KG-1 cells. Colony and cluster formation by KG-1 (hatched boxes) and normal human mononuclear, nonadherent marrow cells (dotted boxes) is shown after the cells were cultured with conditioned medium from KG-1 cells exposed to \(10^{-7}-10^{-9} M\) TPA. The values are expressed as a mean percent of control where control represents maximal CSF-stimulated KG-1 and normal human myeloid colony and colony formation. In the presence of CSF and TPA, KG-1 (\(1.5 \times 10^6\) cells/plate) formed a mean 65 ± 9 (± SD) colonies and clusters and normal human mononuclear, nonadherent marrow cells (\(1.5 \times 10^6\) cells/plate) formed an average of 142 ± 7 colonies and clusters. Values are expressed on semilogarithmic plot and represent the mean of 8 separate experiments.
TPA ENHANCED LEUKEMIC CLONAL GROWTH

DISCUSSION

The data presented shows that phorbol diesters in low concentrations act synergistically with CSF from various sources in enhancing KG-1 clonal proliferation. The ability of the various phorbol diesters to stimulate KG-1 growth paralleled their reported ability to promote tumors in mice. Other growth factors, such as insulin, epidermal, and fibroblast growth factors, did not effect CSF-stimulated clonal growth of KG-1 cells. The mechanism by which the phorbol diesters stimulate the clonal growth of KG-1 cells is not clear. Phorbol diesters probably do not have a direct colony-stimulating activity because TPA produced very little stimulation of colony formation in the absence of CSF. The study showed that conditioned medium from TPA-exposed KG-1 cells stimulated low numbers of KG-1 and normal human myeloid colonies. This cluster and colony formation was due possibly to the induction of a small amount of CSF activity by the KG-1 cells. This might suggest a self-stimulation model of leukemic cell proliferation by phorbol diesters.41 The model does not explain, however, the data that TPA increased KG-1 clonal proliferation in cultures containing maximally stimulating concentrations of CSF.

Another explanation for the results is that active phorbol diesters enhance clonal proliferation by specific or nonspecific binding to the KG-1 cells that induces the cells to become more responsive to CSF. Investigators have shown that phorbol diesters can interact synergistically with other growth factors such as insulin, somatomedin, and epidermal, fibroblast, and platelet-derived growth factors to stimulate DNA synthesis in cultures of transformed mouse and human cells.31,22 Studies have also found that TPA alters the epidermal growth factor receptors on mouse and human transformed cells.42,43

Several recent studies suggest that TPA enhances normal murine myeloid colony formation.14,24,25 The present investigation found that normal human myeloid colony growth was stimulated only slightly by TPA in the absence of CSF and not at all in the presence of CSF. This difference in results may reflect a species variation to phorbol diesters or a difference in culture technique. Likewise, it is not clear why TPA enhanced KG-1 clonal growth but not normal human myeloid colony formation. Because the KG-1 cells are a homogeneous, CSF-responsive human myeloid cell line, the cells provide the opportunity to closely examine the TPA enhancement of CSF-stimulated growth.

ACKNOWLEDGMENT

I would like to thank Leslie Lowe for superb technical help; Dr. Martin Cline for helpful discussions; Aldons Lusis and David Golde for generously providing the partially purified CSF; and Janice Furst for her assistance in the preparation of this manuscript.

REFERENCES

19. Peterson AR, Mondal S, Brankow DW, Thon W, Heidelberg


25. Fibach E, Marks PA, Rifkind RA: Tumor promoters enhance myeloid and erythroid colony formation by normal mouse hematopoietic cells. Proc Natl Acad Sci USA 77:4152, 1980


Human myelogenous leukemia: enhanced clonal proliferation in the presence of phorbol diesters

HP Koeffler